



*Synthesis of bamboo derived sulfonated solid acid catalyst for the hydrolysis of cellulose and epoxidation of cottonseed oil*

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**ADDIS ABABA UNIVERSITY**  
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**SCHOOL OF CHEMICAL AND BIO ENGINEERING**

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**“Synthesis of bamboo derived sulfonated solid acid catalyst for the hydrolysis of cellulose and epoxidation of cottonseed oil”** and submitted in partial fulfillment of the requirements for Degree of Doctor of Philosophy in Chemical Engineering (Process Engineering Stream) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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## DECLARATION

I hereby declare that the entirety of the work contained in this PhD dissertation is my own original work. To the best of my knowledge, it contains neither material previously published nor written by any other person or material which has been accepted for the award of any other academic degree in any University. Further, I have acknowledged all sources used and cited them in the reference section.

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## **Executive Summary**

Nowadays the use of biomass derived sulfonated catalyst in the place of mineral acid becomes a hot research spot. Biomass derived catalysts own multiple quality regards to environment, reuse of catalyst and in facilitating down-stream process. In this work, bamboo derived sulfonated catalysts were prepared with consecutive carbonization and sulfonation process. The synthesized catalysts were characterized and its activity was evaluated through cellulose hydrolysis and epoxidation of cottonseed oil.

The catalyst was prepared through carbonization of bamboo saw-dust at three different temperatures; 400 °C, 450 °C, and 500 °C and then sulfonated using concentrated sulfuric acid (> 96 %), at 150 °C temperature for 15 h. The synthesized catalyst was characterized by Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) analysis, scanning electron microscopy (SEM), elemental analysis, thermogravimetric analysis (TGA) coupled with mass spectrophotometer, ammonia temperature-programmed desorption (TPD), total acid content (titration method), and surface area analyzer (surface area, total pore volume, and pore size).

The FTIR spectroscopy result of the catalyst indicated a sulfonate group (-SO<sub>3</sub>H) which was not found in the respective char of the catalyst. Moreover, elemental analysis of the catalyst showed significant amount of elemental sulfur in the catalyst. The acid content analysis revealed maximum amount of acid density (0.58 mmol/g) in the catalyst which its char was prepared at 500°C.

The catalyst activity was primarily studied in the hydrolysis of crystalline cellulose. The hydrolysis was performed using autoclave reactor with varying hydrolysis temperature and time. The final product for total reducing sugars were analyzed using dinitro salicylic acid (DNS) method. From this study, the highest of total reducing sugar yield was found (4.0 %) at a temperature of 150 °C over a hydrolysis period of 8 h.

The reuse capacity of the catalyst was tested in three successive hydrolysis reactions and the results showed that the catalyst maintain its activity in each hydrolysis reaction.

The catalyst activity was further studied using microwave reactor in the hydrolysis of crystalline cellulose. The converted cellulose and glucose yield after the hydrolysis were analyzed by total organic carbon (TOC) analyzer and high performance liquid chromatography (HPLC). The combined effects of the synthesized catalyst and microwave reactor in crystalline cellulose hydrolysis resulted higher glucose yield. The effects of the synthesized catalyst on the hydrolysis of crystalline cellulose was studied with varying operating temperature and time. The maximum yield of glucose was found 43.5 % at a reaction temperature of 180°C and 60 minute of reaction time. Similar conversion and glucose yield were attained in second run showing the reusable potential of the synthesized catalyst.

Cellulose hydrolysis operating condition optimization was performed using response surface methodology. Three-factor and three- level Box-Behnken design was employed to study the effects of hydrolysis temperature, hydrolysis time and catalyst to substrate ratio on total converted cellulose and glucose yield. Additionally, prior to the hydrolysis took place, the crystalline cellulose was milled using vibrational mill. The XRD result of the milled cellulose showed that vibrational milling could decrease the crystallinity index from 96.4 % to 59.55 % and the crystallite size from 3.61 nm to 2.09 nm within 60 min milling time. The result of optimization revealed both response best explained in quadratic model. The respective analysis of variance (ANOVA) showed that the proposed quadratic models could be used to navigate the design space. The optimum hydrolysis conditions were found out to be hydrolysis temperature of 175 °C, hydrolysis time of 74 minutes and catalyst to substrate ratio of 1.25 g/g. Under these conditions the total amount of converted cellulose and glucose yield were 79.4% and 61.1%, respectively. Model validation was

performed at the given operating conditions. A cellulose conversion of  $78.5 \pm 0.75$  and glucose yield of  $60.6 \pm 0.4$  were found. These results indicated that the predicted values were in a good agreement with the experimental results.

In addition to running cellulose hydrolysis tests, the synthesized catalyst activity was tested in the epoxidation of cottonseed oil. Bamboo derived sulfonated catalyst (BSC) was used for all epoxidation reactions. The effects operating conditions such as: catalyst loading, epoxidation temperature, and amount of peroxide on the oxirane content were studied. In general, the results of relative percentage conversion of oxirane indicated that the given operating conditions significantly affected it. On the other hand, the maximum amount of relative percentage conversion of oxirane content 36.8 % was obtained at temperature of 60 °C, reaction time of 8 h, catalyst loading of 15 %, and mole ratio of hydrogen peroxide to double bond (DB) of the oil was 2.

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## List of Acronyms

<b>ANOVA</b>	Analysis of variance
<b>ASTM</b>	American standard for testing materials
<b>AV</b>	Acid value
<b>BBD</b>	Box Bhenken design
<b>BC</b>	Bamboo char
<b>BET</b>	Brunauer Emmet Teller
<b>BJH</b>	Barett Joyner Halenda
<b>BSC</b>	Bamboo derived sulfonated catalyst
<b>CC</b>	Cellulose conversion
<b>CI</b>	Crystallinity index
<b>DB</b>	Double bond
<b>DNS</b>	Dinitro salicylic acid
<b>DPE</b>	Deprotonation enthalpy
<b>EA</b>	Elemental analysis
<b>FFA</b>	Free fatty acid
<b>GC-MS</b>	Gas chromatography mass spectrometer
<b>GY</b>	Glucose yield
<b>HPLC</b>	High performance liquid chromatography
<b>HTC</b>	Hydrothermal carbonization
<b>IV</b>	Iodine value
<b>MM</b>	Molar mass
<b>MWR</b>	Microwave reactor
<b>OD</b>	Optical density
<b>RID</b>	Refractive index detector
<b>RPM</b>	Revolution per min
<b>RSM</b>	Response surface methodology

<b>TAG</b>	Triacylglycerol
<b>TC</b>	Total carbon
<b>TOC</b>	Total organic carbon
<b>TPD</b>	Temperature programmed desorption
<b>TRS</b>	Total reducing sugar
<b>UV-VIS</b>	Ultraviolet visible

# Chapter One

## 1. Introduction

### 1.1. Background

Biomass generally refers to organic materials such as wood, grass, algae, agricultural crops and their residues and wastes, including some animal wastes [1]. It is an ideal alternative to fossil resources and the only sustainable source of organic compounds which has been proposed as petroleum equivalent for the production of fuels, chemicals and materials. It can provide a rich source of sustainable commodity products, such as bio-fuels (biodiesel, bio-ethanol, and marsh gas) and chemicals (acetic acid, malic acid, acetone, lactic acid) that are now primarily derived from petroleum [2].

Lignocellulose biomass which is mainly composed of cellulose, hemicellulose and lignin is the main type of biomass that has a potential for the production biofuel and chemicals [3]. One way of lignocellulose utilization for the productions of chemicals and energy is the thermochemical processes such as gasification, pyrolysis and torrefaction [1], [4], [5]. However, these techniques have a number of drawbacks such as intensive energy consumption, require costly operational equipment, and give low yield of desired products. On the other hand, lignocellulose (cellulose) hydrolysis can guarantee the stable production of bulk liquid fuel and bio-chemicals with limited energy, if methods and infrastructures sufficiently advanced [6].

Cellulose hydrolysis can be achieved via mineral acids, enzyme, supercritical water and heterogeneous catalysts [7]–[12]. However, the use of mineral acids, enzymes and supercritical water for cellulose hydrolysis have some short comings [13]. Mineral acids create equipment corrosion, high cost of product separation and possible generation of wastes. Enzymes are expensive and difficult to recycle [13]–[15]. Cellulose hydrolysis using supercritical water creates

undesired degradation of monosaccharide with a fraction seconds, hence generating glucose like monosaccharides is too difficult [15]. Due to these reasons the search of a catalyst which is easily recyclable, low cost of production, environmentally friendly and barely corrode the operating equipment leads to heterogeneous catalysts. Consequently, heterogeneous catalysts such as acid resin, alumina, sulfonated carbon, and noble metal have been widely studied for this type of reaction.

Heterogeneous catalysts like noble metal-supported solid catalysts of Pt/Al<sub>2</sub>O<sub>3</sub> and Ru/C were tested for conversion of cellulose into sugar and which further can be converted to alcohols in the presence of enzymes [11], [16]–[18]. The use of noble metal based solid catalysts for cellulose hydrolysis was effective except the expensiveness of the catalysts[18]. On the other hand, carbon-based sulfonated catalysts were found to be economically feasible for cellulose hydrolysis reaction [12], [19].

Carbon derived sulfonated materials can be synthesized using different carbon sources such as sulfoaromatic compound and biomass sources [20], [21]. When the carbon source purely comes from biomass, the catalyst will be named biomass derived sulfonated catalyst. Biomass derived sulfonated catalysts can be synthesis from activated carbon, lignocellulose, cellulose, sugar, lignosulfonate waste, grass, and agricultural wastes [22]–[25]. The catalysts own the carbon skeleton bearing high density of Brønsted acid sites (-SO<sub>3</sub>H and -COOH) and nearly neutral phenolic -OH groups making it distinct from conventional solid acids catalysts which only have single functional group [26].

Despite their high performances as compared to other heterogeneous catalysts, the amount of glucose yield obtained by using biomass derived sulfonated catalysts was still lower as compared

to mineral acid catalysts (homogeneous catalyst). To improve cellulose hydrolysis efficiency of the catalyst, different techniques were implemented. For instance, Onda et al. reported that they had achieved 40.5% of glucose yield and 42.5% cellulose conversion in the hydrolysis of ball milled cellulose using the catalyst [27]. Similarly, higher glucose yield was reported in the hydrolysis of ionic liquid pretreated cellulose using the catalyst [13], [28]. Some researchers also reported higher monomeric sugar yield in the hydrolysis of microwave pretreated cellulose using the catalyst [29], [30]. Moreover, the use of biomass-derived sulfonated catalyst together with microwave irradiation resulted in higher amount of monomeric sugar yield [15], [31]. Bai et al. have also reported 54.7% yield of xylo-oligosaccharides in the hydrolysis of hemicellulose in the presence of sulfonated biomass catalyst at higher temperature using microwave irradiation [31]. Though hydrolysis of hemicellulose is much easier than crystalline cellulose, their study spotlight the importance of using high temperature microwave irradiation on the hydrolysis process.

Biomass-derived sulfonated catalysts with proper design and use have great potential for efficient conversion of biomass through hydrolysis, esterification and transesterification, dehydration, alkylation, oxathioketalization, acetalization reactions [23], [32]–[36]. The applications of biomass-derived catalyst can also be further expanded in other reactions that uses mineral acids as a catalyst. The main advantages which are considered in applying this catalyst is its recycle ability, free of equipment corrosion and environmentally friendly. Based on this criteria, examining the use of biomass derived sulfonated catalyst in other reaction such as epoxidation of vegetables oil will expand the catalyst uses.

The epoxidation of alkenes is a highly significant chemical reaction because epoxides are important in producing various important industrial products, such as surfactants, epoxy resins,

lubricating oils, textiles, cosmetics, surface coatings, corrosion protection agents, pesticides, detergents, sweeteners, chiral pharmaceuticals, perfumes, and so on [37].

Epoxidation of vegetable oil mainly carried out in industrial scale with the Prileschajew reaction method [38]. In this method the formation of percarboxylic acid (peracetic or performic acid) is generated by the reaction between acetic acid or formic acid with hydrogen peroxide in the presence of mineral acid as a catalyst [39]. The formed percarboxylic acid then reacts with the unsaturated oils to create Oxirane rings [40].

On the other hand, the use of mineral acids such as sulfuric acid presents several drawbacks. Separation and recovery of the mineral acid at the end of the epoxidation reaction is very difficult. Moreover, large amount of waste is generated during neutralization of the catalyst. Furthermore, technical problem such as equipment corrosion can be happened due to the mineral acid [41]. In contrast, such problems are not existed when solid catalysts used in the place of mineral acids [42]. However, most of these catalysts come from transition metal and their synthesis is a bit expensive. Therefore searching a heterogeneous catalyst which can be synthesized in low cost for alkene epoxidation should be continued. From the synthesis point of view biomass derived catalyst can be prepared with low cost compared to those of transition metals [43], [44]. Moreover, the catalyst has been seen successfully substitute mineral acids in a number of reactions [45]. Therefore, it worth to test its activity in the epoxidation reactions of vegetable oil. On top of that, it will incur additional knowledge in the applications of biomass derived sulfonated catalyst.

Ethiopia has ample biomass resources and forest residues, which can be used for the utilization of chemicals and fuels [46]. However, like several low income countries, biomass is directly utilized for the generation of basic energy needs in the form fire woods, charcoal and dung cake [47]. It is known that the consumption wood-fuel has direct relation with deforestations and causes for series

environment impact. Hence, innovative way of utilization of biomass for the production of chemicals and generation of energy is a must. Here in this work, a catalyst synthesized from bamboo was studied for its use for the hydrolysis of cellulose and peracid epoxidation of cottonseed oil.

## **1.2. Problem Statement**

The depletion of fossil fuel, the current climate conditions and continues world energy demands gears the search of renewable, low cost and environmental friendly sources of energy [48]. Due to this, different renewable sources such as wind, solar energy, hydro, geothermal energy and biomass were studied to replace fossil fuel. Among these, the utilization of biomass resources for the replacement of fossil fuel has taken immense attention. Biomass can be used as the source of energy by direct and indirect utilization [1]. Moreover, it can be used as a source of basic chemicals, food and medicine.

Biomass can be utilized indirectly, through the conversion of lignocellulose materials and bio oils. When lignocellulose materials such as cellulosic biomass properly hydrolyzed it can be used for the production of energy and chemical [49], [50]. The formed chemicals further uses either for biofuel production through fermentation or can be modified to other fine chemicals [51]. Similarly, oil from different biomass source can be converted into biodiesel, lubricants and epoxy like chemicals.

Even though cellulose is the most abundant lignocellulose material found in the world, glucose and other fine chemicals production from cellulose is not widely practiced [52]. One of the reason stated for this is the difficulty of cellulose hydrolysis due to its stiff bio-molecules structure and long inter and intra hydrogen bond chains [2], [27]. In general, cellulose is insoluble in water and other organic solvents such as ethanol, acetone and benzene [27], [53].

To tackle this problem, different advanced techniques and equipment were introduced for the conversion of cellulose biomass into chemicals and energies. For instance, supercritical hydrolysis method can convert cellulosic biomass into glucose in short period [54], [55]. In this method, cellulose can be fully hydrolyzed within a split of seconds and little waste is generated to the environment. On the other hand, the energy entail to bring the water into supercritical state and size reduction of biomass to specific range is too expensive [54]. Enzymatic hydrolysis of originally pretreated cellulose with *Cellulases* enzyme has received much attention in academia and industry [56]. Enzymatic hydrolysis of cellulose is more preferable when the synthesized glucose is used for immediate fermentation process. This method seems advantageous as the hydrolysis is performed at ambient temperature and gives high selectivity of glucose. However, it has a number of shortcoming such as high cost of enzymes, long period of hydrolysis time, high cost of equipment and operation, and the reuse of enzymes through recycling are complicated [2]. On the other hand, cellulose can be hydrolyzed with in short residence time and low cost using mineral acids (homogeneous catalysts) such as  $H_2SO_4$ ,  $HCl$  and  $H_3PO_4$ . Mineral acids have shown relatively high catalytic activity in the hydrolysis of cellulosic materials. In addition to this mineral acids can be used in the epoxidation of vegetable oil. Nevertheless, the process creates equipment corrosion, requires costly separation techniques, and produced large amount of waste in the time of separation of the catalyst from the hydrolysate and the epoxidized products [57].

All these short comings with the aforementioned method drive research works to focus on finding catalysts that are environmentally friendly, economic, easily separable from the products and recyclable. On the other hand, solid acid catalysts have an attributes such as recyclability, environmentally friendly, and have also high selectivity and efficiency [2]. Solid catalysts can be easily separated from the liquid mixture after the reaction, thereby allowing the possible reuse of

the catalysts along with minimum equipment corrosion [58], [48]. Moreover, solid acid catalysts have the potential to be easily applied to a continuous flow fixed-bed reactor.

Different solid catalyst were tested for the conversion of lignocellulose biomass and bio oil into valuable chemicals [59]. Metal supported solid catalyst showed an excellent activity in the hydrolysis of cellulose and conversion of some biomasses into value added chemicals [17]. However, synthesizing of these catalysts are expensive and it also needs improvement in the catalytic activity and production costs [60]. Consequently, with the aim of improving the efficiency of cellulose hydrolysis, the search of a solid catalyst which can be produced with low cost, environmental friendly and easily recyclable come across with biomass derived sulfonated solid catalyst.

The use of biomass derived sulfonated catalyst in the hydrolysis of crystalline cellulose and in the utilization of bio oil have been reported vastly. Moreover, biomass derived sulfonated catalyst also used in different reaction substituting mineral acid and expensive metal catalysts such as; Esterification, transesterification, dehydration, transformation, alkylation, acetalization and oxathioketalization. Furthermore, it has a potential to be used in any reaction substituting mineral acids [61], [62].

Even though the catalyst has such a potential, its industrial efficiency in converting crystalline cellulose and other biomass are very low. On the other hand, to improve the industrial efficiency of the catalyst different techniques were applied in the synthesis of the catalyst. For example, different biomasses were tested for the synthesis of the catalyst precursor. In addition to this a number sulfonation techniques were applied to improve the acid density of the catalyst. In contrary, little attention is given for carbonization temperature biomass. Since biomass

carbonization temperature significantly affects the final catalyst structure and functional group density, studying the effects of carbonization temperature will lead to find the best temperature for carbonization of the biomass.

Similarly, the effects of reactor type on catalyst activity has not got enough attention. An autoclave reactor is commonly used for all cellulose hydrolysis reaction. However, the use of microwave reactor for the pretreatment of biomass had shown promising results [30], [63]. Moreover, higher yield of 5-hydroxymethylfurfural and glucose yield were reported in microwave-assisted dilute acid cellulose conversion [64], [65]. Bai et al., reported higher yield of pentose sugar in the combined use of biomass derived sulfonated catalyst and microwave reactor in the hydrolysis of hemicellulose [31]. This result spot light the potential uses of biomass derived sulfonated catalyst when it used in microwave reactor for the hydrolysis of crystalline cellulose. Hence, it is worthwhile to study the activity of the catalyst for crystalline cellulose hydrolysis using microwave reactor.

Another area which are given little attention in crystalline cellulose hydrolysis using this catalyst is cellulose hydrolysis operating condition. So far few works have been done concerning optimization of hydrolysis operating condition. On the other hand, optimization of operating conditions helps to identify which operating conditions significantly affect the hydrolysis process and the best operating conditions for pilot plant test. The efficiency of the catalyst can be further improved, if prior milled cellulose is used for the hydrolysis. However, the frequent used ball-milling techniques for cellulose milling requires longer time. Finding fast milling techniques will improve the demand of the catalyst for cellulose hydrolysis.

Finally, the study on the use of the catalyst in substituting mineral acids catalyzed reaction has to be continued since its avoid the disadvantage comes from the use of mineral acids. Compared to mineral acids solid catalyts in epoxidation of vegetable oil have a number of advantages such as; easy separation from the product, environmentally sound, and it avoids equipment corrosion [42]. Hence, the study of bamboo derived sulfonated catalyst for the peracetic formation in the epoxidation of vegetable oil is an important task.

### **1.3. Objectives:**

#### **1.3.1. General Objective**

The general objective of this research is to synthesize and characterize a bamboo derived sulfonated solid acid catalyst and tests its performance in catalytic hydrolysis and epoxidation of cellulose and cottonseed oil respectively.

#### **1.3.2. Specific Objectives**

The specific objectives of this dissertation are;

- To synthesize bamboo derived sulfonated solid acid catalysts and characterize the chemical composition, phase structure, and examine the activity through cellulose hydrolysis using autoclave reactor.
- To examine the performance (activity, selectivity, and reusability) of the synthesized catalyst through cellulose hydrolysis at higher temperature using microwave reactor.
- To investigate the optimal cellulose hydrolysis operating conditions (hydrolysis temperature, time, and synthesized catalyst loading ratio) by response surface methodology in microwave reactor using vibrational milled cellulose.
- To investigate the performance of carbon based sulfonated solid acid catalyst during epoxidation of cottonseed oil.

### **1.4. Scope of the study**

The aim of this study is to synthesis bamboo derived sulfonated catalyst for the hydrolysis of crystalline cellulose and epoxidation of cottonseed oil. The catalyst activity is applied the

hydrolysis of crystalline cellulose using different reactors. The catalyst further tested in the hydrolysis of vibrational milled cellulose using microwave reactor. Finally, the catalyst was used for the epoxidation of cottonseed oil.

### **1.5. Significance of the study**

Recently the use of biomass derived sulfonated catalyst for the hydrolysis of cellulose has been given immense attention. However, the catalyst activity is more pronounced when it is used in ball-milled cellulose or in the presence of ionic liquid. On the other hand, due to elongated milling time and expensiveness of the ionic liquid these methods needs to be replaced. One way improving the catalyst activity is the use microwave reactor. Hence, one of the significance of this dissertation will be providing basic information on the combined effects of the catalyst and the reactor in crystalline cellulose hydrolysis. Moreover, the introduction of alternative milling system such as vibrational milling technique will shorten the required time for cellulose milling. Consequently, more time will be saved, less energy will be spent, and most importantly the activity of the catalyst will be increased. The catalyst activity will be further improved, if cellulose hydrolysis operating conditions are optimized, which has not been given any attention so far. Finally, replacing mineral acids in the peracetic epoxidation of cottonseed oil will further expand the application of the catalyst.

Hence, to improve the catalyst activity in crystalline cellulose hydrolysis, to optimize the cellulose hydrolysis operating condition, and to expand further the application of the catalyst it is important running cellulose hydrolysis and epoxidation of cottonseed oil using bamboo derived sulfonated catalyst.

## 1.6. Organization of the thesis

This dissertation is composed of eight chapters:

**Chapter 1** provides the background of the study, brief overview of the problem, significance of the study and potential contributions of the study in the area of biomass valorization (crystalline cellulose hydrolysis and epoxidation of cottonseed oil) using bamboo derived sulfonated catalyst.

**Chapter 2** reviews current and past issues related to the potential use of biomass in substituting petroleum and its derivative chemical, the use of lignocellulose biomass in energy and chemical production, cellulose hydrolysis techniques, synthesis of biomass derived sulfonated catalyst, characterization techniques of biomass derived sulfonated catalyst, epoxidation reaction, epoxidation of vegetable oil using solid catalyst, the possible use of bamboo derived sulfonated catalyst for the production of epoxy.

**Chapter 3** outlines the general methodology implemented in the preparation of bamboo derived sulfonated catalyst, in the characterization of the synthesized catalyst, in testing the activity of catalyst through cellulose hydrolysis and vegetable oil epoxidation reactions.

**Chapter 4** describes the synthesis of three bamboo derived sulfonated solid acid catalysts using bamboo chars which were initially carbonized at three different temperatures, characterization and selecting of the best out of the three catalysts, studies the performance of the selected catalyst through cellulose hydrolysis in autoclave reactor, and the use of DNS method for glucose yield analysis.

**Chapter 5** investigates direct hydrolysis of crystalline cellulose (96% of crystallinity with 3.61 nm average crystallite size) at higher temperatures using microwave reactor (MWR) in the presence of bamboo derived sulfonated catalyst.

**Chapter 6** deals about the optimization of cellulose hydrolysis using bamboo derived sulfonated catalyst in MWR through response surface methodology (RSM). Three factor and three level Box-

Behnken design was employed to study the effects of hydrolysis temperature, hydrolysis time and catalyst to substrate ratio on total converted cellulose and glucose yield.

**Chapter 7** investigates the physico-chemical properties cottonseed oil and the use of bamboo derived sulfonated catalyst for the epoxidation of cottonseed oil.

**Chapter 8**, the final chapter of the dissertation, it provides the general conclusion of the study, contribution to science, and recommendations made from the research findings.

## Chapter Two

### 2. Literature Review

#### 2.1. Biomass feed stocks used for fuel and chemical production

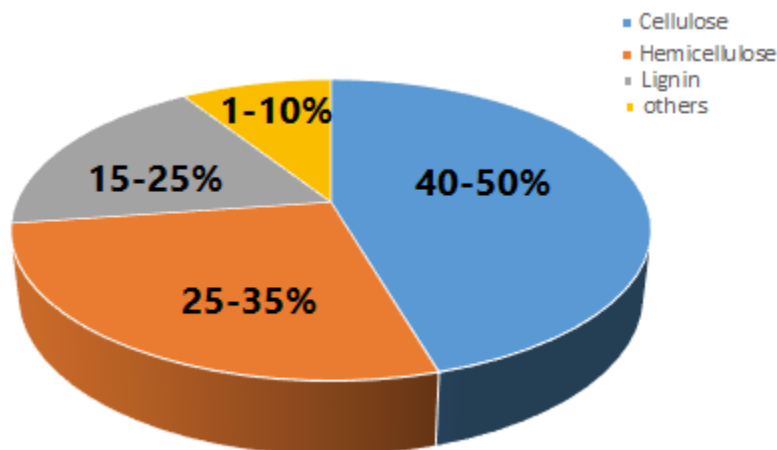
From the chemical viewpoint biomass is a composite material, constituted by a mixture of hemicelluloses, cellulose, lignin extracts and mineral elements [3]. Biomass derived feedstocks, which are used for the generation of fuel and chemicals can be classified into three groups: starchy feedstocks, triglyceride feedstocks, and lignocellulosic feedstocks [6]. Starchy feedstocks are mainly composed of glucose polysaccharides, which are easily decomposed into monosaccharides sugar, making it the best feedstock for the production of bio-ethanol. On the other hand, the use of starchy feedstocks for energy and chemical production is limited because many of starchy biomass are also used for food consumption [66].

Triglycerides feedstocks are comprised of fatty acid and glycerol from both animal and plant sources [67]. Vegetables oils, algal oil and some waste oils are the main source of triglycerides. Triglycerides mainly used for the production of bio-diesel and some fine chemicals like epoxy resin which was previously produced only from petroleum [67]–[70]. Some of the triglycerides sources are also used as human feed, and considering the expansion of the current food demand it is better to search non-edible triglycerides sources or waste vegetable oil to avoid the competition with human feed.

The presence of triglycerides and starch are limited to some crops and vegetables and most of them are also used for human consumption. The production of energy and chemicals using these feedstock will bring about unnecessary competition with food resulting in food shortage and land scrambling. This in turn makes the production cost of energy and chemicals from these sources expensive. In contrast, lignocellulose biomass which can grow in a little effort in any land exists

in abundant supply of it with low-cost, which can guarantee stable production of bulk solid, liquid and gaseous fuel, and chemicals through thermochemical and bio-chemical conversion under gasification, pyrolysis, torrefaction and hydrolysis [49], [51], [71]. Lignocellulose biomass is a collective name given for bio based macro molecules such as cellulose, hemicellulose, and lignin [72]. These macromolecules are the main constituent of the structural materials which is produced by plants to create cell walls, leaves, stems and stalk.

Lignocellulose is mainly composed of cellulose, hemicellulose and lignin and also contains minor amount of minerals and extractive [73]. As shown in figure 2.1 the major constituent of lignocellulose biomass is cellulose and it accounts for 40-50%, whereas hemicellulose and lignin shared 25-35% and 15-20% of the constituent of lignocellulose biomass respectively.



*Figure 2-1 Lignocellulose constituent [74]*

## **2.2. Potential of lignocellulos for the production of energy and chemicals**

The ambition in the utilization of lignocellulose biomass for the substitution of non- renewable energy and chemical sources is more focused on the unlocking of the lucrative potential of cellulose for the production of chemicals and energy. One of the reason is cellulose is the most abundant organic molecule found in the earth. Globally, it is estimated that the annual synthesis of

cellulose by plants nears to  $10^{12}$  ton [74]. It is a fibrous, tough, water-insoluble substances, which is found in the protective cell walls of plants particularly in stalks, stems, trunks and all wood portions of plant tissues [75]. Parallel to this the majority of composition of lignocellulose biomass is cellulose. Cellulose by itself is currently used as; reinforcing component in bio-composite materials [76], pharmaceuticals [77], textile [78], paper and pulp industries [79]. In addition to this, cellulose can be hydrolyzed to glucose, which is the base for synthesis of bioethanol through fermentation and the production of platform chemical through dehydration and rehydration reaction [80].

However, this doesn't mean that other constituent of lignocellulose such as hemicellulose and lignin cannot be used as renewable sources [6]. In fact, hemicellulose consists of short and branched hydrocarbons which can easily be hydrolyzed to give both five and six carbon sugars. Composition of hemicellulose such as pentose and hexoses varies from plant to plants, for example hemicellulose found in hard wood rich in five-carbon sugars those from soft wood more delivered six carbon sugars [81]. On the other hand, if production of bio-ethanol is the main aim of the hemicellulose hydrolysis, multiple type of yeasts will be required as the hydrolysate is composed of pentose and hexoses sugars [82]. Hemicellulose can be a source of platform chemicals such as xylose if hydrolyzed over proper catalyst. Xylose can be converted to xylitol through hydrogenation and it also gives furfural by acid dehydration. Xylitol and furfural used in many of chemical, food, and pharmaceuticals industries [83].

Lignin, a second to cellulose as the most plentiful renewable carbon source on Earth, is a complex and highly cross-linked aromatic polymer [84]. It is a macromolecule whose typical molecular weight exceeds 10,000. The presence of aromatic compound makes lignin higher value fuel additives and can significantly enhance the competitiveness of bio-refinery technology [17]. On

the other hand, due to its structure, lignin is more difficult to process, extract hydrolyze or react than cellulose or hemicellulose.

Lignin has a potential to be used as a transportation fuel if properly degraded over noble metal catalyst [17]. Carbonization of lignin using super- and subcritical solvents could generate chars, gaseous and liquid products [85]. The utilization of lignin for chemical production still needs improvement both in technology and production cost.

In general, lignocellulose conversion for sustainable energy and chemical production has great potential due to their low cost and high availability. Study in these areas should be geared towards to wide-open the conversion of cellulose to glucose and other monomeric sugars through cellulose hydrolysis because cellulose is the most abundant resource compared to both hemicellulose and lignin.

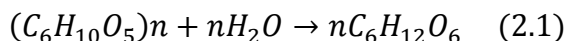
In addition to cellulose, triglyceride feedstock can also be used to produce chemicals of high importance. Both edible and non-edible triglycerides, such as *Jatropha*, castor, cottonseed oil, soya bean oil and other are widely used for the production of biodiesel, epoxy and other fine chemicals [86]–[88]. The annual production of biodiesel from triglycerides is increasing continuously throughout the world due to a number of reasons.

Further search on the substitution of petroleum derived chemicals from triglyceride (vegetable oil) leads to epoxidized products. Epoxidized oils contain epoxide groups or oxirane rings, which come from the modification of the unsaturation present in the vegetable oils by epoxidation reaction [69]. Epoxides are very crucial chemicals because they can undergo a number of reactions to produce different chemical products [70]. Previously, epoxy oil was commercially produced from non-renewable petroleum sources. However, the finding of vegetable oil potential for the production of epoxy oil shifts the commercial production to vegetable oil. Despite of this, the

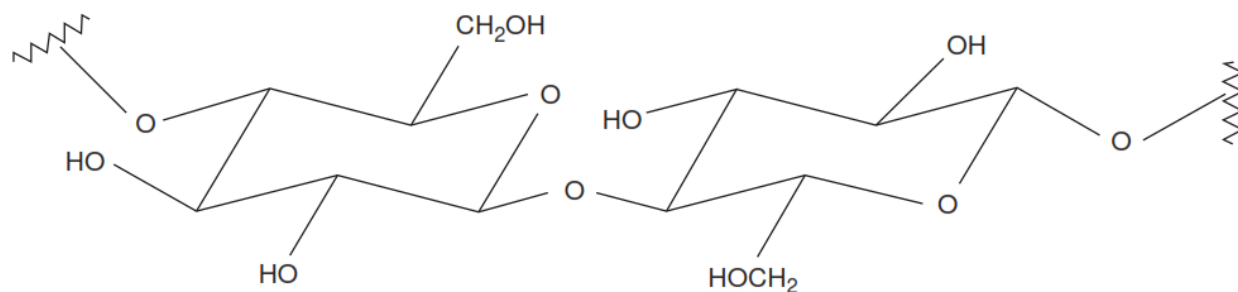
production of epoxy oil from triglycerides mainly conducted with percarboxylic acids in the presence of mineral acid catalyst. All in all this thesis will focus on the synthesis of bamboo derived sulfonated catalyst and testing its activity in the hydrolysis of crystalline cellulose for ultimate production of fermentable sugar (glucose). Furthermore, the activity of the catalyst will be tested for the production of epoxy group using cottonseed oil.

### **2.3. Conversion of cellulose into glucose**

Utilization of cellulose for the production of biofuel as well as plat form chemicals starts from synthesizing of glucose from cellulose, since cellulose is composed of a homo-polymer consisting of many glucose units connected through  $\beta$ -1,4-glycosidic bonds [74]. Glucose and other oligosaccharides can be recovered by breaking down of this bond through hydrolysis reaction. Hydrolysis literally means reaction with water. It is a chemical process in which a molecule is cleaved into two parts by the addition of a molecule of water [89]. One fragment of the parent molecule gains a hydrogen ion ( $H^+$ ) from the additional water molecule. However, under normal conditions, only a few reactions between water and organic compounds occur. Generally, strong acids or bases must be added in order to achieve hydrolysis where water has no effect. Cellulose hydrolysis is a process related to the breakage of  $\beta$ -1, 4-glycosidic bonds in the reaction as given by equation 2.1.



For the case in which there are no by-products or side reactions, glucose is the major product of cellulose hydrolysis [49].



*Figure 2-2 Fragment (repeating unit) of a cellulose chain [75]*

Although the synthesis of glucose and other water soluble oligomers are possible through hydrolysis reaction of cellulose, it is a very difficult process as the cellulose structure is composed of a robust crystal structure with high chemical stability. Two general methods used to hydrolyze celluloses are mechanical (supercritical hydrolysis) and chemical (enzymatic and acid hydrolysis) hydrolysis [49].

### **2.3.1. Mechanical (Supercritical) hydrolysis**

Cellulose can be hydrolyzed into glucose when it combines with water at state of supercritical phase. In such a supercritical state, the process would have a temperature and pressure around 400 °C and 230 bar respectively, on top of these the water would be expected to act as an acid, and it can hydrolyze the cellulose into glucose [58]. Supercritical hydrolysis method can convert cellulosic biomass which is derived from hardwood, softwood, agricultural residue, grass, algae, paper, waste lignocellulosic biomass, thermally processed cellulosic biomass, chemically processed cellulosic biomass, or combinations [54],[55]. The size of the biomass best suited for the process is in the maximum of 500 µm.

When it is compared to conventional hydrolysis method (enzymatic and acid), supercritical hydrolysis of cellulose is more environmental sound process than acid hydrolysis and it requires small period of time in contrast to enzymatic hydrolysis, however the energy entail to have the

aforementioned pressure, temperature and size reduction of biomass to specific range is too expensive [54].

