PREPARATION AND CHARACTERIZATION OF CARBOXYMETHYLCELLULOSE FROM NATURAL RESOURCE

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A project report submitted in partial fulfilment of the requirements for the award of the degree of Bachelor of Engineering (Hons) Petrochemical Engineering

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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

I certify that this project report entitled **"PREPARATION AND CHARACTERIZATION OF CARBOXYMETHYLCELLULOSE FROM NATURAL RESOURCE"** was prepared by **VANESA CHU WAN YI** has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of Engineering (Hons) Petrochemical Engineering at Universiti Tunku Abdul Rahman.

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ABSTRACT

In this study, sugarcane bagasse is the raw material used to produce cellulose. SCB was treated using a strong alkali to eliminate lignin and hemicellulose. The treatment was carried out at three different process parameters, at various temperature, duration and concentration of the alkali solution. The effects of the three process parameters were characterized and investigated through its crystallinity, thermal stability and carbohydrate content. With the most effective and productive selection of parameters, the treated SCB was converted to carboxymethylcellulose (CMC) by two steps, alkalization and etherification. First, treated SCB was reacted with NaOH then followed by the addition of sodium monochloroacetate (SMCA) in isopropanol where carboxymethylation occurs. The slurry is purified by using distilled water and hot ethanol for several times. The residue is dried in an oven until a constant weight is obtained. The characterization was done to analyse the amount of cellulose recovered successfully, predict the structure and estimate their thermal properties. Approximately 39.0 – 47.0 % w/w of treated SCB was recovered from sugarcane bagasse using NaOH. The amount of carbohydrate calculated in SCB biomass is approximately 45 % w/w of cellulose content. After carboxymethylation process, the CMC produced obtained has a degree of substitution (DS) of 0.59. The structure and thermal properties of treated SCB and CMC were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC) and High Pressure Liquid Chromatography (HPLC). The findings of the research are promising. Furthermore, the extraction process could be simplified into a single step to recover cellulose from plant biomass.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS / ABBREVIATIONS

NH_3	Ammonia
AGU	Anhydroglucose units
$Ca(OH)_2$	Calcium hydroxide
CO_2	Carbon dioxide
CMC	Carboxymethylcellulose
DS	Degree of Solubility
FTIR	Fourier Transform Infrared Spectroscopy
GPC	Gel-Permeation Chromatography
HPLC	High Performance Liquid Chromatography
HCl	Hydrochloric acid
HNO_3	Nitric acid
H_3PO_4	Phosphoric acid
KBr	Potassium bromide
KOH	Potassium hydroxide
NaOH	Sodium hydroxide
Na_2SO_3	Sodium sulphite
SCB	Sugarcane bagasse
SMCA	Sodium monochloroacetate
H_2SO_4	Sulfuric acid
TGA	Thermogravimetric analysis
UV-Vis	Ultraviolent Visible Spectroscopy
XRD	X-ray Powder Diffraction

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background

Nowadays, researchers have put their attention on sustainable and non-toxic materials from biomass for sustainable development of living environment. Biomass generally refers to organic matter such as grass, algae and agricultural wastes including corn cob, rice husks, coconut husks, palm kernel cake and sugarcane bagasse. These organic matters are commonly referred to as lignocellulosic biomass as they are rich in cellulose, hemicellulose and lignin. Most of the plant biomass have the following compositions on dry basis, lignin has a range of 15 – 35 %, hemi-cellulose with 25 – 40 % and 40 – 50 % of cellulose present (Bajpai, 2016). Cellulose is one of the major component in biomass and it is a renewable resource. Cellulose are chains of sugar molecules of naturally occurring polysaccharides that are bonded together in plants. It acts as the structural basis to provide strength in plant cells (Chen, 2014).

With an increased activity in the modern agricultural sector, agricultural wastes have increased drastically which has led to environmental concern. A very huge amount of biomass is generated from agriculture waste annually. The increasing environmental concerns have forced the researchers to obtain useful industrial materials from plant biomass (Saini et al., 2014). When biomass are used as feedstock, it has attractive potentials for large-scale production industries. In this

case, the main focus is to recover cellulose from lignocellulose biomass and convert to a derivative of cellulosic compound such as methylcellulose (MC), hydroxypropylmethylcellulose (HPMC), hydroxyethylcellulose (HEC) and carboxymethylcellulose (CMC) (Jia et al,. 2016).

Sugarcane bagasse (SCB) is a potential feedstock for the production of CMC as it is abundantly available lignocellulosic product which containing high cellulose content. Statistics shows that a sum of about 54 million tons of dry SCB residue has been produced annually throughout the world (Cueva et al., 2017). SCB is a fibrous residue of sugarcane stalks that are left after it has been crushed, juice extracted from the sugarcane. In the sugarcane mill, these complex SCB are the main by-product which are generated in large quantities. Due to its availability and inexpensive cost, it can be further processed to produce value added biomaterials (Sahu et al., 2016). Commonly, SCB consists of lignin, pectin, hemicellulose and cellulose with compositions in dry basis about 14-25% of lignin, 22-30% of hemicellulose and 43.6-55% of cellulose (Hasan and Sauodi, 2014).

Cellulose is a linear polysaccharide polymer consisting of glucose monosaccharide unit with high molecular weight. The presence of cellulose in plants is to give a rigid cell wall and structural support. These glucose monomers are associated in cellulose molecule structure due to strong various intermolecular and intramolecular linkages of hydrogen bonds (Guilherme et al., 2015). In the presence of hydrogen bonds, cellulose does not melt or dissolve in organic solvents, restricting its applicability. Cellulose shows a large variability and complexity in its molecular arrangement that embedded with matrix of lignin and hemicelluloses.

There are several ways to extract cellulose from lignocellulosic biomass. It is categorised into chemical, physiochemical, mechanical and enzymatic treatments. Chemical treatment involved utilization of acid, alkali and organic solvent while mechanical treatment includes high-intensity ultrasonication. Physiochemical treatment uses steam explosion in the process and enzymatic treatment depends on the enzymes used for extraction (Brodeur et al., 2011). Among these treatment methods, alkali treatments are more favourable since the cost of chemicals are less,

produce less by-products and are more environmentally friendly (Bensah and Mensah, 2013).

Cellulose is converted into useful derivatives especially carboxymethylcellulose (CMC) to improve its applicability. CMC is a linear, long-chain and water-soluble fibre at room temperature. It is a man-made modified cellulose prepared by two main reaction, naming alkalization and carboxymethylation under heterogeneous conditions. The alkali catalysed reaction of cellulose with sodium hydroxide (NaOH) followed by methylation of sodium monochloroacetate (SMCA) eventually produce CMC as end product (Gulati et al., 2014). CMC gained sufficient scientific attention, especially due to its polyelectrolyte character and multifunctional properties. As the result, CMC has gained its importance in the market today. The worldwide production of CMC has been estimated to be over 300,000 metric ton annually (Kalia et al., 2011). CMC exhibit useful properties as adhesive, adhesion, stabilizer, suspending agent, surfactant, thickener, thermal gelation and forming films resistant to oils, greases, and organic solvents. In addition, they are kinetically and thermodynamically stable and easy to be prepared (Singh and Singh, 2012). CMC is important for its water-soluble properties where it is widely applied throughout pharmaceuticals, cosmetics, detergent, food, oil drilling, paper, paint, textiles, construction, adhesives as well as ceramics industries (Oun and Rhim, 2015)..

The physical and chemical properties of CMC are mainly determined by the purity of cellulose, distribution, degree of polymerization and degree of substitution (DS). DS refers to the number of the carboxymethyl groups in the molecular unit of the anhydrous glucose units (AGU). AGU represents a single sugar molecule in cellulose polymer, when all hydroxyl groups are substituted, producing a maximum DS of 3 (Bono et al., 2009). With the increasing demand of CMC in the market, recovery of cellulose from the natural resource, sugarcane bagasse in this research, could provide significant contribution in related industry for sustainable development of living environment.

1.2 Problem Statement

Sugarcane bagasse (SCB) is a potential feedstock for the CMC production. Cellulose content in SCB is embedded with pectin, lignin and hemicellulose. In order to extract the cellulose content, dewaxing, delignification and the removal of hemicellulose process has to be carried out. Dewaxing is the process of removing pectin content from the SCB (Abdel, 2014). The effective process of delignification and removal of hemicellulose could simplify the cellulose extraction process. Direct extraction of cellulose without treatment is not applicable as the lignin and hemicellulose inhibits the extraction process (Karp et al., 2016). Chemical treatment is one of the common method that used for lignin and hemicellulose removal by using acid, alkali or organic solvents.

Among the three types of chemical treatments of sugarcane biomass, acid hydrolysis is known as one of the most common and conventional method. In industries, the procedures of cellulose extraction usually involves acid hydrolysis treatment, bleaching and purification to remove the non-cellulosic materials. The use of acids such as sulphuric acid, hydrochloric acid and phosphoric acid normally operate at high concentration or at high temperature (Aboody, 2013). However, there are several problems that have arisen from the current cellulose extraction process in the industry. The most crucial problem is the high cost of acids and high concentration of acid which can cause corrosion to the reactors and other equipment. This requires frequent maintenance and the fee is costly (Balan, 2014). With the problems stated, acid hydrolysis process would need a corrosive resistant equipment. Acid hydrolysis could generate side products as well which requires tedious purification processes (Brodeur et al., 2011).

On the other hand, alkali chemicals such as sodium hydroxide are used as it provides a higher rate of process with simple procedures with relatively lower cost compared to other methods. Due to the corrosive nature of strong base, an adequate material for the reactor is required in order to withstand the required operating conditions (Balan, 2014). This process can be further improved by reducing the corrosiveness using a weak base such as lime, known as calcium hydroxide

($Ca(OH)_2$). Among other alkali chemicals, lime is considered to have the lowest cost and produces very less or no inhibitors during the process (Lee et al., 2014). It is found to improve the yield and effectiveness of the process at the moment.

Most of the aforesaid extraction method involves high usage of chemicals and operating conditions, which lead to complex purification process and generation of huge amount of chemical wastes. Therefore, it is important to develop an effective process pathway to reduce the complexity of the current extraction methods using biomass waste sugarcane bagasse. At the same time obtaining the highest yield of cellulose from SCB through an economical method. The factors such as wastes, toxicity, solvent recovery and environmental friendly, are taken into consideration to minimize any harmful substances that may produce. To achieve the goals, extraction under low concentration of alkaline offer alternative solution. The treatment could be assisted with ultrasonic irradiation to improve the extraction efficiency. The extracted cellulose could be converted to CMC via carboxymethylation process.

1.3 Aims and Objectives

The focus of the study is based on the following objectives:

i) To collect and characterise the sugarcane bagasse.

ii) To extract the cellulose from sugarcane bagasse.

iii) To produce carboxymethylcellulose from the treated SCB.

1.4 Scope of Study

The scope of the study for this research are as follows:

i) Preparation of sugarcane bagasse for cellulose extraction process. The preparation mainly focused to obtain dry and small particle size of sugarcane bagasse. The characteristics of sugarcane bagasse is studied and analysed.

ii) Study and develop extraction methods to extract cellulose from sugarcane bagasse. The ultrasonic treatment is combined with the extraction method to enhance the extraction process.

iii) Study the structure of treated SCB and predict its structure and thermal properties. Analyse the yield of cellulose for production of carboxymethylcellulose from sugarcane bagasse.

iv) Evaluate the CMC products using cellulose recovered from sugarcane bagasse. The evaluation mainly focused in determining the degree of substitution and viscosity results obtained through standard analytical procedure.

CHAPTER 2

LITERATURE REVIEW

2.1 Feedstock of Cellulose Production

Over the last decade, research have proved that the use of agricultural waste has grown significantly. The common plant biomass from agricultural wastes includes palm kernel cake, coconut husks, rice husks and cotton linters. These cellulosic wastes have been increasing overtime but not much action has been done. When this problem arise, communities across the country started to organize "Waste to Wealth" recycling campaign. This program did not only help in fighting the incinerators which causes pollution but also to reduce the need of disposal and stimulate economic growth (Ng et al., 2012).

The properties of the cellulose macromolecule is able to improve by chemical functionalization to produce cellulose derivatives for various applications. The main sources of cellulose for industrial uses are palm kernel cake (contain 65 % cellulose in dry basis) (Ng et al., 2012) and cotton linters (contain 90 % cellulose in dry basis) (Oun and Rhim, 2015). Although these two sources are commonly used today but they are discouraged due to high production cost and harmful environmental concerns (Diego, 2009). Therefore biomass rich in cellulose such as corn cob, wheat straw and sugarcane bagasse could be the alternative chemical feedstock (Taherzadeh and Karimi, 2008). Today, many of these cellulosic biomass were deeply investigated, however the use of sugarcane bagasse (SCB) as a natural resource for the production of CMC has not yet been widely explored in industrial scale.

The cultivation of sugarcane in Malaysia is not as huge as other agricultural crops such as rubber and oil palm. However, SCB is one of the agricultural waste that can be easily found in some states of Malaysia (Baharuddin, 2011). During crushing and extraction of sugarcane juice in the sugar mill industry, an enormous quantity of SCB is generated. It accounts about 22% of the 54 million tons of SCB produced worldwide and it has not been utilized for any further downstream operations (Sanchez et al., 2011). In societies, most of the sugar refineries dispose SCB either by open burning or dumping which in turn leads to environmental pollution. Previous reports on the commercial methods of disposal have been suggested mainly focused in production of fired clay brick (Ali et al., 2016, Kadir et al., 2014). Since SCB consists significant cellulose content, about 43.6-55% in dry basis, the use of SCB as the primary source to produce cellulose fibres is promising (Hasan and Sauodi, 2014). The content of lignocellulosic materials of other agricultural wastes are shown in Table 2.1 below.

Table 2.1: The Content of Cellulose, Hemicellulose and Lignin in Agricultural Waste

Agricultural Waste	**Cellulose (%)**	**Hemicellulose (%)**	**Lignin (%)**	**Reference**
Cotton Linters	90	-	< 2	Oun and Rhim, 2015
Palm Kernel Cake	20-40	10-35	23-52	Ng et al., 2012
Wheat Straw	30	50	15	Sun, 2004
Sugarcane Bagasse	43.6-55	22-30	14-25	Hasan and Sauodi, 2014

Palm kernel cake is produced after oil is extracted in palm oil mill. It is common to extract cellulose from the palm kernel cake and make use of all the parts for different purposes or products. However, there is a significant drawback where it requires to go through quite a number of processes before the palm kernel cake can be further process into other useful products. These processes include grinding, oil extraction, hot water treatment, delignification, the removal of hemicellulose and oxidation, which is very time-consuming (Bono et al., 2009). Wheat straw is generally produced during the harvesting process and are normally sent for open burning (Lawther et al., 1995). SCB are abundantly available as well, therefore the cost of obtaining these materials are low. However, the complex structure which makes treatment and extraction of cellulose complicated (Singh and Singh, 2012). There are quite a number of methods to reduce the complexity of the extraction methods but some of which requires high production cost.

SCB is considered as a potential resource. It has attractive composition of bagasse fibre and a net calorific value of about 8,000 kJ/kg, with a moisture content of 50 mass % and ash content in the range of 4 – 5 mass %. It is therefore utilized as a fuel in boilers in the sugar mills to generate steam and electricity (Mothé and Miranda, 2009). SCB is characterized as a low-density fibre and wide particle size distribution. It is used primarily in the sugar mill supplying ample energy required for sugar production process. There has been a significant interest in converting this residue into value-added derivatives. The obtained cellulosic fraction can be converted to cellulose derivatives like esters, which serve in wide range of applications in many industrial applications, including food, pharmaceutical and paint industry (Johar et al., 2012).

2.2 Biochemical Profile of Sugarcane Bagasse

Sugarcane bagasse consists of two carbohydrate fractions, cellulose and hemicellulose, embedded in a lignin matrix as shown in Figure 2.1. There is also relatively low content of pectin binding with lignin, conferring recalcitrance to hydrolysis. In the cell wall of SCB, lignin and hemicelluloses provides protection against chemical or biological degradation (Abdel, 2014).

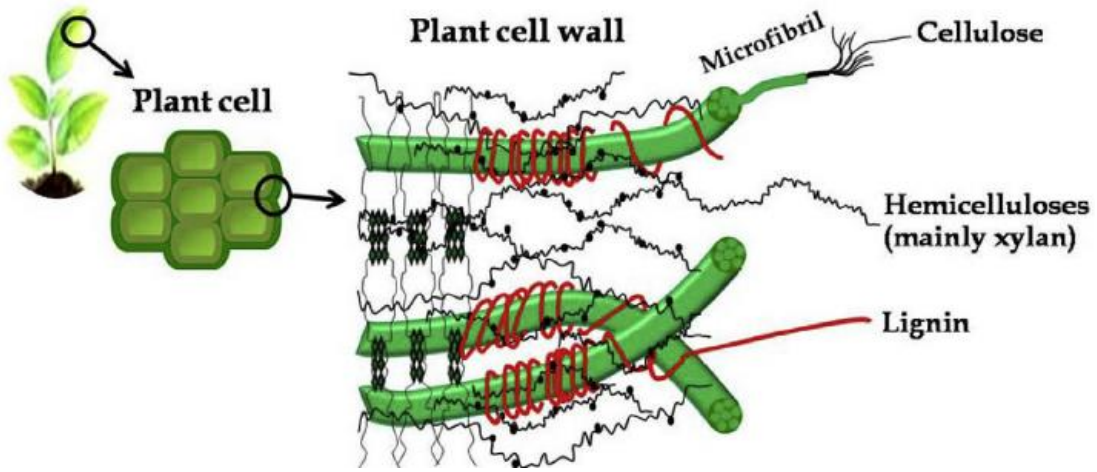

Figure 2.1: Network of Cellulose, Hemicellulose and Lignin in a Lignocellulosic Biomass (Pason et al, 2006)

Lignin present in the walls of SCB gives support to its structure and resistance against oxidative stress. It is most recalcitrant to biodegradation. While pectin is responsible for adhesion to neighbouring cells. It has an important role of controlling the penetration of other enzymes into the biomass network as they are thought to be responsible for determination of cell wall porosity, limiting the size and dimensions of enzymes to penetrate the wall (Rezende et al., 2011).

Cellulose is a linear polymer composed of glucose monomers linked by glycosidic bonds. These monomers are linked together by hydrogen bonds, intra- and inter-molecular Van der Waals forces. The structure of cellulose has a major part of crystalline region and partial chains of amorphous. In the latter conformation,

cellulose is more susceptible to enzymatic degradation and has a higher molecular weight compared to hemicellulose (Meada et al., 2011).

2.3 Cellulose Extraction in Industry

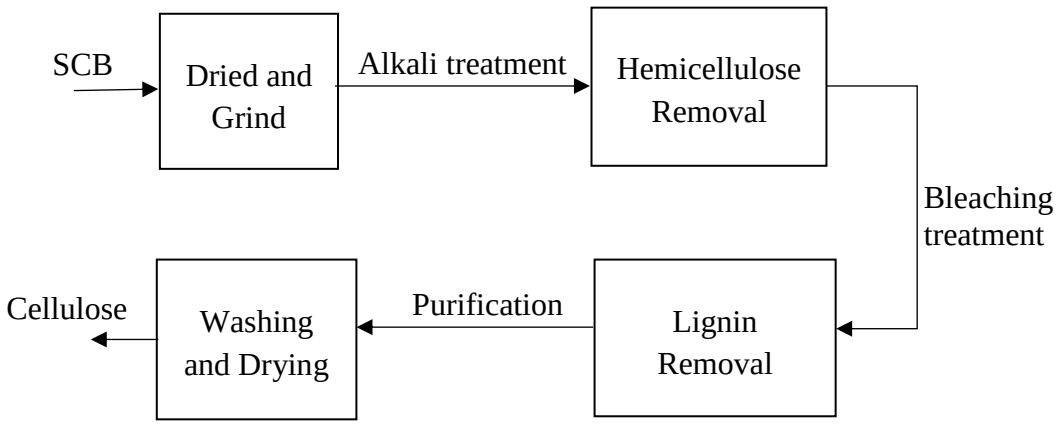

Figure 2.2: Process Flow Diagram of Cellulose Extraction in Industries (Song and Chew, 2016)

In lignocellulosic materials, lignin, pectin, hemicelluloses and cellulose fibre are embedded together. The process of extracting pure cellulose from the structure requires several steps as shown in Figure 2.2 above as it has a complex structure of cell wall. In industries, when biomass have collected, the juice or oil are first extracted. Then the biomass is washed thoroughly in running water to minimize any microbial attack (Brodeur et al., 2011). The biomass needs to be dried completely and grinded into small pieces to increase its surface area. The pieces of dried biomass is then treated with strong acid or alkali such as H_3PO_4 or NaOH to extract non-cellulosic binding materials like hemicelluloses and lignin (Xu, Li and Mu, 2016). The treatment is always operated at boiling temperature for an hours. After that, the treated biomass is separated, washed and dried at ambient conditions. Then it is followed by bleaching process to obtain white cellulosic fibres. The colour of biomass decolourises when it is broken down into simpler compounds using sodium chlorite ($NaClO_2$). This reaction produces chlorine dioxide which is toxic and

corrosive. Advanced technologies were developed, regulating the corrosive gas by addition of weak acids to form buffer and maintain the pH (Abdel, 2014). The cellulose extracted is washed thoroughly, dried and kept for other productions.