### **2.3.2. Chemical hydrolysis**

#### ***2.3.2.1. Enzymatic hydrolysis***

Enzymatic hydrolysis of cellulose with cellulases enzyme after pretreatment of cellulose has received much attention in academia and industry [56]. Cellulases are a mixture of endoglucanases, exoglucanases and  $\beta$ -glucosidase, and catalyze the degradation of cellulose to oligomers according to the following proposed mechanism: (1) endoglucanase arbitrarily attacks  $\beta$ -1, 4 glycosidic bonds in the long chain polymer, and free non reducing ends are created; (2) exo-glucanases, act on the non-reducing ends to liberate cellobiose from the glucan chain ; and (3)  $\beta$ -glucosidase, which splits cellobiose into glucose monomers [90], [91]. The activity of cellulases enzymes depends on substrate concentration, end-product inhibition, reaction temperature, time and pH of the reaction. Since cellobiose inhibits cellulase activity, it is important to remove it immediately as it is formed [92].

Enzymatic hydrolysis of cellulose is more preferable when the synthesized glucose is used for fermentation process. This method seems advantageous as the hydrolysis is performed at ambient temperature and gives high selectivity of glucose. However, it has a number of shortcomings such as high cost of enzymes, long period of residence time and reuse of enzymes through recycling are complicated [2].

#### ***2.3.2.2. Acid (Direct) hydrolysis***

Chemical transformations of substrates using homogeneous catalysts are always attractive since these methods prevail over the mass transfer limitations to allow efficient interactions between substrates and catalysts active sites [50]. Cellulose with its robust crystalline structure is generally

resistant to hydrolyze in water [58]. As such, the hydrolysis of cellulose usually involves the use of both inorganic liquid mineral acids such as  $\text{H}_2\text{SO}_4$  and  $\text{HCl}$ ,  $\text{H}_3\text{PO}_4$  and organic acids such as various carboxylic acids and p-toluene sulfonic acid. It is a reaction in which hydrogen cations ( $\text{H}^+$ ) and hydroxide anions ( $\text{OH}^-$ ) from the splitting of molecules of water react with polymeric cellulose molecules, thereby yielding hydrolytic products by depolymerization [93].

Cellulose hydrolysis in the presence of acids primarily yields reducing sugars, which are a class of compounds that have an open chain with an aldehyde or a ketone group. More importantly, various products such as glucose, cellobiose and oligosaccharides can be readily formed from liquid acid-catalyzed hydrolysis of cellulose as shown in figure 2.3.

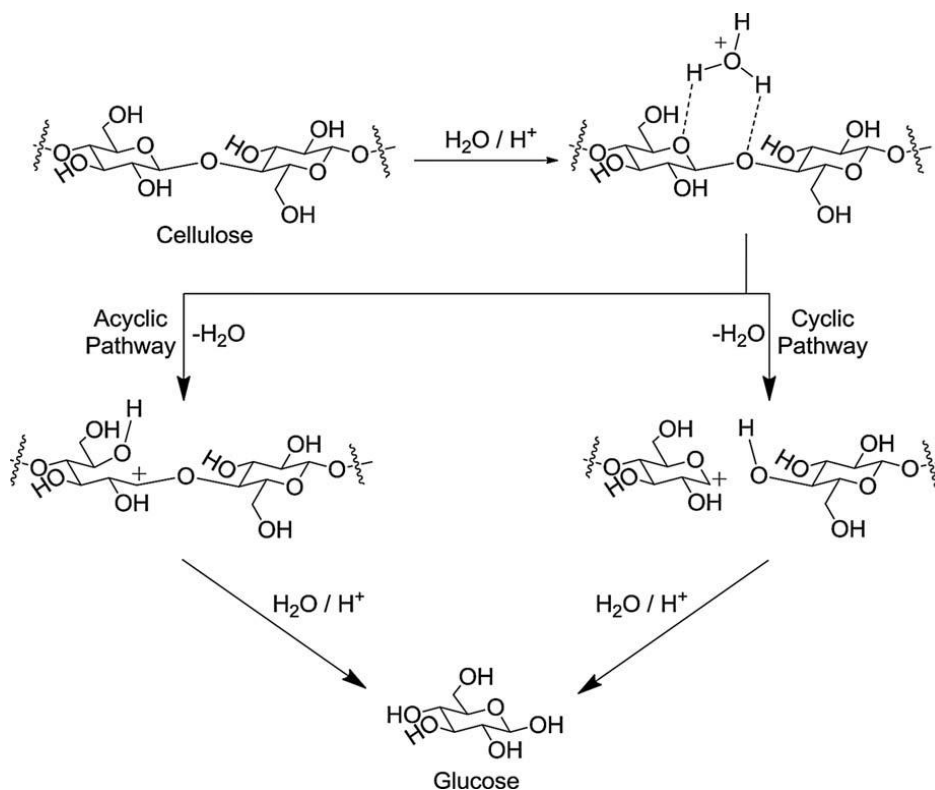


Figure 2-3 Mechanism of acid catalyzed cellulose hydrolysis into glucose [50]

Moreover, using a multistep fractionation process, various useful chemicals and potential fuels can also be directly obtained from acid processing of cellulose [94].

Despite the relatively high catalytic activity of  $\text{H}_2\text{SO}_4$ ,  $\text{HCl}$ , and  $\text{H}_3\text{PO}_4$  in the hydrolysis of cellulosic materials, by and large their uses are still uneconomical because the process suffers from equipment corrosion, costly separation process, and high generation of wastes in the time of separation of the catalyst from the hydrolysate [57].

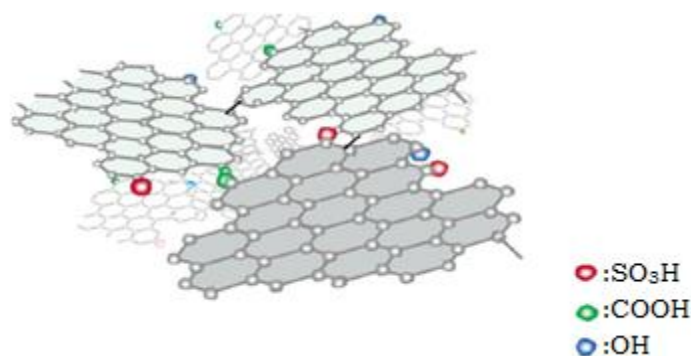
All these shortcomings with the aforementioned method derive research works to focus on finding catalyst which can be synthesized with low cost, easily separated from the hydrolysate and recyclable. Solid acid catalysts are an ideal candidate for such type of quality, indeed a number of solid acid catalyst used for cellulose hydrolysis as well as in other biomass utilization reaction. Unlike mineral acids, solid acid catalysts have several technical and economic 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 minimum of corrosion [58], [48]. Moreover, solid acid catalysts have the potential to be easily applied to a continuous flow fixed-bed reactor.

#### **2.4. Hydrolysis of Cellulose using solid acid catalyst**

A solid acid catalysts defined as ‘solid materials having acidic properties because of the presence of either Brønsted acid sites or Lewis acid sites or both’. Solid acid catalyst includes materials such as zeolites, ion exchange resins, functionalized mesoporous silica, functionalized carbons, functionalized and supported metal oxides, heteropoly acids and others [50].

Solid acid catalysts with proper design and use have great potential for efficient conversion of biomass through hydrolysis, esterification and transesterification vegetables oils. Among a series of typical solid acid catalysts carbonaceous solid acid catalysts (carbon based sulfonated material) showed remarkably higher activity and glucose selectivity in cellulose hydrolysis reaction [95].

The catalysts own the carbon skeleton bearing high density of Brønsted acid sites ( $-\text{SO}_3\text{H}$  and  $-\text{COOH}$ ) and nearly neutral phenolic  $-\text{OH}$  groups as shown in Figure 2.4. The presence of carboxylic and phenolic hydroxyl groups make carbonaceous sulfonated catalyst differs from other solid acid catalysts, that bear single functional group [96]. Consequently, the presence of these functional groups increases the catalyst activity indifferent reactions. On top of that, it has high hydrothermal stability because its surface composed with hydrophobic planes [96]. Moreover, the carbon skeleton is stable and insoluble in most acidic/basic conditions as well as organic solvents [27].



*Figure 2-4 Structure of carbon based sulfonated catalyst [97]*

On the other hand, the catalyst has low activity in crystalline cellulose hydrolysis due to diffusion limitations and thus gives low yield of glucose compared to mineral acid catalysts. Hence to avoid such limitation prior treatment of cellulosic material to reduce its size and to decrease the crystallinity before running the hydrolysis reaction is advisable [27], [13]. Furthermore, the use of water as hydrolysis media facilitate cellulose conversion as stated by a number of research work. However, excess presence of water in the hydrolysis reaction can poison the catalyst by forming a solvation layer around the acid sites of the catalyst by forming hydrogen bonding [98]. On the other hand, excess presence of water had a positive effect on the breakage of  $\beta$ - 1, 4 glycosidic bonds and intra-molecular hydrogen bonds in insoluble cellulose, thereby leading to more

hydrolytic products. *Yamaguchi* and co-workers [99] revealed that the amount of water comparable to the solid catalyst weight could lead to a maximum yield of glucose in the heterogeneous catalytic hydrolysis reaction of cellulose. It becomes absolutely critical to design and develop biomass derived sulfonated catalyst that are hydrothermally stable and tolerate water poisoning.

## **2.5. Synthesis of biomass derived sulfonated catalyst**

Carbon based sulfonated materials can be derived in two main routes; incomplete carbonization of sulfoaromatic compound and/or sulfonation of incompletely carbonized organic matter [26]. Sulfoaromatic compounds can produce higher amount of  $-\text{SO}_3\text{H}$  groups ( $> 4 \text{ mmol/g}$ ), but in large scale production has a safety issue as the carbonization of sulfoaromatic compounds conducted in the presence of sulfuric acid at temperature of 473 K. When incompletely carbonized organic matter (biomass) sulfonated in concentrated  $\text{H}_2\text{SO}_4$  it can give carbonaceous sulfonated catalyst [21]. The amount of  $-\text{SO}_3\text{H}$  group found in the sulfonation of incompletely carbonized organic matter ( $< 2.5 \text{ mmol/g}$ ) is lower than sulfoaromatic compound. However, sulfonation of incompletely carbonized organic matter is preferred to prepare carbonaceous sulfonated solid catalyst because it doesn't bring any safety issue during preparation.

The first biomass derived sulfonated solid acid catalyst was prepared by sulfonation of incompletely carbonized low molecular sugar [21]. Incomplete carbonized organic matter can be prepared from sugars, cellulose, starch, lignocellulosic and other biomass sources [13], [100]–[103]. Biomass derived sulfonated catalysts which were synthesized from different biomass sources are stated in Table 2.1. As shown in Table 2.1, the versatile use of biomass derived sulfonated catalyst makes it the more preferable type of solid acid catalyst to be studied.

Table 2-1 possible uses of biomass based sulfonated carbon catalyst

No	Catalysts that can be substituted by carbon based sulfonated catalyst	Reactions that can be catalyzed by carbon based sulfonated catalyst	Reference	
1	Concentrate and dilute H <sub>2</sub> SO <sub>4</sub> , HCl, NaOH	Hydrolysis reaction for glucose and other lower molecular sugar production	Microcrystalline cellulose	[104]
			Hemicellulose	[81]
			Cellobiose	[103]
			Lignocellulose Biomass	[105], [106]
			Cyclohexayl acetate	[107]
2	NaOH, H <sub>2</sub> SO <sub>4</sub> , KOH, Lipase	Esterification and transesterification reaction for production of biodiesel	Alcohols	[32]
			Vegetable oil	[61]
			Organic acid	[23]
3	Tyrosine, InCl <sub>3</sub> , CrCl <sub>3</sub>	Dehydration and transformation reaction, of monosaccharide and biomass into in the synthesis of furfural and HMF	Fructose, Hexose and lignocellulose	[34], [108], [109]
4	Mineral acid	Alkylation	Phenol	[35]
5	Mineral acid	Oxathioketalization	Cyclohexanone and Mercaptoethanol	[36]
6	Sulfuric acid	Acetalization	Benzaldehyde	[23]
			Glycerol	[110]

Biomass derived sulfonated catalysts can be synthesized in a number of ways, the most common are: one step (pot) synthesis and/or successive carbonization and sulfonation methods as shown in Figure 2.6.

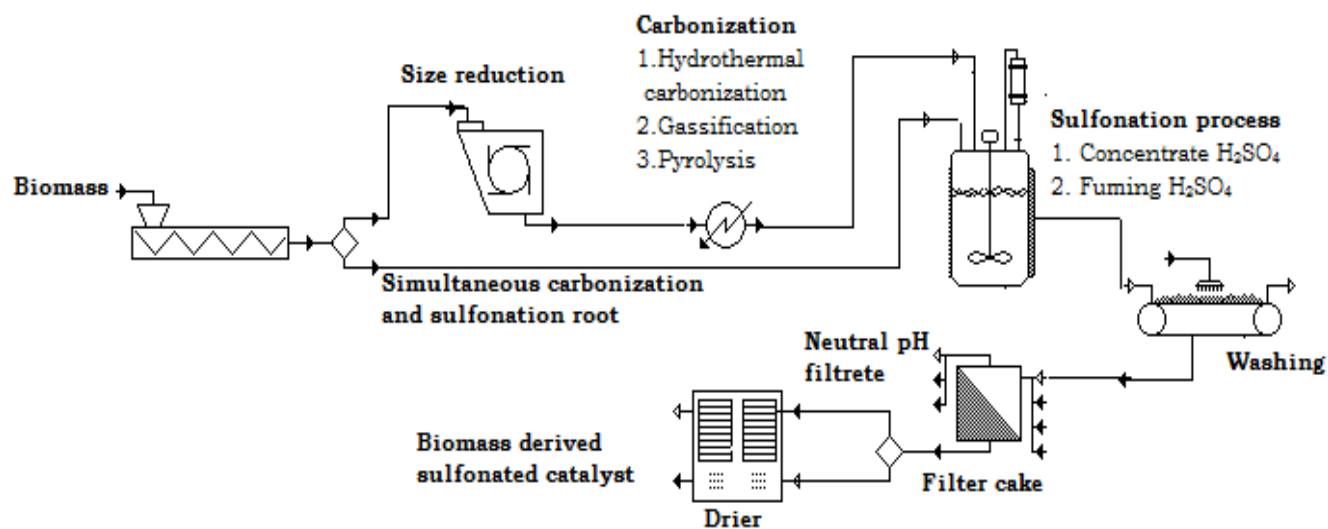


Figure 2-5 Conventional method of biomass derived sulfonated catalyst synthesis

### 2.5.1. One step (pot) preparation of biomass derived sulfonated catalyst

In one step synthesis method the carbonization of biomass as well as the sulfonation is conducted at the same time in well-sealed reactor in the presence of sulfonating agent such as P-toluene sulfonic acid, concentrate sulfuric acid, and hydroxyethyl sulfonic acid as shown in Table 2.2.

The main advantage of this method is relatively needs low energy, short period of preparation, and limited number of equipment for the synthesis. On the other hand, this method to be effective to chemically attach the  $-SO_3H$  in to the precursor, only when single compound like pure cellulose, glucose, and cellobiose are used as carbon precursor or it has to be thoroughly milled biomass. The other disadvantage is, it needs to use higher amount of concentrated sulfuric acid to carbon weight ratio compared to the successive carbonization and sulfonation method.

*Table 2-2 Selected list of one pot synthesis of biomass based sulfonated catalyst*

Carbon precursor	Sulfonating agent used	Surface Area (m <sup>2</sup> /g)	Total acid density (mmol/g)	Amount of SO <sub>3</sub> H group (mmol/g)	Catalytic activities tested reactions	Reference
Glucose with resorcinol	P-toulene sulfonic acid	97	3.23	1.39	Acetylaziation of benzaldehyde	[23]
Glucose	P-toulene sulfonic acid	<1	2	1.3	Dehydration of fructose	[111]
Distiller grain	Concentrate sulfuric acid	-	1.73	0.89	Esterification of acetic acid and butanol	[112]
Kneafcore powder	Concentrate sulfuric acid	-	1.91	0.15	MCC hydrolysis	[113]
Furaldehyde	Hydroxyethyl sulfonicacid	-	2.4	2.25	Esterification of acetic acid and butanol	[36]
Bamboo powder	P-toulene sulfonic acid	1.2	3.2	1.9	Dehydration of fructose	[109]

### **2.5.2. Synthesis of biomass derived catalyst by successive carbonization and sulfonation**

Synthesis of biomass derived sulfonated catalyst by successive carbonization and sulfonation method is the most popular one as it allows to use a cheap biomass as a source carbon precursor. Because of this many researchers use this method to prepare the catalyst from different biomass sources. Here in this method the biomass should be carbonized first to generate the char (disorganized amorphous carbon structure) through different carbonization techniques such as gasification, hydrothermal carbonization, and pyrolysis. The char from the carbonization process can be used as a catalyst for certain reaction [114]. However, certain reaction particularly for

valorization of biomass require the presence of strong acid [26], [61], [62]. One of the techniques uses for introducing this strong acid sites into biomass char is sulfonation. The addition of sulfonic acid group or the corresponding salt or sulfonyl halide group (-SO<sub>2</sub>Cl) chemically into an organic compound is stated as sulfonation [115].

The catalyst activity mainly depends on the quantity of the sulfonate groups present in the end of the sulfonation process. One of the factor which influences the quantity of the sulfonate group is the structure and the content of the carbonized char. Carbonization operating condition such as temperature, time, temperature ramp and biomass source are the main factor control the final state of the char [116], [117]. In addition to these, ways of carbonization such as gasification, hydrothermal carbonization and pyrolysis significantly affect the yield and functional groups of the chars. The other important factor affect the quantity of sulfonate group is the sulfonation process of the char.

#### ***2.5.2.1. Biomass carbonization***

In successive carbonization and sulfonation way of synthesizing biomass derived catalyst, the first task is incomplete carbonization of the biomass or formation of a char. When a plant or/and animal residues is heated in a confined environment with a temperature of less than 700 °C, a carbon rich material (char) is generated [118],[114]. Once the char is produced with intent to use as soil amendment it is called biochar [118]. The conversion of biomass to char can be achieved through dry environment in solid form either using pyrolysis or gasification or in slurry form in wet environment by hydrothermal carbonization (HTC). The char formed through HTC is known as hydro-char [119].

The char generated from gasification method is relatively small and as the main aim of the gasification is generating synthesis gas, light hydrocarbon, liquid fuel synthetic natural gas small

amount of char [120]–[122]. The char from the gasification less likely used for sulfonation process this is due to the leftover from the process counted only 10 % of the total input to the gasification which needs a lot of biomass should be gasified and the second reason is the gasification temperature reaches 700 °C and the carbon structure at this temperature will be less dis-organized which will need a lot of effort to chemically attach the -SO<sub>3</sub>H group [123].

In hydrothermal carbonization carbon materials from different sources like carbohydrates, organic matter and waste biomass are treated in the presence of water at a temperature (150 -300 °C) [124]. To keep the water in liquid phase, the pressure must be raised and which become harsh condition for the carbon material to maintain its insolubility [125]. Furthermore other physico-chemical changes such as dehydration, de-polymerization which appears in organic molecule will be inevitable and at last through precipitation the char will be formed. The char can be separated from other gaseous product and bio-oil by first cooling down the reactor to room temperature and applying physical separation techniques such as filtration and/or centrifugation [62], [126]. The HTC technique is more preferred when the given biomass is relatively wet and which is difficult to handle neither in pyrolysis or gasification which needs prior drying of the biomass [127]. Low surface area char is obtained during HTC process [96] but the surface area can be significantly improved by treatment of the biomass in the process of HTC or post carbonization treatment [128]. However, treated char has less tendency to sulfonate in sulfur compound and the final catalytic activity would be lowered [108].

Pyrolysis is a thermochemical process in which, biomass is changed in to char and other bio-oil and non-condensable gas by thermally heating the biomass in the presence of little oxygen [129], [130]. When pyrolysis of biomass is run a number of reaction will be takes place simultaneously as well as orderly [129]. These reactions are divided either in two or in three main stages of

pyrolysis process [130]. The initial stage is the evaporation of free moisture which includes dehydration of free moisture which is existed in the biomass. Then the primary decomposition stage will be followed, in this stage volatilization that includes decarboxylation and dehydrogenation reactions is managed. At the third stage secondary decomposition of compounds that were created at the secondary stage will be performed. At this stage de-polymerization, isomerization, aromatization and charring reaction would be occurred, and it gives the char and the bio-oil [131].

Compared to gasification higher yield of char is generated in pyrolysis. On the other hand, it has an advantage over hydrothermal carbonization as it uses multiple types of biomass for the production of char regardless of the pretreatment the biomass. This makes biomass char production through pyrolysis is the most preferable one. The char from pyrolysis of biomass uses as precursor of solid acid catalyst which is used for the valorization of biomass itself. On the other hand, the yield and product composition of the char formed during pyrolysis is dependence on several factors such as operating condition (heating rate, pyrolytic temperature, and vapor residence time), biomass source and degree of pretreatment level as stated below.

#### **A. Influence of operating condition during biomass carbonization**

The effects of the reaction conditions such as heating rate, pyrolytic temperature, vapor residence time and atmospheric condition of pyrolysis have a significant impact in the yield, product distribution as well as physico-chemical properties (surface area, conductivity aromatic structure and others) of the final product. The dominant factors in the operating conditions of biomass pyrolysis are heating rate and temperature, however, the impact of residence time and others become significant when combining both temperature and heating rate [130]. Longer vapor

residence with low pyrolytic temperatures leads higher solid product whereas, lesser vapor residence time with lower temperature reduces the char.

Higher heating rate increase gaseous product reducing the char yield, this is due to the higher heating rate leads immediate fragmentation of biomass and possible secondary reactions. On the hand, lower heating rate avoids secondary reactions and avoids thermal cracking this will give higher char yield. Generally the effects of heating rates more noticeable with varying pyrolytic temperature [132].

The yield of the solid product is higher at low pyrolytic temperature since secondary reaction doesn't exist at low temperature [133]. At higher pyrolytic temperature secondary reaction and thermal cracking of the char is started so, it minimizes the char yields. Optimized char and bio-oil obtained at temperature of range 400-550 °C and above this temperature gaseous product will be increased. On the other hand, when biomass carbonized at higher temperature, the final solid product will have relatively higher surface area. However, at higher temperature more hetero-atoms will be removed from the char and it becomes difficult to attach the -SO<sub>3</sub>H group to the char during the sulfonation period. Hence, it is important to put a tradeoff between the surface area and catalytic active sites when the carbonization temperature of the char is assigned.

### **B. Influence of biomass constituent on final property of biomass char**

As a precursor of solid acid catalyst more of solid product from pyrolysis of biomass is preferred. Biomass pyrolysis product yield and quality are directly depending on the composition of biomass. As the main constituent of biomass lignin, cellulose, hemicellulose and small amount of extractives and minerals existed in varied ratio. Quantitative existence of these compounds depend on several factors such as biomass type, growth environment and maturation time. So, different biomass leads to different nature and quality of pyrolysis product [85]. A biomass with high amount lignin leads

to have more of solid product whereas, more cellulose, hemicellulose and extractives presence in biomass leads to more of liquid product. Therefore, when a biomass is considered for a precursor of solid acid catalyst, it is better to select more lignin constituent once. Mineral matter content of the biomass also affect the distribution the pyrolysis product. A mineral content in biomass significantly affects the biomass pyrolysis yield and product distribution due to the mineral in the biomass act as a catalyst [73], [134].

### **C. Influence of biomass pretreatment during biomass carbonization**

The effects of biomass pretreatment before pyrolysis has been studied in detail previously [135] and when biomass is pretreated it will be changed significantly and consequently the yield and product distribution of the pyrolysis will be altered. A number of pre-treatment techniques such as physical, thermal, chemical and biological are currently applied before pyrolysis of the biomass [135]. Physical pretreatment include size reduction and it will favor liquid product during pyrolysis. On the other hand, chemically untreated biomass will give excess amount solid product due to catalytic activities of the inorganic mineral existed in biomass [73]. Generally, when the operating condition of the pyrolysis process varies, the effects of pretreatment on biomass become more visible.

#### ***2.5.2.2. Biomass char sulfonation***

The prepared biomass char can then be sulfonated using different sulfonating agent. The most common are concentrate sulfuric acid (96%) and fuming sulfuric acid [97], [103], [110]. In typical sulfonation procedure the synthesized char is sulfonated using fuming or concentrate sulfuric acid at a given temperature and time with frequent stirring [97]. In addition to this, to avoid any oxidation reaction nitrogen gas will be shield throughout the sulfonation process.

Since fuming sulfuric acid contain large amount of  $-SO_3H$  group, using fuming sulfuric acid as sulfonating agent creates more  $-SO_3H$  groups in the catalyst compared with concentrate sulfuric

acid [21], [136]. On the other hand, handling of fuming sulfuric acid needs extra care and it makes large scale synthesizing of the catalyst using this method costly. Sulfonating using concentrate sulfuric acid (96%) is the most common method applied in many studies. One of the reason for this is the handling of concentrate sulfuric acid easier compared to fuming sulfuric acid. In addition to this, the amount of  $-SO_3H$  group existed in the final catalyst depends on operating condition of the sulfonation such as sulfonation temperature, residence time of sulfonation, and heating system [32], [96]. A temperature of  $150\text{ }^\circ\text{C}$  and residence time of 15 h have been mentioned for sulfonation of biomass char in most research works. However, in some studies it was found out the sulfonation temperature were above and below the stated temperature and time [13], [107]. Gonzalez and coworkers showed that using a microwave based heating system for sulfonation of biomass char could be achieved with lower sulfonation temperature ( $100\text{ }^\circ\text{C}$ ) and time (4h) [88], [102]. Better sulfonation with low residence time and temperature in microwave heating is mainly due to MW irradiation as it creates similar temperature rise for both outer surface and central constituents, whereas the conventional heating starts from the outer surface and goes to the center creating non uniformity in the heating process affecting the sulfonation. Furthermore, the MW irradiation might create collisions between the char and the sulfonate group and this might increase the grafting process of  $SO_3H$  in to the char [15]. Nevertheless, in conventional heating sulfonation, applying lower temperature will results in less degree of sulfonation (less amount of  $-SO_3H$  group grafted on the char), whereas very high sulfonation temperature leads to destabilize the catalyst [137]. To assign best sulfonation temperature it is necessary to perform sulfonation tests for specific biomass char at temperature range of  $100\text{-}200\text{ }^\circ\text{C}$ .

### **2.5.3. Characterization techniques of biomass derived sulfonated catalyst**

Characterization of a heterogeneous catalyst refers to the measurement of its ‘characteristics’, which are those physical and chemical properties of the catalyst assumed to be responsible for its performance in a given reaction. Therefore, the prepared catalyst can be characterized before and after the intended reaction and this will help to understand relationships among physical, chemical, and catalytic properties, (relating catalyst structure and function; composition, pore size, surface area, particle size, and strength).

Surface area analysis of a biomass derived sulfonated catalyst can be conducted through nitrogen adsorption-desorption isotherms, as the total surface area, the pore volume and the pore size distribution of such porous materials has significant effect on its final catalytic performance [138]. The other important characterization techniques is its surface texture and morphology [139]. Such characterization can be performed using Scanning Electron microscope (SEM) [140]. SEM coupled with EDX can also be used to analysis the elemental composition of the periphery of the given catalyst. The analysis performed by SEM compared with bulk elemental analysis result provides crucial information about the surface functional property of the catalyst [96]. If similar result in elemental composition is found in both cases, the surface and bulk functional property of the given catalyst is uniform. Knowing surface chemistry and structure of solid catalyst is extremely important, as the surface is the location of active sites and the interface at which chemisorption, surface reaction and desorption take place [141], [142]. Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and acid content analysis can be used to evaluate the surface chemistry and the structure of the given catalyst [143]. Acid density, distribution, and strength of the given catalyst can be measured using temperature program desorption (TPD) [144].

The stability of the prepared catalyst can be evaluated using thermogravimetric analysis (TGA) techniques. It uses heat to force reactions and physical changes in materials.

## **2.6. Biomass derived sulfonated catalyst for hydrolysis of cellulose**

Evaluating the catalytic activity is another attribute of biomass derived sulfonated catalyst which can be evaluated through different reactions. Biomass derived sulfonated catalyst activity have been tested using different reactions, cellulose hydrolysis have frequently used by researchers. On the other hand, the catalytic activity can be also evaluated in unique reactions like epoxidation of vegetable oil. When the activity of the catalyst becomes promising, it is important to assess its reuse potential. The reuse potential of this catalyst can be measured based on intended reactions. In the case of esterification of vegetable oil the reuse potential evaluated by separating the catalyst from the product and the unconverted reactants. However, in the case of hydrolysis the procedure differs as separating the catalyst from the final product and the unconverted reactant is a little difficult hence to evaluate the reuse potential of the catalyst, the method applied by Onda and coworkers was implemented in most cellulose hydrolysis reaction [27]. Some researchers also used the conventional method to evaluate the reuse capacity of the catalyst in cellulose hydrolysis with minor modification of the catalyst. One of the possible modification is the synthesis of magnetic based biomass derived sulfonated catalysts as stated by some researchers [145], [146].

The activity of biomass derived sulfonated catalyst have been tested in different chemical reaction by replacing those of mineral acids and expensive metal based solid acid catalysts [2], [59], [147]. The catalyst activity observed from those reactions was promising and with the support of further studies this catalyst will be used in industrial level. Hence, the research in biomass derived sulfonated catalyst should be geared towards to the elimination of the drawbacks associated with the use of this catalyst and a continual test of this catalyst in different reaction.

One of the drawback of the use of biomass derived sulfonated catalyst, for example in the area of cellulose hydrolysis is lower cellulose conversion and glucose yield. The crystalline structure of the cellulose is the main reason for low cellulose conversion and glucose yield [75], [148]. Moreover, the type of reactor used for the crystalline cellulose hydrolysis also affects the activity of the catalyst. Commonly, autoclave reactor is applied in most cellulose hydrolysis reactions, which its heating system goes from the surface of the reactor to the reactant in a gradient way. This creates non-uniform heat distribution inside the reactor and result in inefficient heat transfer to the intended purpose.

The problem associated with the rigidity of cellulose can be solved by pretreating the cellulose using different techniques prior to the hydrolysis reaction. The other method which can improve the activity of the catalyst in the hydrolysis of crystalline cellulose is replacing the autoclave reactor in microwave reactor. Some studies revealed that, the use of microwave reactor in the hydrolysis of some type of lignocellulose resulted a promising results. Similar effects of microwave reactor reported in dilute acid catalyzed crystalline cellulose hydrolysis. Therefore, implementing microwaves reactor with biomass derived sulfonated catalyst for crystalline cellulose hydrolysis could deliver higher yield of glucose.

Another area should be addressed in the hydrolysis of crystalline cellulose using biomass derived sulfonated catalysts is optimization of the hydrolysis operating conditions. Little works has been done so far concerning optimization of crystalline cellulose hydrolysis using biomass derived sulfonated catalyst. However, optimization of selected operating conditions will help to identify the best operating conditions in which the catalyst could act. Therefore, studies of biomass derived sulfonated catalyst in crystalline cellulose hydrolysis should give an attention for optimal operating conditions.

The application of biomass derived sulfonated catalyst still continued in a number of reactions replacing mineral acids. To exploit the potential use of the catalyst, it needs to be tested in a number of reactions, which mainly use mineral acids as a catalyst. Such kind studies using the catalyst will broaden its applications. Therefore, parallel to improving its catalytic activity, studies of the applications of the catalyst in previously untested reactions has to be continued. With the aim of broadening the applications area of the catalyst, in this dissertation work epoxidations of cottonseed oil is performed using biomass derived sulfonated catalyst.

## **2.7. Epoxidation of vegetable oils**

Epoxidation of plant oils, commonly termed “vegetable oils,” is a commercially important reaction because the epoxides obtained from these renewable raw materials and their transesterification product have applications as plasticizers and polymer stabilizers [149]. Owing to the high reactivity of the oxirane ring, epoxides also act as a raw material for a variety of chemicals, such as alcohols, glycols, alkanol amines, carbonyl compounds, olefinic compounds, and polymers, e.g., polyesters, polyurethanes, and epoxy resins. Epoxidation of long-chain olefins and unsaturated FA derivatives of vegetable oils such as soybean, linseed, rapeseed, olive, corn, sunflower, melon seed, and cotton seed is carried out on an industrial scale [150].

### **2.7.1. Epoxidation Methods**

Epoxidation of vegetable oil can be performed both enzymatically as well as chemically. Enzymatic epoxidation proved to be effective for the epoxidation of vegetable oils with aqueous hydrogen peroxide. Immobilized lipases and esterases showed high activity to convert fatty acids and their methyl esters to percarboxylic acids using  $H_2O_2$  as oxidant [151]. In chemical epoxidation method, there are basically four known technologies to produce epoxides from olefins:

1. Epoxidation with percarboxylic acids (Prileschajev), the most widely used method in industry. The percarboxylic acid is formed *in situ* by the reaction of carboxylic acid and hydrogen peroxide, catalyzed by acids or by enzymes [152], [153].
2. Epoxidation with organic and inorganic hydro peroxides, which includes hydrogen peroxide epoxidation as well as transition metal-catalyzed epoxidation [154].
3. Epoxidation with halohydrins, using hypohalous acids (HOX) and their salts as reagents for the epoxidation of olefin with electron-deficient double bonds [69].
4. Epoxidation with molecular oxygen [69].

Epoxidation with percarboxylic acids (or per-acids) is generally catalyzed by soluble mineral acids like sulfuric, tungstic or molybdic acids. The acid is used to catalyze the transformation of a carboxylic acid, e.g. acetic, 3-chlorobenzoic and trifluoroacetic, to the respective percarboxylic acid. Epoxides are produced by the non-catalytic reaction of the percarboxylic acid with the olefin. The final step in the mineral acid catalyzed epoxidation is the neutralization of the acid with an alkali, usually  $\text{Ca}(\text{OH})_2$ , producing salts, which renders this process environmentally unfriendly [155]. Moreover, the presence of strong mineral acids is linked to equipment corrosion problems, therefore more expensive corrosion-resistant construction materials are required for the production plant.

Halohydrins are prepared by the addition of hypohalous acids to olefins, subsequently, their treatment with alkali produces the epoxide [69]. This is a highly environmentally unfriendly system: apart from being a classical stoichiometric synthesis which are characterized by an extensive use of reactants, dihalides, halogen ethers, and salts formation.

Epoxidations with molecular oxygen which are catalyzed by compounds containing elements from groups IV-VIB show high selectivity but low activity [156]. With elements from groups I, VII and

VIII B the epoxidation are more active but less selective. Silver is an unique catalyst for the heterogeneous epoxidation of ethylene with molecular oxygen, but the reaction is mainly restricted to a few substrates like ethylene and butadiene, the epoxidation of other alkenes results in very low yields [157]. Liquid phase oxidations with molecular oxygen are generally free radical autoxidation's, the intermediate alkyl peroxy and alkoxy radicals are largely indiscriminate in their reactivity and selective oxidations are generally observed only with relatively simple substrates containing one reactive group. Thus, although  $O_2$  is a cheap oxidant with no waste problems its scope is limited to a rather small number of simple petrochemicals [158]. In particular case of vegetable oil, the oxidation with  $O_2$  leads to the degradation of the oils to smaller volatile compounds such as ketones and aldehydes as well as short-chain dicarboxylic acids. Therefore, it is not an adequate method to epoxidize vegetable oils, at least not yet.

### 2.7.2. Heterogeneous catalysts for epoxidation of olefins

The first successful trial to heterogenize the catalyst used in this process was the use of acidic resins by E.I. du Pont de Nemours and its coworkers in 1956 [155]. This process requires a relatively large amount of poly styrene sulfonic acid resin, typically 10-15 % wt. % based on the olefin. Disadvantages are the chemical and physical degradation of the resin, which has to be changed after 6-8 runs. The use of a more stable heterogeneous acid catalyst is highly desirable to improve the economics of this approach.