2.4 Treatment of Sugarcane Bagasse for the Extraction of Cellulose

Sugarcane bagasse is treated to decrease the recalcitrance and eventually improve the efficiency of cellulose extraction. Various treatment methods includes dewaxing, delignification and removal of hemicellulose have been explored. The structure of cellulose, hemicellulose and lignin has a strong matrix, which could be ruptured with proper treatment. This leads to formation of disordered structure of cellulose-lignin complex (Kim and Day, 2013). Cellulose constitute structure with a major part of crystalline region and minor part of amorphous region. The crystalline part is resistance to enzymatic hydrolysis. While amorphous region is easily accessible by enzyme and readily hydrolyses by the cellulase enzyme. The goal of treatment is to increase the digestibility of lignocelluloses (Hendriks and Zeeman, 2009).

In order to extract cellulose from natural resource for CMC production, few steps of treatment process is necessary. An effective and economical treatment would need to have a few requirements. First and foremost, cellulose must not be destructed and there should not be any formation of inhibitors and toxic materials. The treatment has to be controlled at minimum energy demand, thus reducing operating cost. Maintaining the consumption of chemicals at minimum where less pollution and residue are produced. There are several methods that have been used for treating lignocellulosic materials. Some of which are chemical treatments, physiochemical treatment, mechanical treatment and enzymatic hydrolysis (Tarkow and Feist, 1969).

2.4.1 Chemical Treatments

2.4.1.1 Acid Hydrolysis Treatment

Acid hydrolysis is one of the most common methods to extract cellulose. Sulfuric acid (H_2SO_4) is most commonly used for its high efficiency in breaking down the rigid structure of hemicellulose and lignin when in contact with lignocellulosic biomass. Other acids such as hydrochloric acid (HCl), phosphoric acid (H_3PO_4) and nitric acid (HNO_3) have also shown promising results. The conditions of the process usually can be performed at temperatures of 120–180 ℃ and residence time ranging from 15 to 60 minutes (Kumar and Sharma, 2017). For concentrated acid hydrolysis treatment, it operates at a low temperature and high acid concentration. Dilute-acid hydrolysis is another common method. With an elevated temperature and low concentration of sulfuric acid, this dilute acid treatment can achieve high reaction rates. It is possible to remove the entire hemicellulose and disrupt lignin entirely. This treatment was conducted either in high temperature of short retention time or vice versa (Anuj et al., 2004). Moreover, it extends the lifetime of a reactor compared to the use of highly concentrated acid. However, the treatment conditions have to be properly monitored as extreme treatment could degrade the cellulose as well. Degradation of cellulose could reduce their degree of polymerization and eventually diminish their outstanding properties such as thermal stability and mechanical strength.

During acid hydrolysis, the process could degrade lignin and hemicellulose but not cellulose. However, problems may arise due to high concentration of acid where equipment may corrode overtime. Therefore it requires neutralization process to remove excess acid. Another drawback of this process is the formation of by-products which requires detoxification to remove the inhibitory (Maurya et al., 2015). The high investment and maintenance costs also reduce the commercial interest in this process as a commercial option.

2.4.1.2 Alkali Hydrolysis Treatment

Alkali treatment refers to the use of sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$) or ammonia (NH_3) to treat lignocellulosic biomass. These strong bases degrade the ester and glycosidic bonds altering the lignin structure, solubilise the hemicellulose, and causes cellulose to swell and decrystallize. The disruption of lignin structure and removal of hemicellulose increases the accessibility of solutions and enzymes to the structure of biomass (Jung et al., 2015). According to Liu and co-workers (2006), treatment with alkali can be performed at ambient conditions, high concentration and low temperatures, where it takes long residence time. It is found that NaOH treatment has resulted in the highest level of delignification at conditions of 90 minutes at 121 ℃. In comparison with acid or oxidative reagent treatments, alkali hydrolysis seems to be more effective in breaking the ester bonds between cellulose, hemicellulose and lignin and the conditions are less severe.

Alkali treatment has proved to be more suitable compared to acid hydrolysis. Although NaOH is corrosive, it causes degradation only on certain metals. Unlike strong acids such as H_2SO_4, causing corrosion to reactors or any machineries made of metals, leading to high maintenance cost (Cabet, 2008). This problem is overcome by using a corrosion resistance material for the reactor to withstand the experimental conditions. In previous reports, researchers usually use strong alkali for treatment due to its better performance in terms of rate of reaction. In order to select a suitable alkaline solution for the treatment of SCB, the degree of delignification and dissolution hemicellulose is analysed to study the effectiveness of the solution.

The raw SCB biomass are usually dried and grinded into small pieces where it is soaked with an alkali reagent. This mixture removes lignin and hemicellulose, followed by a neutralization step removing inhibitors such as furfural and aldehydes. When slurry appears, it is then washed, filtered and reflux with mixtures of nitric acid in ethanol (Park, 2010). The dried sample of cellulose is then further used for CMC production.

There is a huge advantage of using $Ca(OH)_2$ since the cost of chemicals is lower. Alkali hydrolysis is also combined with other treatment methods, such as wet oxidation, steam explosion to enhance the extraction of pure cellulose (Liu et al., 2006). The results showed that relatively pure cellulose is obtained by combining acid and alkali treatment with other physical treatment. Despite that, the use of radiofrequency dielectric heating or ultrasonic has predicted to accelerate the disruption of the lignocelluloses structure. This method results in higher yields of carbohydrates compared to the conventional heating method (Wyman et al., 2009).

2.4.1.3 Organic Solvent Treatment

The treatment process of lignocellulosic biomass could be conducted with organic solvent as well to extract cellulose, known as organosolv. This process has gained importance as it has the ability to dissolve various types of biomass. The dissolution of SCB in organic solvent is heated to temperatures range of 90 ℃ to 130 ℃ to dissolve and decompose the network of lignin and hemicellulose, leaving reactive cellulose in the solid phase. The biomass is repeatedly precipitated and washed with water. The cellulose dissolved does not reduce the degree of polymerization since the chains of cellulose are not degraded (Ruzene et al., 2007).

When lignin is removed, the superficial area and volume increased considerably, facilitating the enzyme accessibility and consequently improving the efficiency of the process. This process uses fewer amounts of chemicals to neutralize the hydrolysate and generates less amounts of wastes compared with other similar processes (Rezende et al., 2007). Sodium hydroxide (NaOH) or sodium sulphite (Na_2SO_3) are catalysts used to reduce the operating temperature and to enhance the delignification process (Weerachanchai and Lee, 2013).

The solvent may also be accompanied with acetic acid developed by hydrolysis of hemicelluloses. Organic solvents such as alcohols, ketones, organic

acids, and ethers are used. However, the cost of solvent and simplicity in recovery of solvent was considered**.** The process of evaporation and condensation were used to separate the applied solvents, recycling them to reduce the cost of operation. These solvents are then removed as it might be inhibitors to the enzymatic hydrolysis (Saputra, 2014). Economically, alcohols with low molecular weight are used since it is favoured over high boiling point alcohols. The benefits of using organic solvent treatment is that lignin can be recovered as a by-product. Organic solvents can be recovered, recycled, and does not produce any toxic materials,

SCB can first be treated with dilute aqueous acid at 100 ℃ for 60 minutes in order to selectively hydrolyse the hemicellulose fraction. The purpose of the second stage is delignification by acid, when ethanol is added and lignin will be recovered. It was found that, 1-ethyl-3-methylimidazolium acetate, an organic solvent has the ability to extract selectively (Sun et al., 2004). The solvent has high solubility for lignin but low solubility for cellulose. Thus, unaltered lignin was solubilise and extracted, yielding a highly degradable cellulose fraction**.** Another benefit of organic solvent is it can be recycled many times, reducing costs (Rezende et al., 2007).

2.4.2 Physiochemical Treatment

2.4.2.1 Steam Explosion

Steam explosion is widely known as hydrothermal process. This treatment method is a thermo-chemical process, where SCB is exposed to steam. This method is seen as an outstanding method as it uses both chemical and physical techniques in breaking structure of SCB. Among the physio-chemical processes, steaming with or without explosion for the removal of hemicellulose has improved the enzymatic digestion (Dungani et al, 2016). In steam explosion, pressure decreases drastically where materials undergo an explosive decompression. Steam explosion occurs at

high temperature and pressure at a very short residence time (Pielhop et al., 2016). The cost of energy is moderate, satisfying the requirements of the process.

Steam explosion treatment has divided into two steps, it is heated to 180 ℃ for the purpose of solubilizing and removing the hemicellulose fraction. It is then followed by a high-temperature pressurized treatment to break down the linkages of carbohydrate. During the process, the reactor is maintained at temperatures and pressures between 160-240 ℃ and 0.7-4.8 MPa respectively, thus disrupting the structure of the fibrils (Brodeur et al., 2011). The combination of steaming and mechanical treatment is possible to disrupt the cellulosic structure effectively. Therefore, the selection of conditions in steam explosion is crucial and should be specify clearly, avoiding any excessive degradation of the properties of cellulose (Dungani et al, 2016). In serious conditions, lower enzymatic digestibility of lignocelluloses may also be observed after steam explosion.

2.4.3 Mechanical Treatment

2.4.3.1 High-intensity Ultrasonication

The high intensity ultrasonic treatment is one of the mechanical method for the extraction of cellulose fibres with hydrodynamic forces. Ultrasonic is usually carried out in a combination with other methods, acid or alkali hydrolysis, to successfully extract the cellulose fibre. These combinations not only increase yield of cellulose but also the efficiency of cellulose extraction (Szczodrak and Fiedurek, 1996). The ultrasound energy from the ultrasonic machine is transferred to the cellulose chains by cavitation process, referring to the formation, growth, and violent collapse of cavities in liquid. The degradation of polymeric sequences of lignin and hemicelluloses can be done using ultrasound (Liu et al., 2006).

A series of processes consisting of chemical treatment and high-intensity ultra-sonication could be done to extract cellulose from SCB. At first, acidified

sodium chlorite solution was used to ensure lignin was fully remove and it is replicated until the colour of the sample has faded. Samples were treated with potassium hydroxide (KOH) to leach hemicellulose, residual starch, and pectin. After a series of chemical treatments, the samples were filtered and rinsed with distilled water until the residues were neutralized (Chen et al., 2011). When samples are stored for later use, it is kept in a water-swollen state to avoid strong hydrogen bonds to generate.

After chemical treatment has been done, the purified cellulose fibres were soaked in distilled water. The solution containing chemical-purified cellulose fibres were treated with ultrasonic generator (frequency of 20–25 kHz) equipped with a cylindrical titanium alloy probe. The ultra-sonication was then conducted for 30 minutes to isolate the fibres (Khawas and Deka, 2016). The effect of ultrasonic intensity on cellulose was investigated at different output power. It has to run at suitable output power to obtain large aggregates of wire-like cellulose fibres. It was found that purity of cellulose increased when ultrasonic output power increases. The degree of crystallization is also affected by the output power of the ultrasonic treatment (Liu et al., 2006).

High intensity ultrasonic waves disperse cellulose by its strong mechanical oscillating power. Within the environment and bubbles of cavitation, fibrils are isolated from cellulose fibres by violent shock waves. However to achieve high dispersion, several parameters such as temperature, pressure, output power, concentration of solvent and intensity are taken into account (Boufi and Khalil, 2010). The impact of the ultrasonic can gradually disintegrate cellulose into its simpler monomers when parameters applied are not suitable.

2.4.4 Enzymatic Hydrolysis Treatment

Enzymatic hydrolysis is an approach to recover cellulose by using enzyme. Enzymatic hydrolysis depends on optimized conditions such as time, temperature, pH, enzyme and substrate, for maximum efficiency. There are several enzymes that only breakdown hemicellulose, some of them are, xylanase, glucuronidase and acetylesterase (Canilha et al., 2012). These enzymes react together on delignified SCB, exhibit better yields of cellulose. Removal of lignin significantly improves the carboxymethylation reaction. With lower lignin content, enzyme loadings can be reduced considerably. However, extensive lignin removals by treatments add cost to the processing line (Yang et al., 2011). Enzyme has a major effect on the hydrolysis efficiency. Although enzyme price has decreased due to intensive research, enzymes loading during cellulose hydrolysis should be minimized. Therefore, understanding the interaction between cellulose and pre-treated biomass is vital to reduce production costs of CMC.

2.5 Production of Carboxymethylcellulose

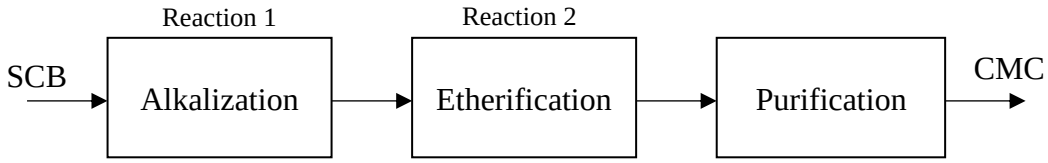

Figure 2.3: Process Flow of Carboxymethylation Production.

Figure 2.3 demonstrated the production of carboxymethylcellulose in industry. It started with alkalization reaction using sodium hydroxide, as written in Equation 2.1. Alkalization reaction normally conducted by adding cellulose powder into isopropanol as solvent at different concentrations of NaOH at 10 %, 15 %, 20 %, 25 % and 30 % w/v (Koh, 2013). The concentration could significantly affect the efficiency of carboxymethylation (etherification) that followed after alkalization. The overall chemical equation are presented in Equation 2.1 and 2.2 below (Abdel, 2014).

Etherification reaction take place by adding SMCA to the reaction mixture under continuous stirring (He et al., 2009). Figure 2.4 demonstrates the substitution of hydroxyl group by carboxymethyl group in CMC production process. The substitution process is always affected by the reaction condition such as SMCA concentration and reaction temperature. Duration of etherification could affect the overall quality of CMC as well. It was observed that longer duration increases the degradation of polymer and reduces the DS value as well (Bono et al., 2009). CMC produced is always purified with several washing steps by using organic solvent and chemicals. In general, the CMC slurry was s soaked in methanol, neutralized with 90 % of acetic acid and then filtered. After that, final product was washed several times by soaking in ethanol to remove undesirable by-products before going through drying (Sun, 2004).

$$R - OH + NaOH \rightarrow R - OH.NaOH$$

(Alkali cellulose) (2.1)

$$R - OH.NaOH + ClCH_2COONa \rightarrow R - O - CH_2COONa + NaCl + H_2O$$

(Carboxymethylcellulose)

(2.2)

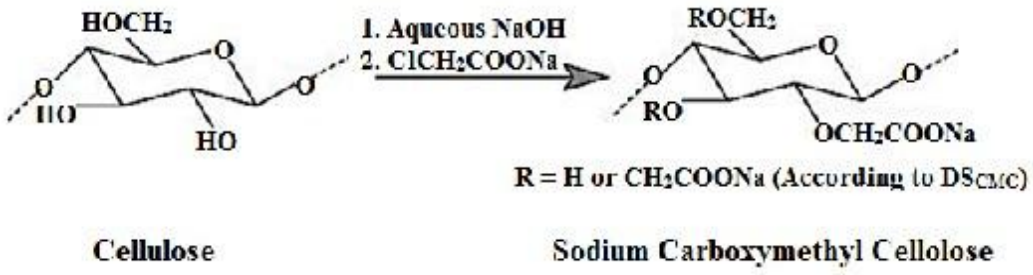

Figure 2.4: Hydroxyl Groups Being Substituted by Carboxymethyl Groups during Carboxymethylation (Konduri et al., 2015)

CHAPTER 3

METHODOLOGY

3.1 Research Flow Chart

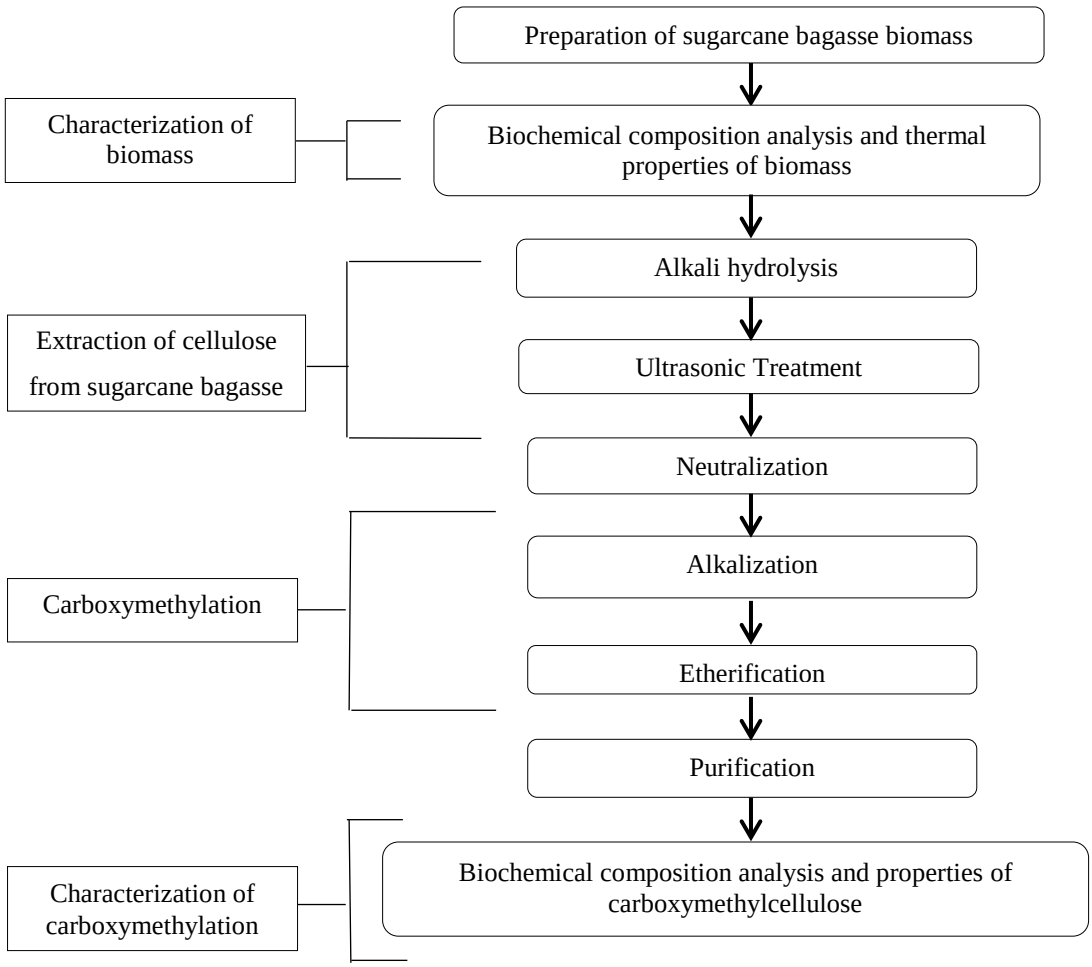

Figure 3.1: Research Flow Chart

3.2 Materials and Chemicals

3.2.1 Chemicals Used in this Study

Table 3.1: Chemicals Used in this Research

Chemical	Source
Sodium hydroxide	Essex, U.K.
Potassium bromide	Essex, U.K.
98 % Sodium monochloroacetic acid	Merck, U.K.
98 % Absolute methanol	Spectrum Chemicals, India
91 % Isopropanol	Parchem, Singapore
95 % Ethyl alcohol	HmbG Chemicals, U.K.

3.3 Procedures of the Experiment

3.3.1 Preparation of Sugarcane Bagasse Powder

The SCB was cleaned and cut into pieces. It was dried in an oven maintaining at temperature of 60 °C for 24 hours. After one day, it is taken out and weighed. The SCB is then continue to dry until it has reach a constant weight, to allow all the moisture in the SCB to evaporate. After that, SCB was blended and grinded into powder form. The SCB powder was screened using a 10 mm mesh and kept in an airtight container for later use.

3.3.2 Treatment to Extract Cellulose

Ultrasonic-assisted alkali treatment has been carried out to remove lignin and hemicellulose from SCB. Strong alkali was used in presence of ultrasonic irradiation as shown in Figure 3.2 below.

Figure 3.2: Set-up of Treatment with NaOH in an Ultrasonic Water Bath

At first, 5 g of SCB was added to 150 ml of NaOH in a Scott bottle. There are three important treatment conditions, temperature, NaOH concentrations, and duration have been investigated. All parameters are kept constant varying one parameter at each time with continuous stirring as shown in the Table 3.2. There are 9 sets of experiment for the study to determine the optimum condition of the cellulose recovery as shown in Table 3.2 – Table 3.4. After the treatment, mixture was filtered and washed with 500 ml of distilled water and 500 ml of hot ethanol for 5 times, removing all the impurities and soluble hydrocarbons. The residue was dried in oven at 60 ℃ for 12 hours until a constant weight is obtained. The recovered SCB is calculated using the following Equation 3.1:

$$Yield\ of\ treated\ SCB\ (\%) = \frac{weight\ of\ treated\ SCB\ obtained\ (g)}{weight\ of\ bagasse\ used\ (g)} \times 100\ \% \quad (3.1)$$

The treated SCB was dried and kept in a polyethylene bag. The colour and the end products were observed and the yield was calculated. The effect of treatment conditions on SCB were further analysed with FTIR, DSC, TGA and HPLC.