Epoxidation with organic and inorganic hydroperoxydes: in homogeneous phase the most active catalysts are soluble compounds of the early transition elements  $Ti^{IV}$ ,  $V^V$ ,  $Mo^{VI}$ , and  $W^{VI}$ . With organic hydro peroxides the activity of this metals is in the order  $Mo^{VI} \gg Ti^{IV} \sim V^V > W^{VI}$  but with anhydrous hydrogen peroxide  $W^{VI}$  catalysts are superior than the others [152]. Some compounds of these metals have been successfully heterogenized but the heterogenization

remarkably changes their relative activity. The epoxidation of olefins with organic hydroperoxydes catalyzed by early transition elements involves a peroxometal mechanism in which the rate-limiting step is oxygen transfer from an electrophilic (alkyl) peroxometal species to the nucleophilic olefin [158]. The metal center does not undergo any change in oxidation state during the catalytic cycle, it functions as a Lewis acid by withdrawing electrons from the O-O bond and thus increasing the electrophilic character of the coordinated peroxide. Therefore, active catalysts are metals which are strong Lewis acids and relatively weak oxidants [158]. Even though metallic catalysts have better epoxidation activity, their synthesis are expensive and more complicated.

On the other hand, biomass derived sulfonated catalyst as stated early has shown its potential to replace mineral acids as well as transition metal in multiple type of reactions. In one of the study which was made by Silva and coworkers shown that biomass based sulfonated catalyst could be used for epoxidation reaction [39]. Their work was only focused on the epoxidation activity comparison between biomass based sulfonated catalyst and cationic resin in cottonseed oil. They reported that 14 % and 34 % oxirane yield for biomass based sulfonated catalyst and cationic resin respectively. On the other hand, there exists a lack of detailed study in the activity of biomass based sulfonated catalyst in the epoxidation of vegetable oil (cottonseed oil). Consequently, additional research works needs to be implemented in the use of the catalyst. One of the area which has to be addressed in the studies are epoxidation operating conditions. Hence, here in this work, the study of selected operating conditions in the epoxidation of cottonseed oil in the presence of biomass based sulfonated catalyst was executed.

## Chapter Three

### 3. General materials and methods

In this chapter the materials and methods employed common to all the preceding four chapters stated. The specific equipment and techniques used in each chapter is stated in materials and methods section of the respective chapter.

#### 3.1. Materials and chemicals

Raw bamboo was collected from the local market; analytic grade concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$  96%) and hydrochloric acid (HCl) from Carlo Erba were used for the preparation of the catalyst. Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), sodium hydrogen carbonate ( $\text{NaHCO}_3$ ), powder phenolphthalein, sodium hydroxide (NaOH), and distilled water were used for the analysis of the acidity and functional group of the synthesized catalyst. Analytic grade gases such as helium (He), nitrogen ( $\text{N}_2$ ), argon (Ar), and ammonia ( $\text{NH}_3$ ) were used for carbonization and further catalyst characterization. Sigma-Aldrich cellulose (acid washed, powder, for column chromatography) with an average particle size of  $51\ \mu\text{m}$  was used as a raw material to test the hydrolysis activity of the synthesized catalyst.

Carbolite carbonizer was used for bamboo char preparation. EA 1112 Flash CHNS/O- elemental analyzer is used for elemental analysis of the samples. Furthermore, the synthesized samples were analyzed with Auto-chem II quadrasorb station I, 65 FTIR (PerkinElmer) analyzer, MiniFlex 300/600 XRD equipment, Sensys Evo TGA-DSC analyzer, JEOL JSM-IT300 scanning electron microscope containing an energy-dispersive X-ray (EDX) detector, micromeritics auto-chem II chemisorption equipment is used for  $\text{NH}_3$ -TPD for analysis the samples.

## 3.2. Methods

### 3.2.1. Bamboo derived sulfonated solid acid catalyst preparation

The first step in the preparation of the biomass derived sulfonated catalyst were carbonization of specific biomass. Bamboo with known species bought from the local market, and cut into a smaller size with a cutter. To remove any dirt associated with it, the pieces were thoroughly washed with distilled water. Then, it was let to sun-dried until the excess water removed. The sun-dried pieces were then ground by wood grinder machine. The pulverized bamboo was sieved, and a particle size between 0.5 and 1.4 mm was collected for further analysis.

The sulfonated bamboo-derived solid acid catalysts were prepared with a modification of previously published methods [21], [97]. A measured amount of bamboo sawdust was taken and pyrolyzed in carbolite carbonizer for a period of 2 h in three different temperatures (400 °C, 450 °C, and 500 °C) with a temperature ramp of 10 °C/min. The carbonization temperatures were selected based on the previous work of Fukuhara et al., in which the generated chars are composed with poor carbon structure so that the sulfonate group will easily attach to it at the time of sulfonation process [103]. Nitrogen gas with specific flow rate was flown to the carbonizer to keep the environment inert. Each of the carbonized char was soaked in 0.01 N HCl for an hour and then washed with a hot distilled water (>80 °C) until the pH of the filtrate became similar to that of the distilled water. Each of the washed char was then oven-dried at 105 °C for 4 h, cooled, and named bamboo char-400 °C (BC-400 °C), bamboo char-450 °C (BC-450 °C), and bamboo char-500 °C (BC-500 °C), where BC is stands for bamboo char and the suffix number represents the carbonized temperature. Each of the bamboo char was further milled to powder, and then 15 g of powder was measured and added into 150 ml of concentrated sulfuric acid (>96%) solution. The suspension was then heated at a temperature of 150 °C for a period of 15 h in the presence of nitrogen gas to give the sulfonated char. The sulfonated char was then cooled to room temperature, and 1000 ml

of distilled water was added. The sulfonated char was filtered, and the retentate was continuously washed with hot distilled water until its filtrate pH became neutral. The washed sulfonated char was oven-dried for 24 h at a temperature of 80 °C and then cooled to room temperature to make it ready for further analysis. The synthesized catalysts were named in general as bamboo-derived sulfonated catalyst (BSC). Since the pyrolysis temperature of each synthesized BC varied, their respective catalyst designated as BSC-400 °C, BSC-450 °C, and BSC-500 °C.

### **3.2.2. Catalyst characterization**

All the synthesized catalysts were initially characterized by elemental analysis and acid content. The results obtained from the above characterization tests were used to select the best BSC from all of the three synthesized catalysts (BSC-400 °C, BSC-450 °C, and BSC-500 °C). The selected catalyst was further characterized by FTIR, XRD, TGA coupled with mass spectrometry (MS), SEM, temperature programmed desorption (TPD), elemental analysis, titration method, and surface area analyzer. Before running any of the aforementioned catalyst characterization, characterization of the pulverized bamboo through proximate analysis was used to identify the quality of the raw material.

#### **3.2.2.1. Proximate analysis of pulverized bamboo**

The proximate analysis of raw biomass was based on the ASTM standards methods (ASTM E871-82, ASTM E1755-01, and ASTM E872-82). The proximate analysis separates the analysis into four groups: (1) moisture content, (2) volatile matter, consisting of gases and vapors driven off during pyrolysis, (3) ash, the inorganic residue remaining after combustion, and (4) fixed carbon, the non-volatile fraction of biomass.

##### **I. Moisture content of pulverized bamboo**

The moisture content was analyzed as follows, initially a petri-dish was taken and dried for 30 min at 105 °C in the oven, and then it was cooled in a desiccator to room temperature and weighed to

the nearest 0.02 g and record as petri-dish weight  $W_p$ . A pulverized bamboo sample was taken and dispersed nicely in the petridish and the weight of the sample and the petri-dish was measured and recorded as initial weight  $W_i$ . Then after the sample and the petri-dish was placed in an oven for 16 h at 103 °C. After the completion of the given time the sample and the petri-dish was removed from the oven and cooled to room temperature in the desiccator. Finally, the sample and the petri-dish was removed from the desiccator and immediately weighed to the nearest 0.01 g and recorded as  $W_f$ . The percent of moisture in the analysis sample was calculated as follows:

$$\text{Moisture in analysis sample, \%} = \left( \frac{W_i - W_f}{W_i - W_p} \right) * 100 \quad (3.1)$$

Where:

$W_p$  is the petri dish weight, g,

$W_i$  is petri-dish and sample initial weight, g, and

$W_f$  is petri-dish and sample final weight, g.

## ***II. Volatile matter***

Volatile matter of the sample analysis was determined by establishing the loss in weight resulting from heating the pulverized bamboo under rigidly controlled conditions. Volatile matter includes all gaseous products of the sample analysis, exclusives of moisture vapor. The analysis was conducted as follows: at first, platinum crucible with closely fitting cover was pre-heated to oxidize and stabilize the weight. Then, the weight of the crucible and its cover was measured to the nearest 0.01 g and recorded as crucible weight  $W_c$ . Approximately 1 g of sample was placed in the crucible, covered and then the total weight of the sample, the crucible, and its cover was measured to the nearest 0.01 g, and recorded as initial weight  $W_i$ . The covered crucible with the sample was placed in the furnace which is heated to 950 °C for exactly 7 min. Then after, the crucible was removed

from the furnace, without disturbing the cover, allowed it to cool in a desiccator. The cooled crucible with its cover and sample was weighed to the nearest 0.01 g and recorded as final weight,  $W_f$ . The total weight loss percent of the analysis sample is calculated as follows:

$$\text{weight loss, \%} = 100 * \left( \frac{W_i - W_f}{W_i - W_c} \right) = A \quad (3.2)$$

Where:

$W_c$  is weight of crucible and cover, g,

$W_i$  is initial weight of the sample, the crucible, and its cover, g and

$W_f$  is final weight of the sample, the crucible, and its cover, g.

Since the total weight loss represents the sum of the volatile matter and the moisture content, the total volatile matter in the sample was calculated as follows:

$$\text{volatile matter in the analysis sample, \%} = A - B \quad (3.3)$$

Where:

$A$  is weight loss %, and  $B$  is moisture % in analysis sample.

### ***III. Ash content***

The ash content is an approximate measure of the mineral content and other inorganic matter in biomass. The ash content of the pulverized bamboo was performed as follows: an empty crucible was taken and placed in a muffle furnace at 600 °C for 3 h. The heated crucible was removed from the furnace and cool to room temperature in a desiccator. The cooled crucible was weighed to the nearest 0.1 mg and recorded as the weight of the crucible,  $m_{cr}$ . Approximately, 1 g of pulverized bamboo, which was initially dried at 105 °C and stored in the desiccator was taken into the crucible, weighed and recorded as,  $m_{cds}$ . The crucible with the sample placed in 600 °C heated muffle furnace for 3 h and then removed and cooled to room temperature in desiccator weighed,

and recorded as mass of the crucible and the ash,  $m_{ash}$ . Finally, the mass percent of ash calculated as follows:

$$\% \text{ ash} = \left( \frac{m_{ash} - m_{cr}}{m_{cds} - m_{cr}} \right) * 100 \quad (3.4)$$

Where:

$\% \text{ ash}$  is mass percent of ash, based on 105 °C oven-dried mass of the sample,

$m_{ash}$  is mass of ash and container, g,

$m_{cr}$  is empty mass of container, g, and

$m_{cds}$  is initial mass of 105°C dried sample and container, g

#### ***IV. Fixed carbon***

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

$$\text{Fixed carbon (\%)} = 100 - (\text{moisture, \%} + \text{ash, \%} + \text{volatile matter, \%}) \quad (3.5)$$

##### ***3.2.2.2. Elemental analysis***

The elemental analysis was performed for the raw bamboo, for all carbonized bamboo char, and respective sulfonated char (catalyst). It 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 was fed into the analyzer along with an excess supply of oxygen. Four calibration points were taken for every chemical component and run in triplicate.

##### ***3.2.2.3. Acid content analysis***

Titration method (acid-base and Boehm) was used to quantify every functional group: sulfonic (–SO<sub>3</sub>H), phenolic (–OH), and carboxylic (–COOH) existed in sulfonated bamboo chars as well as

in bamboo chars based on the method reported by a number of authors [15], [107], [159]. All of the analysis were performed in triplicate as follows.

The total acid density, which is the sum of  $-\text{SO}_3\text{H}$ ,  $-\text{COOH}$ , and  $-\text{OH}$  group, was determined as follows. First, each of the samples were pre-dried in an oven at a temperature of  $105\text{ }^\circ\text{C}$  for a period of 4 h. A 0.05 g of sample was taken from the pre-dried samples and introduced in a solution of 0.01 N NaOH (20ml) and then sonicated for 1h at room temperature. The sonicated mixture was subjected to back titration with 0.01 N HCl using phenolphthalein as indicative. The total acid density of the catalyst ( $N_{total}$ ) was calculated using equation 3.6 as follows

$$N_{total} (\text{mmol/g}) = \frac{V_{\text{NaOH}} (\text{ml}) * N_{\text{NaOH}} - V_{\text{HCl}} (\text{ml}) * N_{\text{HCl}}}{m_{\text{sample}} (\text{g})} \quad (3.6)$$

Where:

$N_{total}$  is the total acid density found in the sample (mmol/g),

$V_{\text{NaOH}}$  is the volume of NaOH (ml),

$N_{\text{NaOH}}$  is the normality of NaOH (N),

$V_{\text{HCl}}$  is the volume of HCl (ml),

$N_{\text{HCl}}$  is the normality of HCl, and

$m_{\text{sample}}$  is the mass of sample taken (g).

The sulfonic group acid density was determined by standard acid-base titration method. A pre-dried sample of 0.05 g was taken and dissolved in 20 ml of 0.01 N of sodium chloride and then sonicated for an hour at room temperature. The solution was filtered using filter paper, and the filtrate was titrated by 0.01 N of NaOH aqueous solution using phenolphthalein as an indicator.

The sulfonic acid density was calculated using equation 3.7 as follows:

$$N_{-\text{SO}_3\text{H}} (\text{mmol/g}) = \frac{V_{\text{NaOH}} (\text{ml}) * N_{\text{NaOH}}}{m_{\text{sample}} (\text{g})} \quad (3.7)$$

$N_{-\text{SO}_3\text{H}}$  is the acid density ( $\text{SO}_3\text{H}$  found in the sample (mmol/g),

$V_{NaOH}$  is the volume of NaOH (ml),  
 $N_{NaOH}$  is the normality of NaOH (N), and  
 $m_{sample}$  is the mass of sample taken (g).

#### **3.2.2.4. Surface area analysis**

The specific surface area and pore structure of the catalysts as well as their respective chars were measured by N<sub>2</sub> isothermal adsorption-desorption method using quadrasorb station I. All samples were initially pretreated with helium gas at a temperature of 200 °C for 24 h in a vacuum. Mass of every sample was measured before and after helium gas treatment and significant mass changes were observed. Nitrogen adsorption was taken place at 77.3 K, then after the isotherm data was generated from the nitrogen volume adsorbed vs. its relative pressure (P/P<sub>o</sub>) between 0.01 to 0.35. Surface area for all samples were calculated by multipoint Brunauer-Emmet-Teller (BET) and pore size and pore volume were calculated using Barrett-Joyner-Halenda (BJH) methods.

The Afforementioned catalyst characterization technique used for the selection of which catalyst could be used for the preceding analysis work. Hence the following catalyst characterization test was only performed for the selected catalyst and its respective char.

#### **3.2.2.5. Fourier Transform Infrared (FT-IR) Spectroscopy**

The FT-IR was used for analyzing the main functional group existed in the selected catalyst, its respective char, and bamboo sawdust. FTIR spectra were measured in spectrum 65 FTIR (PerkinElmer) in the range 4000–400 cm<sup>-1</sup>. The samples for the analysis were prepared as follows: the samples initially milled with ceramic pestle and mortar to powder and then mixed with KBr powder and pressed to a circular 1 mm thick sample and ready to be analyzed in the equipment. The data generated would be converted into excel sheet using essential ft-ir software, then after wavelength vs. transmittance graph were generated. The functioning groups were determined

based on the interpretation of the infrared spectrum obtained by comparing it with the standard spectrum group frequencies.

#### **3.2.2.6. X-ray Diffraction (XRD) Spectroscopy**

The X-ray diffraction (XRD) patterns of the selected catalyst and its respective char were obtained using Mini-Flex 300/600, operated at 40 kV and 15 mA, using Ni-filtered CuK $\alpha$  radiation (1.54059–1.54441). The sample was placed in custom-made silicon wafer sample holder and the measurements were taken continuously from 10 to 80° angles over the range of 2 $\theta$ . The resultant intensity data was processed by using in-built diffraction software Match! Version 3 to graph and analyze the peak position.

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

TGA of the sulfonated char was conducted in SensysEvo TGA-DSC analyzer with a mass detection sensitivity of 0.005 mg. Argon gas with a flow rate of 20 ml/min was introduced to create the inert environment. The sample was heated from room temperature up to 800 °C with a temperature ramp of 20 °C/min. The outlet gas from the TGA was directly connected to SensysEvo mass spectrometer, also containing a thermal conductivity detector.

#### **3.2.2.8. Scanning Electron Microscopy (SEM) Analysis**

The surface morphology, as well as quantification of elemental composition of selected sulfonated char and its initial char, were observed and quantify using JEOL JSM-IT300 scanning electron microscope containing an energy-dispersive X-ray (EDX) detector.

#### **3.2.2.9. Temperature-Programmed Desorption (TPD) Analysis**

TPD analyses for the samples were performed in a micromeritics autochem II chemisorption equipment. The samples were initially pretreated with helium (150 °C flow rate of 30 ml/min for 2 h). Then, after the sample was cooled to 40 °C, ammonia adsorption was performed introducing a flow rate of 30 ml/min for 2 h of 10 % of ammonia in helium gas. Then, the excess of ammonia was desorbed by passing helium gas at flow rate of 30 ml/min for a period of 1 h. Finally, the

desorption of ammonia was run by heating the samples from 40 to 900 °C over a period of 2 h with flowing pure helium (flow rate of 30 ml/min).

## Chapter Four

### 4. Synthesis and characterization of BSC

#### Abstract

*In this work, biomass derived sulfonated solid catalysts were synthesized by consecutive carbonization and sulfonation of bamboo biomass. Three catalysts were prepared based on three different carbonization temperature. Characterization tests such as physisorption analysis (surface area, total pore volume, and pore size), CHNS, and acid content analysis were performed for all the three catalysts. The result obtained from these three tests were used for the selection of the best catalyst for further analysis. Other characterization tests such as FTIR, XRD, SEM, TGA-MS, and TPD were performed for the selected catalyst. The activity of the catalyst was tested through hydrolysis of crystalline cellulose using autoclave reactor. The hydrolysate were analyzed using DNS assay method in spectrophotometer. The characterization result showed that the synthesized catalyst contained carboxylic (COOH), phenolic -OH and most importantly sulfonate (-SO<sub>3</sub>H) groups. The surface area of the catalyst was 41.8 m<sup>2</sup>/g and its acid density and elemental sulfur were 0.58 mmol/g and 2.1 % respectively. The maximum yield of total reducing sugars found out to be 4.0 % at hydrolysis temperature of 150°C and hydrolysis period of 8 hrs.*

**Keywords:** Sulfonated bamboo catalyst, Cellulose, Hydrolysis, reducing sugars, Hydrolysis, Autoclave reactor.

#### **4.1. Introduction**

Biomass derived sulfonated catalyst is a bio based or ‘green’ catalyst derived from natural source such as glucose, starch, cellobiose, cellulose, lignin, and lignocellulose, plant residue and agricultural wastes. It is considered as a low-cost material since it is mainly derived from biomass that exists abundantly [160].

Bamboo categories as one of a few selected of biomass which are eminently renewable resource and with favorable conditions displays prodigious growth rates and early maturity [161]. Once successfully planted, they keep on rhizoming, shooting and maturing every year, hence sustainable utilization can be implemented without damaging ecological environment. Therefore, applying bamboos for the synthesis of biomass based sulfonated solid catalyst is environmentally benign.

Higher activity of bamboo derived sulfonated catalyst in the hydrolysis of hemicellulose is reported by Bai and coworkers [31]. Similarly, Li, et al. described the use of bamboo derived magnetic catalyst for the conversion of corncob into furfural [162]. Both works exhibited that bamboo derived sulfonated catalyst has a capacity to hydrolyze biomass. On the other hand, a few work has been reported in the use of this catalyst for crystalline cellulose hydrolysis. Hence, synthesizing bamboo derived sulfonated catalyst and evaluating its catalytic activity in crystalline cellulose hydrolysis will incur additional information about the catalyst.

In this work, bamboo derived sulfonated catalyst was synthesized in successive carbonization and sulfonation using pulverized bamboo. The synthesized catalyst was characterized by FT-IR spectroscopy, XRD, TGA-MS, SEM-EDX, CHNS, TPD, physisorption and acid titration method. The activity and selectivity of the catalyst were evaluated in the hydrolysis of crystalline cellulose using autoclave reactor.

## 4.2. Materials and Methods

### 4.2.1. Materials

Sigma-Aldrich cellulose (acid washed, powder, for column chromatography) with an average particle size of 51  $\mu\text{m}$  was used as a raw material to test the hydrolysis activity of the synthesized catalyst. Analytical grade 3, 5-dinitrosalicylic acid, potassium sodium tetra-hydrate ( $\text{C}_4\text{H}_4\text{KNaO}_6 \cdot 4\text{H}_2\text{O}$ ) and methyl blue was used for the analysis cellulose hydrolysate.

Drying oven (intercontinental equipment) used for moisture content analysis of pulverized bamboo. *Nabertherm* (30-3000  $^\circ\text{C}$ ) furnace was used for volatile matter and ash content analysis of the pulverized bamboo. Carbonization of pulverized bamboo was executed by carbolite GHC 1200  $^\circ\text{C}$  horizontal three zone tube furnace. MSH-20D hot-plate stirrer was used for the sulfonating process. The hydrolysis of the cellulose was performed using *Sonaclav* autoclave reactor. *Spectro UVD-3200*, spectrophotometer used to analyze the hydrolysate, and *Jenway* model 3510 digital pH meter used for measuring of pH.

### 4.2.2. Methods

#### 4.2.2.1. Catalyst preparation and characterization

Catalyst preparation and characterization were conducted according to section 3.2.1 and 3.2.2 respectively.

#### 4.2.2.2. Cellulose hydrolysis

The activity of the synthesized catalysts were tested using cellulose hydrolysis. All cellulose hydrolysis reactions were conducted by modifying the method stated by Onda et al. Accordingly, the hydrolysis reactions were performed in a batch mode in 15 ml Pyrex glass tube filled with measured amount of distilled water, cellulose powder and synthesized catalyst, and it was tightly sealed with cap and Teflon material to avoid any leakage. The glass tubes were immersed in 500 ml borosilicate beaker which was half -filled with distilled water. The beaker then was placed in

*Sanoclav* autoclave reactor and heated at temperatures of 120°C, 135 °C and 150 °C with autogenous pressure different hydrolysis period. After each reaction completed, the autoclave left to cool for some times and the Pyrex tubes were taken out and the separation of the hydrolysate from the unreacted cellulose and the catalyst were carried out by filtration. The filtrate was then subjected to total reducing sugar (TRS) analysis using 3, 5-Dinitrosalicylic acid (DNS) acid method.

#### ***4.2.2.3. Estimation of reducing sugars by DNS method***

The amount of total reducing sugar found in the filtrate was measured using 3, 5-dinitrosalicylic acid method [163]. 1 ml from the filtrate sample was mixed with 4 ml of DNS reagent and heated in boiling water for 5 minute. Then its absorbance (optical density) was measured at a wavelength of 540 nm using Spectro UV-VIS (Spectro UVD-3200) spectrophotometer. The TRS concentration was calculated by using a calibration curve of the standard glucose solution. Finally, the total reducing sugars yield was calculated as follows equation 4.1 [53].

$$TRS \text{ Yield (\%)} = \frac{\text{amount of TRS} \times 100}{\text{Dry cellulosic biomass weight}} \quad (4.1)$$

### **4.3. Results and discussion**

#### **4.3.1. Catalyst characterization**

##### ***4.3.1.1. Proximate analysis of pulverized Bamboo***

The proximate analysis of the pulverized bamboo was performed in accordance to section 3.2.1.

The analysis is performed in triplicate and the result obtained is shown in Table 4.1. The detailed calculation part is also included under appendix-A.

*Table 4-1 Proximate analysis of pulverized bamboo*

Proximate analysis	Component (%)
Moisture content	9.96±0.49
Volatile Matter*	80.97±0.84
Ash content *	1.6±0.14
Fixed Carbon*	17.43±0.91

\*Based on dry basis

The proximate analysis result of the pulverized bamboo determines the distribution of its contents. The moisture content of the pulverized bamboo in the wet basis is 9.96 %. Similar result of moisture content for pulverized bamboo was reported by other scholars [164]. This result indicated that applying pyrolysis techniques for carbonization of the pulverized bamboo is more convenient [165]. The volatile matter of the pulverized bamboo ranges from 80.13 to 81.81 %. Higher volatile contents implies higher weight loss during the carbonization process of the pulverized bamboo. The ash content of the pulverized bamboo found out to be 1.6 % as shown Table 4.1. This result is in agreement with previous studies conducted by other scholars [166]. Lower ash content in biomass is desirable for the synthesis of biomass derived sulfonated catalyst for two reasons; the first one is catalytic effect of the mineral will be limited in the time of carbonization hence higher biomass pyrolysis yield will be found and the second one is no interference of with the sulfonation process. In general ash in char is undesirable and taken as impurity. The amount of fixed carbon which is found in the pulverized bamboo indicates the presence of sufficient elemental carbon for the carbonization process for the synthesis of bamboo based sulfonated catalyst. In general, the proximate analysis result of the pulverized bamboo indicates, the use of bamboo material for the preparation biomass derived sulfonated catalyst is suitable.

#### 4.3.1.2. Elemental analysis of Carbonized bamboo

The elemental analysis results of the bamboo chars are shown in Table 4.2. The elemental content of the carbonized chars were analyzed at different carbonization temperature. As shown in Table 4.2 the fixed carbon content of biochar increases with increments of carbonization temperature. The fixed carbon will further increase if the carbonization temperature also increases, but the char become harder due to plane growth and stacking carbon sheet. This hinders the fixation of the sulfonate group on surface of biochar during the sulfonation process [97]. In contrary, the elemental content of hydrogen and oxygen decreases when the carbonization temperature increases. The decrement of hydrogen and oxygen with increment of temperature can be attributed to the cleavage of weak bonds within the pulverized bamboo structure [167]. On the other hand, relative increases in Nitrogen content was observed in samples of biochar with temperature increment.

Table 4-2 Elemental analysis of carbonized bamboo

Sample	Nitrogen (%)	Carbon (%)	Hydrogen (%)	Sulfur (%)	Oxygen (%)
BC-400°C	0.15±0.05	77.44±0.81	3.49±0.08	0.00	18.84±0.67
BC-450°C	0.14±0.04	79.88±0.85	3.18±0.07	0.00	16.78±0.74
BC-500°C	0.33±0.05	84.48±0.78	2.80±0.07	0.00	12.38±0.66

This is due to the incorporation of N into complex structures resulting in the formation of heat resistant functional groups [168]. Sulfur was not detected in all the carbonized char which indicates little sulfur existed in the given bamboo. In general, the ultimate analysis result of the generated biochar reveals the material is suitable for the synthesis of biomass derived sulfonated catalyst.

#### 4.3.1.3. Elemental analysis result of bamboo derived sulfonated catalysts (BSC)

The elemental analysis results of all BSC were performed using CHNS/O analyzer and the results are shown in Table 4.3. As shown in the table, all the BSC are rich with elemental carbon, however compared with their counterpart BC (Table 4.2) less amount of elemental carbon is reported. Lower carbon content of the BSCs' is associated with the decomposition of aromatic carbons structure at the time of sulfonation process. On the other hand, the oxygen content in all BSC were found out to be higher than their counter part BC. The main reason for the increment of oxygen content in BSC is the sulfonation process, which is an oxidation process, resulting incorporation of elemental oxygen in BSCs'. Unlike BCs' elemental sulfur is detected in all BSCs', as a result of the sulfonation process. The existence of sulfur on the surface of BSC was further confirmed using SEM-EDS (Appendix –A) analysis as shown for BSC-500 °C in Table 4.3. It is also important to note that comparable results of sulfur were obtained by the CHNS (2.10 %) and EDS technique (2.11 %).

Table 4-3 Elemental analysis result of BSC

Sample	Nitrogen (%)	Carbon (%)	Hydrogen (%)	Sulfur (%)	Oxygen (%)*
BSC-400°C <sup>a</sup>	0.325	59.46	2.7	1.86	35.72
BSC-450°C <sup>a</sup>	0.51	64.28	2.65	1.93	30.63
BSC-500°C <sup>a</sup>	0.52	65.68	1.88	2.10	29.82
BSC-500°C <sup>b</sup>	-	66.61	-	2.11	29.33

<sup>a</sup>Elemental analysis by CHNS/O analysis method

<sup>b</sup>Elemental mass % analysis by EDS

\*O content is calculated by difference

Knowing that CHNS/O and EDS techniques are associated with bulk and surface analysis respectively, the equivalent amount of sulfur reported by both techniques indicated that sulfonation process mainly occurred on the surface of the bamboo char. Similarly, Shimin Kang and his

coworkers suggested, chemically attaching of sulfur in the form  $-\text{SO}_3\text{H}$  group is entirely surface phenomena [161].

#### 4.3.1.4. Acid group titration results of BC and BSC

The acid density analysis determined by direct and back titration method for evaluating the sulfonic ( $-\text{SO}_3\text{H}$ ) and total acid group ( $\text{SO}_3\text{H} + \text{COOH} + \text{OH}$ ) for BC and BSC are reported in Table 4.4. As shown in the table no acid group related to  $\text{SO}_3\text{H}$  group is detected in BC samples unlike the BSC samples which imparts  $\text{SO}_3\text{H}$  group acidity. The results indicate that the sulfur group found in all BSC samples are derived from the typical sulfonation process. Moreover, higher total surface acidity was reported for all the BSC as compared to BC. For example, additional acidic density accounted for the BSC-500 °C sample was found to be 1.43 mmol/g.

*Table 4-4 Total and  $\text{SO}_3\text{H}$  acid density of BC and BSC samples*

Samples	Total acid density (mmol/g)	$\text{SO}_3\text{H}$ (mmol/g)
BC-400°C	1.8	-
BC-450°C	1.9	-
BC-500°C	1.71	-
BSC-400°C	3.92	0.44
BSC-450°C	3.84	0.52
BSC-500°C	3.71	0.58

This was calculated indirectly by subtracting of BSC sulfonic acid density (0.58 mmol/g) and BC-500 °C total acid density (1.7 mmol/g) from BSC total acid density (3.71 mmol/g). Higher acid density in the BSC samples might come from the sulfonation process as it induced additional acidic group probably by the formation of  $\text{SO}_3\text{H}$  group and new  $\text{COOH}$  groups or exposing the existing inaccessible site to the surface. On the other hand, the amount of  $\text{SO}_3\text{H}$  density found in the BSCs'

varies as BSC-500 °C > BSC-450 °C > BSC-400 °C. The amount of SO<sub>3</sub>H directly related with initial carbonization temperature of the pulverized bamboo. The main reason might be at lower carbonization temperature the carbonized char will have relative loose structure. Even though this loose structure promotes more sulfonate group to be attached during the sulfonation process, it tends to run off/leach from the carbon particles during washing step [161]. On the other hand, increasing the carbonization temperature above 500 °C will lead to harder carbon materials due to the generation of plane growth and stacking of the carbon sheets [97]. On top of this increasing carbonization temperature removes some of the heteroatoms which are important in the facilitating of the sulfonation process.

#### ***4.3.1.5. Nitrogen physisorption analysis***

The surface area, pore diameters, and pore volumes of BC and BSC samples were measured using N<sub>2</sub> physisorption analysis. Accordingly, the results of the surface area, total pore volume and average pore size of the BC and BSC samples are shown in Table 4.5. As shown in the table, the surface area of BC's increased with the increment of carbonization temperature. Temperature increment in general facilitates the removal of more volatile matter from the char favoring high surface area formation. On the other hand, the surface area of each BSC was lower than its counterpart BC. The sulfonation process using sulfuric acid is accompanied by oxidation reaction. Hence, part of the char is expected to disappear after the sulfonation process. Therefore, the surface area of the sulfonated products were lower compared to theirs counterpart chars. In general, the surface area of biomass derived sulfonated catalyst depends on a number of factors such as carbonization temperature, time, size of biomass, pretreatment conditions and others. With the given carbonization temperature, time and sulfonation operating conditions, the surface area results found here are comparable with previous research works [15], [169].

Table 4.5 showed that, all BC and BSC samples average pore size lays in mesoporous region. The pore volume and pore size of BC's increased with carbonization temperature. On the other hand, the BSC's pore size and pore volume were lower than their counterpart BC's. Lower pore size and volume for all BSCs was due to the incorporation of  $-SO_3H$  group in the BC's pores during the sulfonation process. Moreover, the accompanied oxidation reactions during sulfonation process significantly damaged the pore volume and size created during carbonization process [101].

The result found from elemental analysis, acid density, surface characterization showed that BSC-500 °C has outmost surface and morphological property to be applied for hydrolysis of cellulose. In addition to this, according to preliminary cellulose hydrolysis tests conducted using three synthesized catalyst (BC-400 °C, BC-450 °C and BC-500 °C), the highest glucose yield found using BC-500 °C. Hence, BSC-500 °C was selected for further analysis.

*Table 4-5 Surface characterization of BC and BSC*

Sample	Surface area (m <sup>2</sup> /g)	Total pore volume ( *10 <sup>-2</sup> cc/g)	Average pore size (nm)
BC-400°C	14.76	1.91	2.56
BC-450°C	22.2	2.56	3.82
BC-500°C	54.61	6.11	5.41
BSC-400°C	9.7	1.67	2.04
BSC-450°C	13.3	2.2	2.98
BSC-500°C	41.8	5.65	4.48

#### **4.3.1.6. FTIR Analysis**

The FTIR spectra of the BC-500 °C (a) and BSC-500 °C (b) are shown in Figure 4.1. The two samples show a broad band at 3400 cm<sup>-1</sup> which is assigned to O-H stretching. Similarly, two bands at 2900 cm<sup>-1</sup> and around 2850 cm<sup>-1</sup> are also observed in both samples which are assigned to SP<sup>3</sup>

C-H stretching. A characteristics bands at  $1600\text{ cm}^{-1}$  and  $1730\text{ cm}^{-1}$  ascribed to C=C and C=O stretching vibrations, respectively. The BSC spectrum shows vibration bands at  $1428\text{ cm}^{-1}$  and  $1036\text{ cm}^{-1}$  which are assigned to O=S=O and SO<sub>3</sub>H stretching, respectively [104]. The FT-IR results confirmed that the synthesis catalyst was composed of polycyclic aromatic carbon sheets with randomly attached -SO<sub>3</sub>H, -COOH and phenolic -OH groups and were likely promote acid-catalyzed reactions [31].

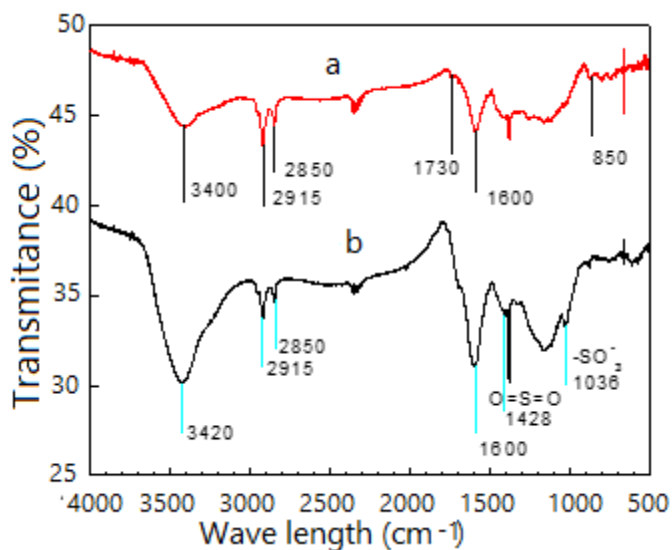
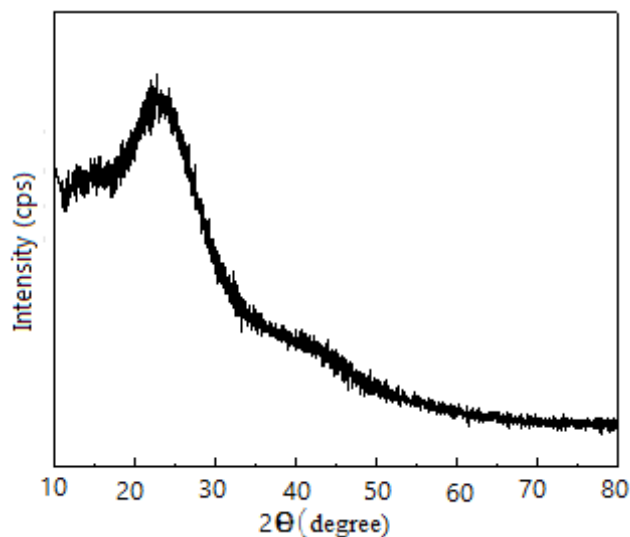


Figure 4-1 FTIR spectra of BC-500°C (a) and BSC-500°C (b)

#### 4.3.1.7. XRD

The XRD pattern of the sulfonated bamboo char is depicted in Figure 4.2. A broad but weak diffraction peak is observed between  $10^\circ$  and  $35^\circ$ , which is mainly associated with amorphous carbon composed of aromatic carbon sheets oriented in a considerably random fashion [170].



*Figure 4-2 XRD pattern of BSC-500°C*

#### **4.3.1.8. TGA coupled with MS**

The TGA curves for the BC-500 °C and the BSC-500 °C are shown in Figure 4.3. The gas from the TGA was further analyzed by the mass spectrometry (MS), and the curve was analyzed based on the specific compound identified from the MS which is shown in appendix B. Generally, both curves show three zones of decomposition. From temperature between 50 and 200 °C, water vapor and low-temperature volatile matter were removed from both samples. The second zone cover from a temperature of 200–550 °C, H<sub>2</sub>O, and CO were released from both samples which are attributed to the decomposition of the carboxylic and hydroxyl groups, respectively. In addition to this, for the BSC-500 °C in this temperature range, the release of sulfur groups was also observed. The final mass loss observed between 550 and 750 °C is due to the removal of hydrogen which is leading to the formation of aromatized residue [171]. The release of hydrogen at this stage shows that incomplete carbonization of the bamboo at the 500 °C. The total weight loss for sample BC and BSC-500 °C were 20 % and 55 %, respectively (Figure. 4.3).

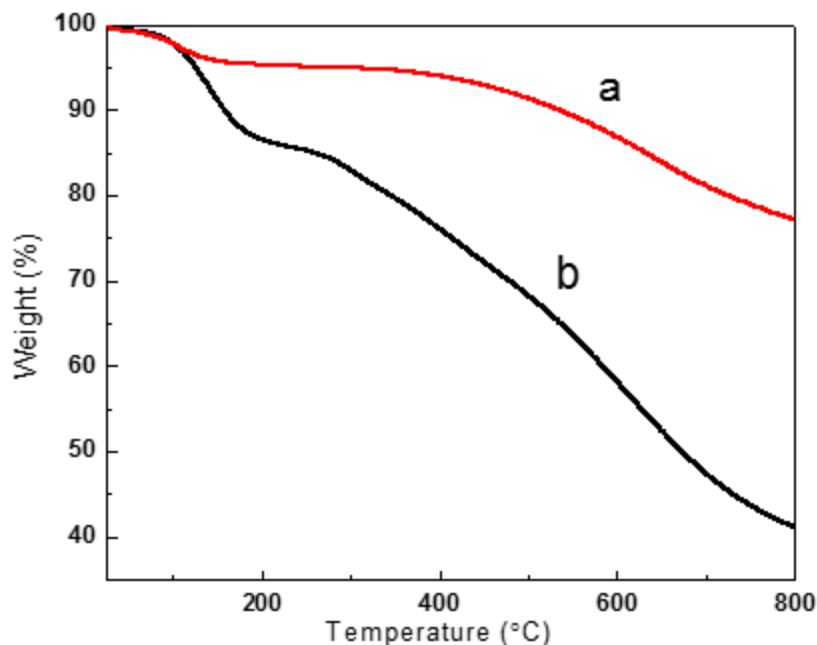
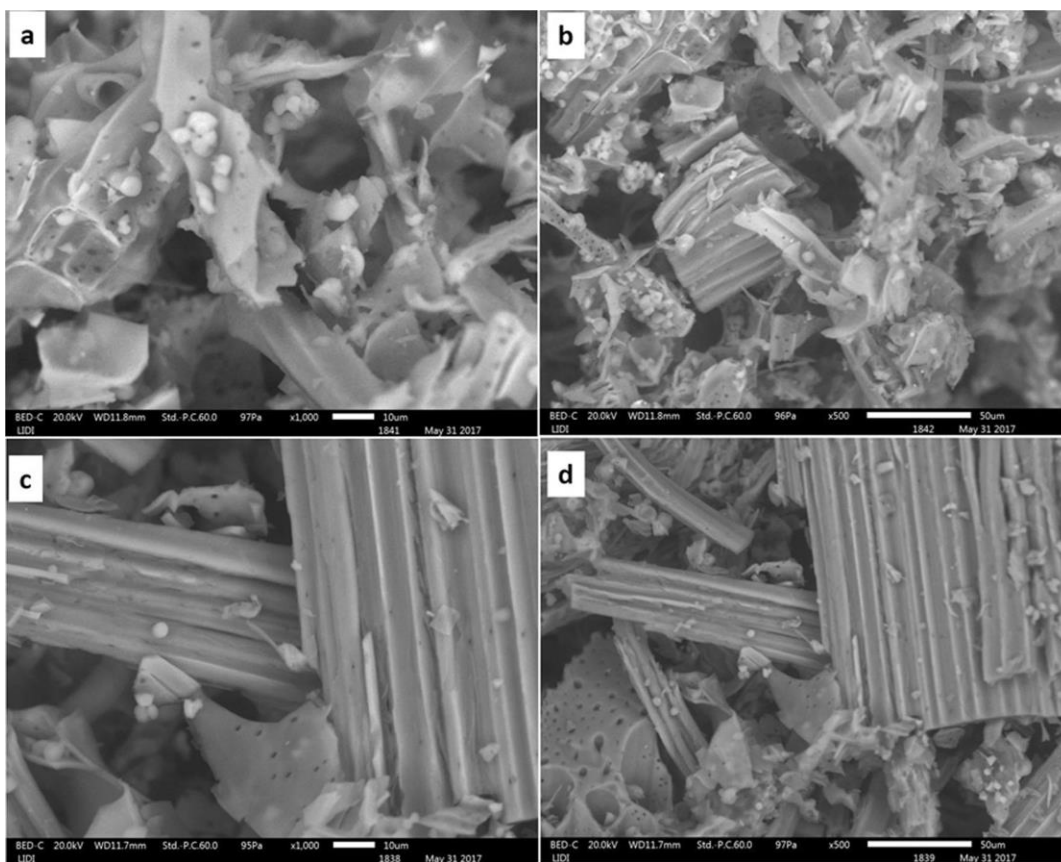


Figure 4-3 The TGA profiles of BC-500 °C (a) and BSC-500 °C (b)

The drastic weight loss associated with BSC-500 °C indicates an important increase in the functionalization of the surface contributing further to the decomposition of carbon material. Moreover, the MS was used to monitor the release of different species such as SO, SO<sub>2</sub>, SO<sub>3</sub>, and lactone groups, and small variations were detected, which show the release of these species in a very small quantity at a temperature around 200 °C.

#### 4.3.1.9. SEM-EDS

The SEM image of the BC-500 °C and the BSC-500 °C samples is shown in Fig. 4.4. A set of irregular aggregate with mainly of an external small size pores can be observed for the BC-500 °C sample. On the other hand, compared to BC-500 °C, the SEM image of the BSC-500 °C is composed of less amount of irregular aggregates and a set of bar shape bundle composed with a number of large size pores. The presence of fewer aggregates and smaller size pores in the BSC-500 °C is due to the sulfonation and oxidation process of the irregular aggregates.



*Figure 4-4 SEM image of BC (a, b) and BSC catalyst (c, d)*

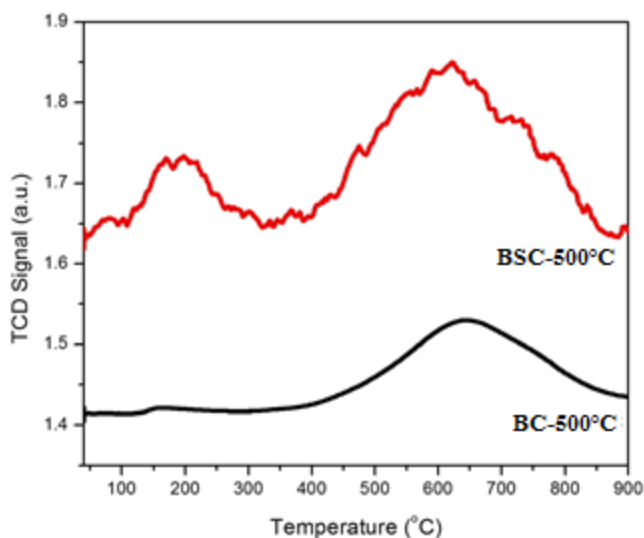
The use of SEM is not only limited to produce images but also can analysis elemental composition of the external surface structure the given samples when coupled with energy-dispersive X-ray spectroscopy (EDS). Accordingly, the EDS result of both BC-500 °C and BSC-500 °C sample, shown in the appendix-C, results in similarity with the CHNS elemental analysis method.

#### **4.3.1.10. TPD**

The ammonia temperature-programmed desorption (NH<sub>3</sub>-TPD) profiles for BC-500 °C (a) and BSC-500 °C (b) are depicted in Figure 4.5. As described in Figure 4.5a, the NH<sub>3</sub>-TPD profile of BC-500 °C has shown two peaks at 157.6 °C and 650 °C. The first peak (the broader peak) which is shown at 157.6 °C attributed to desorption of NH<sub>3</sub> from the weak acidic sites of BC (COOH and OH). On the other hand, the second peak which is shown in the NH<sub>3</sub>-TPD profile of BC-500 °C

clearly showed a broad peak at 650 °C which is probably associated with the release of thermally decomposed species such as hydrogen as also showed by the TGA-MS profile. Similarly, BSC-500 °C exhibited two distinct peaks at temperature of 198.4 °C and 650 °C. In contrast to the BC-500 °C, the existence of clear peak at 198.4 °C in the BSC-500 °C TPD-NH<sub>3</sub> profile is due to the desorption of NH<sub>3</sub> from the acidic sites (including –SO<sub>3</sub>H group) of the catalyst. The broad peak located at 650 °C is similar to that of the BC-500 °C which does not relate to the NH<sub>3</sub> desorption from the acidic sites of the catalyst, but it was due to the release of decomposition products. Similar results were reported for the TPD-NH<sub>3</sub> analysis of starch-based sulfonated solid acid catalyst by other authors [101].

The acidic sites of the BSC-500 °C, where ammonia was adsorbed, are associated with sulfonic and carboxylic groups. As expected, the TPD profile showed that BSC catalyst has higher number of acidic sites due to the sulfonation process. Moreover, NH<sub>3</sub> was desorbed at slightly higher temperatures (198.4 °C) than that of BC-500 °C (156.5 °C) and it shows slight increase in acid strength of the former. Further quantification of the acidic site was performed by analyzing the peaks and calculating the amount of NH<sub>3</sub> desorbed per gram of the catalyst, an indirect measurement for the total acid density. Different standards of NH<sub>3</sub>/He with distinct flow rates were used for calibration purpose to find the relationship between amounts of ammonia desorbed (mmol) and area of peaks. It was found out that the amounts of NH<sub>3</sub> adsorbed on BC-500 °C and BSC-500 °C were 0.05 and 0.781 mmol NH<sub>3</sub>/g respectively. The higher amount of ammonia desorbed from BSC-500 °C proves the existence of higher number of acidic sites at relatively higher strength as compared to BC-500 °C. Moreover, the results showed weak –COOH and -OH groups are also capable of adsorbing ammonia with very small quantity.



*Figure 4-5 NH<sub>3</sub>-TPD analysis of BC-400 °C and BSC-500 C°*

It is also important to note that the NH<sub>3</sub> -TPD result estimated the total amount of NH<sub>3</sub> desorbed from BSC-500 °C to be 0.781 mmol /g NH<sub>3</sub>. Part of this ammonia was attached on the sulfonic groups and part on newly created acidic sites. Assuming 1:1 stoichiometry between ammonia and sulfonic groups and all sulfonic groups are occupied by ammonia, it would require 0.581 mmol of NH<sub>3</sub> to completely occupy sulfonic groups and the rest (0.2 mmol NH<sub>3</sub>) probably was adsorbed on newly formed acidic sites.

#### **4.3.2. Hydrolysis of Cellulose using BSC-500 °C catalyst**

The actual cellulose hydrolysis with biomass derived sulfonated catalyst is a heterogeneous reaction. The reaction mechanism is similar to mineral acid hydrolysis as shown in Figure 4.6. The reaction involves the splitting of the water molecule into hydrogen cations (H<sup>+</sup>) and hydroxide anions (OH<sup>-</sup>). The H<sup>+</sup> then attacks the oxygen atom in 1,4-β-glycosidic bond of the cellulose and creates cyclic carbonium cation. This step is considered as rate-determining step [172]. At last, glucose is formed when the generated cyclic carbonium cation reacts with the split OH<sup>-</sup>.

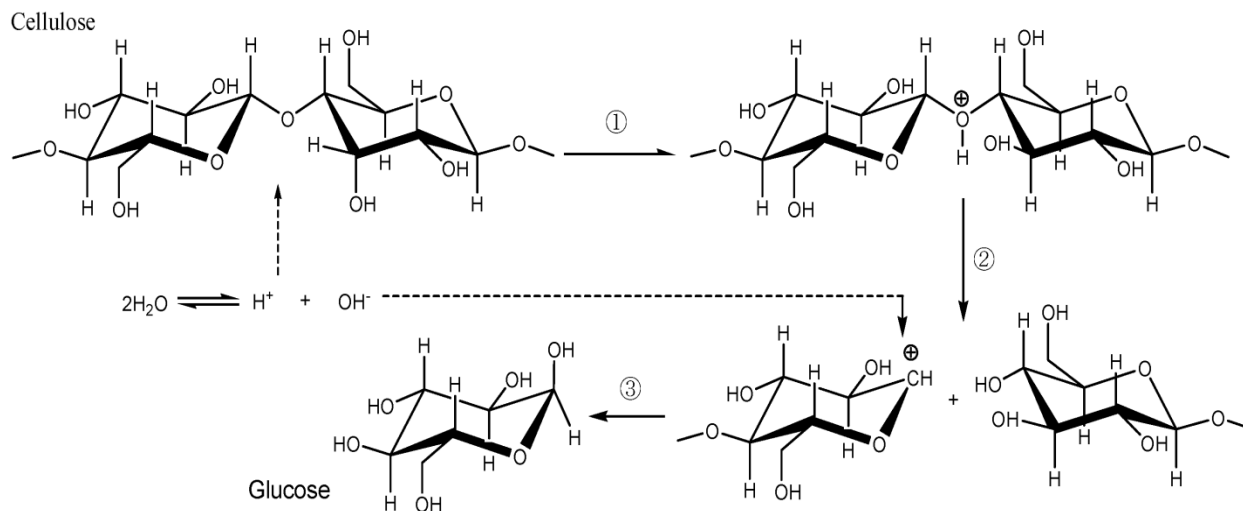


Figure 4-6 Mechanism of breakage of 1, 4-β-glycosidic bonds in the presence of solid catalyst [58]

On the other hand, when cellulose hydrolysis take place in the presence of either in liquid or solid acid the influence of non-reactions factors such as crystallinity, diffusion barrier and physical conformation represents major resistance. However, some of these resistance can be addressed by varying the operating conditions of the hydrolysis.

The hydrolysis of cellulose in *Sanoclave* reactor was examined in the presence of BSC-500 °C. All hydrolysis reaction was conducted using a crystalline cellulose i.e. initially neither treated in ball milling nor in ionic liquid. Major operating conditions such as hydrolysis temperature, hydrolysis time and catalyst loading ratio to cellulose were studied independently. On the other hand, constant amount of water (10 ml) was taken in all hydrolysis reaction.

#### 4.3.2.1. Effects of hydrolysis temperature and time

The hydrolysis of cellulose catalyzed by BSC-500 °C was examined for different hydrolysis temperature and time shown in Figure 4.7. The reaction was conducted in Sonaclave autoclave reactor (0.25g of cellulose, 0.25 g of BSC-500°C and 10 ml of water). In general, the maximum yield of total reducing sugar (TRS) found to be small due to the crystallinity of cellulose, however,

within this small range it is clear that the yield of TRS depends both on hydrolysis temperature and time.

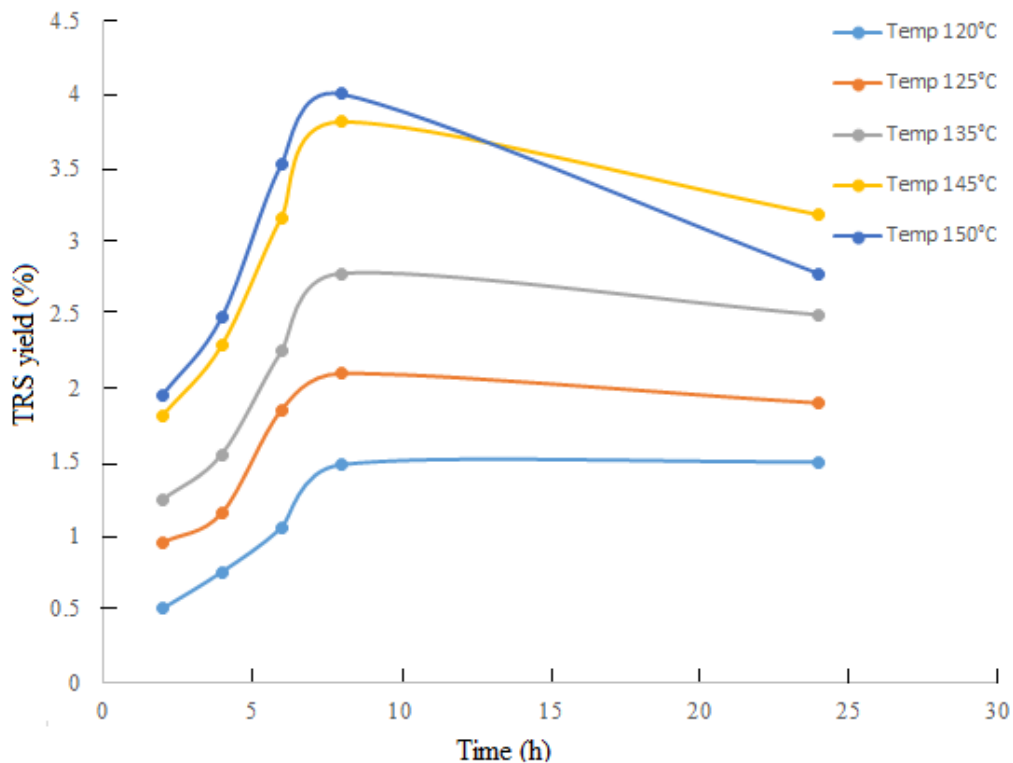


Figure 4-7 Effects of hydrolysis temperature and time on total reducing sugars obtained by hydrolysis of crystalline cellulose catalyzed with BSC-500°C.

When the hydrolysis was conducted at 150 °C, TRS yield of 4 % was found at 8 h reactions time. On the other hand, when the hydrolysis temperature lower to 145 °C, the TRS maximum yield became 3.81 % at 8 h hydrolysis time. At 120 °C, a maximum TRS yield of 1.48 % was found at 8 h hydrolysis time. From the results it can be seen that, reaction temperature highly influence cellulose hydrolysis. Similar to temperature, hydrolysis time in cellulose hydrolysis reaction has an effect on TRS yield. As shown in the figure increasing hydrolysis time further, slightly decreases the TRS yield. The main cause of the decrement of TRS yield is due to its' degradation

into products like hydro methyl furfural (HMF), levulinic, formic acid and insoluble humins due to longer hydrolysis period [173].

#### ***4.3.2.2. Effect of catalyst loading on the cellulose hydrolysis using BSC-500°C***

The effects of catalyst loading on crystalline cellulose hydrolysis is shown Table 4.6. As shown in the table, in the absence of the catalyst, a TRS yield of 0.45 % was obtained at temperature of 150 °C and 8 h reaction time. In contrary, TRS yield of 4.01 % was found, when cellulose hydrolysis was conducted in the presence of 0.25 g of the catalyst. Moreover, the presence of small amount of synthesized catalyst in cellulose hydrolysis gave higher TRS yield compared to the one which is performed in the absence of the catalyst. These results confirmed that the presence of the catalyst during cellulose hydrolysis reaction facilitates the conversion of cellulose into TRS.

Table 4.6 also showed that increasing catalyst loading increases the TRS yield. When the catalyst loading in cellulose hydrolysis reaction is increased from 0.025 g to 0.25 g the TRS yield is increased regardless of other operating conditions. This mainly happen due to the increment of catalyst loading brings more active site to involve in the hydrolysis reactions. Similar results were reported by *Guo. H* and coworkers [174]. On the other hand, further increasing the catalyst loading to 0.5 g created two opposite effect on the TRS yield. In short hydrolysis periods such as 2 and 4 h, when the catalyst loading increased to 0.5 g, the TRS yields were increased to 2.23 and 2.64 %, respectively. However, in long hydrolysis periods (6, 8, and 24 h) the catalyst loading increments lowered the TRS yields. It is probable that large active site with longer hydrolysis period facilitates not only the hydrolysis of cellulose into reducing sugars, but also the degradation of reducing sugar into other products through isomerization, dehydration and rehydration reactions [169].

*Table 4-6 Effects of catalyst dosage on the total reducing sugars (TRS) obtained by the hydrolysis of cellulose with BSC-500 °C*

Catalyst amount (g)	Reaction time (h)	TRS yield (%)
0	8	0.45
0.025	2	0.88
	4	1.08
	6	1.22
	8	1.68
	24	1.34
0.125	2	1.07
	4	1.75
	6	2.18
	8	2.88
	24	2.52
0.25	2	1.92
	4	2.48
	6	3.82
	8	4.01
	24	2.29
0.5	2	2.23
	4	2.64
	6	2.32
	8	3.58
	24	1.85

*Other operating conditions (0.25 g cellulose, 10 ml water, and temperature 150 °C)*

#### ***4.3.2.3. Catalyst recyclability***

Reuse capacity of the BSC-500 °C catalyst in terms of activity and stability was carried and the results are shown in Figure 4.8. Since separation of the catalyst from the unconverted cellulose was difficult, the reuse capacity and stability of the catalyst was studied using the method proposed by Onda et al. [27]. The experiments were conducted with two different test tube (a and b). As stated in section 4.3.2.2, the maximum TRS yield was found at 150 °C and 8 h hydrolysis time, the recyclability of the catalyst was performed at these conditions. 10 ml of water and 0.25 g BSC-500 °C was added in both test tubes and the reaction conducted with the given operating conditions. After the reaction was completed, test tube a was taken and the unconverted cellulose and catalyst were separated from the resultant solution using filter paper. 0.25 g of fresh cellulose was added in the filtrate solution, and then the reaction was carried out at 150 °C for 8 h. The TRS yield of the second run was 4.58 % which is slightly increased from the first run (4.01 %). This results indirectly confirmed that no or very low eluted of  $\text{SO}_4^{2-}$  was found in the filtrate of test tube a after the first run. Hence, BSC-500°C was stable in the given operating conditions.

On the other hand, after the first run, fresh cellulose was added into the resultant solution with the BSC-500°C H catalyst, and then the 2<sup>nd</sup> reaction was carried out. After the completion of the second run, the reaction was repeated once again by adding 0.25 g fresh cellulose with similar conditions. Figure 4.7 shows the TRS yields of each test tube b runs were 4.01, 7.76, and 11.35 % for the first, second and third run respectively. These results assured that the catalyst can be used repeatedly with a small loss of activity.

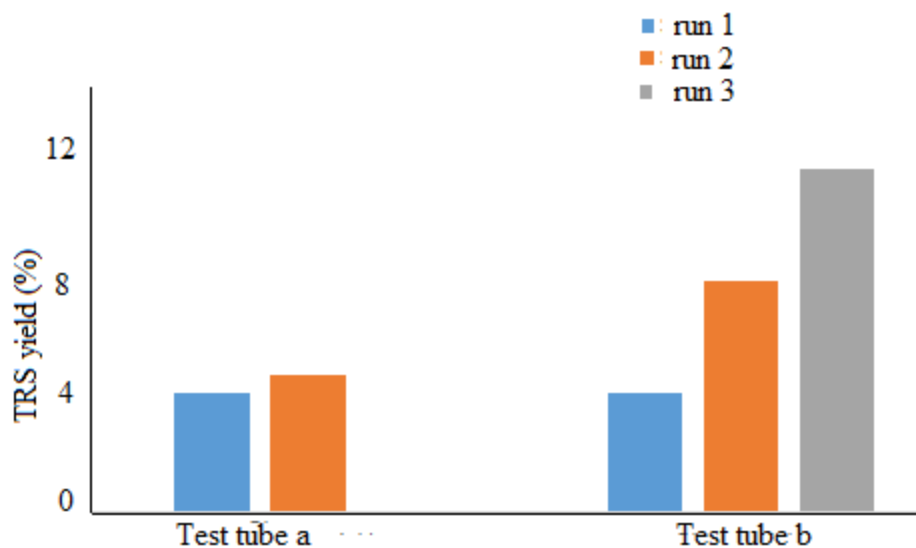


Figure 4-8 Stability (a) and reuse (b) capacity of BSC-500 °C

#### 4.4. Conclusion

Bamboo derived sulfonated catalyst (BSC) were successfully synthesized from bamboo which was initially pyrolysed in three different temperature and then sulfonated in concentrated sulfuric acids. The synthesized catalysts were characterized with different characterization techniques and all catalysts were contain  $\text{SO}_3\text{H}$ ,  $\text{COOH}$ , and  $\text{OH}$  group. Comparison between the synthesized catalysts were conducted in elemental analysis, acid group's presence and surface area analysis. The activity of the BSC-500 °C catalyst was examined through crystalline cellulose hydrolysis reaction. The TRS yield, however, was very low compared to previously reported mineral acid catalyzed cellulose hydrolysis. Even-though the TRS yield was low, the results showed that hydrolysis reaction was influenced by operating conditions such as temperature, time and catalyst dosage. At last, catalyst stability and reusability analysis showed the catalyst can be used repeatedly with a small lose in its activity.

## Chapter Five

### 5. Study on cellulose hydrolysis performance of BSC in microwave-heated reactor at elevated temperatures

#### Abstract

*In this work, the combined effects of microwave reactor and bamboo-derived sulfonated catalyst in the hydrolysis of previously untreated cellulose (96% of crystallinity with 3.61 nm average crystallite size) at higher temperature were studied. The maximum yield of glucose found was around 43.5% at a reaction temperature of 180 °C and 60 min of reaction time, using a microwave reactor. Similar conversion and glucose yield were attained in second run showing the reusable potential of the catalyst. Moreover, the combined effects of the catalyst and the microwave reactor gave a higher yield of glucose from crystalline cellulose. The catalyst has shown its potential to convert glucose further into platform chemicals like hydroxymethylfurfural, levulinic acid, and formic acid.*

**Keywords:** Sulfonated bamboo catalyst, Cellulose, Glucose, Hydrolysis, Microwave-reactor.

## 5.1. Introduction

The synthesis and characterization of bamboo derived sulfonated catalyst (BSC) successfully performed in the previous chapter. Nevertheless, when the catalyst was examined in the hydrolysis of crystalline cellulose, lower amount of TRS yield was found. Similar results were reported in other biomass based sulfonated catalysts, when they were used for the hydrolysis of crystalline cellulose [27]. In general, the amount of TRS yield obtained by using these catalysts were lower compared to mineral acid catalysts in crystalline cellulose hydrolysis. To enhance the efficiency of biomass derived sulfonated catalysts, researchers have tried employing different pretreatment mechanisms to reduce the crystallinity of cellulose prior to or during the hydrolysis reaction. For instance, Onda et al. reported that they have achieved 40.5 % of glucose yield and 42.5 % cellulose conversion using carbon-based catalyst after pretreating the cellulose through ball milling [27]. Similarly, higher glucose yield was reported in the hydrolysis of ionic liquid pretreated cellulose using carbon-based catalyst [13], [28]. However, enhancing the glucose conversion using pretreatments like long hour ball milling (48 h) and ionic liquid makes the process energy intensive and economically less feasible.

Alternatively, some researchers reported that, using microwave reactors both for the pretreatment and hydrolysis of cellulose has created higher result in TRS yield [29], [175]. Moreover, the use of biomass-based sulfonated catalyst together with microwave irradiation has also been carried out [15]. Wu et al. studied the effect of microwave irradiation in cellulose hydrolysis in the presence of biomass-based sulfonated catalyst [63]. The researchers employed a 30-min ball-milled mixture of cellulose and the catalyst with microwave irradiation at 90 °C. A 16 % of glucose yield was attained which was better than the result obtained using conventional heating. On top of this, the

amount of glucose yield obtained can be increased, if elevated temperature is used in the hydrolysis reactions.

Here in this work, the hydrolysis of cellulose (96% crystalline) in the presence of bamboo-derived sulfonated catalyst (BSC-500 °C) using microwave reactor (single chamber *synth-Wave*) at elevated temperatures was studied. The effects of reaction temperature and time on the cellulose conversion, glucose yield, and soluble product distribution in the presence of BSC-500 °C using microwave reactor were thoroughly investigated. In addition to this, the activity of selected solid acid catalysts in the hydrolysis of cellulose were compared with BSC-500 °C. Furthermore, the activity of BSC-500 °C catalyst in the microwave-heated reactor was also compared with conventionally heated autoclave reactor for cellulose hydrolysis reaction.

## **5.2. Materials and Methods**

### **5.2.1. Materials**

Sigma-Aldrich cellulose (acid washed, powder, for column chromatography) with an average particle size of 51 µm was used for hydrolysis reactions. Anhydrous D-glucose, hydroxymethylfurfural (HMF), levulinic acid, acetic acid, lactic acid, and formic acid from Merck and Sigma-Aldrich were used to prepare the standard for the high-performance liquid chromatography (HPLC), whereas Merck sulfuric acid was used to prepare the mobile phase for HPLC and pure nitrogen gas was used to create the vacuum throughout the hydrolysis reaction. The hydrolysis reactions were mainly conducted in single chamber *synthWave* microwave reactor. Similarly, the activity of BSC-500 °C for cellulose hydrolysis using a conventional 100 ml autoclave reactor (*Snap-tite* mini autoclave reactor) was also determined. What-man filter paper was used to separate the hydrolysate from the unconverted cellulose and BSC-500 °C. In addition to this the hydrolysate was further filtered using 2-micron syringe filter before it was analyzed in both in HPLC and total organic carbon analyzer. Siemens D5000 diffractometer was used for the

X-ray diffraction (XRD) measurement for the cellulose sample. A *Synth-wave* single reaction chamber microwave synthesis system was used for microwave reaction and stainless steel 100 ml *Snap tite* mini autoclave with servo motor was used for the conventional hydrolysis of cellulose. Shimadzu total carbon analyzer (TOC-L CSN ver. 1.04.00) was used to measure the total organic carbon from the hydrolysis product and Agilent high-performance liquid chromatography (HPLC) with refractive index detector (RID) and ICSep-COOREGEL 87H3 column was used to quantify the concentration of glucose and other product in the final solution of the hydrolysis.

## 5.2.2. Methods

### 5.2.2.1. X-ray diffraction and analysis techniques of cellulose sample

The XRD measurements were performed using a Siemens D5000 diffractometer (Bragg-Brentano para-focusing geometry and vertical  $\theta$ - $\theta$  goniometer) fitted with a curved graphite diffracted beam monochromator, incident and diffracted beam Soller slits, a  $0.06^\circ$  receiving slit, and scintillation counter as a detector. The angular  $2\theta$  diffraction range was between  $3$  and  $60^\circ$ . The data were collected with an angular step of  $0.03^\circ$  at  $5$  s per step and sample rotation. A low background Si (510) wafer was used as sample holder. Cu Ka radiation was obtained from a copper X-ray tube operated at  $40$  kV and  $30$  mA. Approximately  $0.25$  mg of powdered cellulose was pressed at  $3$  tons during  $10$  s to produce a pellet of  $15$  mm diameter and  $0.2$  mm of thickness. The pellet was placed onto the sample holder to be analyzed. The modified Segal method was used to measure the crystallinity index ( $C_I$ ) as shown in equation 5.1 [176].

$$C_I(\%) = \frac{I_{Cel} - I_{Am}}{I_{Cel}} \times 100 \quad 5.1$$

Where  $I_{Cel}$  refers to the sum of peak intensities that appear in the range of  $2\theta = 10-27^\circ$  and  $I_{Am}$  is the intensity of the amorphous peak ( $2\theta = 18^\circ$ ). All diffractograms were fitted with the TOPAS software (V4.2, Bruker AXS GmbH, Karlsruhe, Germany). The software uses the Pawley method

[177] in which five reflections were employed ((-110), (110), (102), (200), and (004)) to fit all the observed diffractogram. An anisotropic peak broadening of these 5 reflections were adjusted with spherical harmonics model [178] and a Lorentzian function in order to improve even more the goodness of fit. Once all 5 reflections were fitted with this model, the corresponding crystallite size was calculated with the modified Scherrer equation, according to Stokes & Wilson [179], and the integral breadth for each reflection as shown in equation 5.2. The instrumental contribution to the peak width was calculated by describing different component of the diffractometer using the fundamental parameter approach [180].

$$\tau = \frac{\lambda}{\beta \sin \theta} \quad 5.2$$

Where,  $\beta$  is the mean integral breadth and  $\lambda$  is the wavelength used. The background was regarded as a straight line with slope. The amorphous part of the sample was assigned to a pseudo-Voigt peak at  $2\theta = 18^\circ$  with refinable peak width. The cell parameters for cellulose were refined for each sample.

#### **5.2.2.2. Cellulose hydrolysis**

The hydrolysis reactions were commonly conducted in single chamber Synth-Wave microwave reactor within glass vials which were placed on five places rack and easily be mounted in the microwave reactor. The microwave was adjusted to work for all of the analysis at 30 bar pressure and 800 W power with 50 % of its maximum stirrer capacity. In typical reactions, 0.25 g of cellulose combined with 0.25 g of BSC-500 °C was placed in the glass vial; then, 10 ml of water was added. Then after, the glass vials (which were covered with a loose fit Teflon caps to ensure pressure equalization) were mounted in the five places rack of the Teflon made microwave's chamber which contained 300 ml of distilled water. Afterwards, by assigning the specific operating condition (temperature and time) in the external terminal, the hydrolysis was run. In each of the

hydrolysis, 10 min of the total reaction time was assigned for the microwave to attain the operating temperature. To confirm the reproducibility of the results, all experiments were performed in replicates and standard deviation for each experiment was calculated.

Similarly, the activity of BSC-500 °C for cellulose hydrolysis using a conventional 100 ml autoclave reactor (Snap-tite mini-autoclave reactor) was also determined. In the autoclave reactor, 1 g of BSC-500 °C combined with an equal mass of cellulose and 40 ml of distilled water was used. In this way, the performance of autoclave reactor and microwave reactor was compared at reaction temperature of 150 °C.

The activity of the BSC-500 °C in cellulose hydrolysis using microwave reactor was also compared with other solid catalysts such as amberlyst-15,  $\gamma\text{Al}_2\text{O}_3$ , MgO/F-20% (which was synthesized in our lab), activated carbon, and bamboo char (BC-500 °C) at operating condition of 180 °C and 60 min.

Finally, the reuse and stability of the catalyst for the specific hydrolysis condition using the microwave reactor was also analyzed based on the method stated by Onda et al. [27]. The reaction was performed at 180 °C and 60 min reaction. In a typical procedure, after the first hydrolysis runs using BSC-500 °C catalyst at selected temperature and reaction time, fresh cellulose was added into the resultant solution with the BSC-500 °C catalyst; then, the second reaction was run. In each run, 1 ml of the filtrate was taken from each resultant solution and glucose yield was evaluated.