Table 3.2: Treatment at Various Temperatures

Expt No.	SCB (g)	Temp (℃)	[NaOH] (M)	NaOH Vol (ml)	Duration (hr)
1	5	60	0.5	150	2
2	5	70	0.5	150	2
3	5	80	0.5	150	2

Table 3.3: Treatment at Various Concentrations of NaOH

Expt No.	SCB (g)	Temp (℃)	[NaOH] (M)	NaOH Vol (ml)	Duration (hr)
4	5	80	0.5	150	2
5	5	80	0.75	150	2
6	5	80	1.0	150	2

Table 3.4: Treatment at Various Duration

Expt No.	SCB (g)	Temp (℃)	[NaOH] (M)	NaOH Vol (ml)	Duration (hr)
7	5	80	0.5	150	1
8	5	80	0.5	150	2
9	5	80	0.5	150	3

3.3.3 Synthesizing CMC from Cellulose

Dried cellulose powder of 5 g was added to a volume of 150 ml isopropanol in Scott bottle. Then 20 ml of 17.5 w/v % aqueous NaOH was added drop wise into the solution (Bono et al., 2009). This is the alkalization process. The mixture is then stirred continuously for an hour at 30 ℃ using a hot plate. 6 g of SMCA was dissolved in isopropanol and added to the alkali mixture. It was shaken in a water bath horizontal shaker for 2 hours at 50 ℃ where carboxymethylation takes place. After 2 hours, the mixture was filtered and soaked in methanol overnight. In following day, the slurry was purified by washing and filtering it with hot ethanol for several times to remove all the soluble impurities. The CMC obtained from SCB was then filtered and dried at 60 ℃ to a constant weight and kept it in a desiccator for subsequent characterization process.

3.4 Instrumentation of Analysis to Characterise SCB, Treated SCB and CMC

3.4.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is used to determine the functional groups of cellulose and synthesized CMC. The samples were grinded and added to potassium bromide, KBr, compressing it into pellet form. FTIR spectra analysis was measured within the wavelength range of 400–4000 cm^{-1} (Johar et al., 2012).

3.4.2 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) was performed to study the degradation characteristics of the treated SCB and CMC. The thermal stability of samples were measured and recorded in a nitrogen gas atmosphere at a flow rate of 20 ml min^{-1}.

The samples were heated from room temperature of 30 ℃ to 800 ℃ at a heating rate of 20 ℃ min^{-1} (Edreis and Yao, 2016).

3.4.3 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) is used to measure heat flow at different transitions of a material. With this test, the melting point, glass transition point and crystallization temperature can be measured. The dried powdered sample is weighed between 2-10 mg and placed in an empty aluminium pan covered by a lid with a pin hole to allow purging of nitrogen gas. It is then put on the holder to press and seal the pan and lid. An empty pan and lid are also sealed and used as reference in the experiment. The tests were operated at a flow rate of 20 ml min^{-1}. The pans were heated at a rate of 10 ℃ min^{-1} from 25 ℃ to 400 ℃ (Khristova et al., 2006). The DSC results were then analysed to characterise the onset, peak and enthalpy changes.

3.4.4 High Performance Liquid Chromatography (HPLC) for Carbohydrate Content Analysis

High Performance Liquid Chromatography (HPLC) is used to determine the amount of cellulose after treatment process. 300 mg of sample was added into boiling tube that containing 3 ml of 72% sulphuric acid, and then heated for an hour in a water bath maintaining at temperature of 30 ℃ and stirred continuously. It is then transferred to a Scott bottle and 84 ml of deionised water was added. The solution was then heated in an autoclave machine at 121 ℃ for 1 hour.

After the end of heating, solution was allowed to cool and was neutralised to a pH of 3 and centrifuged at 3000 rpm for 30 minutes. The top layer of sample was diluted and filtered through a syringe filter for HPLC analysis. The HPLC column, Rezex Roa Organic Acid H^+, was used and maintained at 56 ℃ and 0.0025 M

sulphuric acid was used as mobile phase, with pump flow rate of 0.6 mL/min. Calibration curves were constructed using standard of simple sugars such as mannose, xylose, arabinose and dextrose to determine the sample composition. The quantification of cellulose was determined based on the calibration line where cellulose breaks down into simple sugars.

3.4.5 Determination of Degree of Substitution (DS) of CMC

A mass of 0.35 g of CMC powder is weighed and wrapped with filter paper. It is placed in a crucible and heated in the furnace for 30 minutes at 600 ℃ (Bono et al., 2009). Then, it is transferred into a petri dish and placed in an oven for 12 hours at 100 ℃ to remove moisture completely. The sample is then put in a beaker added with 17.5 ml of 0.1 N H_2SO_4 solution and 125 ml distilled water (Cobbett et al., 2007). This mixture is boiled for 30 minutes. After the mixture has cooled, it is transferred to a 250 ml conical flask. The solution is added with 3 drops of phenolphthalein indicator and titrated with 0.1 N NaOH for colour change, from colourless to pink. The experiment is repeated to obtain the average volume of base required to neutralise the acid solution. Also, a blank test is carried out for reference and comparison.

Degree of substitution is the amount of hydroxyl groups of cellulose that substituted with carboxymethyl groups of sodium monochloroacetate. The value of degrees of substitution obtained are categorised into two qualities in Indonesian National Standard (SNI), there are those used for food additives and those for non-food products (Alizadeh Asl, 2017). The Equation 3.2 and 3.3 below is used to calculate the degree of substitution of CMC.

$$Degree\ of\ substitution\ (DS) = \frac{0.162 \times B}{1 - 0.080 \times B} \qquad (3.2)$$

$$B = 0.1 \times \frac{b}{G} \qquad (3.3)$$

Where, b = consumption of 0.1 N H_2SO_4 in ml,

G = weight of CMC in g

3.4.6 Determination of Viscosity of Carboxymethylcellulose

Ubbelohde type capillary viscometer was used in the experiment to measure the viscosity of CMC. In this experiment, 0.1 M sodium chloride (NaCl) was used to obtain an accurate viscosity of CMC product produced. 5 ml of the sample was carefully introduced into it. The viscosity of 0.5 % aqueous solution of CMC synthesized were immersed in a 30.0 ± 0.1 ℃ water bath. The time of flow, t, was recorded to calculate the viscosity, η of the solution. (Minagawa, 2001)

The viscosity of a solution is a measure of its resistance to gradual deformation by shear stress, which is due to intermolecular cohesive forces. The formula is shown in Equation 3.4 below. The cohesive forces can be influenced by concentration of CMC, degree of substitution and temperature (Bhattarai, 2012).

$$\eta = \rho k t \qquad (3.4)$$

Where η = Viscosity of the solution in cP

ρ = density of the solution in g cm^{-3}

k = viscometer constant in mm^2 s^{-2}

t = flow time in s

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Sugarcane Bagasse Biomass Characterization

Sugarcane bagasse was collected and characterized before undergoing any processes to identify the components in the biomass and to predict its properties. The results obtained are used for comparison after it is investigated. The biomass was sent for several tests which includes fourier transform infrared spectroscopy (FTIR), high pressure liquid chromatography (HPLC), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA).

Figure 4.1 shows the FTIR spectrum of sugarcane bagasse biomass. Based on FTIR spectrum, the grounded SCB has a broad band at 3415 cm^{-1} representing the bands of OH-stretching vibration where molecules are intermolecularly bonded in the forms of hydrogen bonds (Johar et al., 2012). The band at 2918 cm^{-1} shows the existence of C-H stretching. The observed band at 1735 cm^{-1} is attributed to carbonyl group, C=O stretching of the acetyl and ester groups in the hemicellulose of biomass (Moth é and Miranda, 2009). While the absorption band at 1637 cm^{-1} is assigned to the bending of water molecules (Alizadeh, 2017). The presence of peak at 1509 cm^{-1} indicates aromatic symmetric of lignin whereas the absorption band at 1431 cm^{-1}, represents the symmetric deformation of methyl group of cellulose, $-CH_2$ (Oun and Rhim, 2015). Besides, bending vibration at 1377 cm^{-1} is also assigned to –CH groups (Singh and Singh, 2012). The spectrum of the absorption band at 1256 cm^{-1} refers to C-O-C vibrations. In addition, the bands at 1163 cm^{-1} and 1045 cm^{-1} are in

connection with C-O-C and C-O stretching of cellulose and hemicellulose (Abdel, 2014). Furthermore, bands ranging from 600 – 900 cm^{-1} represents the C–O–C stretching vibration of the glycosidic linkages in the cellulose components (Singh and Singh, 2012).

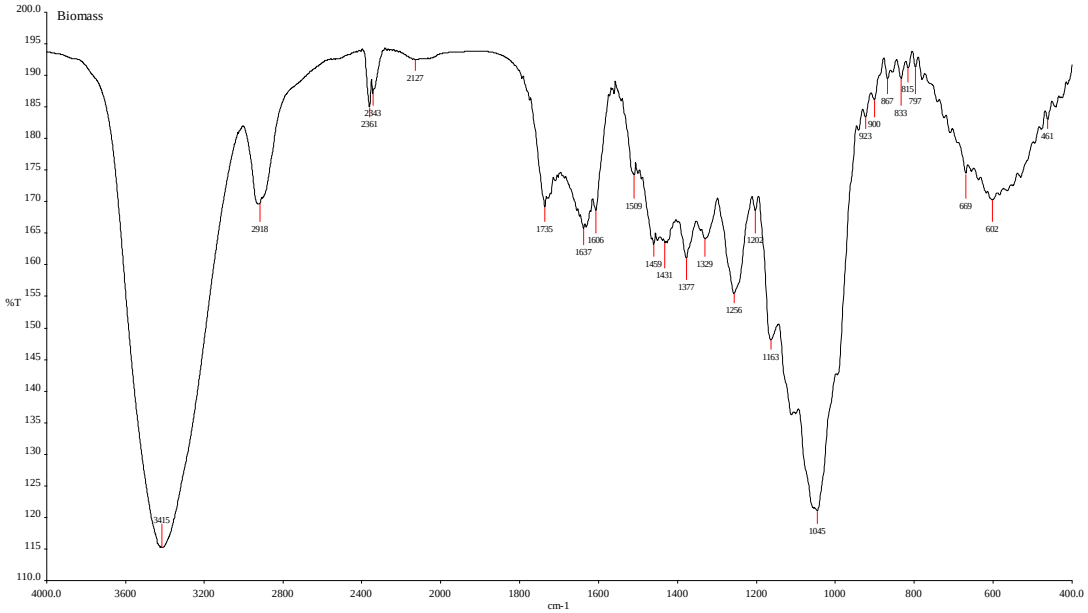

Figure 4.1: FTIR Spectrum of Sugarcane Bagasse Biomass

Based on HPLC graph, area of each peak was calculated to estimate the total cellulose content in the biomass, which has been broken down into sugar molecules via acid hydrolysis treatment. From Figure 4.2, the peak at residence time of 7.354 min shows the presence of water molecules while the peaks at residence time of 9.903 min and 10.536 min shows the presence of sugar (Ball and Bullock, 2011). By comparing with standards of four different sugars, namely xylose, mannose, dextrose and arabinose, the sugars present in biomass are dextrose and xylose. The graphs of HPLC against concentration was plotted as seen in Appendix A for further calculation using the calibration equation. From the calculation, the area obtained is used to calculate amount of dextrose present at peak 9.903 min, which is approximately 0.0836 g of dextrose. This calculation is done based on dextrose calibration standard. Besides, there is another peak at retention time of 10.536 min indicating the presence of xylose. It can be concluded that the SCB biomass consists of dextrose and xylose. The sample calculation of carbohydrates content in biomass

is shown in Appendix B. The total amount of sugars calculated in 0.3 g of SCB biomass is approximately 0.1352 g, which is about 45 % w/w of cellulose content. According to Dondi and co-workers (2008), the peak of cellubiose and water molecules overlapped at residence time of 7 min. Therefore, the cellulose content in the biomass should be higher.

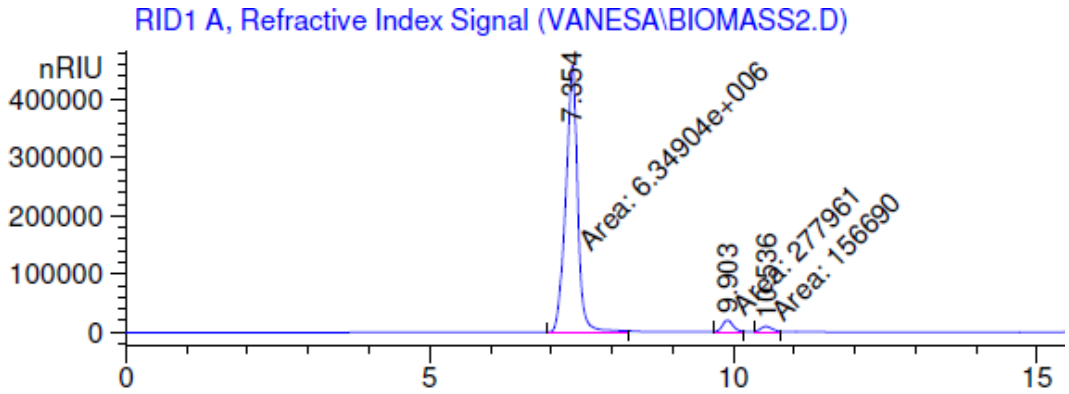

Figure 4.2: HPLC Analysis of Sugarcane Bagasse Biomass

The thermal properties of biomass can be predicted from the graphs obtained from DSC and TGA testing. Figure 4.3 shows the DSC result, an endothermic peak of biomass detected has an integral area of approximately 1195.03 mJ. In DSC curves, the integral area represents the crystallinity of the sample. The larger the area of integral shows that the sample has lower crystallinity (Bertran and Dale, 1986). The SCB biomass has an enthalpy of 170.72 Jg^{-1}. The higher the value of enthalpy obtained, more amount of energy is required to break the bonds of the structure (Bertran and Dale, 1986).

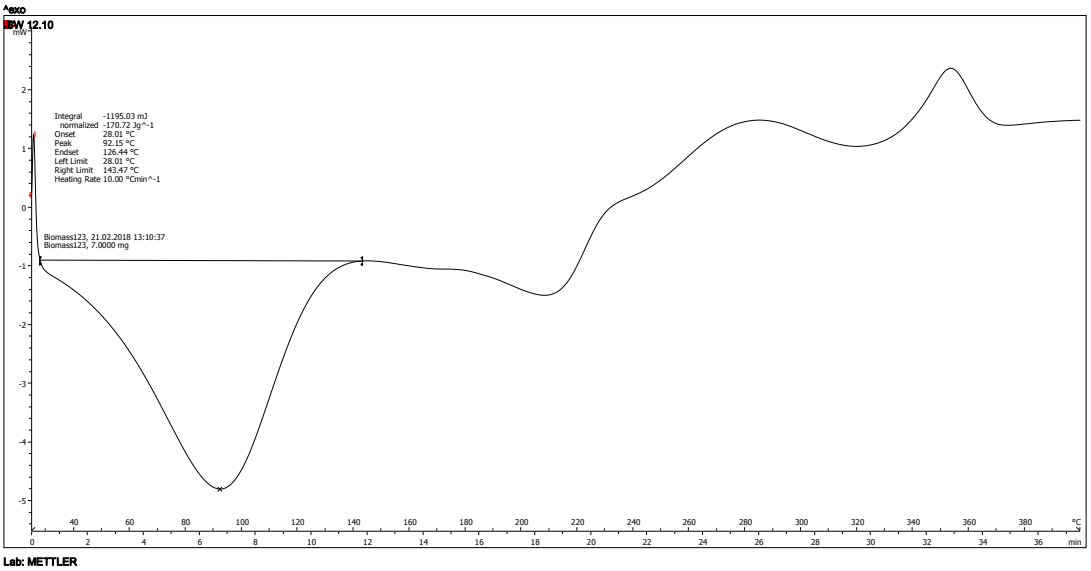

Figure 4.3: DSC Thermograph of Sugarcane Bagasse Biomass

In the TGA graph as shown in Figure 4.4, thermal stability of SCB biomass can be predicted in the presence of nitrogen gas. When temperature start to increase from room temperature to 100 ℃, the vaporisation of water occurs in the lignocellulose fibres. The removal of water causes the reduction in weight (Watkins et al., 2015). As temperature further increases, a sharper weight drop is observed. In lignocellulosic materials, the decomposition temperature of pectin, lignin and hemi-cellulose is much lower compared to cellulose (Khawas and Deka, 2016). At approximately 20 min, sample slowly reaches its thermal stability at range from 350 ℃ – 460 ℃. This reveals the removal of lignocellulosic materials in SCB. The residence time shows the duration it can tolerate as temperature increases before the sample fully decompose.

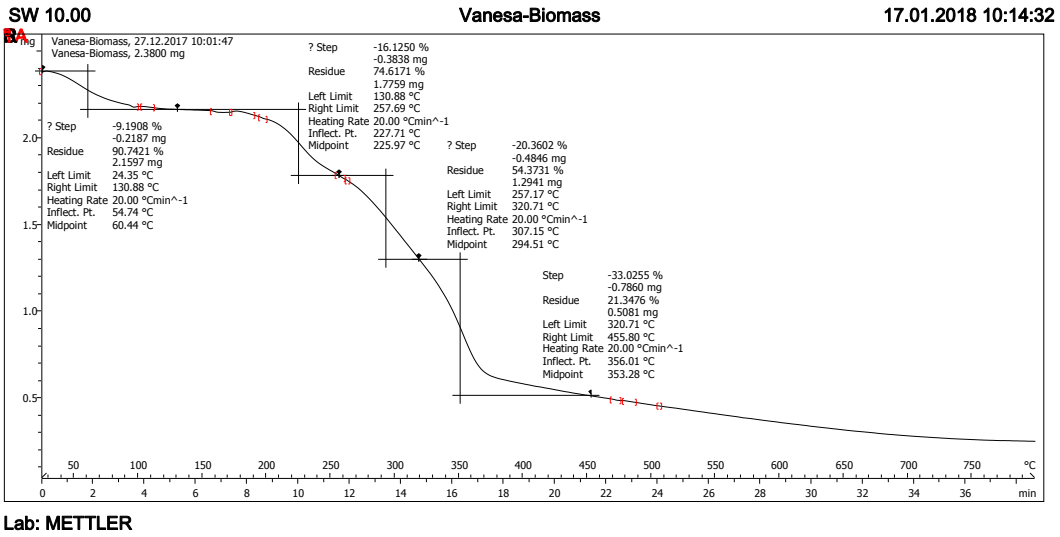

Figure 4.4: TGA Thermograph of Sugarcane Bagasse Biomass

4.2 The Content of Treated SCB under Ultrasonic-assisted Sodium Hydroxide Treatment

The treatment process of SCB biomass was conducted to allow the penetration of alkaline solution to extract cellulose. The removal of pectin, lignin and hemi-cellulose are necessary by dissolution with sodium hydroxide solution and it is assisted with the use of an Elma S 180 (H) ultrasonic water bath. This alkali treatment and ultrasonication process can cause collapsing of cell structure and lignocellulosic materials, allowing cellulose to be extracted (Wang et al., 2005). Ultrasonic treatment is to induce micro-streaming effect and also to increases the frequent interactions of sample and solvent by the cavitational bubble collapse (Santos et al., 2009). This can result in the destruction of cell wall, lignin, and hemi-cellulose in the SCB biomass. In addition, this treatment could improve the extraction of cellulose at lower temperatures (Bhat, 2011). It is then followed by a series of washing and filtering steps with distilled water and hot ethanol to remove soluble impurities as shown in Figure 4.5. The residue decolourised as it removes the green pigments of plants and further removes lignin to obtain a higher purity of cellulose.

Figure 4.5: Treated SCB Before and After Washing with Distilled Water and Hot Ethanol

The effect of the treatment parameters included temperature, concentration of NaOH solution and duration. Each parameter varies on three different values to further analyse and calculate the yield of cellulose obtained. From the calculation using the Equation 3.1, alkalization and ultrasonic treatment process recovered approximately 39.0 – 47.0 % (w/w) of the treated SCB. Table 4.1 shows the yield of treated SCB from each experiment at different parameters. When treatment parameters were varied, the yield of treated SCB were studied. It shows that the treatment duration affects the most in this experiment.