#### **5.2.2.3. Analysis of cellulose hydrolysate**

After the hydrolysis reaction, the reactor was cooled to room temperature. The mixture from the glass vial was immediately filtered and the filtrate collected and diluted using measured amount of distill water. The dilute solution then passed through 2  $\mu\text{m}$  disc filter, the filtrate then analyzed using total carbon (TC) analyzer. The TC analyzer analyzed the amount of carbon existed in the filtrate in the form of total organic carbon and inorganic carbon. The value of the total organic

carbon (TOC) was taken and used for to evaluate the conversion cellulose with the following equation (5.3).

$$Cell_{conv}(\%) = \frac{TOC(mg/l) \times Volume\ of\ filtrate(l) \times (\frac{1\ g}{1000\ mg})}{mass\ fraction\ of\ carbon \times given\ mass\ of\ cellulose(g)} \times 100 \quad 5.3$$

Where, Cell<sub>conv</sub> stands for total amount of converted cellulose.

The yield of glucose and other obtained products were measured using high-performance liquid chromatography (HPLC) with refractive index detector (RID) and IC Sep ICE-COREGEL 87H3 column. Anhydrous D-glucose, hydroxymethylfurfural (HMF), cellobiose, levulinic acid, acetic acid, lactic acid, and formic acid from Merck and Sigma-Aldrich were used to prepare the standard solutions for the high-performance liquid chromatography (HPLC), whereas Merck sulfuric acid was used to prepare the mobile phase for HPLC. The retention time from the reference standards were used to identify each of the hydrolysis product. The amount of glucose yield was calculated using the following equation (5.4).

$$Glucose\ Yield\ (\%) = \frac{mole\ of\ glucose\ (HPLC)}{mole\ of\ charged\ cellulose\ (initial\ fd)} \times 100 \quad 5.4$$

## 5.3. Results and Discussion

### 5.3.1. XRD patterns and crystallinity index of cellulose sample

The XRD patterns of the cellulose sample used for hydrolysis depicted in Figure.5.1 showed typical crystalline cellulose structure. The XRD pattern showed diffraction peaks of cellulose crystalline around 15.8°, 22.32°, and 34.46°. From the XRD pattern the crystallinity index (CI) and average size of the crystallite were found out to be 96.4% and 3.61 nm, respectively.

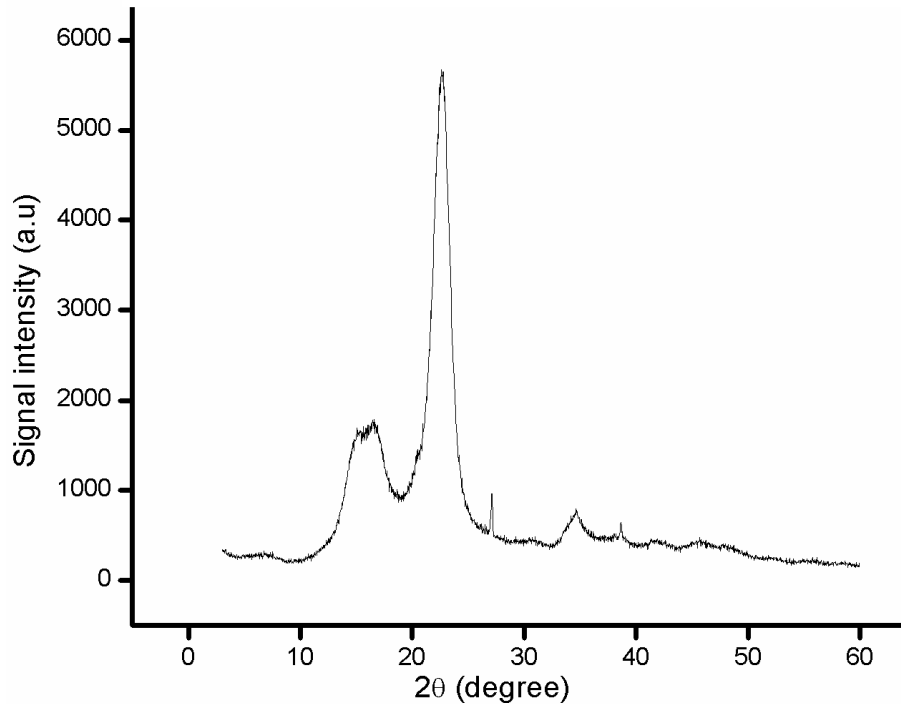


Figure 5-1 XRD patterns of cellulose

### 5.3.2. Cellulose hydrolysis performance of BSC- 500 °C using microwave reactor

#### 5.3.2.1. Comparative study of microwave and autoclave reactors on the hydrolysis efficiency

The cellulose used for the hydrolysis was found to be 96.4 % crystalline with an average crystal size of 3.61 nm, and it was not subjected to any kind of pretreatment for the reduction of crystallinity and average crystal size before the hydrolysis. The comparative results for the performances of microwave and the conventional reactor (autoclave) are shown in Table 5.1. The hydrolysis was performed with similar operating conditions, and to have a meaningful cellulose conversion and glucose yield from the autoclave reactor, higher hydrolysis time was employed.

As shown in Table 5.1, the cellulose conversion in microwave reactor increases from 3 to 12.31 % as the time of reaction proceeded from 30 to 120 min. On the other hand, the cellulose conversion in autoclave reactor increased from 6.78 to 8.72 %, when hydrolysis time increased 240 min and 1440 min respectively. The results showed that during the hydrolysis reaction, the

cellulose conversion was improved by using microwave compared to autoclave reactors, at lower hydrolysis time. Conversely, using the microwave reactor, the glucose yield increases to 10.8 % as the time of reaction increased to 120 min, unlike the maximum of 4.1 % glucose yield obtained at 480 min using autoclave reactor. This shows that the type of reactor affects not only the cellulose conversion but also the glucose yield. The glucose yield in the microwave increased with the cellulose conversion; likewise, the autoclave showed the increment of glucose yield with the increase of cellulose conversion at first but with increment of the hydrolysis time, the glucose yield started to decrease regardless of the amount cellulose conversion. Apart from glucose, small amount of other products such as formic acid, levulinic acid, and trace amount of hydroxymethyl furfural HMF and cellobiose were detected in both reactors. Formic acid (1.5%) and levulinic acid (0.75%) were detected at 150 °C and 120 min in the microwave reactor.

Table 5.1 also shows the selectivity of glucose with respect to the converted cellulose. The result indicate that using microwave reactor, at 150 °C and 120 min of reaction time, the selectivity of glucose with respect to converted cellulose was found to be 87.7 %. However, only 55.5 % of glucose selectivity was obtained using autoclave reactor at similar operating conditions. The results indicate that high hydrolysis efficiency can be achieved by using microwave reactor. The effect might be explained by the rapid heat generation of the microwave and the presence of hot spots that probably accelerates the transformation of cellulose [181]. This in turn leads to high glucose yield at the shorter reaction time, inhibiting further dehydration and rehydration of glucose into other products. On the other hand, the high conversion and yield obtained in microwave reactors can be attributed to the ability of microwave to promote the BSC-500 °C catalyst activity through reduction of the cellulose crystallinity [105]. This fact is supported as the hydrolysis

performed in the absence of catalyst using microwave reactor (at 150 °C and 120 min) resulted in 0.75 % glucose yield, somehow contributing to de crystallization of cellulose.

*Table 5-1 Cellulose conversion and glucose yield in different reactors*

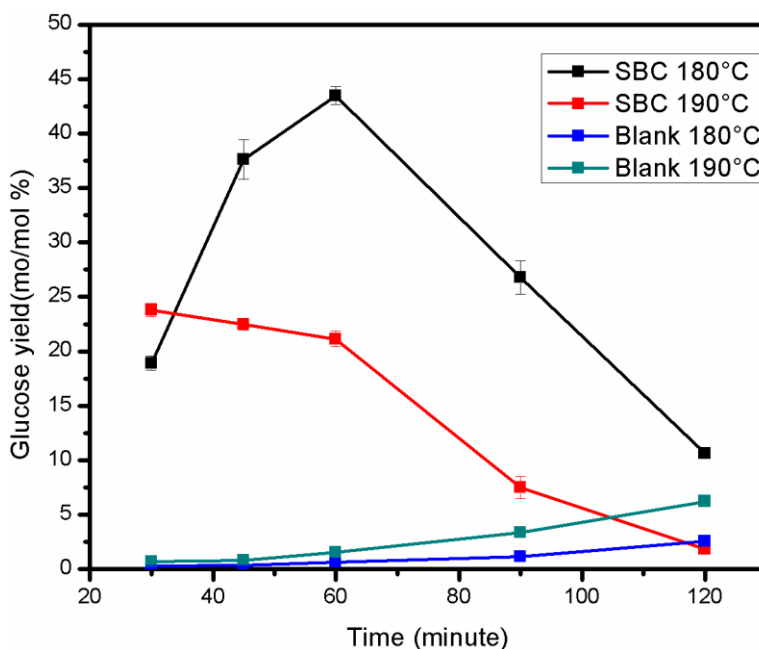
Time (min)	Converted cellulose (w/w %)		Glucose yield (mol/mol %)		Selectivity of glucose per Cellulose converted (%)	
	Microwave reactor	Autoclave reactor	Microwave reactor	Autoclave reactor	Microwave reactor	Autoclave reactor
30	3±0.16	nd	0.32±0.01	nd	10.66	Nd
45	5.7±0.12	nd	3.5±0.18	nd	61.4	Nd
60	6.01±0.16	nd	4.15±0.39	nd	69	Nd
90	10.3±1.08	nd	8.09±0.33	nd	78.54	Nd
120	12.31±0.68	nd	10.8±0.88	nd	87.73	Nd
240	nd	6.78±0.34	nd	2.52±0.17	Nd	37.1
480	nd	7.4±0.29	nd	4.11±0.18	Nd	55.5
1440	nd	8.52±0.17	nd	2.24±0.12	nd	26.29

Typical reaction condition (catalyst, 0.25 g; cellulose, 0.25 g; water, 10 ml; temperature, 150 °C and microwave power, 800 W), nd: not determined

### **5.3.2.2. Cellulose hydrolysis using microwave reactor**

The influence of the microwave reactor in the hydrolysis process was investigated with and without the presence of BSC-500 °C at a temperature of 180 °C and 190 °C plus hydrolysis period between 30 and 120 min, and the result is shown in Figure 5.2. The glucose yield in the presence of BSC is much higher than the blank (control-non-catalytic) for both reaction temperatures at a given reaction time. Maximum glucose yield of 43.5 % was obtained at 180 °C and 60 min, whereas only trace amount of glucose was found from the non-catalytic hydrolysis. In the non-catalytic hydrolysis (at 190 °C temperature) when the reaction period became longer, the glucose

yield started to increase continuously and maximum glucose yield of 7.5 % was obtained at 120 min of reaction time. The glucose yield increment can be directly associated with the microwave ability of converting the reaction media into acidic, consequently, initiating auto-hydrolysis of cellulose into glucose [63]. However, the glucose yield was lower compared to the hydrolysis conducted in the presence of BSC-500 °C. The possibility of leaching of acidic group into the aqueous solution which would then catalyze the reaction homogeneously was investigated as per Onda et al [27]. In this case, the first hydrolysis was run in the presence of BSC-500 °C and then the resultant solution was filtered to remove the catalyst and residues. Then, an equal amount of fresh cellulose was added to the filtrate and the hydrolysis was conducted at similar reaction condition. It was found out that the glucose yield of this second run was very low showing that the contribution of leached acid in the hydrolysis reaction is insignificant when compared to the BSC-500 °C catalyst.



*Figure 5-2 Influence of the microwave reactor on glucose yield with and without BSC (reaction conditions 0.25 g, cellulose; sulfonated bamboo char 0.25 g; 10 ml of water, 180 °C and 190 °C hydrolysis temperature and MP = 800 W).*

### 5.3.2.3. Efficiency of BSC-500°C compared with other heterogeneous catalysts

Comparison on the activity of cellulose hydrolysis between BSC-500 °C and other heterogeneous catalysts (BC-500 °C, Amberlyst15, MgO/F-20 %, activated carbon, and  $\gamma$ -Al<sub>2</sub>O) was performed at operating conditions of 180 °C and 60 min, and the results are shown in Figure 5.3.

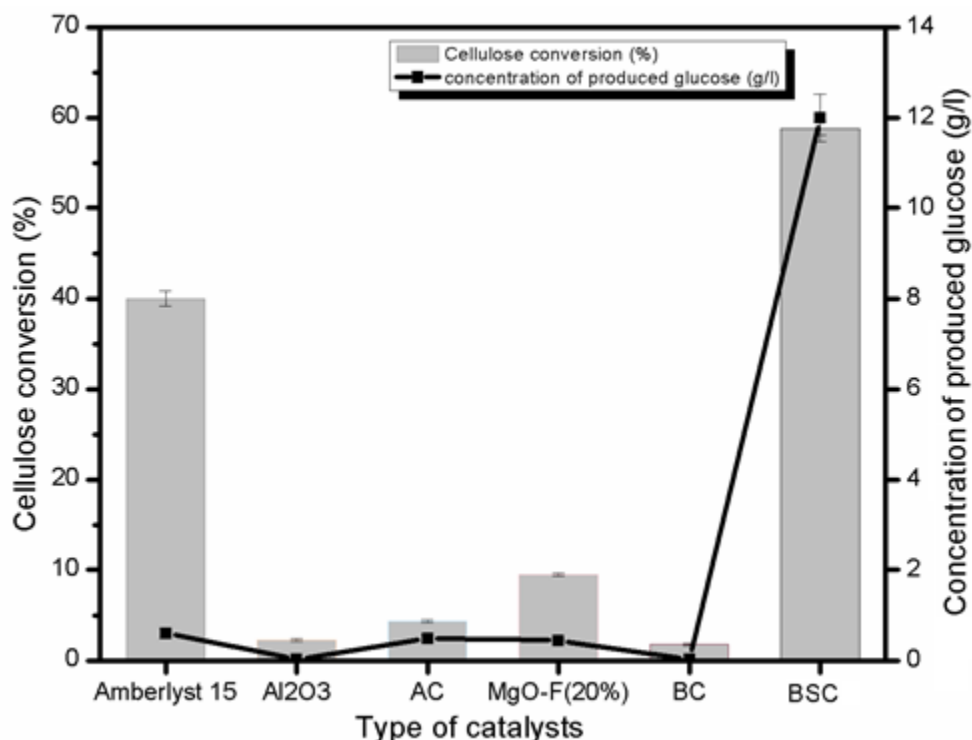


Figure 5-3 Comparative study of BSC catalyst with other solid acid catalyst in microwave reactor (reaction conditions  $T = 180^{\circ}\text{C}$ ,  $t = 60$  min; mass of catalyst and cellulose 0.25 g each; volume of water 10 ml and  $MP = 800$  W)

Amberlyst 15 showed relatively higher cellulose conversion (40 %) compared to other catalysts. However, its selectivity toward glucose was very small and product analysis from the HPLC showed that significant quantity of converted cellulose was instead changed to levulinic acid. It is also important to note that the Amberlyst resin disintegrated/degraded into small resins at the end of reaction. On the other hand, the BC -500 °C (the bamboo char) has lower cellulose conversion and traces of glucose yield in spite of the presence of weak acidic sites such as carboxylic and phenol groups. MgO/F-20 % catalyst with basic and acidic functional groups, which resulted in

cellulose conversion (10 %) and very low glucose yield (2.01 %) which is slightly higher than Amberlyst 15 catalyst. The hydrolysis performance of activated carbon was also analyzed, despite of having highest surface area (1778 m<sup>2</sup>/g) compared to BSC-500 °C catalyst (13.2 m<sup>2</sup>/g), which delivered very low cellulose conversion (4.3 %). Previously, Onda and coworkers showed that  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> catalyst's potential to convert ball-milled pretreated cellulose into water-soluble products. However, in this work, it barely converted the crystalline cellulose (2.28 %) and only traces of glucose were detected. In general, BSC-500 °C has shown superiority both in cellulose conversion and glucose yield as compared to these catalysts. The superior activity of the BSC-500 °C does not lie on the amount of acid group it contains since Amberlyst 15 has higher acid group but failed to deliver effective hydrolysis of cellulose. On the other hand, the surface area of the BSC-500 °C is too low to bring such kind of hydrolysis effect, but it contains different acidic and phenolic groups which makes it distinct from the rest [104]. Hence, the unique attribute of the BSC-500 °C probably comes from the simultaneous existence of sulfonic and the phenol and carboxylic groups [27], [104].

#### **5.3.2.4. Effect of reaction temperature and time on hydrolysis efficiency**

The effect of temperature and time of hydrolysis on the performance of BSC-500 °C (cellulose conversion and glucose yield) using the microwave reactor was further studied, and the results are shown in Figure 5.4. Figure 5.4 a shows the influence of temperature and time on cellulose conversion. As is shown in the figure, except for reaction performed at 190 °C, when the reaction temperature increased, the amount of cellulose converted also increased. Similarly, at the beginning of the reaction, high cellulose conversion was obtained as the temperature increased. This might be because, at a higher temperature, the microwave radiation deeply absorbed in the crystalline structure destroys some parts of the crystallinity of the cellulose structure, thereby

facilitating the hydrolysis [182]. At a higher temperature such as 190 °C, above 70 % of cellulose conversion was observed within 30 min of reaction. In contrast, at a lower temperature (150 °C) and time (30 min), very low cellulose conversion (3 %) was observed, and this is due to the high crystalline nature of cellulose resisting degradation [183]. In this case, only cellulose with amorphous and disordered crystallinity was able to be hydrolyzed by BSC-500 °C.

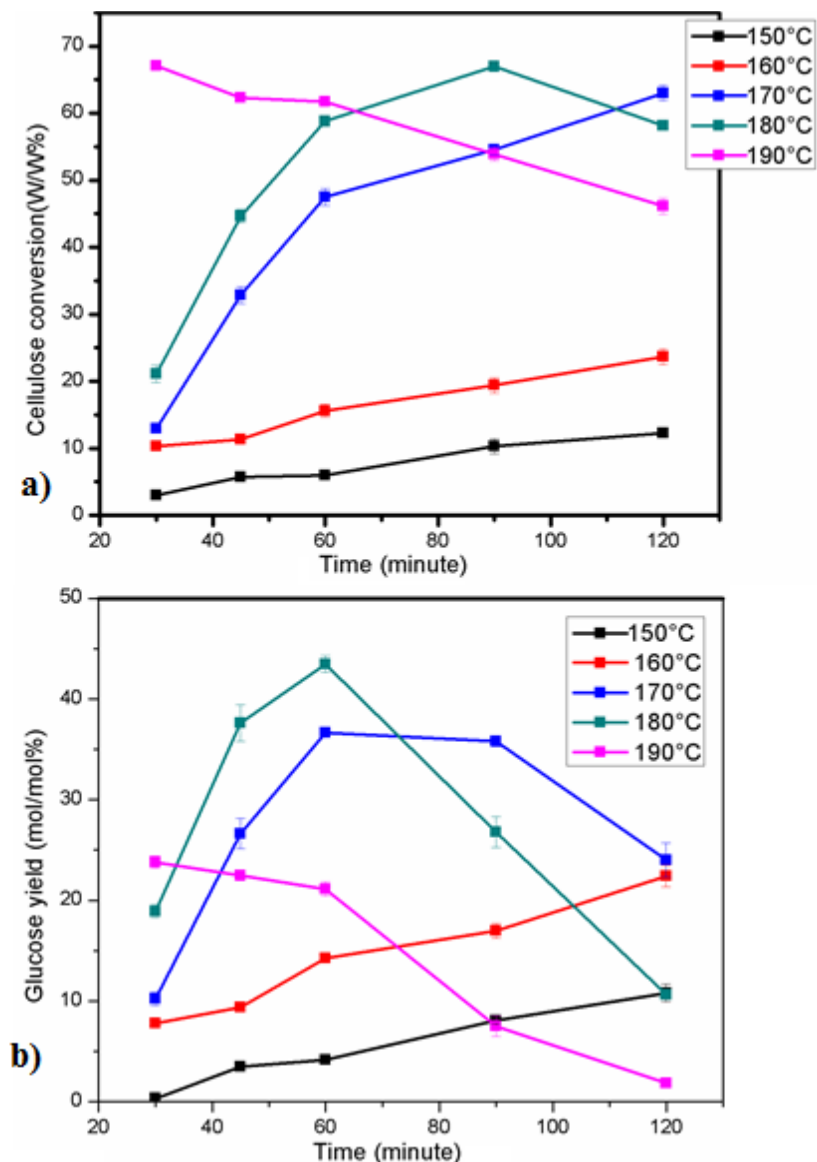


Figure 5-4 Effect of reaction temperature and time on cellulose conversion (a) and glucose yield (b) (0.25 g, cellulose; 0.25 g, sulfonated bamboo char, 10 ml of water and 800 W of microwave power)

Figure 5.4 a also shows cellulose hydrolysis reactions performed at temperature of 150 °C, 160 °C, and 170 °C, and the result specified that when the hydrolysis period increased, the amount of cellulose converted as well as glucose yield were increased. In contrast, increasing the hydrolysis period at a higher temperature such as 190 °C drastically reduced the cellulose conversion. The low performance of the BSC at higher temperature and longer period of hydrolysis could be associated with BSC deactivation or the disappearance of organic carbon from aqueous solution via solid or gas formation. Since cellulose conversion was measured indirectly by measuring total organic carbon, any alteration of its concentration would affect the cellulose conversion value. Based on this, as more and more cellulose are converted into glucose, the glucose will start to undergo further degradation reaction through consecutive dehydration and rehydration process. These consecutive reactions create other products like hydroxymethylfurfural (HMF), cellobiose, acids (levulinic and formic), and most importantly, insoluble humins (which is difficult to quantify the amount of carbon in it) [173]. Hence, the formation of insoluble humins could be the main reason for the decrement of cellulose conversion as the humins is insoluble in water posing difficulty to measure its carbon content by TOC analyzer. The highest amount of cellulose conversion obtained using microwave reactor was 70.5 % at 190 °C and 30 min of reaction.

The cellulose hydrolysis efficiency in terms of glucose yield at a given temperature and reaction time is further depicted in Figure 5.4 b. For reactions performed at 150 °C, 160 °C, and 170 °C, it has been shown that the glucose yield increased with time of reaction. Whereas for reactions performed at 180 °C and 190 °C, different scenarios were observed. For instance, at 180 °C initially the glucose yields increased as the hydrolysis time increased maximum glucose yield of 43.5 % found at 60 minute. However, further increment of reaction time in resulted in the decline of glucose yields. For reaction performed at 190 °C, maximum glucose yield of 24 % was obtained

in the first 30 minute of hydrolysis time. When the hydrolysis time further increased the glucose yields started to decreased.

To sum it up, extended hydrolysis period increased the glucose yield for reactions performed at temperature range 150–170 °C, but not too high temperature operated reactions. This implies that the glucose degradation is probably favored at higher temperatures and extended reaction time. Maximum glucose yield of 43.5% was obtained for reactions performed at 180 °C and 60 min of reaction. Figure 5.5, further shows the concentration of different products identified for the hydrolysis reaction performed at the temperature of 180 °C at different times of reaction. At the start of hydrolysis (30 min), only glucose was detected and its amount significantly increased with time until 60 min of reaction.

Further increasing of time lowers glucose yield due to the speed up glucose degradation rate than its formation so that its concentration starts to decrease sharply. As a result of glucose degradation, reverted product-like (cellobiose) and other derivatives of glucose such as HMF, levulinic, and formic acid were appeared. It is believed that glucose would first derivate into HMF then to levulinic, to formic acid, and finally to insoluble humins.

Hence, during glucose degradation process, the concentration of levulinic acid and formic acid showed a steady increment with time, whereas the concentration of cellobiose and HMF does not vary that much despite the sharp decrement in the concentration of glucose. This shows that the rate of conversion of HMF into levulinic acid and formic acid are very high using BSC. It is also important to note that, at longer reaction times, the degradation of glucose was higher but the formation of HMF and other derivative products and quantitative emergence were not equivalently high as of the glucose sharp decrement. This implies that part of glucose and other products are converting and forming insoluble humins as also described in previous section [184].

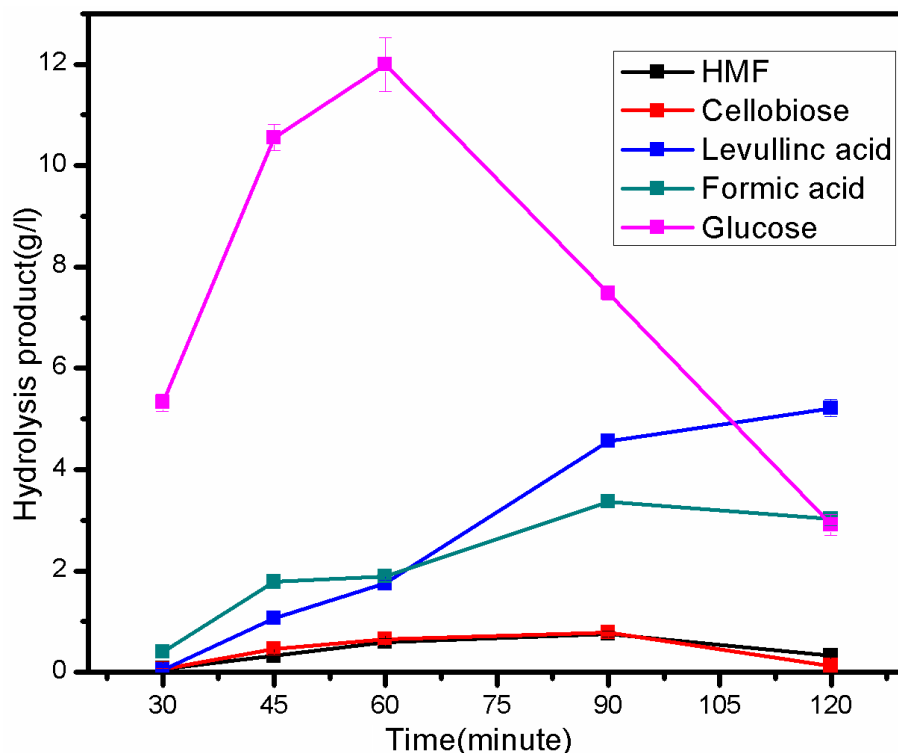


Figure 5-5 Concentration of glucose and selected glucose degradation products vs time of reaction (reaction conditions; 0.25 g, cellulose; sulfonated bamboo char 0.25 g, 180 °C and 10 ml of water)

Further investigation was done to check the BSC-500 °C capacity for the formation of these by-products, by feeding pure glucose and catalyst into microwave reactor (results not shown). In this reaction, the BSC-500 °C catalyst was able to produce similar water-soluble products as the cellulose hydrolysis reaction. This shows that BSC-500 °C catalyst has also a potential to produce this platform chemicals from glucose.

#### 5.3.2.5. BSC-500°C catalyst reusability study

The reuse potential of BSC-500 °C catalyst using microwave reactor has been checked at an operating condition which gave maximum glucose yield. The amount of cellulose used for the initial reaction was 0.25 g. Measuring the exact amount of the unconverted cellulose is difficult due to the presence of other insoluble products such as humins. As a result, different protocols were used to evaluate the reusability performance of the catalyst. The reuse potential was tested

by adding fresh cellulose (0.25 g) onto the first resultant solution from the first run (hydrolyzed product and BSC-500 °C catalyst). After the completion of the second hydrolysis, the solution was filtered and the glucose yield of the hydrolyte measured and compared with the first resultant solution. The glucose yield of the second solution (24 g/l) was found out to be almost double to the initial resultant solution (12 g/l). This implies that the BSC-500 °C catalyst can be used once again without significant loss of its activity.

#### **5.4. Conclusion**

In summary, hydrolysis of untreated crystalline cellulose (96.4 % crystallinity) in the presence of bamboo derived sulfonated catalyst using microwave reactor has been performed. It has been possible to achieve a maximum glucose yield of 43.5 %, at 180 °C and 60 minute of reaction time. The result was found to be remarkably high compared to other reports on the hydrolysis of untreated cellulose using conventional heating with bamboo catalyst and/or other solid catalysts. The relative high conversion of crystalline cellulose was attributed to the combined effect of the sulfonated bamboo catalyst and the high temperature microwave heating. This study demonstrated that the combined use of biomass derived sulfonated catalyst and high temperature microwave heating can significantly hydrolyzed crystalline cellulose without the aid of any pretreatment processes.

## Chapter Six

### 6. Microwave reactor based optimization of cellulose hydrolysis in the presence of bamboo derived sulfonated catalyst using response surface methodology

#### Abstract

*The optimization of cellulose hydrolysis under microwave reactor (MWR) in the presence of bamboo derived sulfonated catalyst was studied through a response surface methodology (RSM). Three-factor and three-level Box-Behnken design was employed to study the effects of hydrolysis temperature, hydrolysis time and catalyst to substrate ratio on total converted cellulose and glucose yield (the responses). Quadratic models were proposed for both responses. The respective analysis of variance (ANOVA) showed that the proposed quadratic models could be used to navigate the design space. The optimum hydrolysis conditions were found out to be hydrolysis temperature of 175 °C, hydrolysis time of 74 min and catalyst to substrate ratio of 1.25 g/g. Under these conditions the total amount of converted cellulose and glucose yield were 79.4% and 61.1%, respectively. Validation of the models indicated the predicted values were in a good agreement with the experimental results.*

**Keywords:** Microwave reactor, Sulfonated catalyst, Cellulose hydrolysis, Response surface methodology, Box-Behnken design, Optimization.

## 6.1. Introduction

In previous chapter, the effects of microwave reactor in the hydrolysis of crystalline cellulose in the presence bamboo-derived sulfonated catalyst was studied. Accordingly, some of the findings were published [185]. Promising results of total converted cellulose and glucose yield in a short period were found for cellulose (96% crystalline) hydrolyzed in a microwave reactor. Based on the results found in the previous chapter, the use of microwave reactor combined with BSC-500 °C catalyst in the hydrolysis of crystalline cellulose is promising to be implemented in pilot scale production. On top of this, the combined use of microwave reactor and BSC-500 °C catalyst on total cellulose conversion further pronounced, if prior ball-milled cellulose was used in the hydrolysis reaction [15].

It is known that, degree of crystallinity of a cellulose reduce when it is properly milled. Lowering the crystallinity of the cellulose will facilitates the conversion of the cellulose immensely. Furthermore, cellulose with lower degree of crystallinity can be hydrolyzed at mild hydrolysis operating conditions (temperature and time). Similarly, hydrolysis of milled cellulosed in microwave reactor in the presence of BSC-500 °C catalyst is expected to result higher cellulose conversion (mainly glucose) compared to crystalline cellulose.

On the other hand, to effectively reduces the crystallinity of cellulose under ball-milling, it requires longer milling period (48 h) [27]. Conversely, longer ball-milling period for the pretreatment of cellulose makes the techniques less attractive when it comes to large scale productions of glucose from cellulose hydrolysis. Hence, it is important to search other milling type which could be used for crystalline cellulose milling, and can give similar result with ball milling, but within a short period of time. Among different type of milling, vibrational milling was selected for this work for crystalline cellulose milling. It is expected that, the substitution of

vibrational milling in the place of ball milling for cellulose milling will have the potential to shorten the milling time because it has high vibrational speed.

In addition to searching advanced milling system with the combined use of BSC-500 °C and microwave reactor further the hydrolysis reaction can be promoted at optimal cellulose hydrolysis operating conditions. Hence, optimizing the cellulose hydrolysis operating conditions will lead to identifying the best operating conditions. Previously, the influence of stirring rate, amount of water, and cellulosic reactant on the hydrolysis of cellulose and lignocellulose material using biomass-derived sulfonated catalyst was investigated through a conventional reactor (oil-bath heating system) [99], [186]. However, the interaction effects of important operating parameters like hydrolysis temperature, time and substrate to catalyst ratio in the hydrolysis of cellulose using sulfonated solid catalysts have been barely studied. Moreover, the previous studies focused only on the conventional type reactor with an oil-bath heating system in which its mode of heat transfer to the reaction is slow compared to microwave heating. Therefore, to identify the best operating conditions for maximum cellulose conversion and glucose yield output, it is important to make numerical optimization of cellulose hydrolysis reaction.

RSM is an empirical optimization technique for evaluating the relationship between the given factors and the responses (experimental output). RSM is usually used with the combination of factorial design method such as BBD and central composite. Implementing RSM with BBD can significantly reduce the total number of experiments without decreasing the accuracy of the optimization [187]. Moreover, BBD can avoid the combinations of extreme treatment conditions [188]. The combinations of extreme temperature and time during cellulose hydrolysis would lead to the degradation of converted cellulose into insoluble humin, which is difficult to quantify [189], [190].

In this work, cellulose (initially milled by vibrational milling) hydrolysis using BSC-500°C in microwave reactor was performed and optimized in terms of hydrolysis temperature, hydrolysis time and substrate to catalyst ratio to obtain the maximum amount of converted cellulose and glucose yield using response surface methodology (RSM) with Box-Behnken design (BBD).

## **6.2. Materials and Methods**

### **6.2.1. Materials**

BSC-500 °C catalyst which was synthesized and characterized in previous section was used for the hydrolysis reactions, Sigma-Aldrich cellulose powder (acid washed, powder, for column chromatography) which was initially pretreated using a vibrational mill for 1 h was used for the optimization of the hydrolysis process. Analytical grade anhydrous D-glucose, hydroxymethylfurfural (HMF), levulinic acid, cellobiose, acetic and formic acid from Sigma-Aldrich were used to prepare the standard for the high-performance liquid chromatography (HPLC). Furthermore, Merck sulfuric acid was used to prepare the mobile phase for the HPLC. Pretreatment of cellulose powder was performed using Retsch mix milling (MM 2000) equipment with two 12 mm zirconium oxide made grinding ball. X-ray diffraction (XRD) measurement for cellulose powder before and after pretreatment of vibrational milling was performed by Siemens diffractometer D5000. A Synth-wave single reaction chamber microwave reactor was used for cellulose hydrolysis reaction. Shimadzu total carbon analyzer (TC) was used to measure the total organic carbon from the hydrolysis product. Furthermore, Agilent made high-performance liquid chromatography (HPLC) with refractive index detector (RID) and ICSep-COOREGEL 87H3 column was employed to quantify the concentration of glucose and other product in the final solution of the hydrolysis.

## **6.2.2. Methods**

### **6.2.2.1. Cellulose milling**

The crystalline cellulose was initially milled using benchtop Retsch mix laboratory vibrational mill (MM 2000) before the hydrolysis reaction. The milling process was conducted by measuring 3.14 g of cellulose powder and by introducing it into a 25 ml grinding jar that was equipped with a push-fit lid made of zirconium oxide and protective stainless steel jacket. Moreover, two 10 g of balls made of zirconium oxide (12 mm of diameter) were used for the milling process. The milling did run for a period of 5, 15, 30, and 60 min with a constant vibrational frequency of 900 min<sup>-1</sup>. When the milling period was more than 15 min, the cellulose showed a tendency to attach with the ball. To avoid such problem, the milling ball was cleaned after every 15 min of milling time for milling period above 15 min.

### **6.2.2.2. X-ray diffraction and analysis techniques of cellulose sample**

The XRD (X-ray diffraction) measurements for all samples were made based on section 5.2.2.

### **6.2.2.3. Cellulose hydrolysis and analysis of its hydrolyte**

Cellulose hydrolysis was performed in a single chamber *synth-wave* microwave reactor using glass vials which were placed on five places rack and introduced in the microwave reactor. For all analysis, the microwave was adjusted to work at 30 bar of pressure, 800 W power, and 50% of its maximum stirrer capacity. In all hydrolysis run, measured amount of milled cellulose (for 60 min), sulfonated bamboo catalyst and 10 ml of distilled water were mixed in glass vials (which were covered with a loose fit Teflon caps to ensure pressure equalization) and then placed on five places in a rack. The rack was mounted in a 300 ml distilled water contained Teflon made microwave's chamber. Afterward, by assigning the specific operating conditions (temperature, time, and microwave power) in the external terminal, the hydrolysis was run. For the given hydrolysis period, 10 min out of the total reaction time was assigned for the microwave to attain the operating

temperature. After completion of the hydrolysis, the microwave was left to cool for a couple of minute and the resultant solution was filtered and the unreacted cellulose and the BSC catalyst were separated from water-soluble products. The cellulose conversion was evaluated from the total organic carbon content of the filtrate using equation. 5.3. The total organic carbon of the filtrate was measured using Shimadzu total organic carbon (TOC-L CSN ver. 1.04.00) analyzer. The amount of glucose and related cellulose hydrolysis products such as hydroxymethylfurfural (HMF), cellobiose, levulinic and formic acid were measured by HPLC. Dilute sulfuric acid was used as a mobile phase. The standard solutions of glucose, HMF, cellobiose, levulinic and formic acid were prepared and run every time with the filtrate of the hydrolysis product during HPLC analysis. The retention time took by each standard was used to identify the type of products found in the samples. The peak area found at the given retention time was converted into concentration (g/l) by initially prepared patrons. Finally, the glucose yield was calculated using Equation. 5.4.

#### **6.2.2.4. *Box-Behnken experimental design***

The BBD with RSM was used to optimize the hydrolysis conditions to obtain the maximum amount of converted cellulose and glucose yield. Implementing RSM with Box-Behnken design for optimization comprises four main steps [187]: (i) Generate a statistical experimental plan based on the independent variables and execute the experiment according to the plan. (ii) Propose a mathematical model according to the responses of the experimental results and elaborate the result of analysis of variance. (iii) Check the accuracy of the model through diagnostic plots (iv) Execute response analysis of the model and predict optimal conditions, and confirm the model through running an experiment

Based on the results found from the previous study of the authors [185]; the workable range of the independent variables identified as hydrolysis temperature, ' $X_1$ ', (165-175 °C), hydrolysis time,

' $X_2$ ', (45-75 min), and catalyst to substrate ratio ' $X_3$ ', (1-1.5 g/g). A three-factor with three-level Box-Behnken experimental design in response surface methodology (RSM) was used to optimize the selected parameters to have a maximum amount of converted cellulose and glucose yield. The range and level of the variable is shown in Table 1. The three factors  $X_1$ ,  $X_2$ , and  $X_3$ , were prescribed into three levels, coded +1, 0, and -1 for high, intermediate, and low value, respectively. The three test variables coded according to equation 6.1 as follows.

$$xi = \frac{Xi - Xo}{\Delta X} \quad i = 1,2,3 \quad 6.1$$

Where  $xi$  is the coded variable,  $Xi$  is the actual value of an independent variable,  $Xo$  is the actual value of the independent variable at the center point and  $\Delta X$  is the step change value of an independent variable.

*Table 6-1 Coded and actual levels of the three independent factor for Box-Behnken design*

Independent variables	Symbol of actual value	Coded and Actual value		
Hydrolysis reaction temperature (°C)	T	(-1)165	(0)170	(+1) 175
Hydrolysis reaction time (min)	t	(-1) 45	(0) 60	(+1) 75
Catalyst to Cellulose loading; C (g/g)	w/w	(-1)1	(0) 1.25	(+1) 1.5

Using Box-Behnken design, employing the statistical package Design Expert 10.0., the total numbers of the experimental runs were found to be 17 and five of the experimental runs out of the 17 were a replicates at the center point of the experiment. To avoid any bias, the sequence of the experimental runs were randomized. The relation between the independent and the response variables was assumed to be quadratic, and its general form of expression is given in equation 6.2.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad 6.2$$

Where  $Y$  is the response (amount of total converted cellulose or glucose yield),  $b_0$  is offset term,  $X_1$ ,  $X_2$ , and  $X_3$  are the independent variables,  $b_1$ ,  $b_2$ , and  $b_3$  are linear terms,  $b_{12}$ ,  $b_{13}$  and  $b_{23}$  are interaction terms, and  $b_{11}$ ,  $b_{22}$ , and  $b_{33}$  are quadratic terms. The goal of this design experiment is to determine conditions for a maximum amount of cellulose conversion and glucose yield with optimized hydrolysis temperature, time, and catalyst to substrate ratio. Based on this design, the number of the experimental run chosen is suitable for the assessment of quadratic response surface and to generate a second-degree polynomial model, which is used optimizing a process using a small number of experimental runs.

### **6.3. Results and Discussion**

#### **6.3.1. X-ray diffraction patterns comparison of raw and milled cellulose**

The XRD characteristics of the raw cellulose powder treated in Retsch vibrational mix mill for a period of 5, 15, 30 and 60 min and the XRD patterns of each treated cellulose was compared. Figure 6.1 shows the XRD diffractograms of raw and milled cellulose for different milling time. The 5 min vibrational milled cellulose has almost similar diffractogram patterns with the raw cellulose (Figure 6.1). However, a significant decrease of the crystalline index ( $C_{rl}$ ) is observed after the five min of milling process (from 96.4 % to 85.7 %). On the other hand, the crystallite size only slightly changed (3.61 nm to 3.59 nm) as it is shown in Table 6.2. The diffractogram of cellulose milled for 15 min (Figure 6.1) showed a decrease in the intensity of the diffraction peak around  $2\theta = 22^\circ$  compared to the peak of the raw cellulose at the same diffraction angle. However, the change in its  $C_1$  value was not significant compared to the  $C_1$  value of the 5 min milled cellulose. However, the corresponding milling process led crystallite size to decrease from 3.61 nm to 3.28 nm.

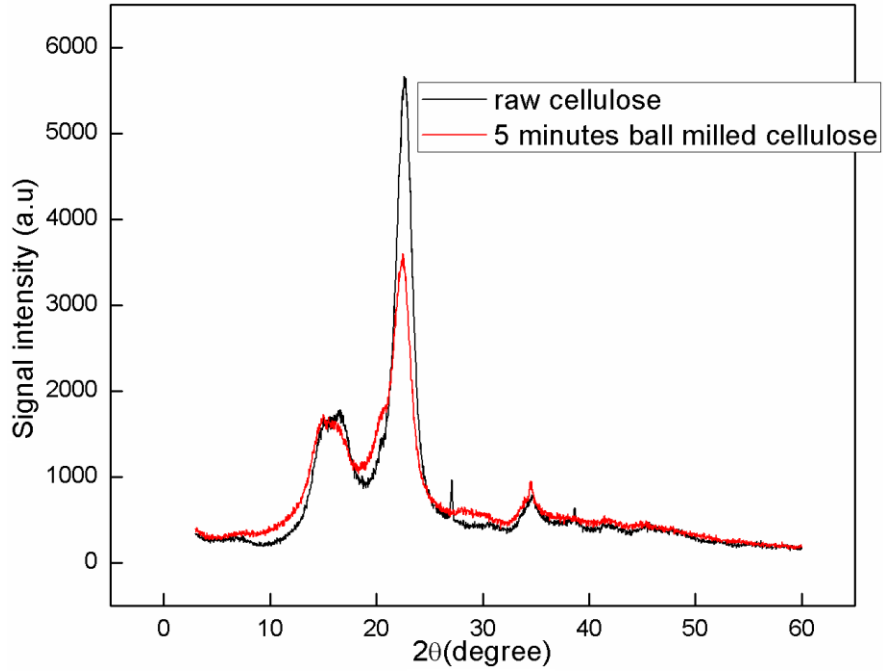


Figure 6-1 XRD patterns of raw cellulose compared with 5 min vibrational milled cellulose

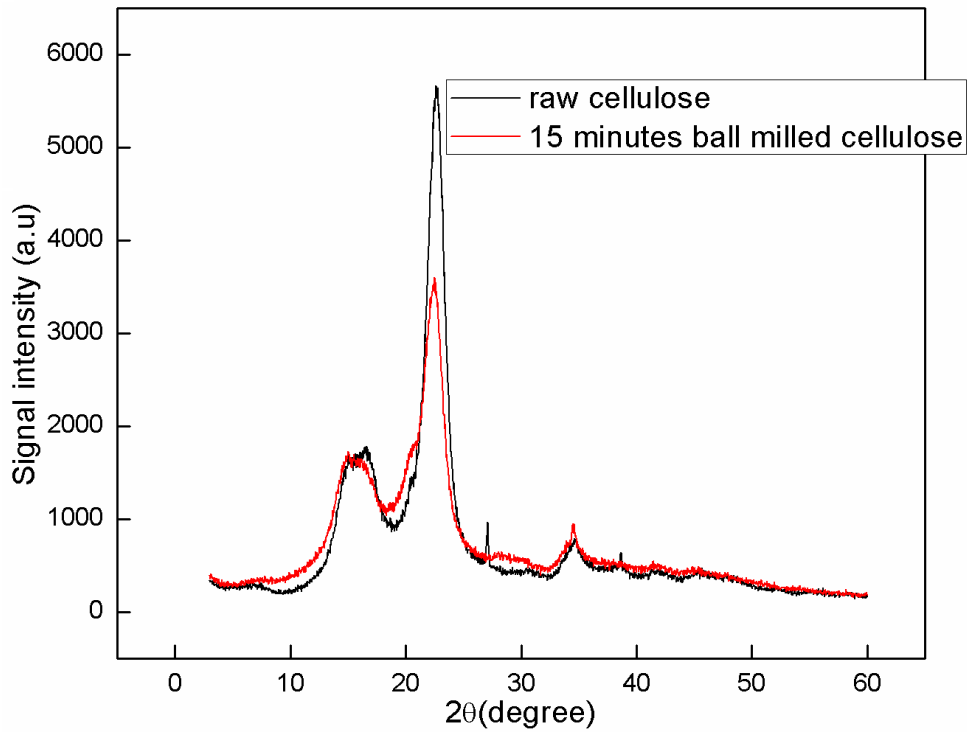
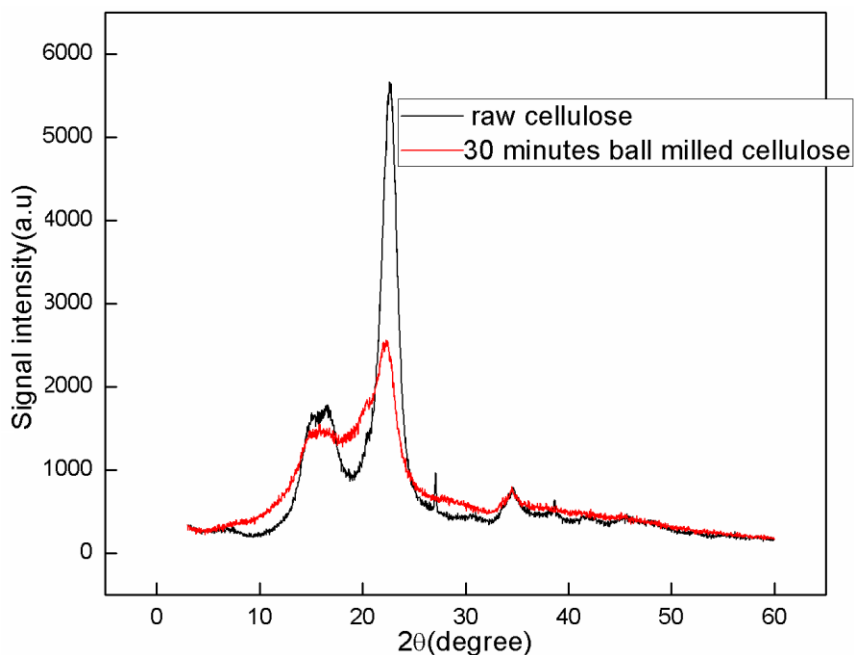


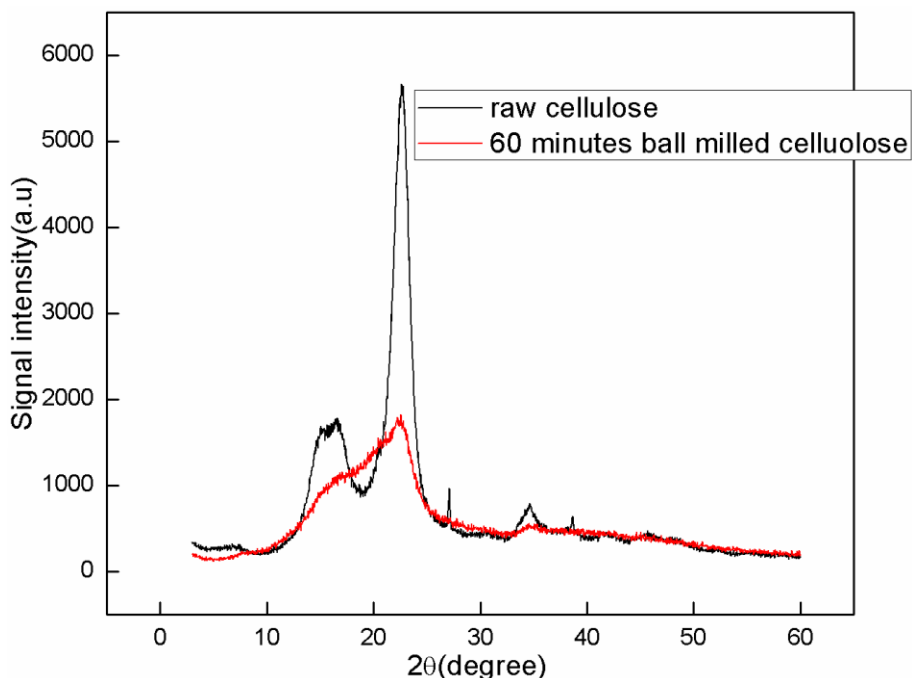
Figure 6-2 XRD patterns of raw cellulose compared with 15 min vibrational milled cellulose

Figure 6.3 shows the XRD patterns of cellulose which was milled for 30 min. The peaks of the diffractogram at  $2\theta = 22^\circ$  for the 30 min vibrational-milled cellulose are much lower than the raw cellulose. Furthermore, the size of the crystalline is decreased up to 2.42 nm. However, the change observed in the  $C_I$  after 30 min milling is not significant as is shown in Table 6.2.

Figure 6.4 represents x-ray patterns of raw cellulose and 60 min -milled cellulose. Some of the diffraction peaks which existed in raw cellulose practically disappeared after the 60 min vibrational-milled cellulose. Moreover, the most intense diffraction line located in raw cellulose around  $2\theta = 22^\circ$  exhibited a lower signal intensity (the peak height became significantly lower and broader). The  $C_I$  value was found to be 59.55 % which significantly lower compared to the  $C_I$  of the raw cellulose.



*Figure 6-3 XRD patterns of raw cellulose compared with 30 min vibrational milled cellulose*



*Figure 6-4 XRD patterns of raw cellulose compared with 60 min vibrational milled cellulose*

In addition to this, the crystallite size also decreased to 2.09 nm. The  $C_{rl}$  value and the crystallite size attained in 60 min vibrational milling is comparable to the previous work of Onda et al. who used ball mill to mill cellulose for a period of 48 h with a rotational speed of 60 rpm [27]. Furthermore, the amount of total converted cellulose as well as glucose yield found after the hydrolysis of 60 min vibrational milled cellulose is higher compared to the authors' previous work., in which only raw cellulose used in the place of milled cellulose but the rest are (operating conditions, catalyst and reactor) are similar to the current work. Therefore, applying vibrational milling as one of the techniques to use for the reduction of the crystallinity of raw cellulose is convenient.

*Table 6-2 Crystallinity index and crystalline size of cellulose vibrational milled at different time*

Ball milling time (min)	Crystallinity index (%)	Crystallite size (nm)
0	96.4	3.61
5	85.7	3.59
15	84.4	3.28
30	80.4	2.42
60	59.55	2.09

### **6.3.2. Hydrolysis of pretreated cellulose**

Crystalline cellulose which was pretreated for 60 min in the vibrational mill had been used for the hydrolysis reactions. The experimental condition for the hydrolysis is stated in Table 6.3, other operating conditions such as stirrer speed (50 % of the maximum), water (10 ml), microwave power (800 W) and 30 bar of pressure. Cellulose conversion value and the glucose yield from the experiment were taken and run in the design expert software. The statistical analysis of the results stated as follows.

#### **6.3.2.1. Model fitting and analysis of variance (ANOVA)**

Single and interaction effects of the independent factors on the amount of total converted cellulose and glucose yield were studied by employing the Box-Behnken design with RSM. A total of 17 experimental run according to Box-Behnken design were performed and the actual and predicted value of the responses along with the experimental conditions are summarized in Table 6.3.

The experimental data was analyzed by Design-Expert 10.0.1 software and the quadratic models are suggested to best fit both responses. The result of the analysis of variance (ANOVA) for the response surface quadratic model both for cellulose conversion and glucose yield are given in

terms of F and p-value (Table 6.4). The three factors designated as temperature ( $X_1$ ), time ( $X_2$ ), and catalyst to substrate ratio ( $X_3$ ).

*Table 6-3 Box-Bhenken design, experimental data for 3-level and 3-factor response surface analysis along with model fitted values*

Run No.	Actual Factor			Cellulose Conversion (%)		Glucose yield (%)	
	Hydrolysis temperature (°C)	Hydrolysis time (min)	Catalyst loading ratio (g/g)	Actual value	Model fitted value	Actual value	Model fitted value
1	165	75	1.25	60.6	60.664	47.54	47.958
2	170	45	1.5	60.1	59.923	49.05	49.416
3	170	60	1.25	70.58	70.364	54.25	54.232
4	165	60	1.5	58.52	58.657	47.09	46.915
5	165	45	1.25	55.72	55.759	44.85	44.658
6	165	60	1	58.45	58.209	45.25	45.197
7	175	45	1.25	64.8	64.735	52.6	52.181
8	170	45	1	59.43	59.631	48.25	48.493
9	170	60	1.25	70.25	70.364	54.2	54.232
10	170	60	1.25	69.42	70.364	53.12	54.232
11	170	60	1.25	70.89	70.364	54.24	54.232
12	170	75	1	67.2	67.376	53.32	52.953
13	175	60	1.5	74.256	74.497	58.12	58.172
14	170	75	1.5	72.05	71.848	57.49	57.246
15	170	60	1.25	70.68	70.364	55.35	54.232
16	175	75	1.25	79.54	79.500	60.98	61.171
17	175	60	1	70.32	70.182	54.5	54.675

As it is shown in table 6.4, both total amount of converted cellulose (CC) and glucose yield (GY) models are significant. Furthermore, all model terms for CC response are significant. In this case  $X_1, X_2, X_3, X_1X_2, X_1X_3, X_2X_1, X_1^2, X_2^2$  and  $X_3^2$  are significant model terms.

Table 6-4 ANOVA of regression model for cellulose hydrolysis (CC and GY)

	CC		GY	
	F-value	P-value	F-value	P-value
Model	343.83	< 0.0001	82.02	< 0.0001
$X_1$ -Temperature (T)	1654.41	< 0.0001	413.63	< 0.0001
$X_2$ -Time (t)	827.48	< 0.0001	145.31	< 0.0001
$X_3$ -catalyst to cellulose loading ratio,C	48.52	0.0002	26.16	0.0009
$X_1X_2$	103.96	< 0.0001	15.57	0.0043
$X_1X_3$	15.98	0.0052	1.65	0.2402*
$X_2X_3$	18.68	0.0035	5.46	0.0476
$X_1^2$	91.48	< 0.0001	25.20	0.0010
$X_2^2$	156.23	< 0.0001	7.72	0.0240
$X_3^2$	133.61	< 0.0001	12.23	0.0081
Lack of Fit	0.31	0.8180*	0.67	0.6457*

\*Not significant model terms

The "Lack of Fit F-value" for the CC model which is not high indicating the good fitness of the model. On the other hand, for GY response model terms of  $X_1, X_2, X_3, X_1X_2, X_2X_3, X_1^2, X_2^2$  and  $X_3^2$  are significant, but the interaction term of  $X_1X_3$  (interaction between temperature and catalyst to substrate ratio) is insignificant, hence it will not be included in the model equation of glucose yield response. The "Lack of Fit F-value" of 0.62 implies the lack of fit is not significant relative to the pure error.

The fitness and suitability of the developed regression models to the experimental results were further analyzed by the coefficients of determination ( $R^2$ ). Table 6.5 explains the regression

coefficient of the quadratic models developed for both responses. An  $R^2$  value of 0.998 is reported for CC model. An  $R^2$  value of 0.998 means that the model can explain 99.8 % of the variation in CC, and only 0.2 % was as a result of chance. In addition to this, the predicted  $R^2$  ( $R^2_{pre} = 0.990$ ) is in a reasonable agreements with the adjusted  $R^2$  ( $R^2_{adj} = 0.995$ ) implying the high correlation between the actual and predicted values of CC. The adequate precision value measures the signal to noise ratio and, a ratio greater than 4 is always desirable. In the case of CC model an adequate precision value of 64.021 is obtained that indicates an adequate signal. Hence, this model can be used to navigate the design space.

*Table 6-5 Coefficient of determinations and adequacy precision (CC and GY)*

Source	Response value	
	CC	GY
R-Squared	0.998	0.988
Adjusted R-Squared	0.995	0.976
Predicted R-Squared	0.990	0.941
Adequate Precision	64.021	31.480

Similarly, for the GY developed quadratic model, the coefficient of determination ( $R^2 = 0.988$ ) indicates an excellent fit, meaning that the fitted model explained about 98.8% of the total variation in the GY. In addition to this, the difference between the adjusted  $R^2$  ( $R^2_{adj} = 0.976$ ) and that of predicted  $R^2$  ( $R^2_{pred} = 0.941$ ) is in reasonable agreement, that means high correlations existed between the actual and predicted value of glucose yield. Finally, the adequate precision of the model is 31.48 hence, the suggested quadratic model is a good fit to the experimental data and can be used to navigate the design space.

### 6.3.2.2. *The Quadratic model equations and selected model diagnostic test*

After applying multiple regression on the independent experimental data, the quadratic models were adequately represented the relationship between both CC as well as GY with those of hydrolysis temperature, hydrolysis time and, a catalyst to substrate ratio. The model equations generated based on the coded factors are written for both the cellulose conversion and glucose yield respectively as follows:-

$$CC(\%) = 70.36 + 6.95 * X_1 + 4.92 * X_2 + 1.19 * X_3 + 2.46 * X_1X_2 + 0.97 * X_1X_3 + 1.04 * X_2X_3 - 2.25X_1^2 - 2.95 * X_2^2 - 2.72X_3^2 \quad 6.3$$

$$GY(\%) = 54.23 + 5.18 * X_1 + 3.07 * X_2 + 1.30 * X_3 + 1.42 * X_1X_2 + 0.84 * X_2X_3 - 1.76 * X_1^2 - 0.98 * X_2^2 - 1.23X_3^2 \quad 6.4$$

Whereas, CC and GY refers to the cellulose conversion and glucose yield, respectively.

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. Moreover, it is useful for identifying the relative impact of the factors by comparing the factor coefficients. Accordingly, the hydrolysis temperature has a higher impact on both cellulose conversion and glucose yield followed by the hydrolysis time, and catalyst to substrate ratio. On the other hand, the equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the level is specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients scaled to accommodate the units of each factor and the intercept is not at the center of the design space. The final equation in terms of the actual factor is given in equation 6.5 and 6.6 as follows.

$$CC(\%) = -2391.78 + 29.10315T - 4.03703t - 34.451C + 0.032867Tt + 0.7732TC + 0.278667tC - 0.09015T^2 - 0.01309t^2 - 43.58C^2 \quad (6.5)$$

\*to avoid results discrepancy, the generated equation is written without making any approximation

$$GY(\%) = -2015.4 + 23.882T - 2.779t + 40.875C + 0.019Tt + 0.225tC - 0.071T^2 - 0.004t^2 - 19.656C^2 \quad (6.6)$$

The model diagnostic test was performed using the predicted and actual values of for both responses as is shown in Figure 6.5.

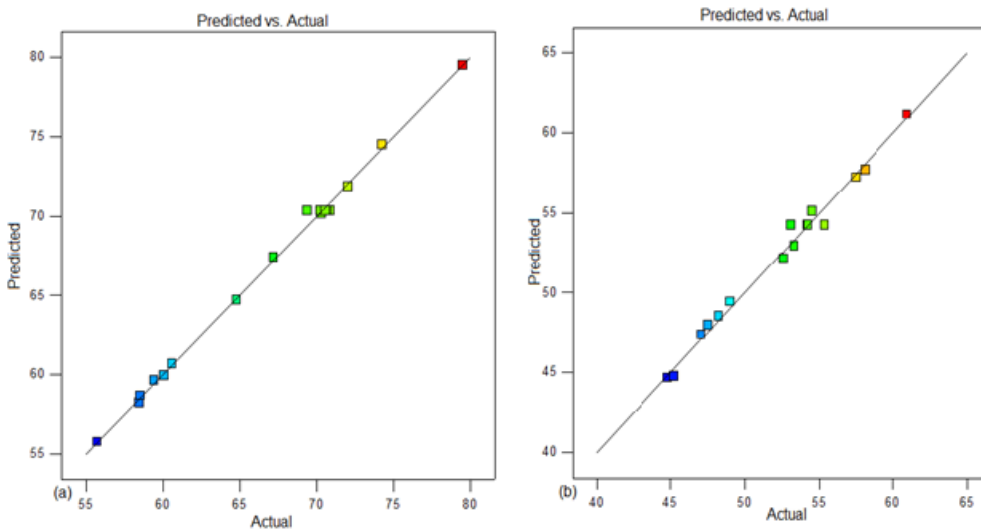


Figure 6-5 Actual and predicted response value of cellulose conversion (a) and glucose yield (b)

Figure 6.5a shows the predicted vs actual value of the cellulose conversion response. All data points are either near to or laid on the  $y = x$  line, which indicates that the model is well fitted.

Similarly, Figure 6.5b shows the predicted vs actual values of glucose yield. Here again, all the

data points lay near to the  $y = x$  line, which indicates the variability of the data is well explained by the generated model.

### **6.3.2.3. Response surface analysis of hydrolysis reaction**

The interaction effects of the independent variables in the given responses can be better understood by studying the three dimensional response surface plots, which are generated from the predicted model equations. Figure 6.6 and Figure 6.7 represent the interaction effects of independent variables on the total converted cellulose and glucose yield respectively. Figure 6.6a, shows the relationship between amount of total converted cellulose with hydrolysis temperature ranges 165-175 °C, hydrolysis time of 45-75 min, and constant catalyst to substrate dosage 1.25 g/g.

As it is shown from Table 5, at hydrolysis temperature of 165 °C and a reaction time of 45 min, the lowest amount of converted cellulose (55.76 %) is obtained. On the other hand, the maximum amount of total converted cellulose (79.5 %) is found when both hydrolysis temperature and time are 175 °C and 75 min, respectively. The result clearly shows that higher temperatures and longer time favors the conversion of cellulose to water-soluble products. Higher temperature and longer time facilitate the breakdown of the hydrogen bond in the cellulose, which in turn leads an enhanced cellulose conversion. However, too high hydrolysis temperature and time will minimize the measured total cellulose conversion by creating water-insoluble humins [189]. To avoid such problem, it is better to limit the maximum temperature and time to 175 °C and 75 min respectively [185], [64]. Figure 6.6b shows the interaction effects of hydrolysis temperature (165 °C- 175 °C) and catalyst to substrate ratio (1-1.5 g/g) on the total converted cellulose at constant hydrolysis time of 60 min. The total converted cellulose is directly related to both temperature and catalyst to substrate ratio. The hydrolysis temperature provide more thermal effects to exist in the hydrolysis reaction hence more hydrogen bond of cellulose is destroyed and this in turn increases

the conversion of cellulose. On the other hand, the increment of catalyst to substrate ratio increased the total converted cellulose, this is due to that the increment of catalyst increased the active surface area of the catalyst in contact with the cellulose. Figure 6.6c shows the interaction effects hydrolysis time (45-60 min) and catalyst to substrate ratio (1-1.5 g/g) at constant hydrolysis temperature of 170 °C. When the catalyst to substrate ratio and hydrolysis time increased, the total cellulose conversion also increased. Cellulose conversion of 72.5 % was found for a hydrolysis time of 75 min and a catalyst to substrate ratio of 1.5. However, when the hydrolysis time was 45 min and the catalyst to substrate ratio was 1 a cellulose conversion of 59.43 % was found. Since the surface area of the given catalyst is too small, increasing its ratio increases the total contact area with the cellulose and this in turn favors more cellulose to convert into water soluble product within a shorter period of hydrolysis time. The total converted cellulose which was found using a catalyst to substrate ratio of 1.5 g/g at a temperature of 170 °C and hydrolysis time of 75 min is similar to the total converted cellulose that was obtained using similar temperatures but with a catalyst to substrate ratio of 1.25 g/g, and lower hydrolysis time (60 min). Therefore applying a catalyst to substrate ratio of 1.25 g/g is an economical choice for the conversion of cellulose using the aforementioned catalyst.

The interaction effects of the given factors on glucose yield are shown using a response surface plot in Figure 6.7. Figure 6.7a indicates the interactions effects of hydrolysis temperature (165 °C-175 °C) and hydrolysis time (45-75 min) on the amount of glucose yield at a constant catalyst to substrate ratio of 1.25 g/g. Similar to amount of total converted cellulose, a minimum amount of glucose yield observed at lower hydrolysis temperature and time. This is due to the fact that, at lower temperature and short hydrolysis time, the breakdown of cellulose is limited and this in turn minimizes the glucose yield.

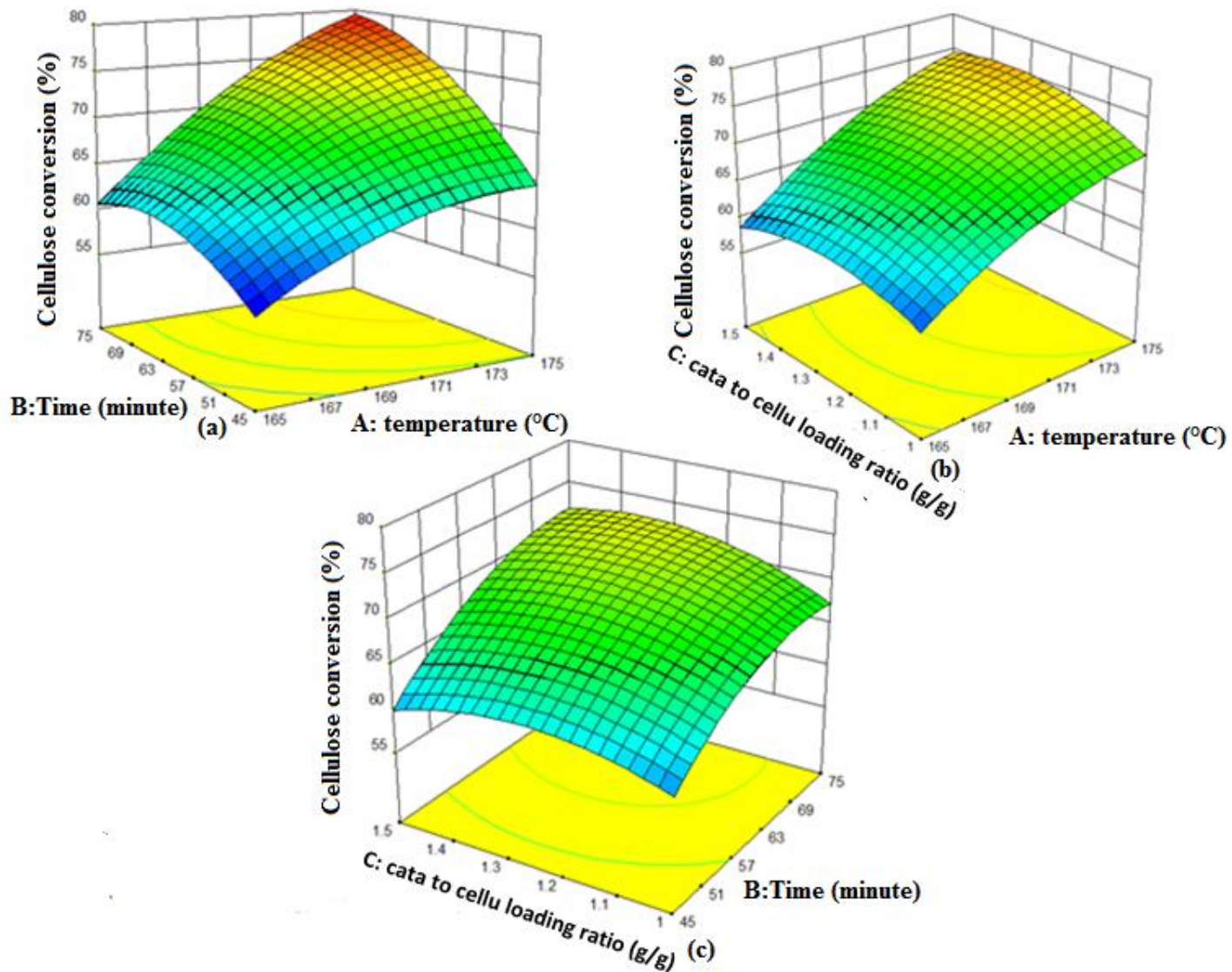


Figure 6-6 Response surface plot of (time and temperature vs cellulose conversion (a)), (cat to cell ratio and time vs cellulose conversion (b)) and (cat to cell ratio and temperature vs cellulose conversion (c))

On the other hand, at certain temperature and hydrolysis time, a simultaneous increment of both factors leads to more cellulose to be converted into water soluble products and this in turn made the glucose yield to be increased. Further increment of temperature and time increased the glucose yield. However, at elongated hydrolysis time and higher temperature the rate of glucose yield

increment was decreased due to the degradation of some part of the formed glucose into HMF, levulinic and formic acid [169].

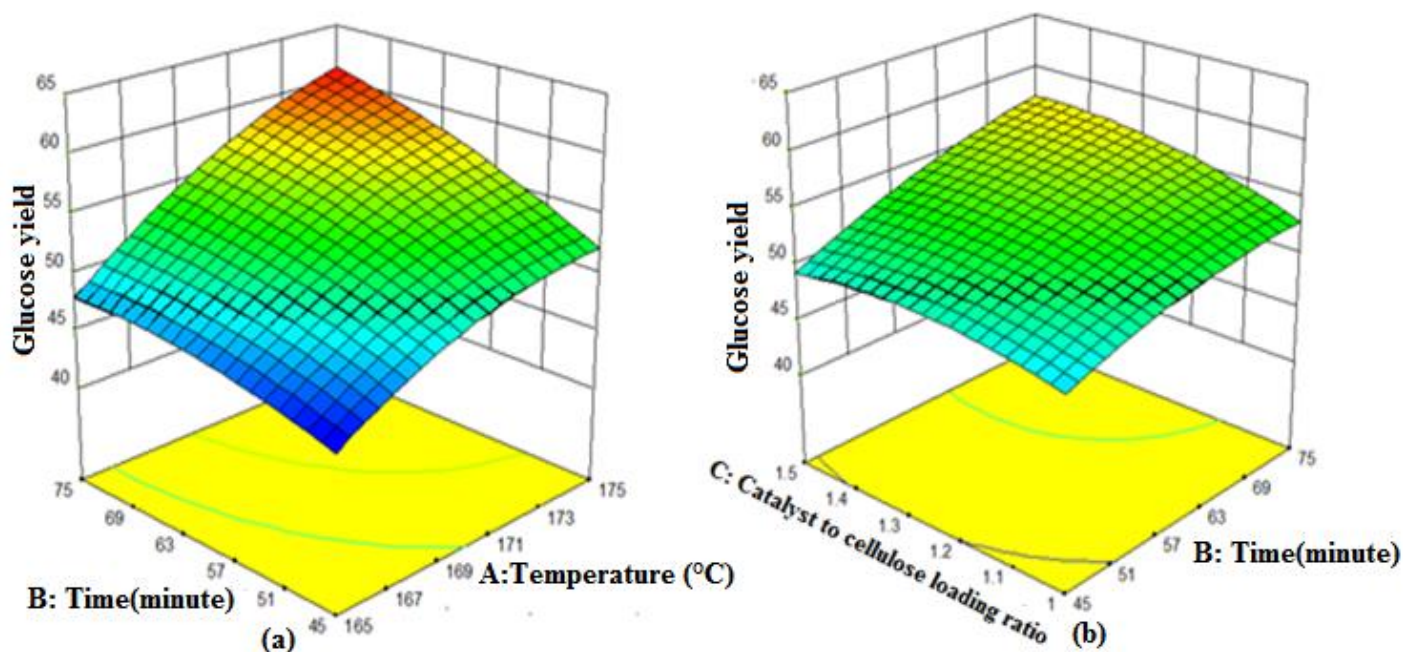


Figure 6-7 Response surface plot of (time and temperature vs glucose yield (a)) and (cat to cell ratio and time vs glucose yield (b))

Hence, the tradeoff between the amount of glucose yield and its selectivity should be taken into consideration when increasing the hydrolysis temperature and time.