Table 4.1: Yield of Treated SCB

Expt No.	**Mass of biomass before treatment (g)**	**Mass of biomass after treatment (g)**	**Yield of treated SCB (%)**
1	5.0021	2.0831	41.64
2	5.0011	2.1124	42.24
3	5.0034	2.0143	40.26
4	5.0085	2.1178	42.28
5	5.0040	1.9939	39.85
6	5.0050	2.0480	40.92
7	5.0030	2.0160	40.28
8	5.0040	2.3618	47.19
9	5.0025	2.3400	46.78

4.3 Effect of Treatment Conditions on SCB

4.3.1 Effect of Treatment Temperature on Total Cellulose Produced

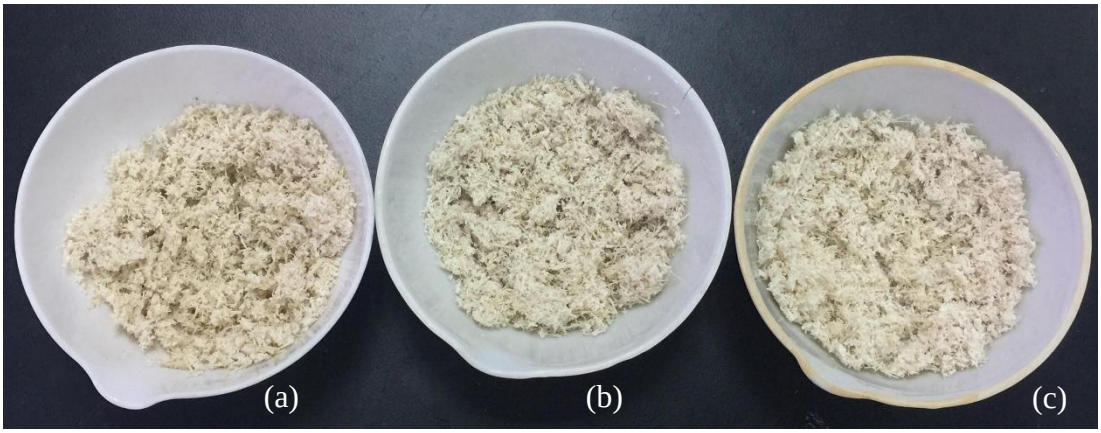

Figure 4.6: Appearance of Treated SCB after Treatment at Various Temperature of (a) 60 °C (b) 70 °C (c) 80 °C

The treatment with ultrasonic hot water bath has known to be one of the effective methods in extraction of cellulose. Figure 4.6 shows that the appearance of the treated SCB have not much of a difference under investigated temperature. However, the biomass which has undergo treatment at 60 °C, has a light brown colour while when temperature increases to 70 °C and 80 °C, it decolorizes to pale yellow and white. This could be due to delignification, where pigments on the biomass was removed. By comparing the three FTIR graphs in Figure 4.7, the presence of components in the treated SCB are similar. The differences between the SCB biomass and treated SCB were compared and presented in Table 4.2. Table 4.2 shows the absorption peaks of SCB and treated SCB and differences were found at wavelengths of 1163, 1256, 1431, 1509 and 1735 cm^{-1} which is not observed in the treated SCB. The absence of these absorption bands indicate that most lignin has been removed (Singh and Singh, 2012).

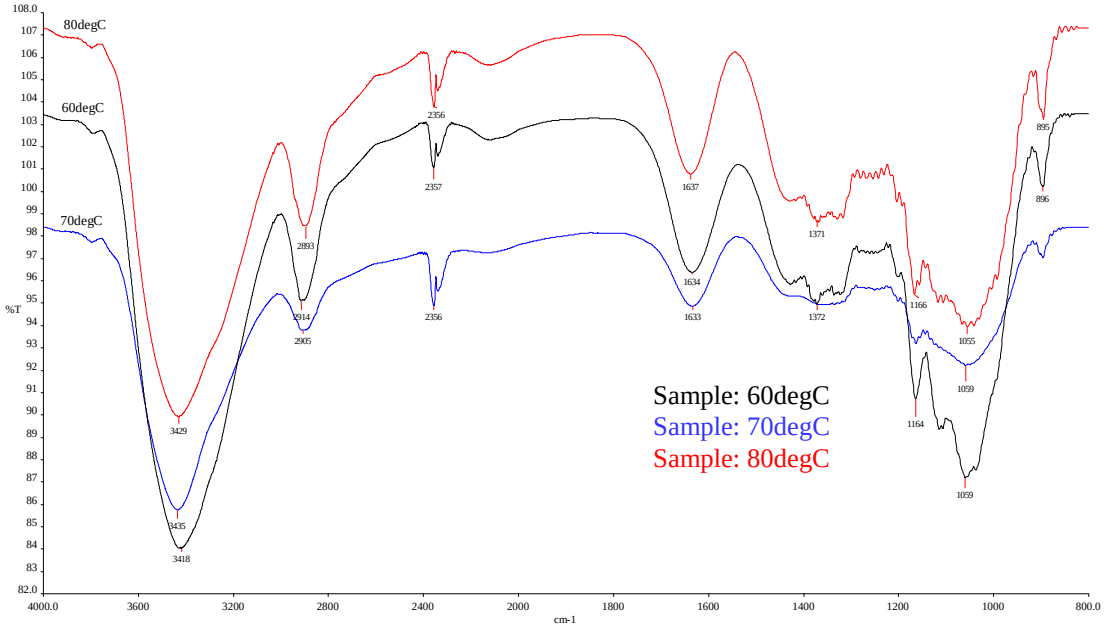

Figure 4.7: FTIR Spectra of Treated SCB after Treatment at Various Temperatures

Table 4.2: Assignment of Main Absorption Bands in SCB Biomass and Treated SCB (Singh and Singh, 2012)

Wave number (cm^{-1})		Assignment
SCB biomass	Treated SCB	
3415	3418	-OH stretching
2918	2905	-CH stretching of CH_2 and CH_3 groups
2361	2356	O=C=O carbon dioxide streching
1735	-	C=O stretching of acetyl or carboxylic acids
1637	1633	Carbonyl stretching with aromatic ring
1509	-	C=C stretching of aromatic ring (lignin)
1431	-	CH_2 bending
1377	1372	C-H deformation
1256	-	C-O stretching of ether linkage
1163	-	C-O-C antisymmetric bridge stretching
1045	1059	C-O symmetric stretching of primary alcohol
900	901	Glycosidic linkages between sugar units

To compare and analyse effect of temperature on SCB, HPLC analysis was used to calculate the cellulose content. From the Appendix C, D and E, the calculation has shown that at 60 °C, 70 °C and 80 °C, the estimated cellulose content are 0.4719 g, 0.5229 g and 0.4680 g respectively as shown in the Table 4.3 below. At 70 ℃, the estimated cellulose content is the highest. The yield of treated SCB increases as the treatment temperature increases from 60 °C to 70 °C. As temperature increases, kinetic energy in the solution increases. This results in the collisions between alkaline solution and the biomass to be more frequent, increasing the surface area of reaction. However, when treatment was carried out at 80 °C the estimated cellulose content decreases. This happens because some of the structure of cellulose may be destroyed or ruptured. The treatment conditions do not only remove lignin and hemicellulose but could remove cellulose as well (Bhat, 2011).

Table 4.3: Yield of Estimated Cellulose at Various Treatment Temperature Based on HPLC Analysis

Temperature (°C)	Total Carbohydrates (wt %)	Mass of estimated cellulose content (g/g)
60	22.34	0.4719
70	25.96	0.5229
80	22.47	0.4680

Figure 4.8 shows the endothermic peaks of treated SCB under various treatment temperature. Smallest area of integral have been found in sample treated at 70 ℃. The area of integral has a value of 1694.74 mJ. This shows that it has the highest degree of crystallinity where the amount of cellulose present is the most. The accessibility of water relates to the amorphous fraction. So, when endotherm is detected between 110 ℃ and 160 ℃, it is due to the loss of absorbed water (Bertran and Dale, 1986). Thus, the enthalpy change from the integral area of this endothermic peak could be belong to the heat of dehydration. To estimate the degree

of crystallinity, the heat of dehydration can relate to obtain fraction of amorphous cellulose in lignocellulosic biomass (Chan et al., 2012).

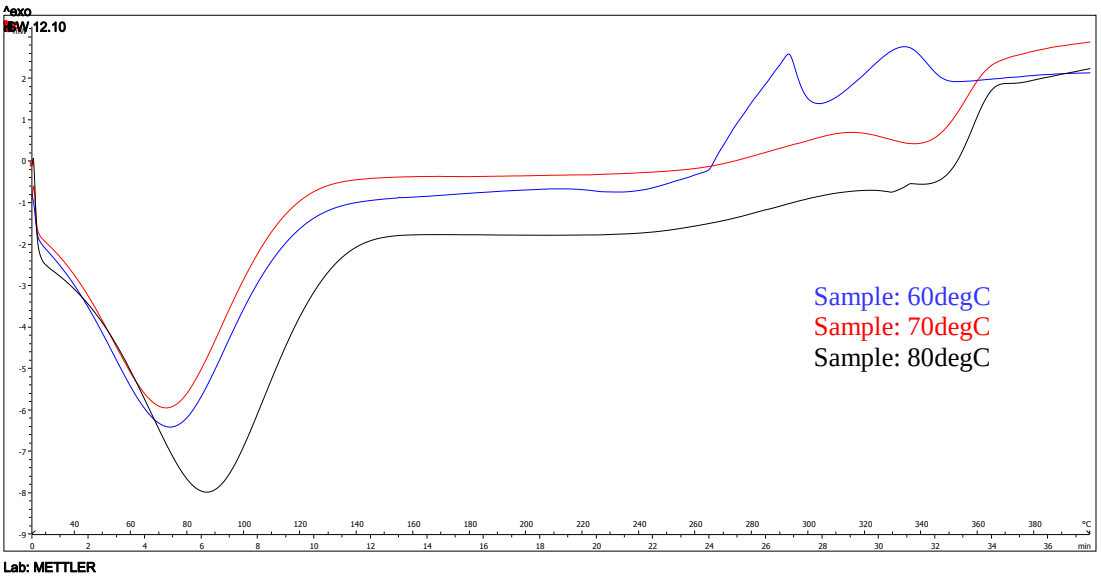

Figure 4.8:DSC Thermograph of Treated SCB after Treated at Various Temperatures

In Figure 4.9, the thermal stability of sample at 70 ℃ and 80 ℃ are similar where the curves mostly stabilize at residence time of approximately 15 min. While at lower temperature of 60 ℃, the sample only has the capability to tolerate increasing temperatures up to 14 min before it fully decompose. Thus, the thermal stability of treated SCB is more stable when treated at higher temperature. Based on the characterization results, the most effective temperature has been compared and presented in Table 4.4.

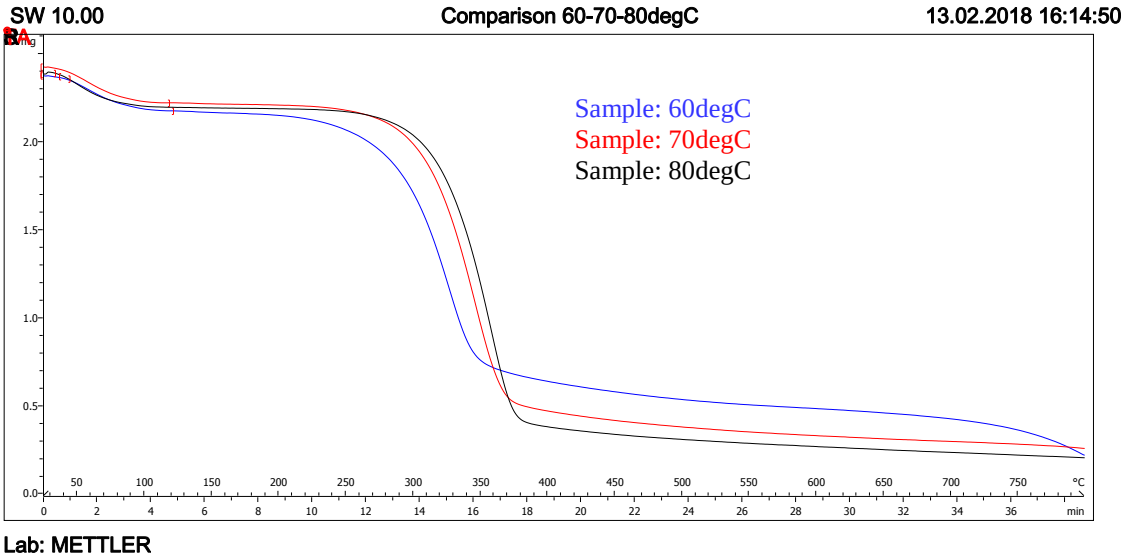

Figure 4.9: TGA Thermograph of Treated SCB after Treatment at Various Temperatures

The selected sample has higher crystallinity, better thermal stability and the estimated cellulose content is higher among the samples compared. Therefore, the temperature of 70 ℃ is most effective and it was selected as optimum condition to produce cellulose for CMC production as shown in Table 4.4.

Table 4.4: Comparison of Treatment Effectiveness under Various Treatment Temperatures

Temperature (°C)	Thermal stability (TGA)	Crystallinity (DSC)	Estimated cellulose content (HPLC)	Selected
60		✓		
70	✓	✓	✓	✓
80	✓			

4.3.2 Effect of Sodium Hydroxide Concentration on Total Cellulose Produced

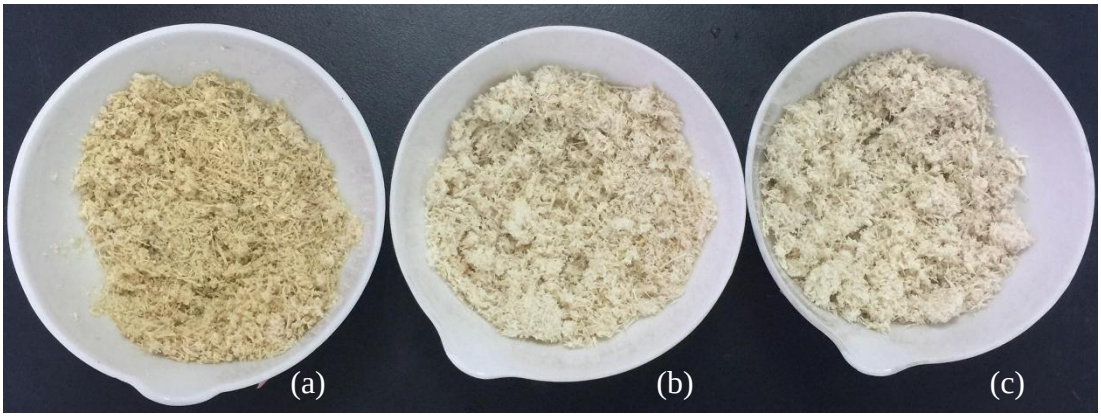

Figure 4.10: Appearance of Treated SCB after Treatment at Various NaOH Concentrations of (a) 0.5 M (b) 0.75 M (c) 1 M

Figure 4.10 shows obvious colour difference between the three samples treated at various sodium hydroxide concentration. It was observed that increasing NaOH concentration could cause more decolourization to the biomass sample. Figure 4.11 shows that the adsorption bands in FTIR result are similar. However, it consists of functional group as explained in Section 4.3.1. It is difficult to analyse the differences of the samples therefore yield of estimated cellulose content is compared.

Based on the HPLC analysis as stated in Appendix F, G and H, the integral area of the peaks were calculated for cellulose content at 0.5 M, 0.75 M and 1 M sodium hydroxide concentrations. The respective values are 0.5716 g, 0.4251 g and 0.3555 g as shown in Table 4.5. The highest mass of treated SCB was found for SCB treated with 0.5 M NaOH. At low concentration there might be incomplete removal of lignin and hemicellulose where solution could not penetrate through to obtain the cellulose present (Bhat, 2011). However at high concentration of NaOH, there may be excessive destruction of lignocellulosic materials where cellulose has also been degraded (Driemeier et al., 2011, Khawas and Deka, 2016).

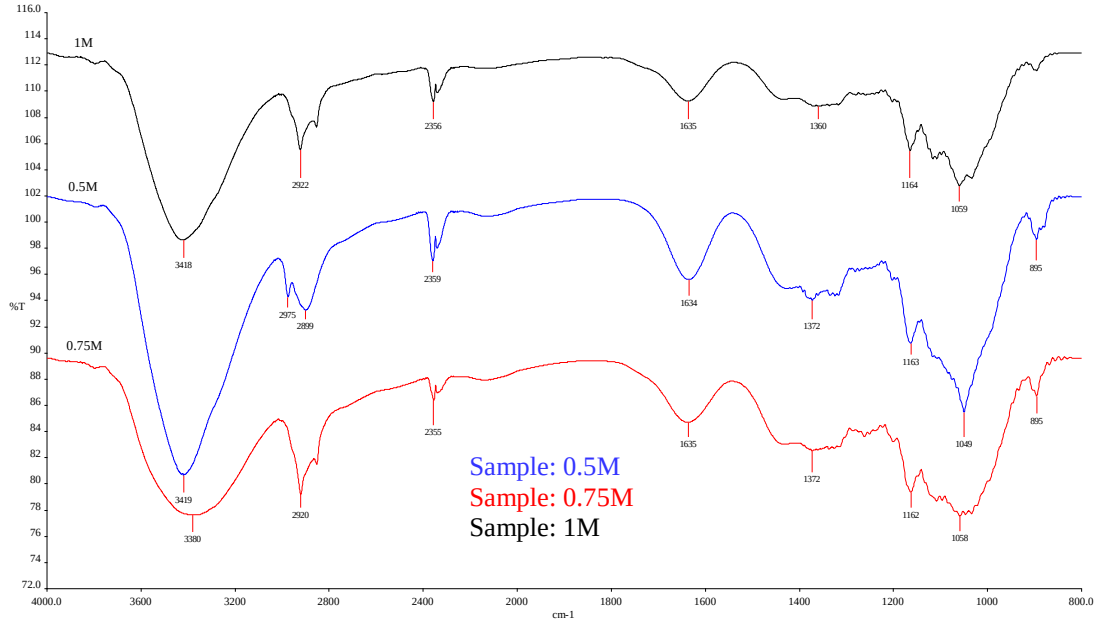

Figure 4.11: FTIR Spectra of Treated SCB after Treatment at Various Concentrations of NaOH

Table 4.5: Yield of Estimated Cellulose Content at Various Concentrations of Sodium Hydroxide from HPLC Analysis

Sodium hydroxide concentration (M)	Total carbohydrates (wt %)	Estimated cellulose content (g/g)
0.5	24.44	0.5716
0.75	21.32	0.4251
1.0	17.36	0.3555

Figure 4.12 shows the DSC results, demonstrates that the integral of 1 M sample has the largest area. This shows that it has the lowest degree of crystallinity compared to the others. On the other hand, SCB treated at 0.5 M and 0.75 M NaOH concentration have almost the same area of integral, indicated both concentration produced samples with higher degree of crystallinity. The reason could be due to the

presence of higher amount of crystalline region in cellulose structure compared to amorphous region (Watkins et al., 2015).

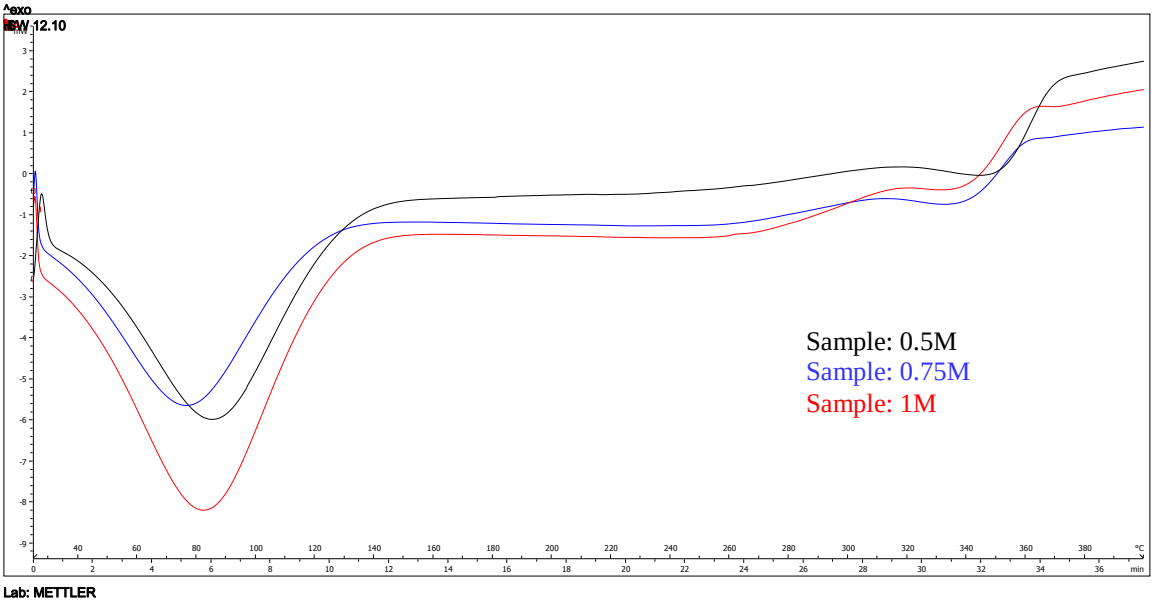

Figure 4.12: DSC Thermographs of Treated SCB after Treatment at Various Concentrations of NaOH

Meanwhile for TGA graphs shown in Figure 4.13, the 0.5 M and 1 M samples have better thermal stability which can tolerate heat longer and stabilise at residence time approximately 18 min. The result suggested that treated SCB requires more heat to break the bonds mostly where cellulose is present. The treated SCB sample treated at 0.75 M has lowest thermal stability. Thus, it requires less heat to break the bonds, indicating less cellulose present in the structure. The characterization results have been compared for treatment under various NaOH concentration. As the result, treatment at 0.5 M sodium hydroxide concentration is the most effective as shown in Table 4.6. The concentration was selected as one of the optimum condition to treat SCB for CMC production.