Figure 6.7b shows the response surface plot for the interaction effect of hydrolysis time (45-75 min) and catalyst to substrate ratio (1-1.5 g/g) at constant temperature of 170 °C on glucose yield. Simultaneous increment of catalyst dosage and hydrolysis temperature showed significant increment in the yield of glucose. This mainly due to increasing catalyst dosage increases the number of active sites involved in the hydrolysis reaction. A further increase in catalyst dosage resulted lower glucose yield rate increment, which may be due to the degradation of the formed glucose by excess active sites of the catalyst [13].

Here it is important to note that among the selected operating conditions; hydrolysis temperature had greatest impact both on cellulose conversion and glucose yield amount. Next to temperature, hydrolysis period highly affected the hydrolysis output. It is known that, the crystallinity of the cellulose greatly reduced at higher temperature and elongated hydrolysis time. This in turn increased the amount water soluble oligomers and glucose yield during the hydrolysis. On the other hand, the effects of the catalyst cannot be ignored, because, without the presence of the catalyst the cellulose wouldn't be hydrolyzed even though higher hydrolysis temperature and time were applied. Consequently, inducing temperature or time could promote the hydrolysis environment, however, after a certain threshold, the surface property dominates the process.

#### **6.3.2.4. *Optimum value of operating conditions for maximum amount of the responses value***

As it has been shown in the previous section, when the generated models tested through the ANOVA statistics and diagnostic graphs it provided a good estimates for the true response. The next step was optimizing the process variables to obtain the highest amount of cellulose conversion and glucose yield in the given design space.

Numerical optimization of cellulose hydrolysis was performed using design expert software 10.1.1 by setting the desired goal of the factors (in range) and responses (maximizing). After analyzing a different combination of the independent variables, the optimum value of the independent factors, which can give the maximum amount of total converted cellulose and glucose yield were selected. Accordingly, the maximum amount of cellulose conversion (79.4%) and glucose yield (61.1 %) were found when the hydrolysis temperature, time and catalyst to substrate ratio were 175 °C, 74 min, and 1.25 g/g respectively. The predicted values of both responses at the above operating conditions were validated using triplicate experiments. The experimental findings showed a cellulose conversion and a glucose yield of  $78.5 \pm 0.75$  and  $60.6 \pm 0.54$  % respectively at the given operating conditions. The experimental results are in good agreement with the predicted maximum

of both responses. Hence, the validation result indicates that the model developed by the BBD design is an accurate model.

#### **6.4. Conclusion**

Hydrolysis of vibrational milled cellulose by microwave reactor in the presence of BSC-500 °C has been optimized by using RSM with Box-Behnken design. Vibrational milling of crystalline cellulose for a period of 1 h can significantly lowered degree of crystallinity (96.4 to 59.55 %) and the crystallite size (3.61 to 2.09 nm). Moreover the time required to bring these changes was relatively short compared to ball-milling. With the incorporation of additional analysis, the above result indicated vibrational milling can be used for the pretreatment of crystalline cellulose. A quadratic regression models were proposed to define the effects of the independent variables and their interaction effects on the given responses. Furthermore, the ANOVA of both responses suggested that the developed quadratic regression models were able to predict both responses with very high level of confidence. A hydrolysis temperature of 175 °C, a hydrolysis time of 74 min, and a catalyst to substrate ratio of 1.25 g were found to be the optimum process conditions to obtain the maximum amount of total converted cellulose and glucose yield. Under these conditions, the amount of total converted cellulose was 79.4 %, whereas the glucose yield was 61.1 %. These results were validated by running triplicate experiments under the optimized conditions and the results from the experiments ( $78.5 \pm 0.75$  and  $60.6 \pm 0.54$  %) were in a good agreement—with the respective predicted values obtained from the developed models.

## 7. Chapter Seven

### Application of BSC for Epoxidation of cottonseed oil

#### Abstract

*In this study, bamboo derived sulfonated catalyst was used for the epoxidation of cottonseed oil in the presence of hydrogen peroxide. The study was conducted by characterizing the selected physico-chemical properties of the given cottonseed oil. Before running any epoxidation experiment, the amount of mole of double bond (DB) existed in the cottonseed oil was analyzed. The epoxidation experiment was conducted in such a way that to study the effects of catalyst loading, temperature, and mole ratio of hydrogen peroxide to mole of DB existed in cottonseed oil. Relative percentage conversion of Oxirane content and percentage conversion of iodine value of the epoxidized product were considered to be the dependent variable. BSC-500°C catalyst was used for all epoxidation reactions. The physico-chemical characterization results of the cotton seed oil indicated the oil contained enough double bonds (0.44 moles of DB in 100 g of cottonseed oil), which could be converted to an Oxirane group. Maximum relative percentage conversion to Oxirane (36.8 %) found out at temperature of 60 °C, reaction time of 8 h, and catalyst loading of 15 %. Although the relative percentage conversion to Oxirane is lower, the catalyst has shown its potential to be used in epoxidation reaction.*

**Key words;** Cottonseed oil, Double bond, epoxidation, Oxirane content, iodine value, biomass derived sulfonated catalyst

## 7.1. Introduction

Epoxy or epoxide are compounds which are described due to the existence of Oxirane or epoxy ring, a three member ring containing an oxygen atom that is bonded with two carbon atom as shown in Figure 7.1 [191]. It can be defined chemically as monomers or oligomers containing two or more epoxy groups in their structure.

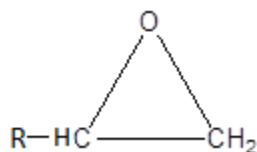


Figure 7-1 Structure of Oxirane group

Epoxidation of plant oils, commonly termed “vegetable oils,” is a commercially important reaction because the epoxides obtained from these renewable raw materials and their transesterification product have applications as plasticizers and polymer stabilizers [149]. Consequently, epoxidation of vegetable oils such as soybean, linseed, rapeseed, olive, corn, sunflower, melon seed, and cotton seed have been frequently carried out in industrial scale [150].

Epoxides are mainly produced by the non-catalytic reaction of the percarboxylic acid with the olefin. Mineral acid such as H<sub>2</sub>SO<sub>4</sub> has been used as a catalyst for the formation of percarboxylic acid formation from the reaction of acetic or formic acid with hydrogen peroxide. However, the use of mineral acids accompanied with drawbacks such as equipment corrosion, waste generation, costly separation and non-recycling [42], [192]. Due to these drawbacks, heterogeneous catalyst were started to practice in the peracid epoxidation reaction.

The most popular heterogeneous catalyst which is used in the epoxidation of vegetable oil is ion exchange resin [193]. A number of ion exchange resins such as *Amberlite IR-122* and *Amberlite IR-120H* with peroxyacid produced in situ used for the epoxidation of wild safflower, canola and soya bean oil [40], [194], [195]. Promising results were obtained in the conversion of ethylenic

unsaturation, however, Oxirane ring opening frequently happens in the final product. One of the reasons for the ring opening is the epoxidation temperature, which is relatively high compared to mineral acids. On the other hand, higher reaction temperature also affects the stability of the ion exchange resin [196]. Other heterogeneous catalyst such as titanium grafted silica, tungsten based catalyst, transition metal complex and rhenium based catalyst were tested and shown a promising result in the conversion of ethylenic unsaturation [42], [197], [198]. Yet, the search of an efficient and effective heterogeneous catalyst for the epoxidation of vegetable oil is still continued.

In this work, bamboo derived sulfonated catalyst was tested for the epoxidation of vegetable oil (cottonseed oil). Cottonseed oil is extracted from cottonseed kernel, it contains approximately 18-25 % of oil [199]. It has polyunsaturated fatty acids in the range of 65-70 % mainly composed with oleic and linoleic groups. The composition of unsaturated fatty acids in the oil makes it the perfect reactant for the Oxirane ring formation through epoxidation reaction. Consequently, the trial of bamboo derived sulfonated catalyst for the formation of percarboxylic group for epoxidation of cottonseed oil will generate additional knowledge in the application of the catalyst.

## 7.2. Materials and Methods

### 7.2.1. Materials

Bamboo derived sulfonated catalyst, which was synthesized based on section 3.1 was used for the epoxidation of cottonseed oil. Refined cottonseed oil is kindly provided from Addis Mojo edible oil factory. The following laboratory grade chemicals: 30 % H<sub>2</sub>O<sub>2</sub>, acetic acid (99 %), formic acid sodium-thiosulphate, KI, toluene, diethyl ether, carbon tetrachloride, KOH, hydrogen bromide, powder phenolphthalein, ethyl alcohol, starch powder, standard Hanus solution, anhydrous sodium sulphate, distil water, and sulfuric acid (96 %) were used for the analysis of the reaction. Thermal oil with high heating temperature was used for the heating media for the reactions.

Agilent GC-7820 gas chromatography equipment was used for the analysis of FA content. MSH-20D hot plate with temperature control and magnetic stirrer was used for the epoxidation reaction. The moisture content of the oil was analyzed in drying oven (intercontinental) where as its dynamic viscosity was measured in elecometer 2300 RV-L viscometer. Equipment such as glass condenser connected with a chiller, three-necked borosilicate glass (100 ml), and a pipette with a controlling nob were used for the epoxidation set-up. At the end of all epoxidation reactions, to separate the final product from the catalysts and the excess reactants separating funnel was used, on top of this the funnel used for washing the final products. Vacuum evaporator applied to separate the diethyl ether from the final product. Petri-dish with anhydrous sodium sulphate were used to remove any trace water which could exist in the product. Finally, borosilicate volumetric flasks with different volume, measuring cylinder, pipette, heating oven with magnetic stirrer and standard weighting balance, and pneumatic glass trough to contain the thermal oil were used in this work.

## 7.2.2. Methods

### 7.2.2.1. *Physico – chemical characterization of refined cottonseed oil*

Before running the epoxidation reactions; selected physico – chemical properties of the cottonseed oil were characterized through a number of tests. The characterization tests includes; moisture content, acid value, density (specific gravity), kinematic viscosity, and iodine value. In addition to this, FA composition of the oil was characterized, since the epoxidation reaction is dependent upon the FA composition of the oil's triglycerides.

### 7.2.2.2. *Moisture content*

The moisture content of the cottonseed oil was analyzed based on ASTM E871-82. In this method the cottonseed oil was heated under specified conditions, and the weight loss was used to calculate the moisture content as stated in section 3.2.1.

### 7.2.2.3. *Acid value*

The acid value can be defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. The analytical importance is that, it is a measured of the amount of fatty acids, which have been liberated by hydrolysis from the glycerides due to the action of moisture, temperature and some enzymes.

The analysis was performed by measuring 10 g well-mixed refined cottonseed oil in 300 ml Erlenmeyer. 100 ml ethanol ether mixture of 1:1(V: V) ratio and 0.1 ml of phenolphthalein were added to the Erlenmeyer. The solution was titrated with standard 0.1 M ethanolic potassium hydroxide solution until permanent faint pink was appeared and persists at least for 10 s. Each analysis was performed in duplicates and the average volume of ethanolic potassium hydroxide solution consumed was taken for the analysis of acid value as follows;

$$\text{Acid Value (AV)} = \frac{56.1 * V * N}{m} \quad (7.1)$$

Where:

V (ml) is the volume of ethanolic potassium hydroxide consumed in the titration;

N (mol/l) is the normality of ethanolic potassium hydroxide solution; m (g) is the oil measured weight; and 56.1 is potassium hydroxide molar mass (g/mol).

#### **7.2.2.4. Density of the refined cottonseed oil**

The density of the refined cottonseed oil was measured using DMA 4100M density meter. The oil was injected using syringe to the density meter and the density meter was recorded for density and specific gravity of the oil (API) at 38°C.

#### **7.2.2.5. Kinematic viscosity of refined cottonseed oil**

Kinematic viscosity of an oil is defined as its resistance to flow and shear due to gravity. Elecometer 2300 RV-L viscometer was used to determine a viscosity of the oil. Approximately 30 ml of refined cottonseed oil heated in water bath to a temperature of 40 °C (equilibrium temperature). After maintaining the equilibrium temperature, the bob of the viscometer inserted in the oil. The correct mode was set for appropriate measuring system and the reading was taken after 60 s measuring time. The measurement was performed in triplicate and the average was taken as the oil dynamic viscosity. The Kinematic viscosity was then calculated using equation 7.2 as follows.

$$\text{Kinematic viscosity} \left( \frac{m^2}{sec} \right) = \frac{\text{Dynamic viscosity}(pa. sec)}{\text{Density} \left( \frac{kg}{m^3} \right)} \quad (7.2)$$

#### **7.2.2.6. Gas chromatography –Mass Spectrometric (GC-MS) analysis of the oil**

GC analysis was performed with Agilent GC – system – 7820A. Sample analysis was carried out on packed column- Agilent Technologies (30 m × 0.250 mm, 0.25 μm). Samples were injected by a sampler injector at an oven temperature of 325 to 350 °C for a total run time of 38 min. The data,

obtained using MS - Agilent Technologies EMS detector and processed using Chem station software, were used to obtain fatty FA composition of the oils.

#### **7.2.2.7. Iodine value determination of a sample**

Iodine value was determined using Hanus method following an official method of AOAC no.920.158, 2000. Sample of 0.50 g added into 500 mL conical flask and dissolved in 10 mL of chloroform. Then after, 25 mL of Hanus Iodine solution was added into the solution using a pipette, then it was left to stand in the dark for 30 min with occasional shaking. The solution was brought from the dark and 10 mL of 15 % KI solution was added on it and thoroughly shaken. To rinse down any free I<sub>2</sub> on stopper previously boiled and cooled, 100 mL of water was added. The free I<sub>2</sub> found in the solution was titrated with 0.1 M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, with gradually adding and constant shaking until the yellow color of the solution turns to colorless. Then after a few drops of 1% starch indicator was added, the titration was continued until the blue colored formed during the addition of starch completely disappeared. The titration stopped and then the volume of thiosulfate taken and assigned as V<sub>s</sub>. Similarly, a blank determination along with the sample performed two times and the average volume consumed by the blank assigned with V<sub>b</sub>. The Iodine value of the sample was calculated as follows;

$$I_2 \text{ number} = \frac{(V_b - V_s) * M * 12.69}{g_{\text{sample}}} \quad (7.3)$$

Where M is the molarity of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>.

#### **7.2.2.8. Determination of Epoxy-Group Oxygen**

The analysis of epoxy group oxygen (Oxirane content) was performed using 6<sup>th</sup> edition of the standard methods for the analysis of oils, fats and derivatives [200]. All the analysis were performed by measuring 0.3-0.5 g of samples to the nearest 0.1 mg in 50 ml conical flasks. Then it was dissolved by 10 ml benzene with stirring magnetic stirrer. Then 0.1 ml of 1 g/l

Hexamethylpararosaniline hydrochloride (crystal violet), which was dissolved in glacial acetic acid was added in the conical as indicator. The titration was begun after adjusting the stopper and the burette in such a manner that the tip of the burette just touched the surface of the solution (in order to avoid loss of hydrogen bromide). The titration was performed with previously standardized (using dry potassium hydrogen phthalate) hydrogen bromide acetic solution. At the initial stage of the titration higher volume of the titrant allowed to mix with the sample. However, when it approach its end point the titrant was added more slowly and finally, the titration was stopped when a bluish-green color appeared persistently for 30 s (the speed of the stirrer was control so as to avoid any splashing). The percentage of epoxy-group oxygen content ( $t$ ) in the sample was given by the formula:

$$t (\%) = \frac{1.6 * V * T}{m} \quad (7.4)$$

Where  $V$  is the number of ml of the standardized hydrogen bromide acetic solution used for the titration,

$T$  is the exact normality of the hydrogen bromide acetic acid solution used.

$m$  is the mass, in gram, of the sample.

From the oxirane content values, the relative percentage conversion to oxirane was calculated using the following expression:

$$\text{Relative percentage conversion to oxirane} = \left( \frac{OO_{exp}}{OO_{the}} \right) * 100 \quad (7.5)$$

Where  $OO_{exp}$  (g /100 g sample) is the experimentally obtained oxirane oxygen and  $OO_{the}$  is the theoretically obtainable maximum oxirane oxygen, which was determined from the following expression:

$$OO_{the} = \left[ \frac{\left(\frac{IV_o}{2A_i}\right)}{100 + \left(\frac{IV_o}{2A_i}\right)A_o} \right] A_o * 100 \quad (7.6)$$

Where  $A_i = (126.9)$  and  $A_o = (16.0)$  are the atomic weights of iodine and oxygen respectively and  $IV_o$  is the initial iodine value of oil sample.

### 7.2.3. Experimental set up

The epoxidation reactions were carried out in a three-necked 100 ml round-bottom flask glass reactor equipped with magnetic stirrer. A glass condenser which was connected with a chiller immersed in one of the three neck so as to avoid any evaporation loss. A 30 % hydrogen peroxide contained burette entered in the second neck for droplet adding of the peroxide at the time of the reaction. The third neck was covered a rubber lid. Then, the whole apparatus kept in thermostatic thermal-oil bath with a temperature control  $\pm 1$  °C. The oil was continuously heated using MSH-D hot plate. Reaction temperature, time and stirring speed of the magnetic stirrer can be adjusted by the external knob of the hot plate. On top of this, external glass thermometer was frequently used to measure the temperature of the oil and the reactor to compare with the reading temperature of the hot plate.

### 7.2.4. Moles of DB (ethylenic unsaturation) in 100 g of cottonseed oil

To measure the appropriate weight of the cottonseed oil, it is important to know the moles of double bond (DB) present in 100 g of the cottonseed oil. This allows to determine the amount of cottonseed oil (in grams) required in the reaction so that given mole of DB is correctly met with the corresponding molar ratios of acetic acid and hydrogen peroxide to be used in the reaction.

The first step in determining the mole DB found in cottonseed oil is analyzing the mole of DB found in the mole of triglyceride (TG). The mole DB found in the mole of TG is determined using following equation.

$$\text{Moles of DB per mole of TG} = \sum \left( \frac{\text{mol DB}}{100 \text{ g oil}} \right) \times \sum \left( \frac{1}{\frac{\text{mol FA}}{100 \text{ g oil}}} \right) \times \left( \frac{\text{mol FA}}{\text{mol TAG}} \right) \quad (7.7)$$

Where the first two terms on the right of Equation (7.7) come from the following. Each unsaturated FA contributes a number of moles of DB for each 100 g of cottonseed oil. This contribution is determined for each type of FA by Equation (7.8) as follows;

$$\frac{\text{mol DB}}{100 \text{ g oil}} = \frac{(\text{number of DB per FA}) \times (\% \text{ FA})}{\text{molar mass of FA}} \quad (7.8)$$

Molar mass (MM) of FA is calculated from the result of FA profile of the cottonseed oil which was found from the GC analysis. The moles of FA in 100 g of cottonseed oil was determined from Equation 7.9 as follows.

$$\frac{\text{mol FA}}{100 \text{ g oil}} = \frac{(\% \text{ FA})}{\text{molar mass of FA}} \quad (7.9)$$

The value of the third term on the right of Equation (7.7) is simply 3, as each triglyceride has three FA chains. Since the quantity of interest is the moles of DB for 100 g of cottonseed oil, it is calculated as follows:

$$\text{Quantity of DB in 100 g of cottonseed oil} = \frac{\text{moles DB}}{\text{mole of TAG}} \times \frac{\text{moles of TAG}}{MM_{TAG}} \times 100 \text{ g} \quad (7.10)$$

The equation for determining the molar mass (MM) of the TAG is as follows:

$$MM_{TAG} = 3 \times MM_{FFA} + MM_{glycerol} - 3 \times MM_{water} \quad (7.11)$$

Where  $MM_{TAG}$  is molar mass of TAG;

$MM_{FFA}$  is molar mass of FA

$MM_{glycerol}$  is molar mass of glycerol (92.09 g/mol)

$MM_{water}$  is molar mass of water (18 g/mol)

### **7.2.5. Epoxidation Procedure**

The epoxidation reactions were conducted in a method similar to Dinda et, al [201]. A measured amount of cottonseed oil (0.463 moles of DB in 100 g oil) was placed in three necked reactor glass. Calculated amount of acetic acid (glacial) and the given catalyst were added to the reactor and the mixture was stirred for 30 min. Then the required amount of aqueous 30 % hydrogen peroxide (based on mole of hydrogen) was added in drop-wise over half an hour by inserting a burette in one of the three necks found in the reactor. The course of reaction time was considered from the completion of hydrogen peroxide addition as being zero time. The reaction mixture was stirred continuously to avoid zones of high peroxide concentration that could lead to explosive mixtures. After the end of every epoxidation reactions the collected samples were placed in separating funnel and let the catalyst to be settled and separated. After the removal of the catalyst, the epoxide product was extracted with diethyl ether and washed with water until it was acid free. Finally, the washed samples were analyzed for Oxirane content and iodine value.

## **7.3. Results and discussion**

### **7.3.1. Physico-chemical properties of cottonseed oil**

The characterization result of the physico-chemical properties of the cottonseed oil is given in Table 7.1. Moisture content of the oil was 0.24 %, which indicates the oil contains very low amount of water. Low amount of moisture preferred as excess moisture can alter the polymerization stage of epoxy reactive system [202]. On top of this, excess moisture in the oil will facilitate its oxidation. The acid value of the oil is in range with the expected value. Since acid value is one of the important parameters related to the oil quality, lower acid value is an indicator for the good quality of the given oil. Viscosity of the oil is found out to be  $36.6 \text{ mm}^2\text{s}^{-1}$ , this result is in comparable with previously analyzed cottonseed oil viscosity [70]. On the other hand, when the viscosity of the oil become high, the epoxidation of the given oil has to be performed in the

presence of an inert solvent to enhance better phase contact by reducing the viscosity of the reaction mixture [193].

*Table 7-1 Selected physico-chemical properties of cottonseed oil*

Properties of the oil	Result
Moisture content (%)	$0.24 \pm 0.01$
Acid value (mg KOH/g oil)	$0.56 \pm 0.025$
Density ( $\text{Kg/m}^3$ ), @ T= 38 °C	$922.2 \pm 1.5$
Kinematic viscosity ( $\text{mm}^2/\text{s}$ ), @ T= 38°C	$36.6 \pm 0.72$
Iodine value( $\text{g I}_2/100 \text{ g oil}$ )	$101.50.01 \pm 2.4$

### 7.3.2. GC-MS analysis of the oil

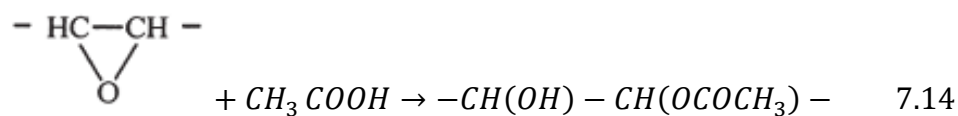
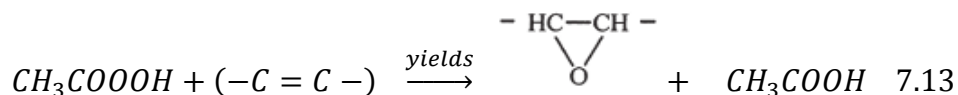
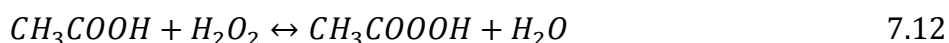
GC-MS analysis result of the cottonseed oil is given in Table 7.2. Moreover, the Table includes the value of moles of DB and FA per 100 g of the oil calculated using equation 7.8 and 7.9. As shown in the Table linoleic acid is the major content of the cottonseed oil. In addition, fair amount of oleic acid was found in the oil. The amount of linolenic acid is too small, despite of this, the given cottonseed oil can be used for the epoxidation reactions. On the other hand, evaluating the amount of moles of DB in the oil is an important task because it is useful to determine the ratios of reacting chemicals required in the epoxidation reaction. Consequently, for 100 g of cottonseed oil moles DB in analyzed using the aforementioned equation and Table 7.2; it was 0.463 moles.

Table 7-2 Fatty acid composition cottonseed oil

FA	% FA	Molar mass (g/mol)	Number of DB	Moles of DB per 100g oil	Moles of FA per 100 g oil
linoleic	55.8	280.45	2	0.398	0.2
Oleic	17.5	282.46	1	0.062	0.062
Palmitic	23	256.42	0	0	0.09
Linolenic	0.3	278.43	3	0.003	0.001
Stearic	2.1	284.48	0	0	0.007
Palmitoleic	0.6	254.41	0	0	0.002
Myristic	0.7	228.37	0	0	0.003
Total	100			0.463	0.365

### 7.3.3. Epoxidation of cottonseed oil

Epoxidation of vegetable oil with peracetic acid formed *in situ* from acetic acid and hydrogen peroxide in the presence of solid acid catalyst involves an acid – catalyzed reactions of peracetic acid formation (Equation (7.12)), an uncatalyzed reaction of epoxy ring formation (Equation (7.13)) and also acid –catalyzed side reactions of epoxy ring cleavage (Equation (7.14)) [203], [204]. The peracetic formation reaction is assumed the rate determining step.



Vegetable oil epoxidation can be affected by different factors such as stirring speed, acetic acid concentration, catalyst concentration, temperature, time, and hydrogen peroxide concentration. In this work stirring speed and acetic acid to DB mole ratio were fixed for all epoxidation reaction. From preliminary study it was identified that when the stirring speed was less than 1000 rpm, less Oxirane content was found in the final product. In contrary, when the stirring speed was above 1000 rpm, the Oxirane content increased significantly. However, the viscosity of the final product became too high. Consequently, separating the final product from the catalyst became challenging. Hence, it was decided to fix the stirring speed at 1000 rpm for all epoxidations reaction. Since the effect of acetic acid to ethylenic unsaturation mole ratio was intensively studied in previous research works, acetic acid to DB mole ratio was fixed to 0.5 for all epoxidations reaction [193], [201], [205].

The effect of other epoxidation variables on the *in situ* epoxidation of cottonseed oil in the presence of bamboo derived sulfonated catalyst (BSC) was examined in the following: weight ratio of catalyst to oil 2-20 %, temperature, 45-75 °C, time, 2-8 h, and H<sub>2</sub>O<sub>2</sub>-to-ethylenic unsaturation mole ratio 1.1-2.5. The Oxirane oxygen content of each sample was determined using the direct method with hydrobromic acid solution in acetic acid, and the iodine value was determined using Hanus method.

#### **7.3.3.1. Effects of BSC loading in the epoxidation of cottonseed oil**

Prior to investigating the effects BSC to oil weight ratio in the epoxidation of cottonseed oil, preliminary study was conducted to evaluate the activity of the catalyst. The result of the study showed that a catalyst to oil ratio lower than 1 % would give few conversion of DB as well as lower amount of epoxy oxygen content. In addition to this, when catalyst like ionic exchange resin and biomass derived sulfonated catalyst applied for epoxidation reaction it were not uncommon to

use higher catalyst loading ratio [39]. Hence, the study for catalyst to oil ratio was conducted by varying catalyst to oil percentage ratio from 2-20 %. Other reactions conditions such as 0.1 mole DB of oil was taken; mole of  $H_2O_2$ : mole DB of oil was 2, mole  $CH_3COOH$ : mole of DB of the oil was taken 0.5; reaction temperature was 60 °C and stirring speed was 1000 rpm. The Oxirane value conversion is shown in Figure 7.2 and percentage conversion of Iodine value is described in Figure 7.3. As shown in Figure 7.2; for catalyst loading 2 and 10 %, the Oxirane content increased throughout the reaction time and the maximum relative percentage conversion to Oxirane was reached 15 % for catalyst loading of 10 %. It is observed that an increase in catalyst loading further in to 15 and 20 % not only increases the initial rate of epoxidation reaction but also brings about an increase in the maximum Oxirane level obtained. Increasing catalyst loading leads to an increase in the active site of the catalyst which involved in the reactions. Similar result was reported in the epoxidation of canola oil using ion exchange resin by *Mungroo* and coworker [206]. Maximum relative percentage conversion to Oxirane both for 15% and 20% catalyst loading was found to be 36.89 % at 8 h reaction time. On the other hand, when the epoxidation period was elongated further, a reduction on Oxirane content was observed. The main reason for this might be the formed Oxirane content was started to convert into product like glycol through ring opening polymerization [193]. On the other hand, in the case of 20 % catalyst loading epoxidation, separation of the catalyst from the reactant was happened to be difficult. Consequently, further increment of catalyst loading above 15 % is not advisable unless the catalyst was used either as a bead or in the form of a pellet.

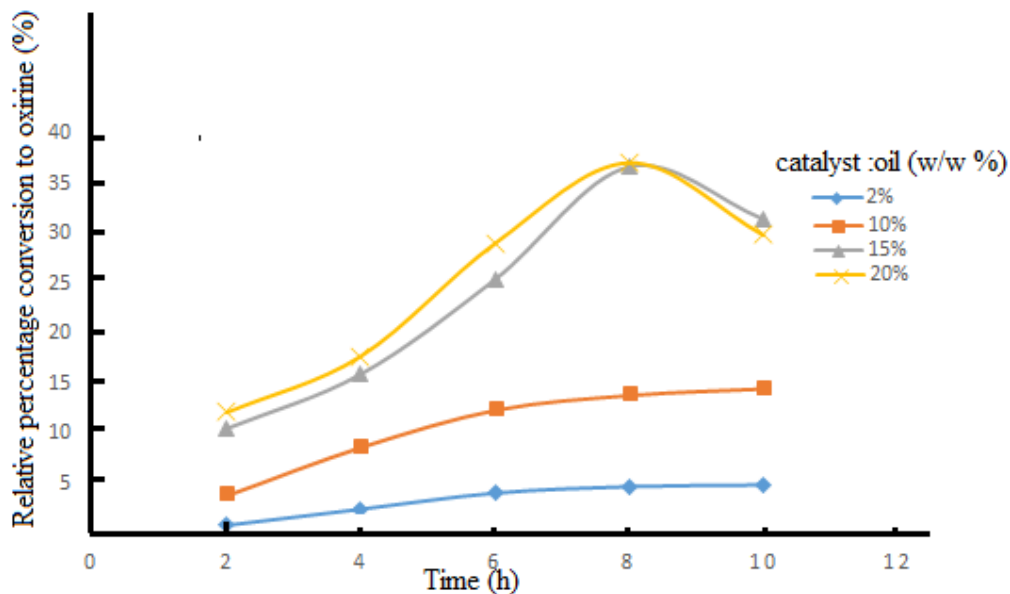


Figure 7-2 Effect of catalyst loading on relative percentage conversion to oxirane (Operating conditions: mole  $H_2O_2$ : mole DB = 2; mole  $CH_3OOH$  : mole DB = 0.5; temperature =  $60^\circ C$  and stirring speed =  $1000 \pm 25$  rev/min).

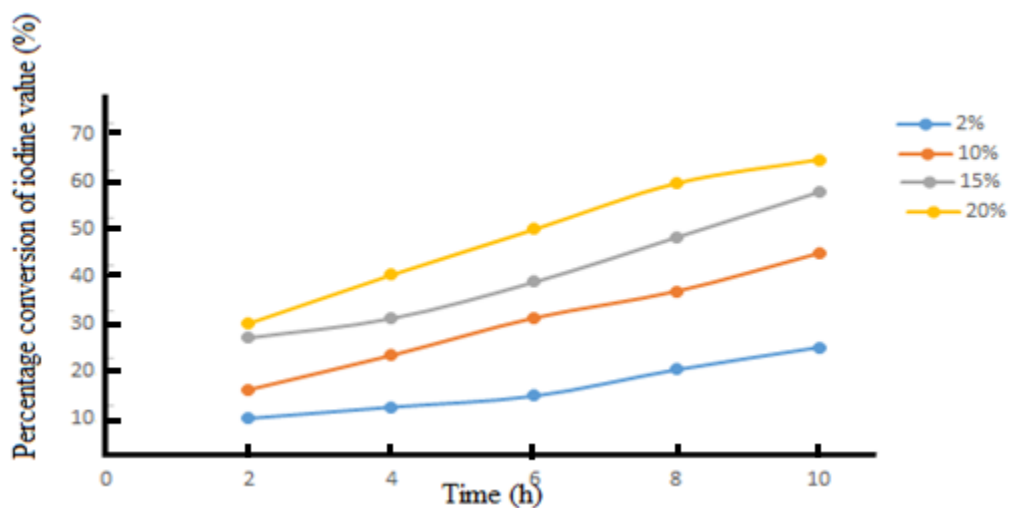


Figure 7-3 Effect of catalyst loading on percentage conversion of Iodine value (Operating conditions: mole  $H_2O_2$ : mole DB = 1.5; mole  $CH_3OOH$  : mole DB = 0.5; temperature =  $60^\circ C$ ; and stirring speed = 1000 rev/min)

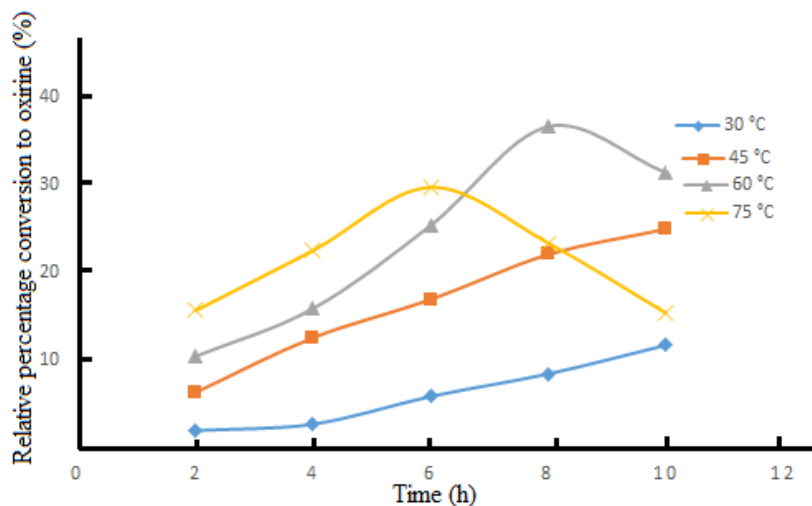
### 7.3.3.2. *Effects of temperature in the epoxidation of cottonseed oil*

To investigate temperature effect in the epoxidation of cottonseed oil, the following operating conditions were used: mole  $\text{H}_2\text{O}_2$  to mole of DB of cottonseed oil was taken 2; mole  $\text{CH}_3\text{COOH}$  to mole of DB of cottonseed oil was assigned 0.5; catalyst loading relative to the oil weight was 15 % and stirring speed of was fixed to 1000 rpm. The influence of temperature on the course of epoxidation was investigated at four different temperatures, namely 30, 45, 60, and 75 °C. The relative percentage conversion to Oxirane and percentage conversion of Iodine value are given in Figure 7.4 and Figure 7.5, respectively. Relative conversion to Oxirane and percentage conversion of Iodine value were found to increase with increasing temperature. Increasing the temperature had a favorable effect on the formation of peroxyacetic acid.

At lower epoxidation temperature (30°C and 45°C) the relative percentage conversion to Oxirane is increased throughout the experimental period. When the temperature was increased to 60 °C and 75 °C, the epoxidation rate are higher at the start of the reaction. Hence, the maximum relative percentage Oxirane conversion was attained in shorter period. Nevertheless, elongated epoxidation time could reduce the Oxirane conversion due to the rate the ring opening of formed epoxy happened. Therefore, making some compromising between epoxidation temperature and time are required.