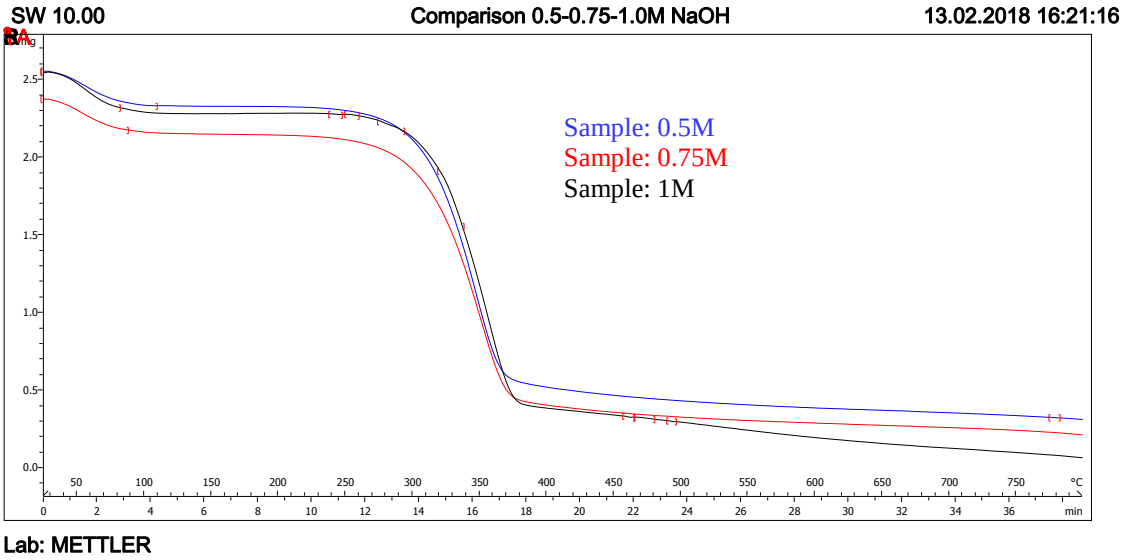

Figure 4.13: TGA Thermographs of Treated SCB after Treatment at Various Concentrations of NaOH

Table 4.6: Comparison of Treatment Effectiveness under Various Sodium Hydroxide Concentration

Sodium hydroxide concentration (M)	Thermal stability (TGA)	Crystallinity (DSC)	Estimated cellulose content (HPLC)	Selected
0.5	✓	✓	✓	✓
0.75				
1.0	✓	✓		

4.3.3 Effect of Duration on Total Cellulose Produced

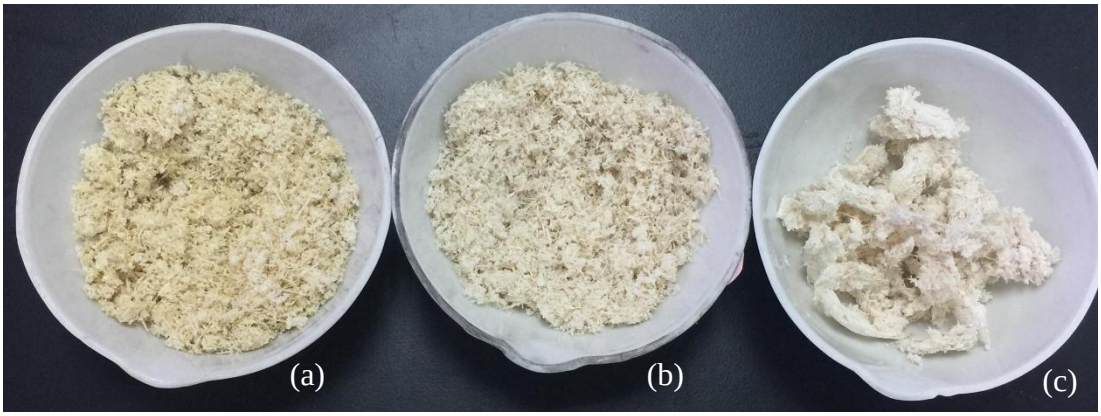

Figure 4.14: Appearance of Treated SCB after Treatment at Various Duration of (a) 1 hour (b) 2 hours (c) 3 hours

Figure 4.14 shows the appearance of treated SCB after undergoing various treatment duration. After the biomass has been treated for 1 hour, the biomass decolourises to light brown colour. As time increases to 2 hours, it has an even lighter colour of pale yellow. At 3 hours, the colour of treated SCB is completely white and the treated SCB tends to clot and stick together.

Table 4.7: Yield of Estimated Cellulose Content at Various Duration

Time (hr)	Total Carbohydrates (wt %)	Estimated cellulose content (g/g)
1	24.52	0.5738
2	35.13	0.8297
3	26.89	0.5421

In Table 4.7, the mass of estimated cellulose content at 2 hours has the highest value with about 0.3 g more than samples undergoing duration of 1 hour and 3 hours. This explains that duration of 2 hours is sufficient for the reaction to occur, removing most of the lignin and hemicellulose, to isolate cellulose completely.

However, the FTIR spectra of the three samples could not be compared much since they have similar absorption of wavelengths as shown in Figure 4.15.

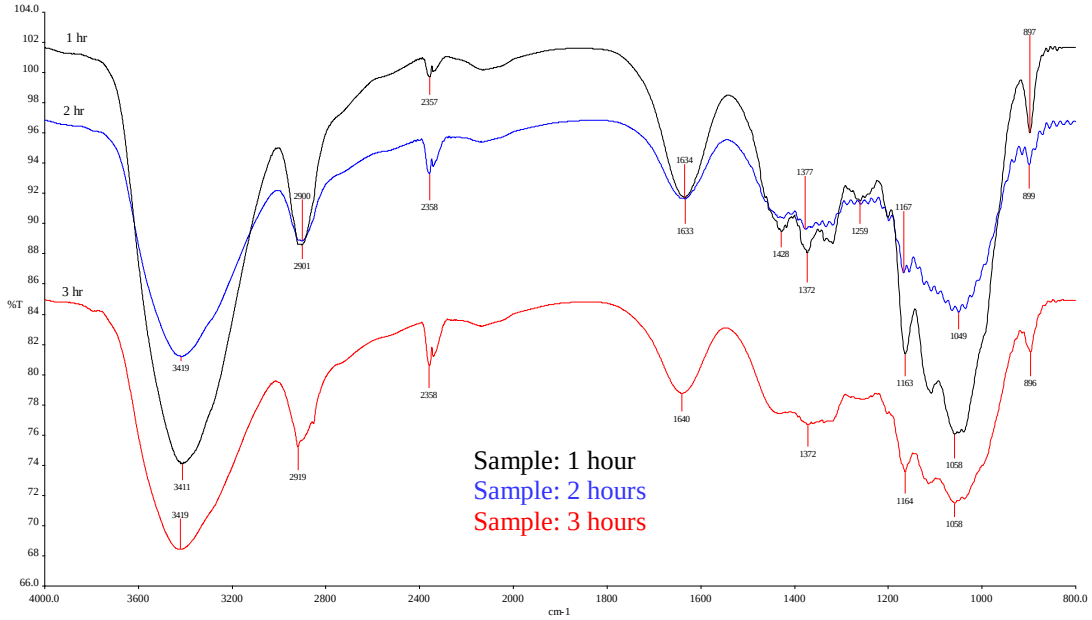

Figure 4.15: FTIR Spectra of Treated SCB after Treatment at Various Duration

The DSC thermograph in Figure 4.16 shows when duration increases, the area of integral increases. This indicates that the degree of crystallinity decreases when samples undergoing longer duration. When the duration is given 1 hour, it might not have sufficient for the solution to penetrate through the lignocellulosic structure to remove all the lignin and hemicellulose. The high crystallinity in this case may be due to the structure of lignin which has not been removed (Driemeier et al., 2011, Khawas and Deka, 2016). When too much time is given for the reaction to react, excessive solubilisation and degradation occurred. Therefore, the duration of 2 hours allows ample time for the alkali solution to penetrate through to remove lignin and hemicellulose and avoid solubilizing of cellulose.

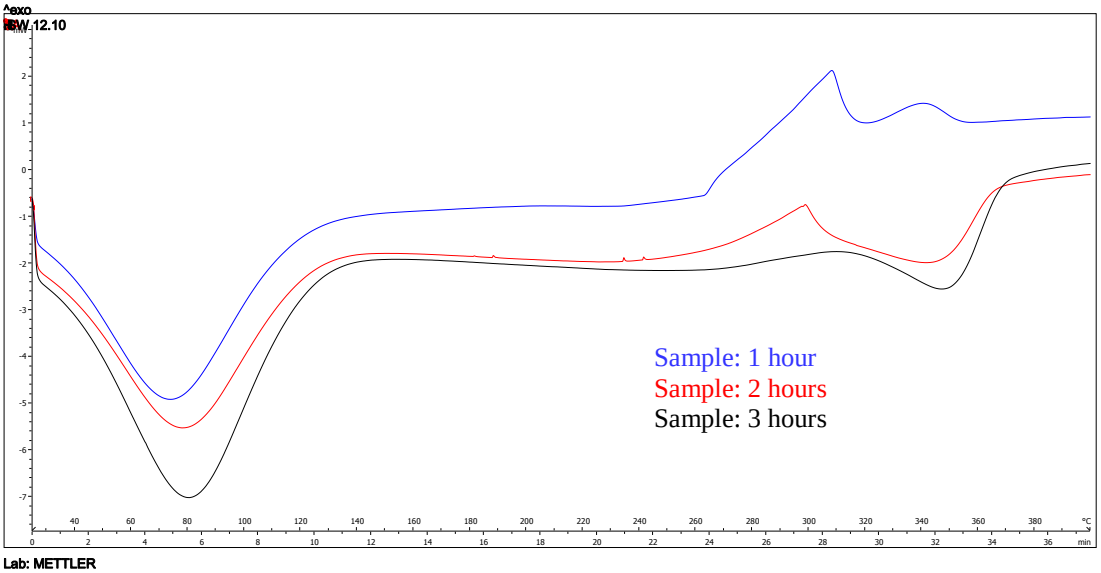

Figure 4.16: DSC Thermographs of Treated SCB after Treatment at Various Duration

For TGA graph in Figure 4.17, the sample that has been treated for an hour has the lowest thermal stability which stabilizes at residence time of 16 min. Biomass treated at 2 and 3 hours have better thermal stability which can tolerate high temperatues better and longer. Both samples stabilizes at residence of approximately 18 min. This indicates that they have better thermal stability and requires more heat to break the structure. This happens when structure is more crystalline where structures of cellulose is present. So, after comparing the results from the characterization tests, one of treatment duration has been selected as optimum condition to treat SCB for CMC production. Table 4.8 has presented the result and treatment duration of 2 hours has shown to be most effective.

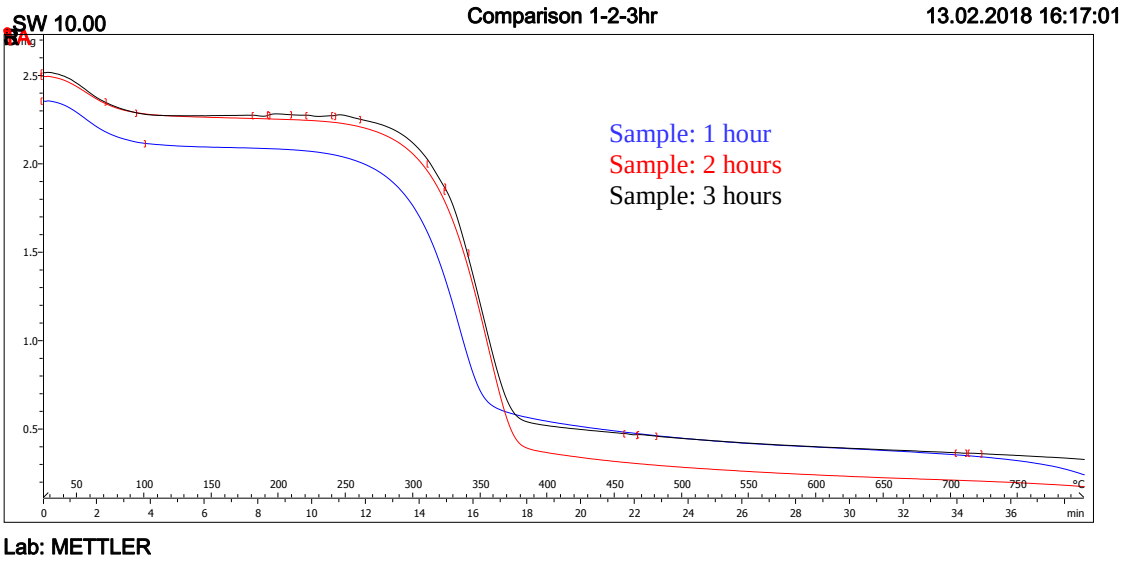

Figure 4.17: TGA Thermographs of Treated SCB after Treatment at Various Duration

Table 4.8: Comparison of Treatment Effectiveness under Various Treatment Duration

Time (hr)	TGA	DSC	HPLC	Selected
1		✓		
2	✓	✓	✓	✓
3	✓			

4.4 Comparison of Cellulose Extraction with Commercialised Process

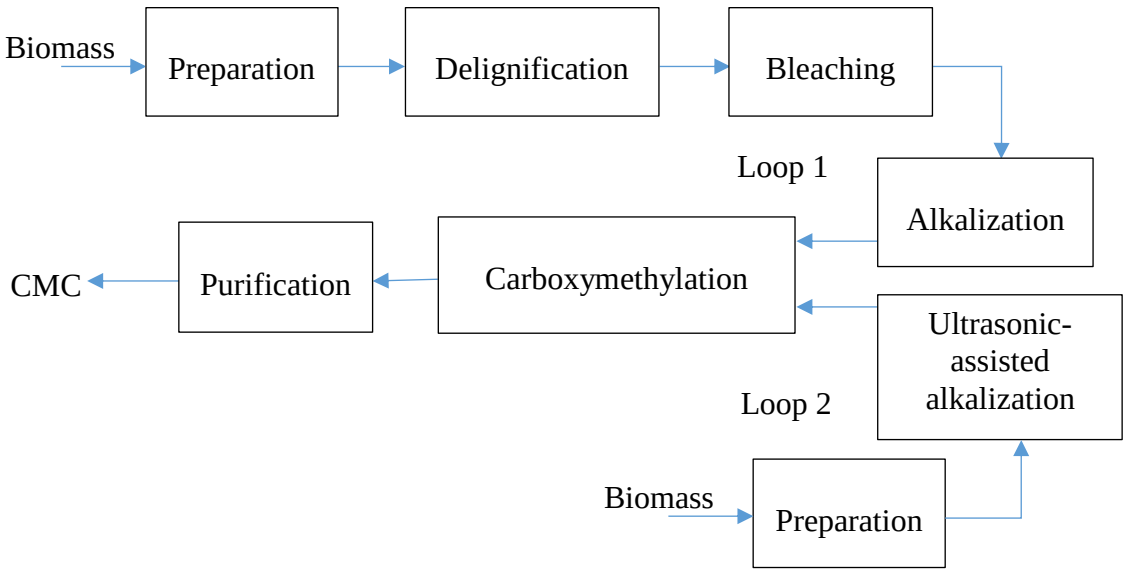

Figure 4.18: The Process Flow Diagram of Commercialized Process (Loop 1) (Hasan and Sauodi, 2014) and Modified Process (Loop 2) (Liu et al., 2006)

Generally, the process of normal commercialized extraction procedures until today has a similar process flow in loop 1 as shown in Figure 4.18 above. The plant biomass is crushed and grinded into powder form. Then delignification takes place to remove lignin. An additional step may be taken into account that is bleaching to remove pectin which is the pigment on lignin and further ensuring lignin is removed completely. Then alkalization treatment takes place to further remove of hemicellulose, isolating cellulose behind. When cellulose is extracted, it undergoes carboxymethylation and purification to produce carboxymethylation.

In this study of research, the process have been modified by the addition of mechanical treatment during the alkali treatment process. A combination of alkali hydrolysis and use of ultrasonicator could improve the extraction of cellulose in many ways. By using ultrasound extraction, it has brought extraction of cellulose to a higher level of enhancement and kinetics (Khawas and Deka, 2016). The ultrasound from the sonicator produces cavitational bubble collapse to enhance mass transfer to allow more frequent interactions between the biomass and solution. This

results in cell wall destruction where solution could penetrate into the structure eventually obtaining better yield (Bhat, 2011).

Besides that, this method of treatment is simple, reliable and inexpensive as it also reduces processing time, chemicals usage and energy consumption. Furthermore, treatment with sonicator has also improve the solution extractability at lower temperatures (Alizadeh, 2017).

4.5 Carboxymethylcellulose Characterization

Figure 4.19 shows the chemical changes in the polymer structure of treated SCB when carboxymethylcellulose has been synthesize from the treated SCB. At some absorption wavelengths, both SCB biomass and carboxymethylcellulose show similar functional groups. The similar functional groups indicates hydroxyl groups at 3400 cm^{-1}, hydrocarbon groups at 1400 cm^{-1}, carbonyl groups and ether groups at 1000-1200 cm^{-1}. However, during carboxymethylation process of the etherification reaction with sodium monochloroacetate, the hydroxyl groups are substituted by CH_2-COONa. This results in the modification of functional groups changing some of the related absorption spectrum (Alizadeh, 2017). The FTIR graph observed in CMC does not have stretching bands at 1509, 1637 and 1735 cm^{-1} wavelengths, representing C=C of lignin, carbonyl stretching and C=O bonds respectively as compared to SCB biomass where lignin and hemicellulose are absent. At 1604 cm^{-1} absorption band, there is a formation of strong band. It indicates and confirms the presence of COONa group of CMC (Singh and Singh, 2012). These absorption bonds are confirmed to be the substitution of carboxymethyl groups in cellulose structure since it is absent in the FTIR spectra of cellulose from bagasse obtained in previous results. The comparison between the assignments of absorption bands are shown in Table 4.9.

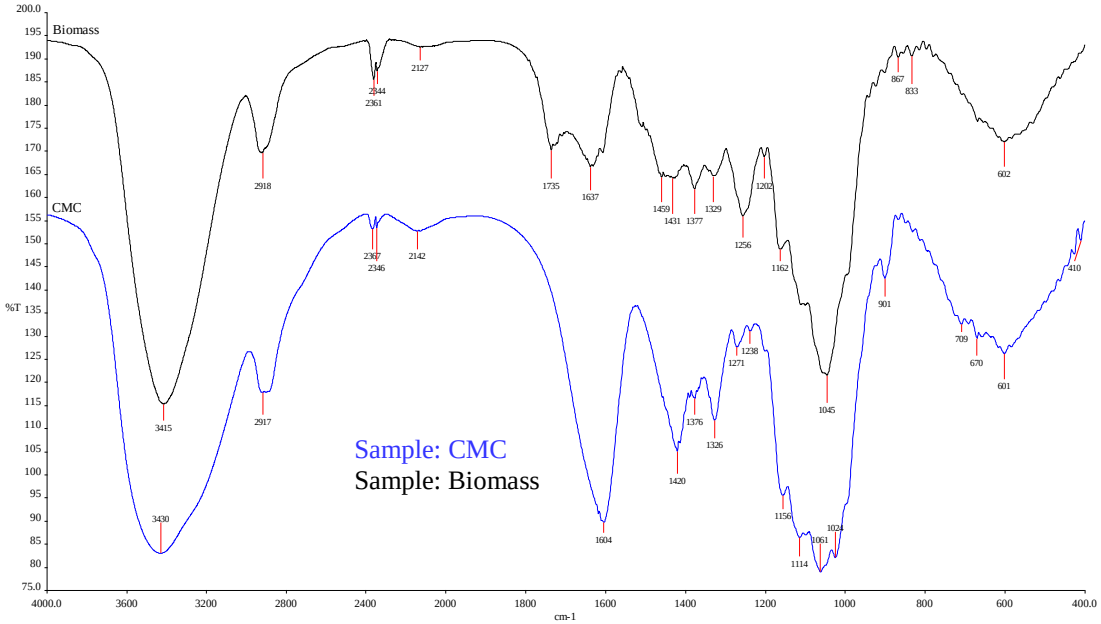

Figure 4.19: Comparison of FTIR Spectra of SCB Biomass and CMC

Table 4.9: Assignment of Main Absorption Bands in SCB Biomass and Carboxymethylcellulose (Singh and Singh, 2012)

Wave number (cm^{-1})		Assignment
SCB biomass	CMC	
3415	3430	-OH stretching
2918	2917	-CH stretching of CH_2 and CH_3 groups
1735	-	C=O stretching of acetyl or carboxylic acids
1637	-	Carbonyl stretching with aromatic ring
-	1604	-COONa group
1509	-	C=C stretching of aromatic ring (lignin)
1431	1420	CH_2 bending
1377	1376	C-H deformation
1256	1238	C-O stretching of ether linkage
1163	1156	C-O-C antisymmetric bridge stretching
1045	1061	C-O symmetric stretching of primary alcohol
900	901	Glycosidic linkages between sugar units

The DSC thermograph in Figure 4.20 compares crystallinity between biomass and CMC. The endothermic peak of the biomass has seen to be having a small area of integral and this indicates that it has high crystallinity due to structures of cellulose. It requires a lot of energy to break the bonds between them. Whereas for the endothermic peak of CMC, the area of integral is much larger after biomass has been pre-treated and processed into modified product. It shows low crystallinity of the product produced and requires only small amount of heat to break the bonds between them (Bertran and Dale, 1986). The crystallinity of biomass and CMC are compared as it will affect the properties of CMC which influences its end use application.

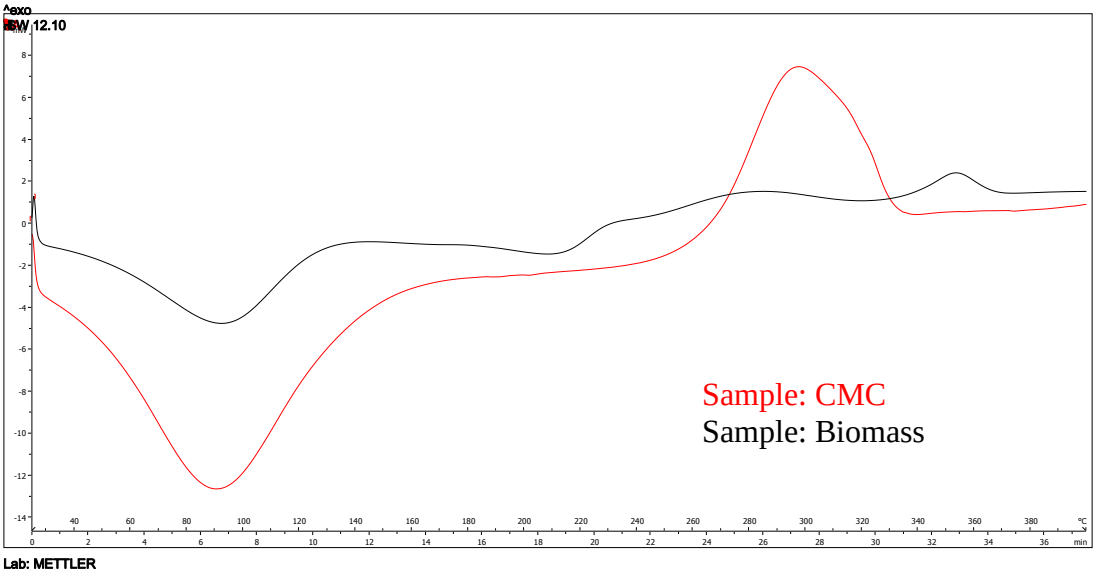

Figure 4.20: Comparison of DSC Thermograph of SCB Biomass and CMC

The degree of substitution of carboxyl group in CMC is determined from potentiometric titration. From the titration between 0.1 N NaOH and 0.1 N H_2SO_4, the average volume of acid used for CMC and blank titration are recorded (Koh, 2013). The degree of substitution calculated is the number of anhydroglucose unit present. In each anhydroglucose unit, there are 3 hydroxyl groups individually (Bono et al., 2009). Based on the analysis, CMC produced from SCB have a DS value of 0.59 with measured viscosity of 2.74 cP. The measured value is agreed well with other reported research using different natural resource (Bono et al., 2009, Shui et al., 2017). The value is quite low since the CMC synthesis process is not at its optimum condition.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, sugarcane biomass was successfully collected and prepared. The results from FTIR showed the presence of cellulose, lignin and hemicellulose in terms of functional groups. Quantitative analysis via HPLC indicated about 45.07 % w/w of cellulose content in collected SCB biomass. While DSC and TGA has shown the predicted crystallinity and thermal stability of the sugarcane.

The extraction of cellulose from sugarcane bagasse has been carried out successfully as well via ultrasonic-assisted alkaline treatment. After a series of experiments, the effect of treatment conditions on products have been studied by carrying various characterization test and the best parameters were selected. The parameters were 0.5 M NaOH at 70 ℃ for 2 hours with an overhead stirrer. Product produced under the treatment parameters have cellulose content about 0.8297 g with comparable thermal stability and degree of crystallinity. The absence of lignin and hemicellulose functional groups in FTIR result proved that both compositions have been removed successfully.

Treatment was carried out on SCB using the optimum parameters prior to CMC synthesis process. The treated SCB, which is the cellulose extracted has been

synthesized to carboxymethylcellulose. The produced carboxymethylcellulose have DS value of 0.59 and viscosity of 2.74 cP. Therefore, the CMC produced is soluble in water to form solution which is not observed for extracted cellulose. Solubility is one of the main properties that should possess by CMC for its application in industry. Carboxymethylcellulose is in high demand today as it is one of most widely used additive in various industries. Thus, in this research, the treatment method of sugarcane bagasse has been modified by adding an additional mechanical treatment which is ultrasonic process. The method helps to reduce the usage of chemicals, lowering the temperature of treatment and improve the collisions for better reaction. This study is vital to overcome environmental problems of large amount of sugarcane bagasse agricultural waste all over the world. This production process could be commercialised as it is an economical way to make use of sugarcane bagasse.

5.2 Recommendation

Sugarcane bagasse is a potential feedstock to produce carboxymethylcellulose due to its high percentage of cellulose in the biomass. Therefore, the study on effective CMC production from sugarcane bagasse is needed. Sugarcane bagasse only requires the extraction of juice unlike palm oil which requires complex processes of oil extraction and complex ginning process of cotton linter. The possible side products formed are also considered to avoid environmental pollution and tedious purification processes. Thus, the mass scale production of cellulose from sugarcane bagasse is a cost effective way to produce CMC.

In addition, the optimum conditions for treating SCB to extract cellulose are essential for cost effective production. Further research and studies can be done on the effect of frequency in ultrasonic water bath to reach its optimum condition including other adjustable environment conditions such as power input and stirring speed. Besides, weak alkali can also be done to test on the effect of cellulose extraction.

Furthermore, characterization methods such as gel-permeation chromatography (GPC) and x-ray powder diffraction (XRD) can be further studied to have better understanding on the properties of the samples. This can improve the accuracy on selecting the optimum parameters. GPC is essential to obtain the molecular weight information of CMC, which is important to determine its strength as binder and thickener. While XRD is use to study the composition, structure and physical properties of the sample to obtain the value of product crystallinity. To sum up, the production of carboxymethylcellulose is important in various industries today, therefore, further research can be done to establish an even more optimum and cost-effective carboxymethylcellulose production from sugarcane bagasse biomass.

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APPENDICES

APPENDIX A: HPLC Analysis Calibration Curve for Simple Sugars

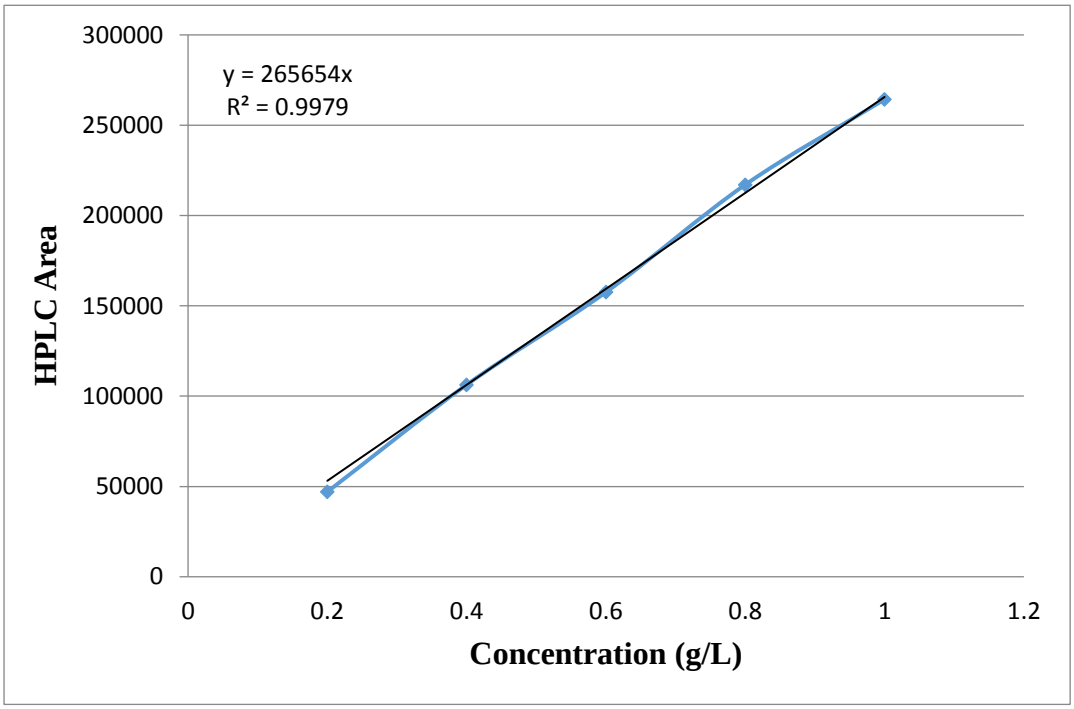

Figure A.1: HPLC Area vs Concentration of Arabinose

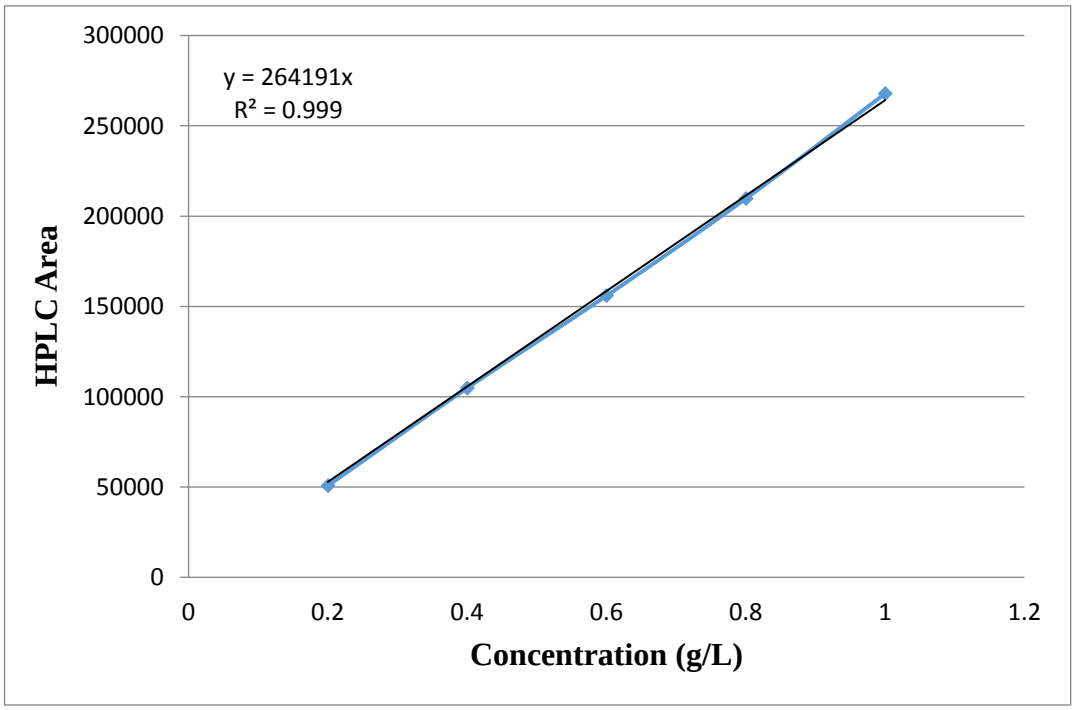

Figure A.2: HPLC Area vs Concentration of Xylose

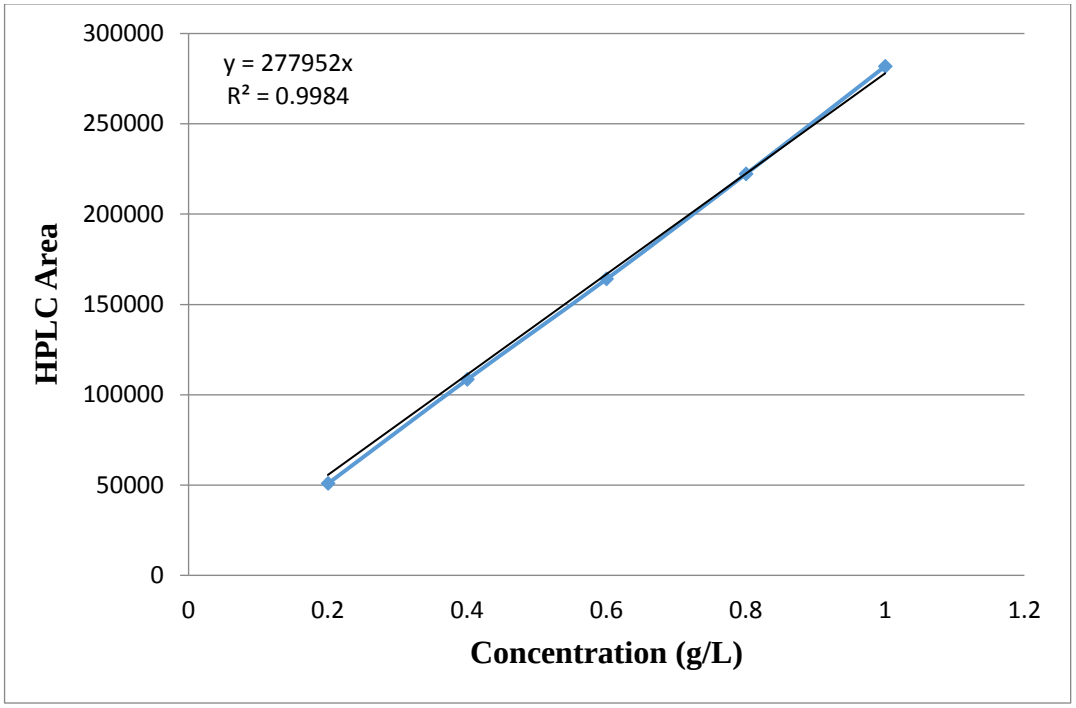

Figure A.3: HPLC Area vs Concentration of Mannose

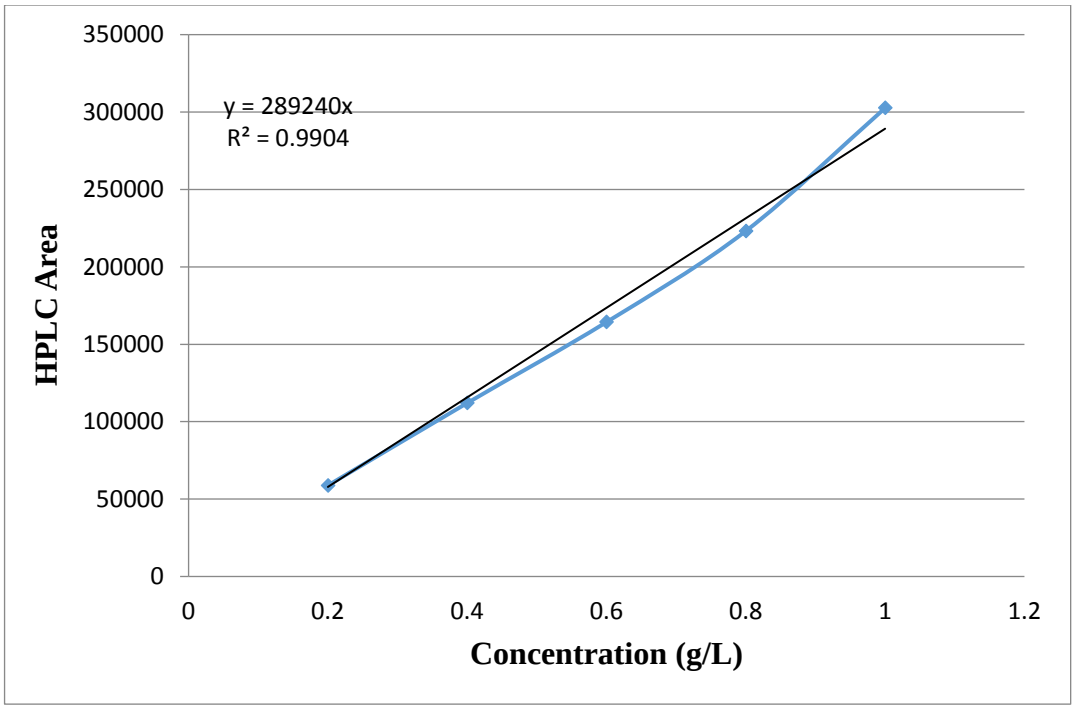

Figure A.4: HPLC Area vs Concentration of Dextrose

APPENDIX B: Sample Calculation of Total Carbohydrates Content in Sample of Biomass

Biomass sample weight = 0.3 g

(I) From calibration curve of HPLC area vs concentration of dextrose:

y = 289240x

HPLC area obtained from sample biomass = 277961

277961 = 289240x

x = 0.9610 g/L

Dextrose content = concentration x total volume

= 0.9610 g/L x 0.087 L

= 0.0836 g

(II) From calibration curve of HPLC area vs concentration of xylose:

y = 264191x

HPLC area obtained from sample biomass = 156690

156690 = 264191x

x = 0.5931 g/L

Xylose content = concentration x total volume

= 0.5931 g/L x 0.087 L

= 0.0516 g

(III) Total carbohydrates content in weight percentage

= [0.0836 g + 0.0516 g] / 0.3g x 100%

=45.07 wt %

(IV) Mass of estimated cellulose content

= 45.07 wt % x 0.3 g

= 0.1352 g of cellulose

APPENDIX C: Sample Calculation of Total Carbohydrates Content in Sample of 60°C

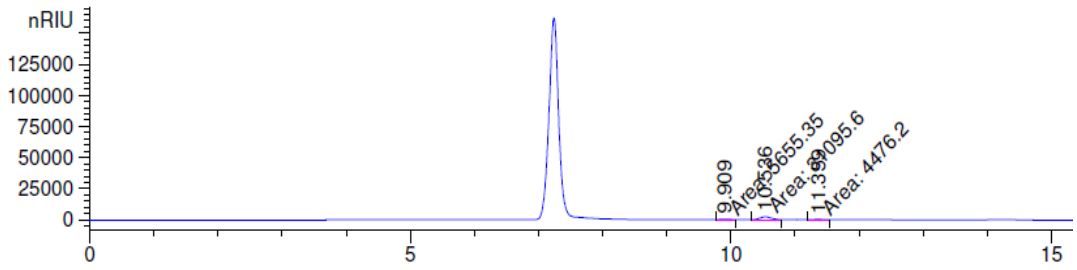

Figure C.1: HPLC Analysis of Treated SCB at 60 °C

Biomass sample weight = 0.3 g

(I) From calibration curve of HPLC area vs concentration of dextrose:

y = 289240x

HPLC area obtained from sample 60 °C = 5655

5655 = 289240x

x = 0.0196 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0196 g/L][5 mL]

M1 = 0.098 g/L

Dextrose content = concentration x total volume

= 0.098 g/L x 0.087 L

= 8.526 x 10-3 g

(II) From calibration curve of HPLC area vs concentration of xylose:

y = 264191x

HPLC area obtained from sample 60°C = 31096

31096 = 264191x

x = 0.1177 g/L

M1V1 = M2V2

M1 [1 mL] = [0.1177 g/L] [5 mL]

M1 = 0.5885 g/L

Xylose content = concentration x total volume

= 0.5885 g/L x 0.087 L

= 0.0512 g

(III) From calibration curve of HPLC area vs concentration of arabinose:

y = 265654x

HPLC area obtained from sample 60°C = 4476

4476 = 265654x

x = 0.0168 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0168 g/L] [5 mL]

M1 = 0.084 g/L

Arabinose content = concentration x total volume

= 0.084 g/L x 0.087 L

= 7.308 x 10-3 g

(IV) Total carbohydrates content in weight percentage

= [8.526 x 10-3 g + 0.0512 g + 7.308 x 10-3 g] / 0.3g x 100%

=22.34 wt %

(V) Mass of estimated cellulose content

= 22.34 wt % x 2.1124 g

= 0.4719 g of cellulose

APPENDIX D: Sample Calculation of Total Carbohydrates Content in Sample of 70°C

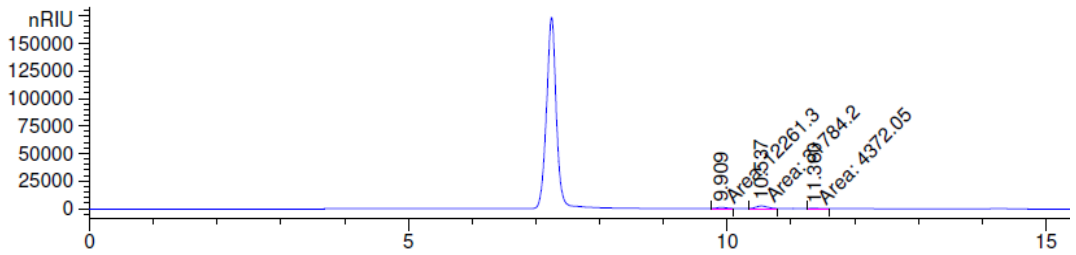

Figure D.1: HPLC Analysis of Treated SCB at 70 °C

Biomass sample weight = 0.3 g

(I) From calibration curve of HPLC area vs concentration of dextrose:

y = 289240x

HPLC area obtained from sample 80°C = 12261

12261 = 289240x

x = 0.0424 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0424 g/L] [5 mL]

M1 = 0.212 g/L

Dextrose content = concentration x total volume

= 0.212 g/L x 0.087 L

= 0.0184 g

(II) From calibration curve of HPLC area vs concentration of xylose:

y = 264191x

HPLC area obtained from sample 80°C = 31784

31784 = 264191x

x = 0.1203 g/L

M1V1 = M2V2

M1 [1 mL] = [0.1203 g/L] [5 mL]