As shown in Figure 7.4 maximum relative percentage Oxirane content (36.89 %) was observed at temperature of 60 °C and 8 h epoxidation time. On the other hand, when the epoxidation temperature was 75 °C the maximum relative percentage Oxirane was 29.5 % (6 h). At similar epoxidation time when the temperature was 60 °C relative percentage Oxirane was 26.3 %. This results suggested that the optimum level of epoxidation could be obtained in shorter time at moderate reaction temperature, 60 °C, at which the epoxy ring cleavage was relatively low. Similar

temperature effects was reported by *Goud* and coworker in the epoxidation of karanja oil using acidic ion exchange resin [207].



*Figure 7-4 Effect of temperature on relative percentage conversion to oxirane (Operating conditions: mole  $H_2O_2$ : mole DB = 1.5; mole  $CH_3OOH$ : mole DB = 0.5; catalyst loading 15 % and stirring speed = 1000 rpm.*

Figure 7.5 indicates the effects temperature on percentage conversion of iodine value within the given reaction time. As indicated in the figure for all temperatures, the percentage conversion of iodine value increased throughout the reaction time. The result found here is expected, as longer period with the given temperature facilitates more conversion of the oil double bond which in turn increased the percentage conversion of Iodine value [207].

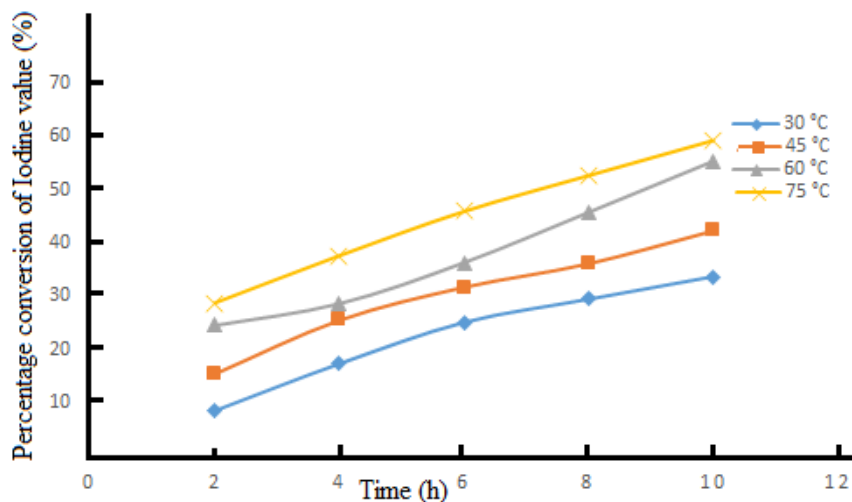


Figure 7-5 Effect of temperature on relative percentage conversion to Iodine value (Operating conditions: mole  $H_2O_2$ : mole DB = 1.5; mole  $CH_3OOH$ : mole DB = 0.5; catalyst loading 15 % and stirring speed 1000 rpm  $\pm 25$ .)

### 7.3.3.3. Effects of $H_2O_2$ to Ethylenic Unsaturation Molar ratio

The molar ratio of  $H_2O_2$  to DB of the cottonseed oil was varied in the range 1.1-2.5, as shown in Figure 6 and 7. Other operating conditions mole  $CH_3OOH$ : mole DB was 0.5; catalyst loading was 15 %; temperature of all epoxidation were conducted 60 °C and stirring speed was 1000 rev/min. When  $H_2O_2$  to DB of the cottonseed oil molar ratio increases from 1.1 to 2, there was a gradual increase in the relative percentage Oxirane conversion along with a corresponding decrease in iodine value as shown Figure 7.6 and 7.7 respectively. However, as can be seen from Figure 7.6, when the molar ratio was increased further to 2.5, the relative percentage Oxirane conversion is decreased with longer epoxidation time. This is due to the fact that higher hydrogen peroxide concentration leads to a higher rate of Oxirane ring decomposition [208]. Similarly, lower relative percentage Oxirane conversion was reported when higher molar ratio of  $H_2O_2$  to ethylenic unsaturation was used in the epoxidation of vegetable oil using acidic ion resin [206].

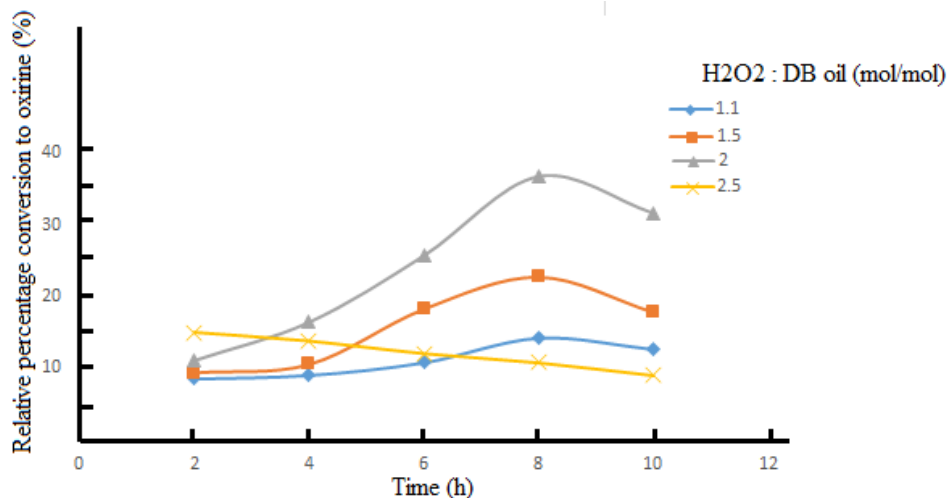


Figure 7-6 Effect of  $H_2O_2$  concentration on relative percentage conversion to oxirane conditions: mole  $CH_3OOH$ : mole DB = 0.5; catalyst loading 15 %; temperature = 60 °C stirring speed = 1000 rpm  $\pm$ 25

The effects mole ratio of  $H_2O_2$  relative the mole ratio of double bond of the cottonseed oil on percentage conversion of Iodine value is given in Figure 7.7. As it was shown in the figure the percentage conversion increases with the increment of  $H_2O_2$  mole ratio. The main reason for this is at higher  $H_2O_2$  mole ratio higher amount of converted double is found.

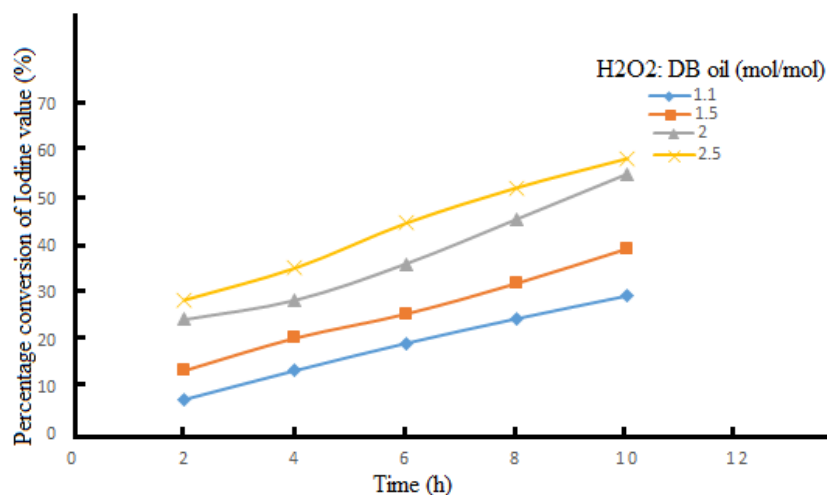


Figure 7-7 Effect of  $H_2O_2$  concentration on percentage conversion of Iodine value (Operating conditions: mole  $CH_3OOH$ : mole DB = 0.5; catalyst loading 15 %; temperature = 60 °C stirring speed = 1000 rpm  $\pm$ 25)

#### 7.4. Conclusion

Bamboo derived sulfonated catalyst previously tested in a number of reactions. At the same time the catalyst has shown its capacity in the substitution of mineral acids and other expensive solid acid catalysts. Here in this work, the epoxidation of cottonseed oil with peracetic acid generated *in situ* was carried out in the presence of acetic acid and bamboo derived sulfonated catalyst. The catalyst activity for the epoxidation of cottonseed oil was studied using three different operating conditions within a given period of time. It was found that the epoxidation occurred optimally at a temperature of 60 °C, stirring speed of 1000 rpm, mole ratio between hydrogen peroxide and DB of the oil and glacial acetic acid and DB of the oil were 2 and 0.5 respectively. Under these conditions 36.89 % relative conversion to Oxirane was found. On the other hand, the result found here is lower than previously used solid catalyst such as acidic ion exchange resin in the epoxidation of cottonseed oil. In addition to this, separating bamboo derived sulfonated catalyst after the epoxidation reactions was very difficult. However, this research showed that if the catalytic activity is improved and simultaneously an easy separating method of the catalyst from the final product is devised the catalyst will have the potential to use in the epoxidation of vegetable oil.

## Chapter Eight

### 8. Conclusion and Recommendation

#### 8.1. Conclusion

In this study bamboo derived sulfonated catalyst was synthesized, characterized and tested its catalytic activity in the hydrolysis of crystalline cellulose and epoxidation of cottonseed oil. The catalyst was prepared in two steps process of carbonization and sulfonation. Characterization results of the catalyst indicated that, the synthesized catalyst contained  $\text{-SO}_3\text{H}$ , OH and COOH groups. Moreover, the synthesized catalyst was identified mainly composed of mesoporous and possessed a maximum surface area of  $41.8 \text{ m}^2/\text{g}$ . The catalyst was shown similar sulfur content both in CHNS and SEM-EDS analysis, which indicates the sulfonation process was not limited to the surface of the bamboo char. The thermal stabilities of the synthesized catalysts were examined by thermogravimetric analysis (TGA) and temperature-programmed desorption (TPD) under air flow. The TGA revealed that only 15 % of the catalyst was lost in the first  $200 \text{ }^\circ\text{C}$ . On the other hand, the TPD indicated the decomposition of  $\text{SO}_3\text{H}$  and COOH group above  $200 \text{ }^\circ\text{C}$ . Hence, the catalyst was thermally stable at the given operating conditions. The reuse capacity test of the catalyst confirmed that the catalyst was chemically stable at least for 3-cycle.

The catalytic performance of the catalyst was examined in cellulose hydrolysis and epoxidation of cottonseed oil. The cellulose hydrolysis performed using conventional reactor showed that the catalyst potential to convert crystalline cellulose into fine chemicals like glucose. Maximum glucose yield of 4.08 was found at 8 h and  $150 \text{ }^\circ\text{C}$  hydrolysis time and temperature respectively. Despite of this, the glucose yield found was too low compared to those of previously reported mineral acids based hydrolysis in similar reactor.

The lower glucose yield resulted from the conventional reactor lead the study to use microwave reactor for the hydrolysis of crystalline cellulose. The preliminary results found using the microwave based hydrolysis of crystalline cellulose were promising. Hence, the effects of selected operating conditions such as hydrolysis temperature and time on the catalyst activity in the hydrolysis of crystalline cellulose were studied. It was found out that, a maximum glucose yield of 43.45 % at temperature of 180 °C and 60 min. On top of that, the synthesized catalyst showed superior activity compared to other solid acid catalysts despite of its lower acid density and surface area. The reuse capacity of the synthesized catalyst was tested in similar operating conditions. The results indicated that the catalyst can be reused for at least three reactions without losing its activity. In this studies, the successful hydrolysis of crystalline cellulose was achieved in the simultaneous use of bamboo derived sulfonated catalyst and microwave reactor. Furthermore, the result obtained was comparable to that of mineral acids, hence it could substitute mineral acids in the hydrolysis of crystalline cellulose with further modification.

In addition to the studies of the effects of hydrolysis temperature and time, optimization of cellulose hydrolysis operating conditions to find maximum glucose yield was performed. The optimization was performed using one hour vibrational milled crystalline cellulose prior to the hydrolysis. The short time vibrational milling brought comparable effect on reducing the crystallinity of the cellulose with that of the longer time taken ball milling. Consequently, higher glucose yield were obtained in the optimization process. Hence, applying vibrational milling to reduce the crystallinity of the cellulose as well as optimizing some of the operating conditions can significantly increases the glucose yield during cellulose hydrolysis.

The application of bamboo derived sulfonated catalyst in the substitution of mineral acid and other expensive heterogeneous catalyst is still continued. Here, in this studies the synthesized catalyst

was tested for the epoxidation of cottonseed oil. On top of this, the effects of selected operating conditions was studied one at a time. In general, the synthesized catalyst had shown its potential in converting ethylenic unsaturation of the cottonseed oil into an oxirane ring.

## **8.2. Recommendation**

The study conduct in this work has met the objectives stated at the beginning. However, further works needs to be addressed before implementing the synthesized catalyst into industrial scale applications. It is recommended that further studies should be pursue to the following area:

*Catalyst synthesis techniques:* synthesis of bamboo derived sulfonated catalyst is a very long process as it needs a series of washing, filtering and filtrate pH testing. Due to this, longer period of time required for the synthesis of the catalyst. Hence, future study in this catalyst has to be geared towards in minimizing the synthesis time.

*Improving catalytic activity:* the result obtained in this studies, specifically in the hydrolysis of cellulose, the ratio of catalyst loading with respect to the cellulose was taken as 1. One of main reason is the acid density group existed in the synthesized catalyst is low, consequently lower glucose yield is obtained when ratio between the catalyst to crystalline cellulose is below 0.5. Therefore, further studies has to be realized in improving the acid density of the catalyst (-SO<sub>3</sub>H). This might starts from creating more aromatic group during carbonization of the biomass, introducing new way sulfonation, or advancing the existed sulfonation techniques.

*Form (shape) of catalyst:* In the preparation and usage of bamboo derived sulfonated catalyst a series of problems had been faced. One of the main problem was handling the final form of the synthesized catalyst. This problem mainly created because of the final prepared catalyst happened to be light in weight and is powdery. It became easily spill to the air due to its lightness and create

difficulty to separate from the product. On top of that, even if it was separated from the product it got stuck in filter paper. If such kind of the catalyst shortcoming is solved, it will be a major success in commercializing it.

*Unlocking reuse potential of the catalyst:* Separation of bamboo derived sulfonated catalyst from the final product reported to be very challenging. Hence to mitigate such a problem it needs to make the synthesized catalyst easily separable from the final product. One of the remedy suggested for this difficulty was magnetizing the synthesized catalyst and conducting the separation of the catalyst from the final solution using a magnet. Therefore, researches in biomass derived sulfonated catalyst synthesis should also focus in this area.

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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

$$\begin{aligned}\text{Weight loss, \%} &= (A-C) / (A-B) \\ &= (22.6135 - 21.8122) / (22.6135 - 21.6124) \\ &= 0.800 * 100\% \\ &= 80 \%\end{aligned}$$

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

### Experiment #1

A. Wt of empty crucible : 36.7273g

B. Wt of bamboo dust : 1.0000gm

C. Wt of bamboo dust and crucible after drying : 37.6242gm

$$\begin{aligned}\text{Moisture content, \%} &= 100 - [(C-A)/B]*100\% \\ &= 100\% - [(37.6242-36.7273)/1.0000]*100\% \\ &= 10.31\%\end{aligned}$$

### Experiment #2

D. Wt of empty crucible : 35.3544g

E. Wt of bamboo dust : 1.0005gm

F. Wt of bamboo dust and crucible after drying : 36.25 30gm

$$\begin{aligned}\text{Moisture content, \%} &= 100 - [(C-A)/B]*100\% \\ &= 100\% - [(36.25 30-35.3544)/1.0000]*100\% \\ &= 10.18\%\end{aligned}$$

### Experiment #3

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

$$\begin{aligned}\text{Moisture content, \%} &= 100 - [(C-A)/B]*100\% \\ &= 100\% - [(35.7761-34.8694)/1.0009]*100\% \\ &= 90.6\%\end{aligned}$$

### 3. Ash content # Bamboo

#### Experiment #1

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

$$\begin{aligned}\text{Ash content amount} &= [((C-A)/B)*100\%] \\ &= [((17.0246-17.0090)/1.0007)*100\%] \\ &= 1.56\%\end{aligned}$$

#### Experiment #2

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

$$\begin{aligned}\text{Ash content amount} &= [((C-A)/B)*100\%] \\ &= [((17.4245-17.4094)/1.0008)*100\%] \\ &= 1.51\%\end{aligned}$$

### Experiment #3

G. Mass of crucible before burning: 16.8616g

H. Mass of sample : 1.0010g

I. Mass of sample + Crucible after burning: 16.8789

$$\begin{aligned}\text{Ash content amount} &= [(C-A)/B]*100\% \\ &= [(16.8789-16.8616)/1.0010]*100\% \\ &= 1.73\%\end{aligned}$$

## Appendix B

### B.1. Adsorption and desorption Isotherm

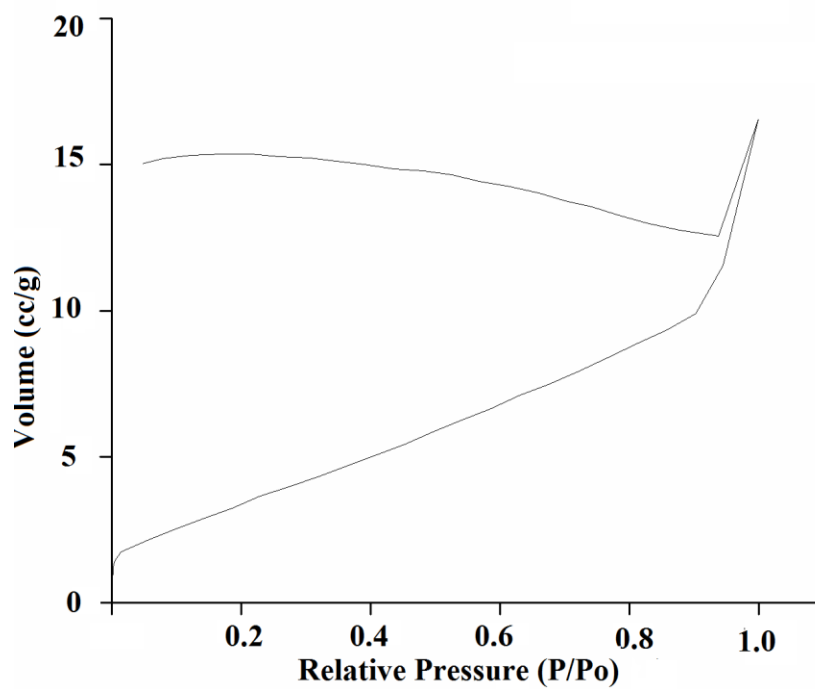


Figure B.1. Adsorption-desorption Isotherm of BSC-500°C

## B.2. TGA-MS analysis SO<sub>2</sub> and SO<sub>3</sub>

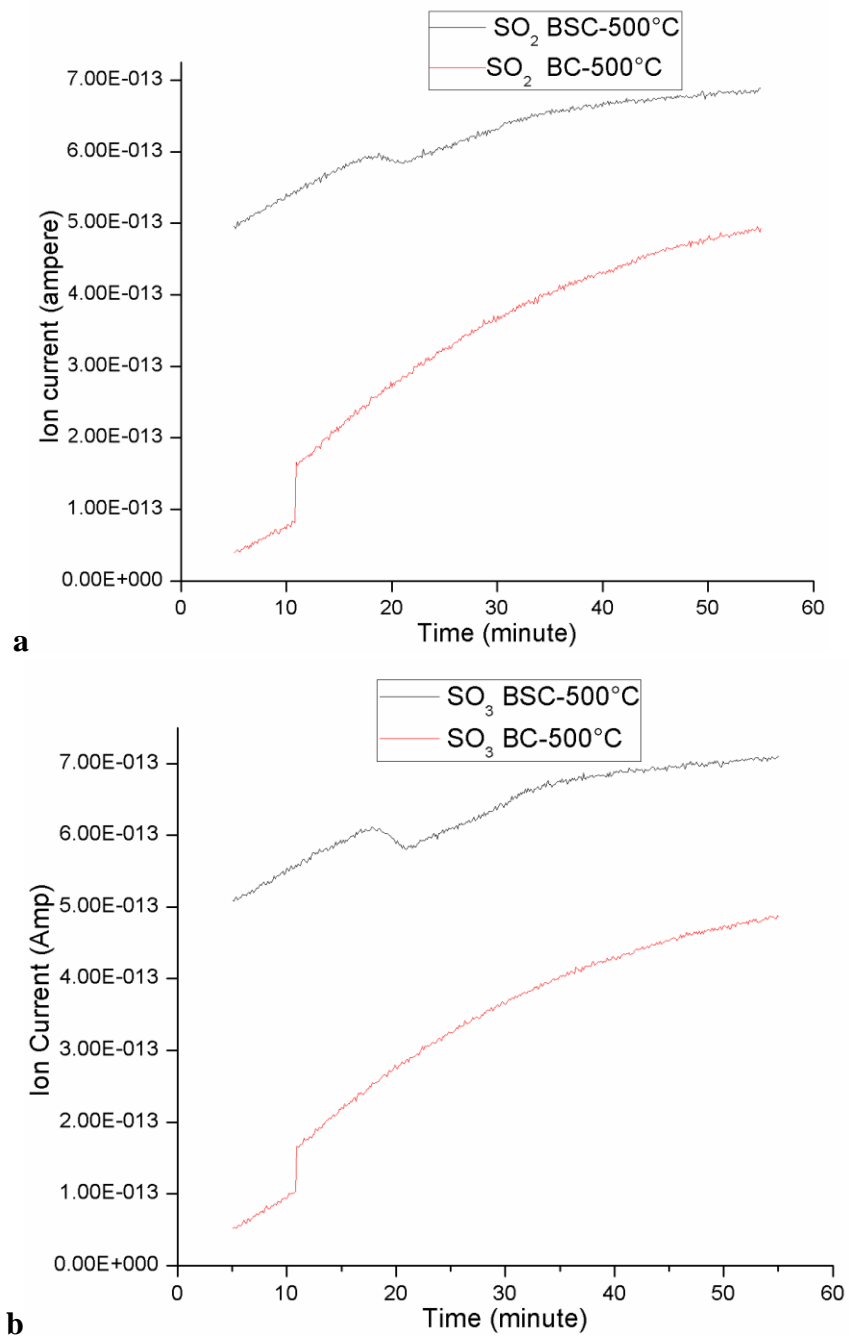


Figure B.2 TGA-MS analysis of SO<sub>2</sub> (a) and SO<sub>3</sub> (b) content BC-500 °C and BSC-500°C



## Appendix C: SEM-EDX analysis for BC-500°C and BSC-500 °C

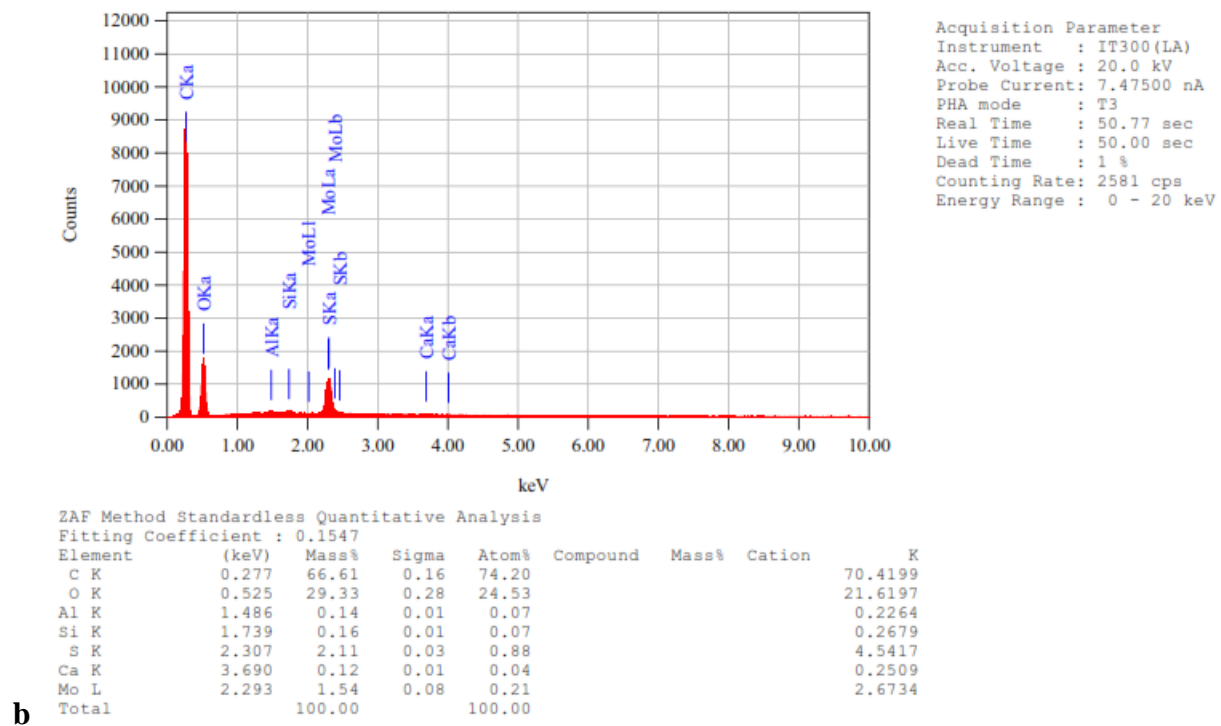
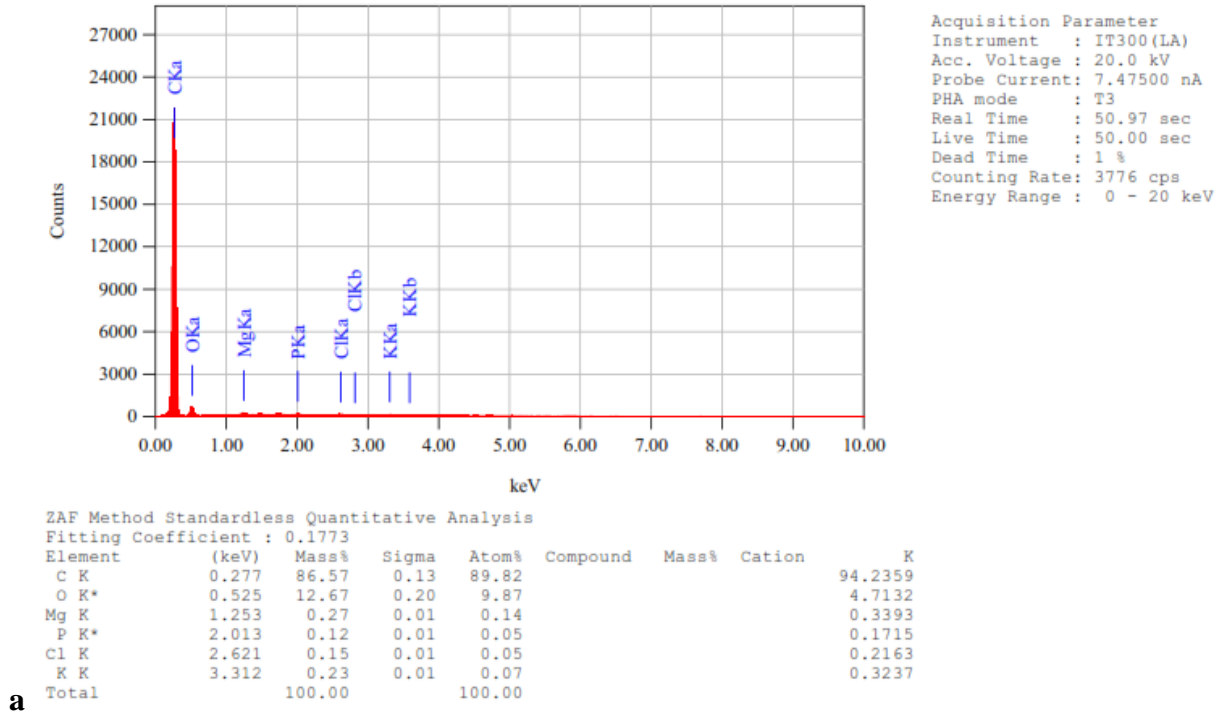


Figure C: SEM-EDX analysis result of BC-500 °C (a) and BSC-500°C (b)

## **Appendix D: Glucose standard curve preparation**

The standard curve of glucose was prepared as follows; firstly 0.1 % standard stock solution of glucose was prepared. 50 ml of distilled water was added in a 100 ml of volumetric flask. It was placed in hot plate and heated for a while. The flask was removed from the hot plate and 0.1 g of D-glucose was added on it. Then after, the flask was further filled with a distill water up to its 100 ml mark. The resulting solution was named a standard stock solution of glucose. consequently, standard curve was prepared by taking 0 (blank), 0.2, 0.4, 0.6, 0.8 and 1 mL of the standard stock solution of glucose into a test tubes and each volume was made up to 3 mL by adding distilled water. About 3 mL of DNS reagent was added in each test tubes and the mixture was heated for 5 min in a boiling water bath. After the development of the color complex, 1 ml of a 40 % potassium sodium tartrate tetra hydrate (Rochelle salt) solution was added in each test tubes (in warm contents) before the mixture is cooled and mixed thoroughly to stabilize the color. After cooling the tubes, the absorbance (optical density) of each test tube solution was measured in UV-vis spectrophotometer at a wavelength of 540 nm. Results of given glucose concentration and respective absorbance is shown in Table D1. A linear regression analysis was performed by taking concentration as independent variable and absorbance (OD) as dependent variables. The generated linear graph is shown in Figure D1. Meanwhile, the concentration of the test samples was analyzed from its absorbance using the given graph.

Table D.1 Concentration vs absorbance (OD) reading @ 540 nm

Sample no	Concentration of glucose (mg/ml)	O.D @ 540 nm (absorbance )
1	0.2	0.5
2	0.4	1.278
3	0.6	1.878
4	0.8	2.4
5	1	2.8

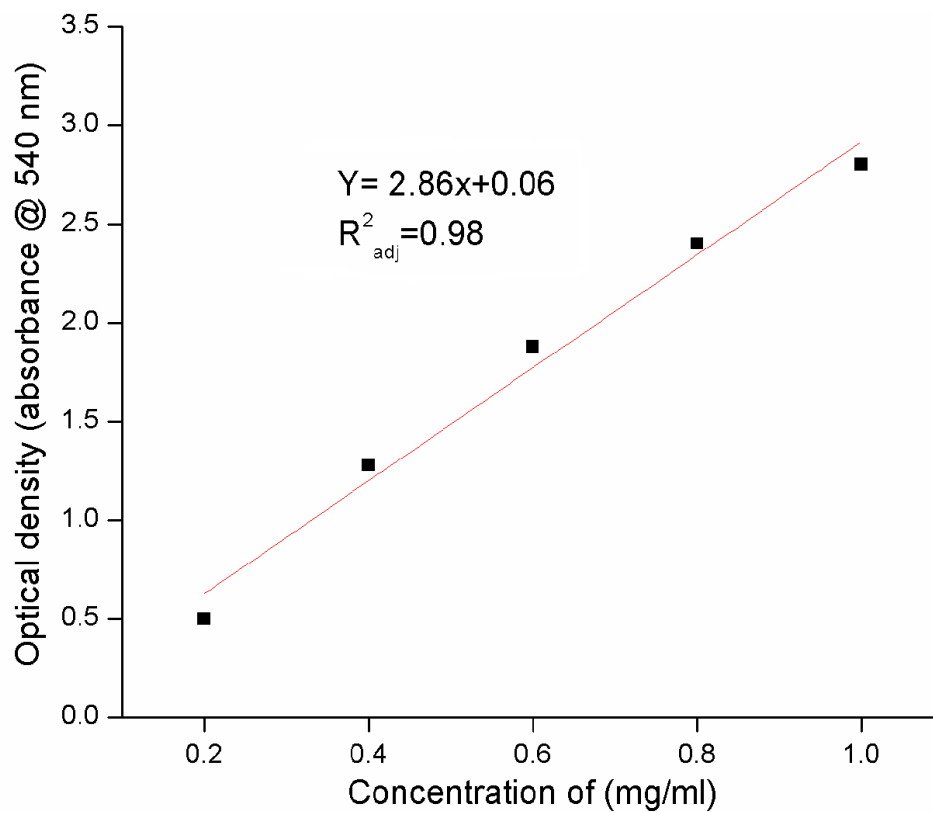


Figure D.1 Standard curve (curve fitted) for glucose vs OD (absorbance @ 540 nm)

## Appendix E

### E.1. Mathematical analysis for moles of Double bond per given mass of cottonseed oil

$$\text{mole of DB in 100 g of cottonseed oil} = \frac{\text{moles DB}}{\text{mole of TAG}} \times \frac{\text{moles of TAG}}{MM_{TAG}} \times 100 \text{ g}$$

Whereas, moles of DB per moles of TAG is evaluated as follows

$$\text{Moles of DB per mole of TAG} = \sum \left( \frac{\text{mol DB}}{100 \text{ g oil}} \right) \times \sum \left( \frac{1}{\frac{\text{mol FA}}{100 \text{ g oil}}} \right) \times \left( \frac{\text{mol FA}}{\text{mol TAG}} \right)$$

$$\text{Moles of DB per mole of TAG} = \frac{0.463 \text{ mol DB}}{100 \text{ g oil}} * \frac{100 \text{ g oil}}{0.365 \text{ mol FA}} * \frac{3 \text{ mol FA}}{TGA} = 3.81 \frac{\text{mol DB}}{\text{mol TAG}}$$

$$MM_{TAG} = 3 \times MM_{FFA} + MM_{glycerol} - 3 \times MM_{water}$$

$$\text{Where } MM_{FFA} = \frac{100 \text{ g cottonseed oil}}{0.365 \text{ mol}} = 273.97 \frac{\text{g}}{\text{mol}}$$

$$MM_{TAG} = 3 * 273.97 \frac{\text{g}}{\text{mol}} + 92.06 \frac{\text{g}}{\text{mol}} - 3 * 18 \frac{\text{g}}{\text{mol}} = 860 \frac{\text{g}}{\text{mol}}$$

$$\begin{aligned} \text{Mole of DB per 100 g of cottonseed oil} &= 3.81 \frac{\text{mol DB}}{\text{mol TAG}} * \frac{\text{mol TAG}}{860 \text{ g}} * 100 \text{ g} \\ &= 0.443 \text{ mole} \end{aligned}$$

### E.2. Theoretical Oxirane content of cottonseed oil (OO<sub>the</sub>)

$$\begin{aligned} OO_{the} &= \left[ \frac{\left( \frac{IV_o}{2A_i} \right)}{100 + \left( \frac{IV_o}{2A_i} \right) A_o} \right] A_o * 100 \\ &= \left[ \frac{\frac{103.5}{2 * 126.9}}{100 + \left( \frac{103.5}{2 * 126.9} \right) * 16} \right] 16 * 100 \\ &= 6.13 \end{aligned}$$

## Appendix F: Photographic images of selected laboratory equipment

**Tubular furnace (Carbolite carbonizer)**



**Sulfonation process**



**Perkin-Elmer FT-IR spectroscopy**



**EA 1112 Flash CHNS/O-analyzer**



**SensysEvoTGA and TPD analyzer**



**GC/MS Instrument**



**MM-200 Vibrational milling**



**UV- Spectroscopy**



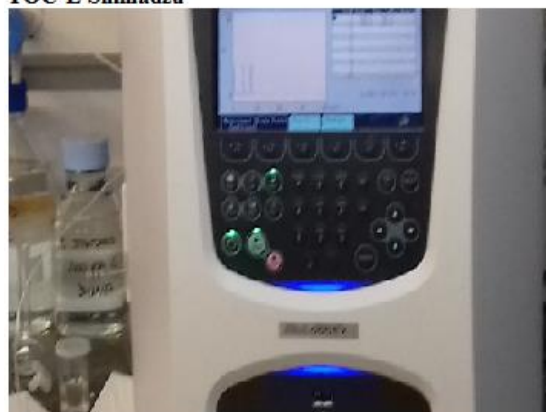
**Agilent 1100 HPLC**



**100 ml Auto-clave reactor**



**TOC-L Shimadzu**



**SynthaWave microwave reactor chamber**