M1 = 0.6015 g/L

Xylose content = concentration x total volume

= 0.6015 g/L x 0.087 L

= 0.0523 g

(III) From calibration curve of HPLC area vs concentration of arabinose:

y = 265654x

HPLC area obtained from sample 80°C = 4372

4372 = 265654x

x = 0.0165 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0165 g/L] [5 mL]

M1 = 0.0825 g/L

Arabinose content = concentration x total volume

= 0.0825 g/L x 0.087 L

= 7.1775 x 10-3 g

(IV) Total carbohydrates content in weight percentage

= [0.0184 g + 0.0523 g + 7.1775 x 10-3 g] / 0.3g x 100%

=25.96 wt %

(V) Mass of estimated cellulose content

= 25.96 wt % x 2.0143 g

= 0.5229 g of cellulose

APPENDIX E: Sample Calculation of Total Carbohydrates Content in Sample of 80 ℃

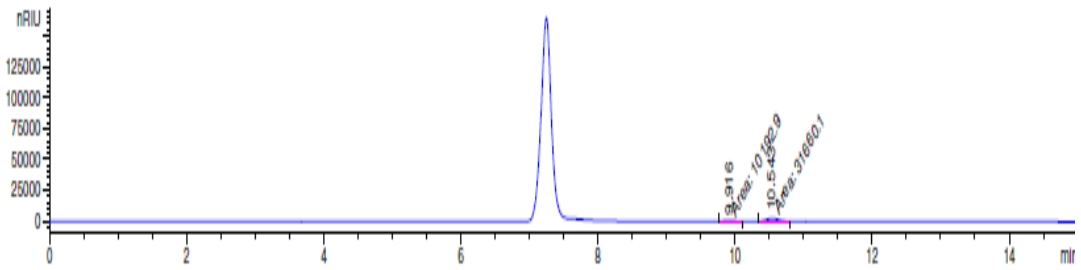

Figure E.1: HPLC Analysis of Treated SCB at 80 °C

Biomass sample weight = 0.3 g

(I) From calibration curve of HPLC area vs concentration of dextrose:

y = 289240x

HPLC area obtained from sample 70°C = 10193

10193 = 289240x

x = 0.0352 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0352 g/L][5 mL]

M1 = 0.176 g/L

Dextrose content = concentration x total volume

= 0.17 6g/L x 0.087 L

= 0.0153 g

(II) From calibration curve of HPLC area vs concentration of xylose:

y = 264191x

HPLC area obtained from sample 70°C = 31660

31660 = 264191x

x = 0.1198 g/L

M1V1 = M2V2

M1 [1 mL] = [0.1198 g/L] [5 mL]

M1 = 0.599 g/L

Xylose content = concentration x total volume

= 0.599 g/L x 0.087 L

= 0.0521 g

(III) Total carbohydrates content in weight percentage

= [0.0153 g + 0.0521 g] / 0.3g x 100%

=22.47 wt %

(IV) Mass of estimated cellulose content

= 22.47 wt % x 2.0831 g

= 0.4680 g of cellulose

APPENDIX F: Sample Calculation of Total Carbohydrates Content in Sample of 0.5 M

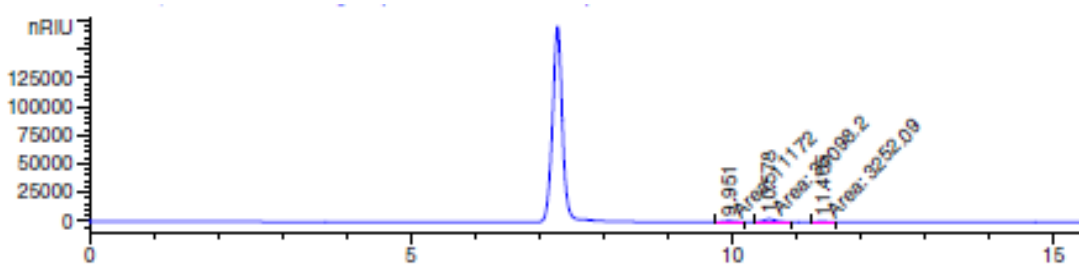

Figure F.1: HPLC Analysis of Treated SCB at 0.5 M

Biomass sample weight = 0.3 g

(I) From calibration curve of HPLC area vs concentration of dextrose:

y = 289240x

HPLC area obtained from sample 0.5M = 11172

11172 = 289240x

x = 0.0386 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0386 g/L] [5 mL]

M1 = 0.1931 g/L

Dextrose content = concentration x total volume

= 0.1931 g/L x 0.087 L

= 0.0168 g

(II) From calibration curve of HPLC area vs concentration of xylose:

y = 264191x

HPLC area obtained from sample 0.5M = 31098

31098 = 264191x

x = 0.1177 g/L

M1V1 = M2V2

M1 [1 mL] = [0.1177 g/L][5 mL]

M1 = 0.5885 g/L

Xylose content = concentration x total volume

= 0.5885g/L x 0.087 L

= 0.0512 g

(III) From calibration curve of HPLC area vs concentration of arabinose:

y = 265654x

HPLC area obtained from sample 0.5M = 3252

3252 = 265654x

x = 0.0122 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0122 g/L][5 mL]

M1 = 0.0610 g/L

Arabinose content = concentration x total volume

= 0.0610 g/L x 0.087 L

= 5.307 x 10-3 g

(IV) Total carbohydrates content in weight percentage

= [0.0168 g + 0.0512 g + 5.307x10-3 g] / 0.3g x 100%

= 24.44 wt %

(V) Mass of estimated cellulose content

= 24.44 wt % x 2.1178 g

= 0.5176 g of cellulose

APPENDIX G: Sample Calculation of Total Carbohydrates Content in Sample of 0.75 M

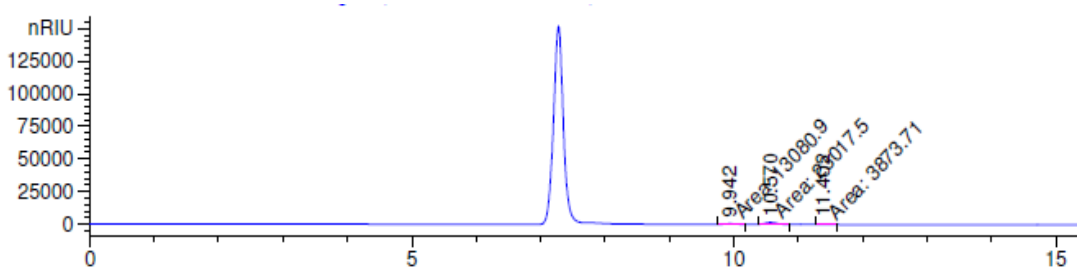

Figure G.1: HPLC Analysis of Treated SCB at 0.75 M

Biomass sample weight = 0.3 g

(I) From calibration curve of HPLC area vs concentration of dextrose:

y = 289240x

HPLC area obtained from sample 0.75M = 13081

13081 = 289240x

x = 0.0452 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0452 g/L][5 mL]

M1 = 0.2260 g/L

Dextrose content = concentration x total volume

= 0.2260 g/L x 0.087 L

= 0.0197 g

(II) From calibration curve of HPLC area vs concentration of xylose:

y = 264191x

HPLC area obtained from sample 0.75M = 23017

23017 = 264191x

x = 0.0871 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0871 g/L][5 mL]

M1 = 0.4355 g/L

Xylose content = concentration x total volume

= 0.4355 g/L x 0.087 L

= 0.0379 g

(III) From calibration curve of HPLC area vs concentration of arabinose:

y = 265654x

HPLC area obtained from sample 0.75M = 3874

3874 = 265654x

x = 0.0146 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0146 g/L] [5 mL]

M1 = 0.0730 g/L

Arabinose content = concentration x total volume

= 0.0730 g/L x 0.087 L

= 6.351 x 10-3 g

(IV) Total carbohydrates content in weight percentage

= [0.0197 g + 0.0379 g + 6.351 x 10-3 g] / 0.3g x 100%

= 21.32 wt %

(V) Mass of estimated cellulose content

= 21.32 wt % x 1.9939 g

= 0.4251 g of cellulose

APPENDIX H: Sample Calculation of Total Carbohydrates Content in Sample of 1 M

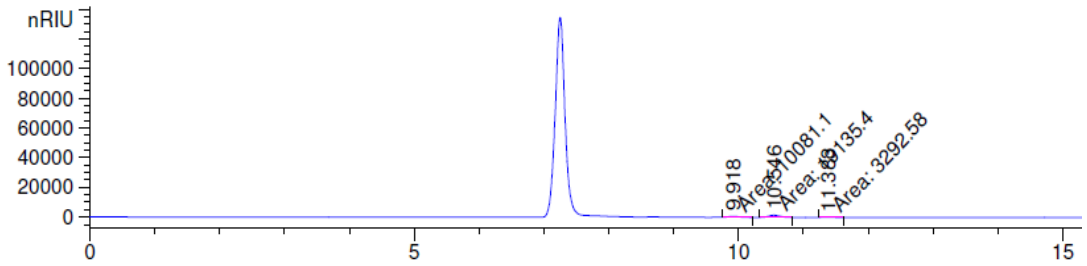

Figure H.1: HPLC Analysis of Treated SCB at 1 M

Biomass sample weight = 0.3 g

(I) From calibration curve of HPLC area vs concentration of dextrose:

y = 289240x

HPLC area obtained from sample 1M = 10081

10081 = 289240x

x = 0.0349 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0349 g/L][5 mL]

M1 = 0.1745 g/L

Dextrose content = concentration x total volume

= 0.1745 g/L x 0.087 L

= 0.0152 g

(II) From calibration curve of HPLC area vs concentration of xylose:

y = 264191x

HPLC area obtained from sample 1M = 19135

19135 = 264191x

x = 0.0724 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0724 g/L] [5 mL]

M1 = 0.3620 g/L

Xylose content = concentration x total volume

= 0.3620 g/L x 0.087 L

= 0.0315 g

(III) From calibration curve of HPLC area vs concentration of arabinose:

y = 265654x

HPLC area obtained from sample 1M = 3293

3293 = 265654x

x = 0.0124 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0124 g/L] [5 mL]

M1 = 0.062 g/L

Arabinose content = concentration x total volume

= 0.0620 g/L x 0.087 L

= 5.394 x 10-3 g

(IV) Total carbohydrates content in weight percentage

= [0.0152 g + 0.0315 g + 5.394 x 10-3 g] / 0.3g x 100%

= 17.36 wt %

(V) Mass of estimated cellulose content

= 17.36 wt % x 2.0480 g

= 0.3555 g of cellulose

APPENDIX I: Sample Calculation of Total Carbohydrates Content in Sample of 1 hour

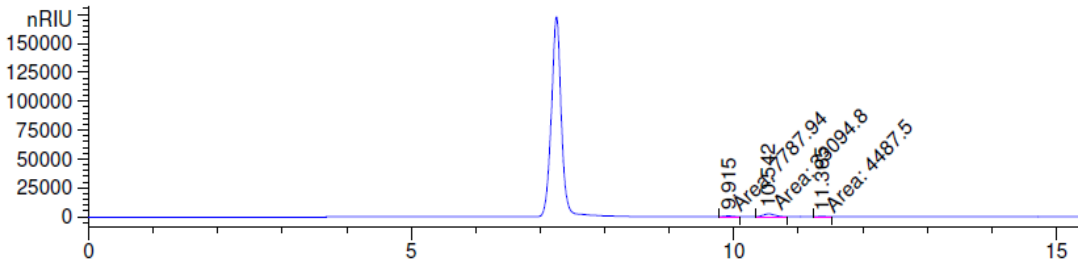

Figure I.1: HPLC Analysis of Treated SCB at 1 hour

Biomass sample weight = 0.3 g

(I) From calibration curve of HPLC area vs concentration of dextrose:

y = 289240x

HPLC area obtained from sample 2 hours = 7787

7787 = 289240x

x = 0.0269 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0269 g/L][5 mL]

M1 = 0.1345 g/L

Dextrose content = concentration x total volume

= 0.1345 g/L x 0.087 L

= 0.0117 g

(II) From calibration curve of HPLC area vs concentration of xylose:

y = 264191x

HPLC area obtained from sample 2 hours = 33095

33095 = 264191x

x = 0.1253 g/L

M1V1 = M2V2

M1 [1 mL] = [0.1253 g/L] [5 mL]

M1 = 0.6265 g/L

Xylose content = concentration x total volume

= 0.6265 g/L x 0.087 L

= 0.0545 g

(III) From calibration curve of HPLC area vs concentration of arabinose:

y = 265654x

HPLC area obtained from sample 2 hours = 4487

4487 = 265654x

x = 0.0169 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0169 g/L] [5 mL]

M1 = 0.0845 g/L

Arabinose content = concentration x total volume

= 0.0845 g/L x 0.087 L

= 7.3515 x 10-3 g

(IV) Total carbohydrates content in weight percentage

= [0.0117 g + 0.0545 g + 7.3515 x 10-3 g] / 0.3g x 100%

= 24.52 wt %

(V) Mass of estimated cellulose content

= 24.52 wt % x 2.34 g

= 0.5738 g of cellulose

APPENDIX J: Sample Calculation of Total Carbohydrates Content in Sample of 2 hours

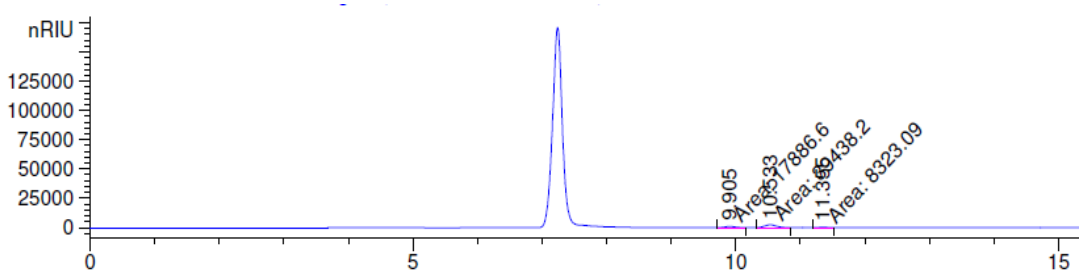

Figure J.1: HPLC Analysis of Treated SCB at 2 hours

Biomass sample weight = 0.3 g

(I) From calibration curve of HPLC area vs concentration of dextrose:

y = 289240x

HPLC area obtained from sample 1hour = 17887

17887 = 289240x

x = 0.0618 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0618 g/L][5 mL]

M1 = 0.3090 g/L

Dextrose content = concentration x total volume

= 0.3090 g/L x 0.087 L

= 0.0269 g

(II) From calibration curve of HPLC area vs concentration of xylose:

y = 264191x

HPLC area obtained from sample 1hour = 39438

39438 = 264191x

x = 0.1493 g/L

M1V1 = M2V2

M1 [1 mL] = [0.1493 g/L] [5 mL]

M1 = 0.7465 g/L

Xylose content = concentration x total volume

= 0.7465 g/L x 0.087 L

= 0.0649 g

(III) From calibration curve of HPLC area vs concentration of arabinose:

y = 265654x

HPLC area obtained from sample 1hour = 8323

8323 = 265654x

x = 0.0313 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0313 g/L] [5 mL]

M1 = 0.1565 g/L

Arabinose content = concentration x total volume

= 0.1565 g/L x 0.087 L

= 0.0136 g

(IV) Total carbohydrates content in weight percentage

= [0.0269 g + 0.0649 g + 0.0136 g] / 0.3g x 100%

= 35.13 wt %

(V) Mass of estimated cellulose content

= 35.13 wt % x 2.3618 g

= 0.8297 g of cellulose

APPENDIX K: Sample Calculation of Total Carbohydrates Content in Sample of 3 hours

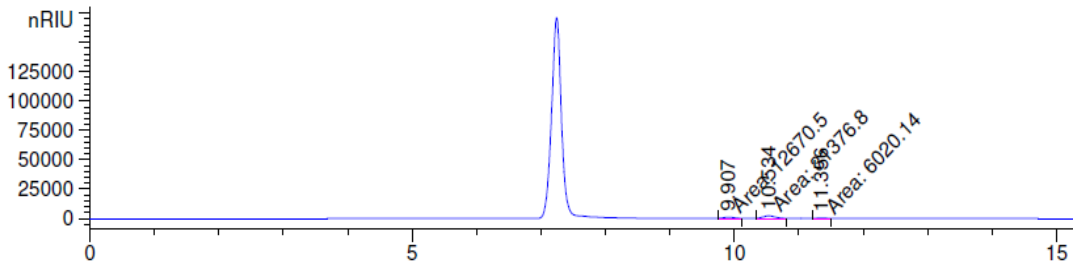

Figure K.1: HPLC Analysis of Treated SCB at 3 hours

Biomass sample weight = 0.3 g

(I) From calibration curve of HPLC area vs concentration of dextrose:

y = 289240x

HPLC area obtained from sample 3 hours = 12671

12671 = 289240x

x = 0.0438 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0438 g/L][5 mL]

M1 = 0.2190 g/L

Dextrose content = concentration x total volume

= 0.2190 g/L x 0.087 L

= 0.0191 g

(II) From calibration curve of HPLC area vs concentration of xylose:

y = 264191x

HPLC area obtained from sample 3 hours = 31377

31377 = 264191x

x = 0.1188 g/L

M1V1 = M2V2

M1 [1 mL] = [0.1188 g/L] [5 mL]

M1 = 0.5940 g/L

Xylose content = concentration x total volume

= 0.5940 g/L x 0.087 L

= 0.0517 g

(III) From calibration curve of HPLC area vs concentration of arabinose:

y = 265654x

HPLC area obtained from sample 3 hours = 6020

6020 = 265654x

x = 0.0227 g/L

M1V1 = M2V2

M1 [1 mL] = [0.0227 g/L] [5 mL]

M1 = 0.1135 g/L

Arabinose content = concentration x total volume

= 0.1135 g/L x 0.087 L

= 9.8745 x 10-3 g

(IV) Total carbohydrates content in weight percentage

= [0.0191 g + 0.0517 g + 9.8745 x 10-3 g] / 0.3g x 100%

=26.89 wt %

(V) Mass of estimated cellulose content

= 26.89 wt % x 2.016 g

= 0.5421 g of cellulose

APPENDIX L: FTIR Spectra of SCB and Treated SCB

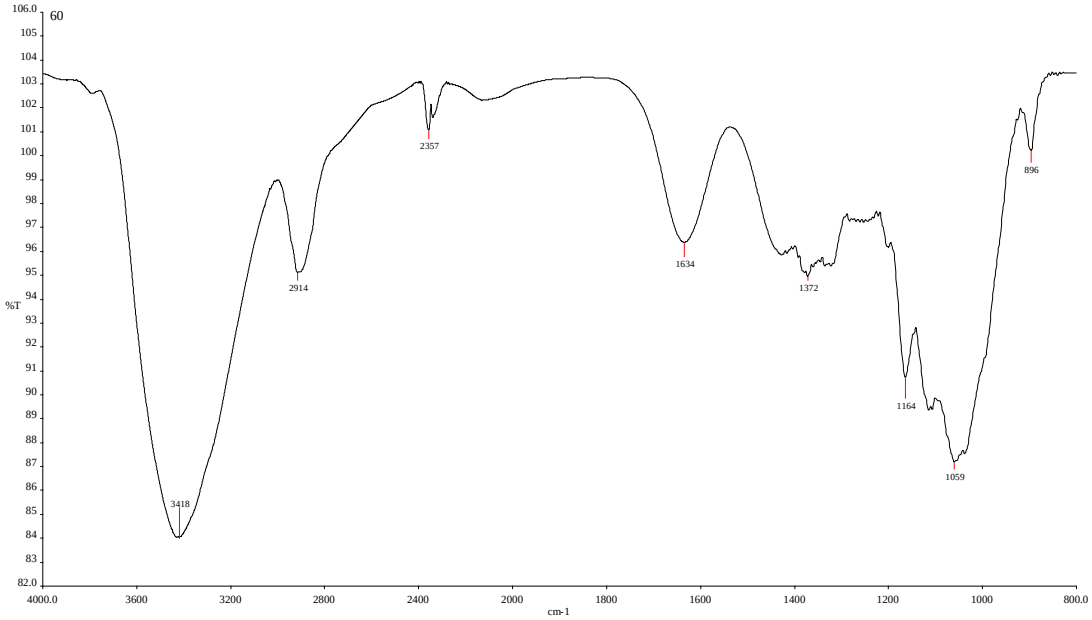

Figure L.1: FTIR Spectra of Treated SCB at 60 °C

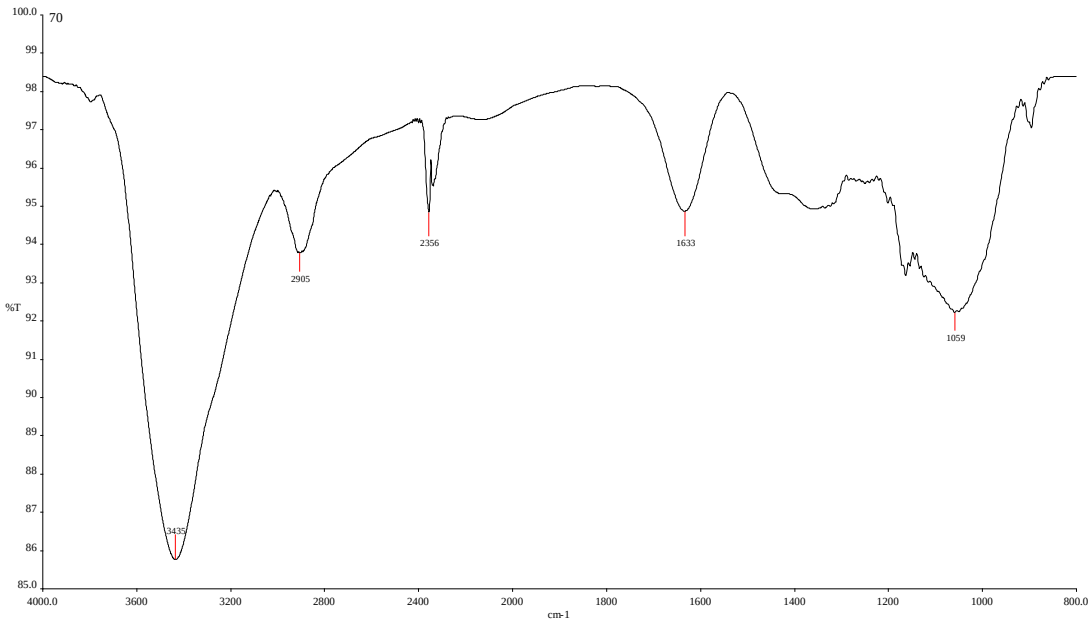

Figure L.2: FTIR Spectra of Treated SCB at 70 °C

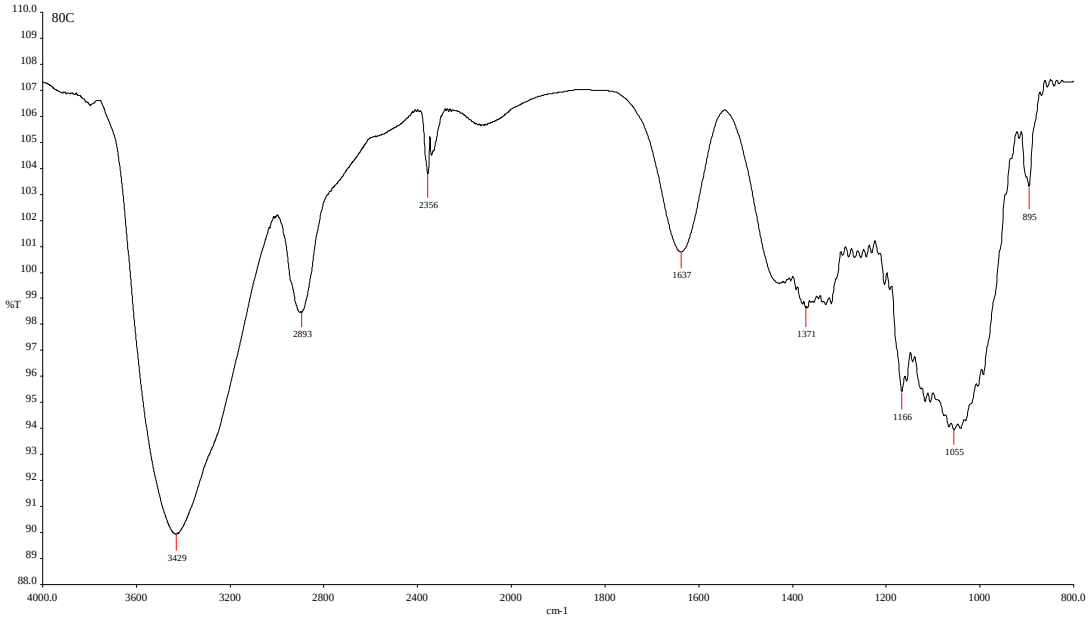

Figure L.3: FTIR Spectra of Treated SCB at 80 °C

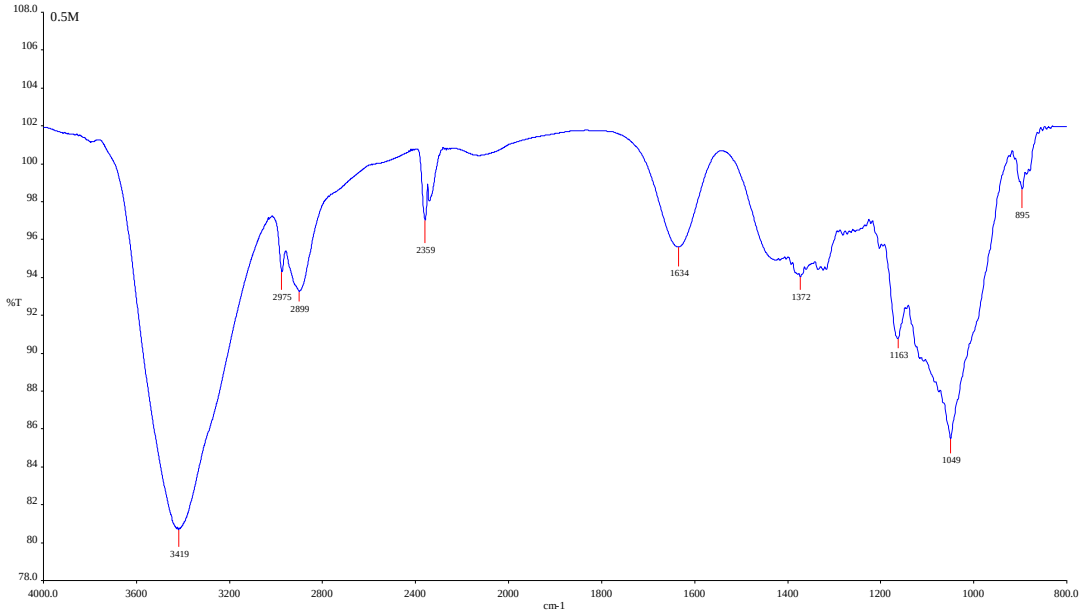

Figure L.4: FTIR Spectra of Treated SCB at 0.5 M

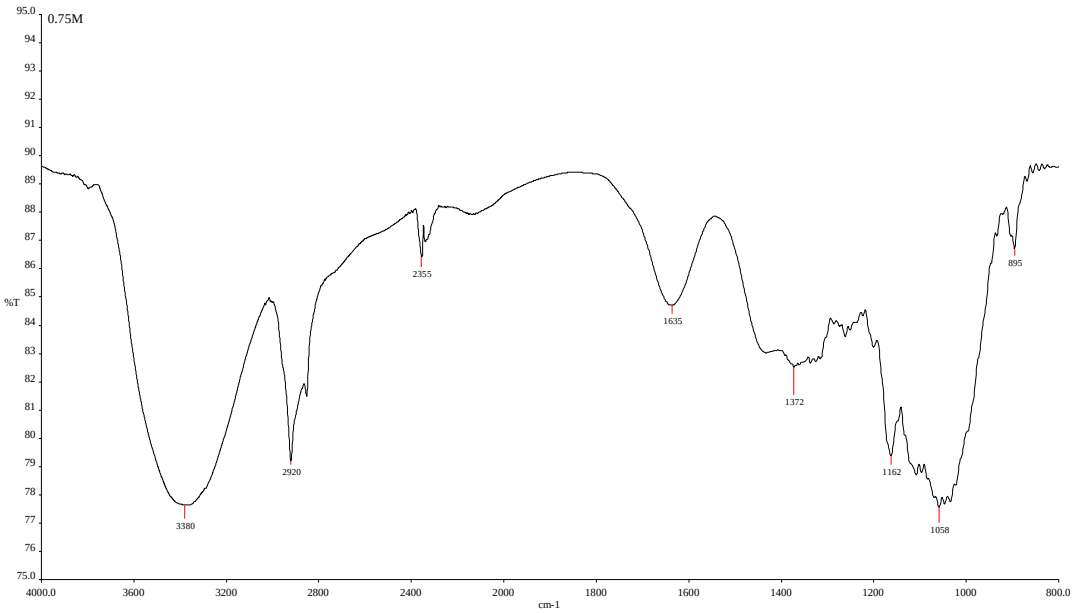

Figure L.5: FTIR Spectra of Treated SCB at 0.75 M

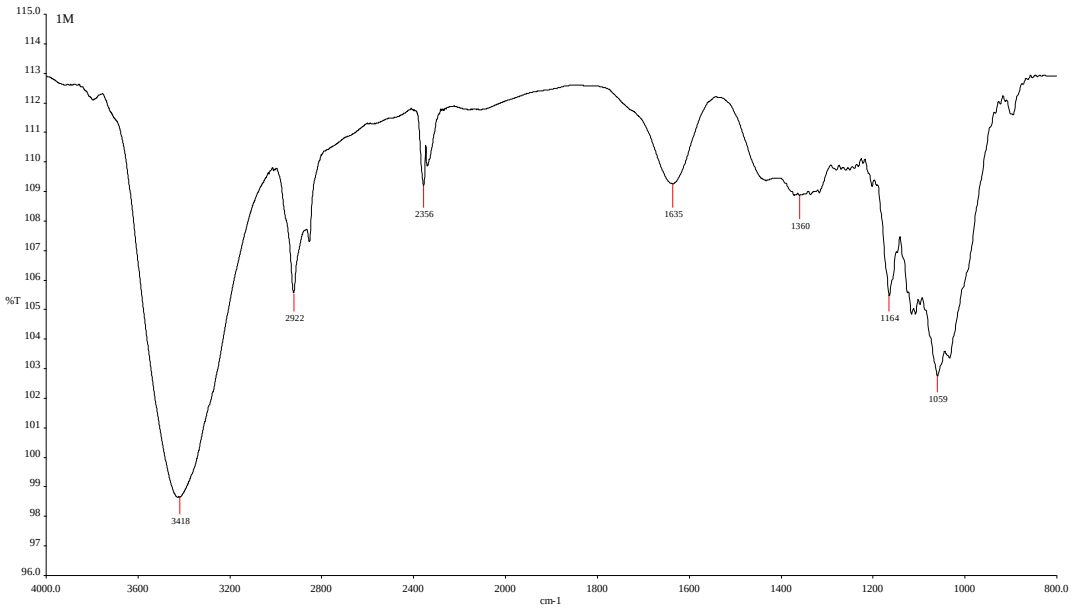

Figure L.6: FTIR Spectra of Treated SCB at 1 M

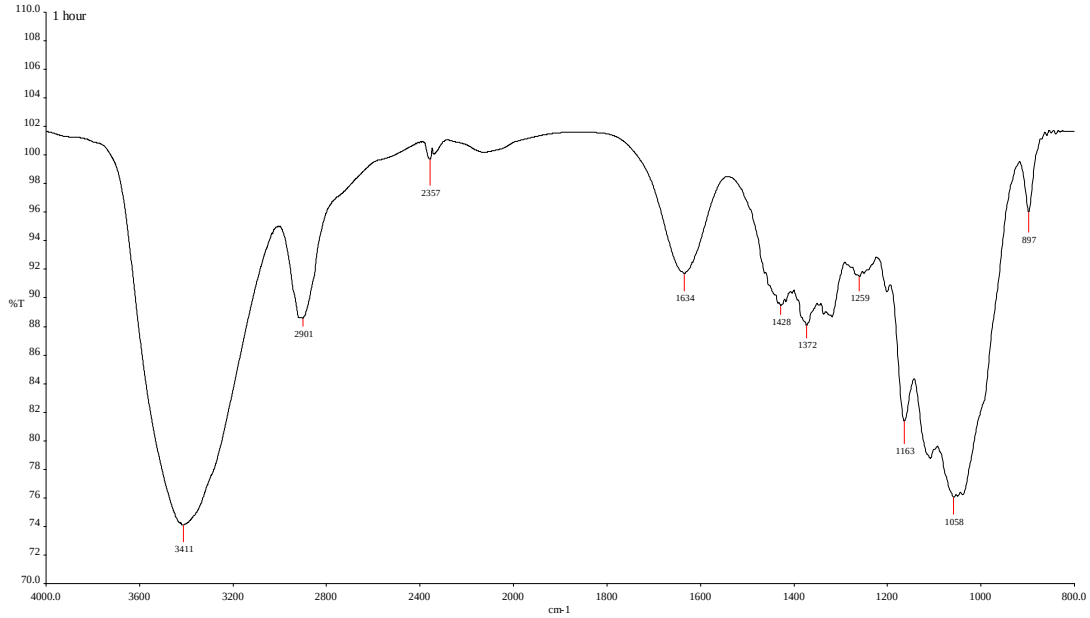

Figure L.7: FTIR Spectra of Treated SCB at 1 hour

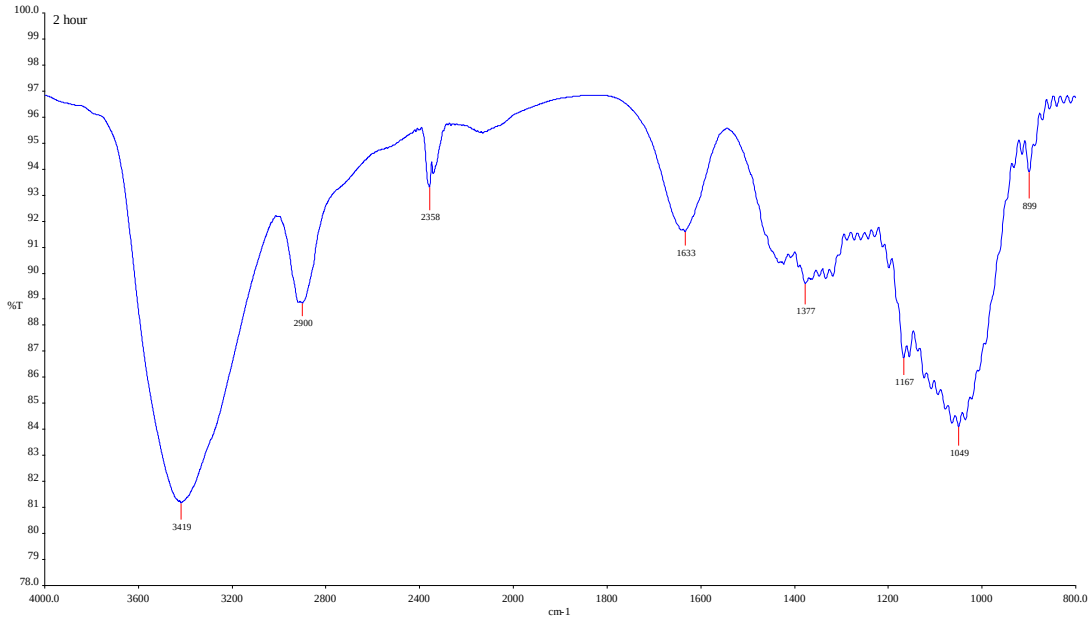

Figure L.8: FTIR Spectra of Treated SCB at 2 hours

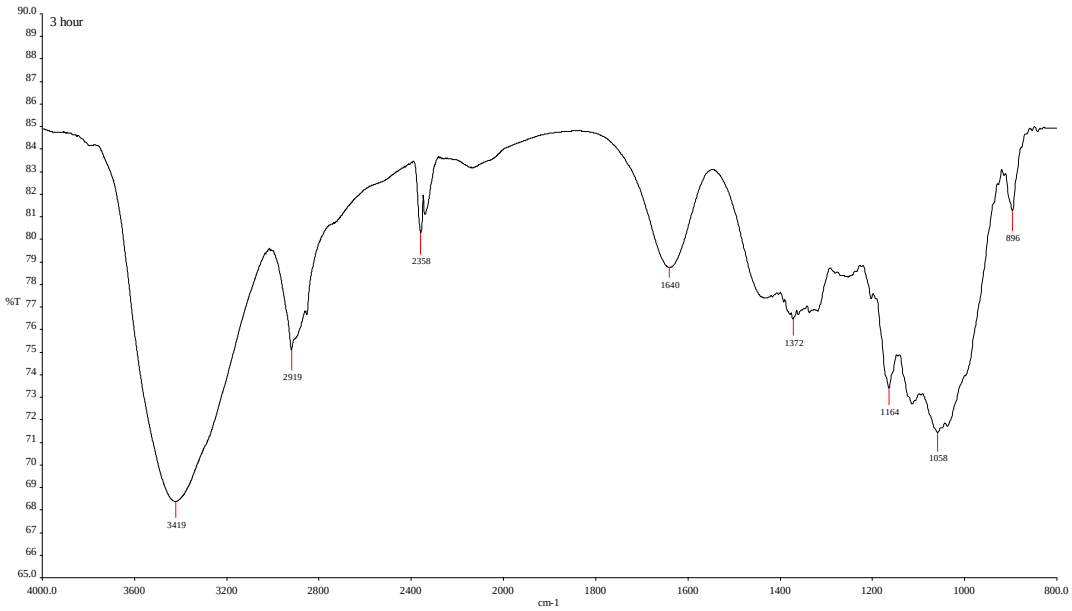

Figure L.9: FTIR Spectra of Treated SCB at 3 hours

APPENDIX M: DSC Thermographs of SCB and Treated SCB

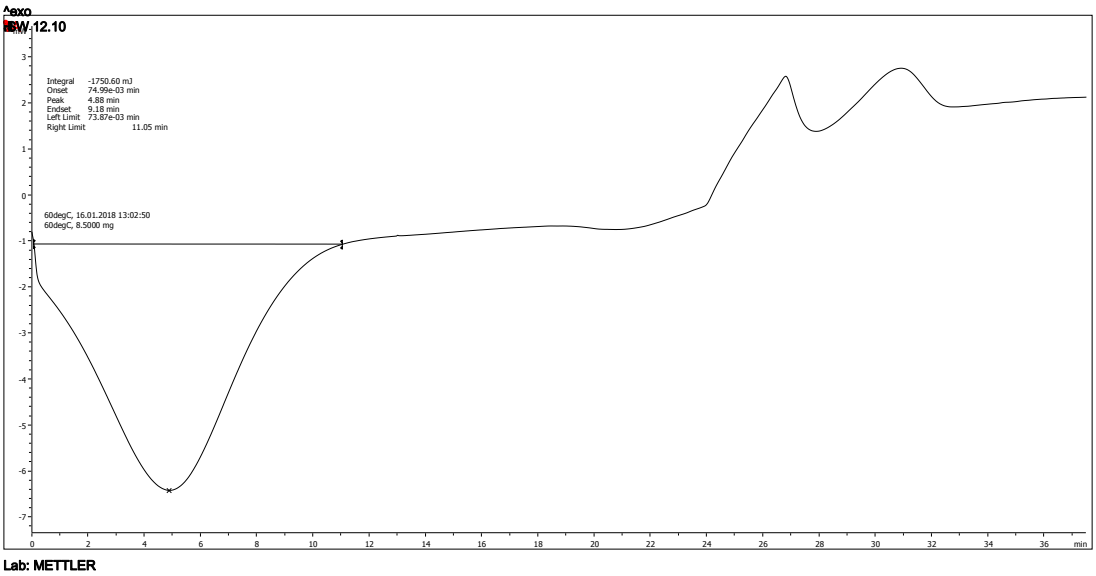

Figure M.1: DSC Thermogram of Treated SCB at 60 °C

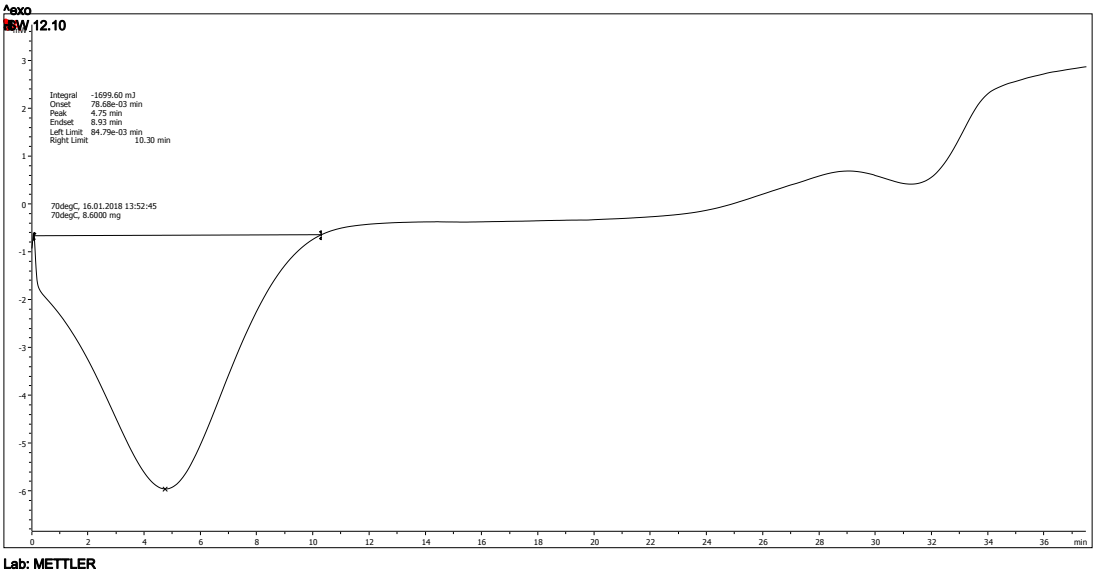

Figure M.2: DSC Thermogram of Treated SCB at 70 °C

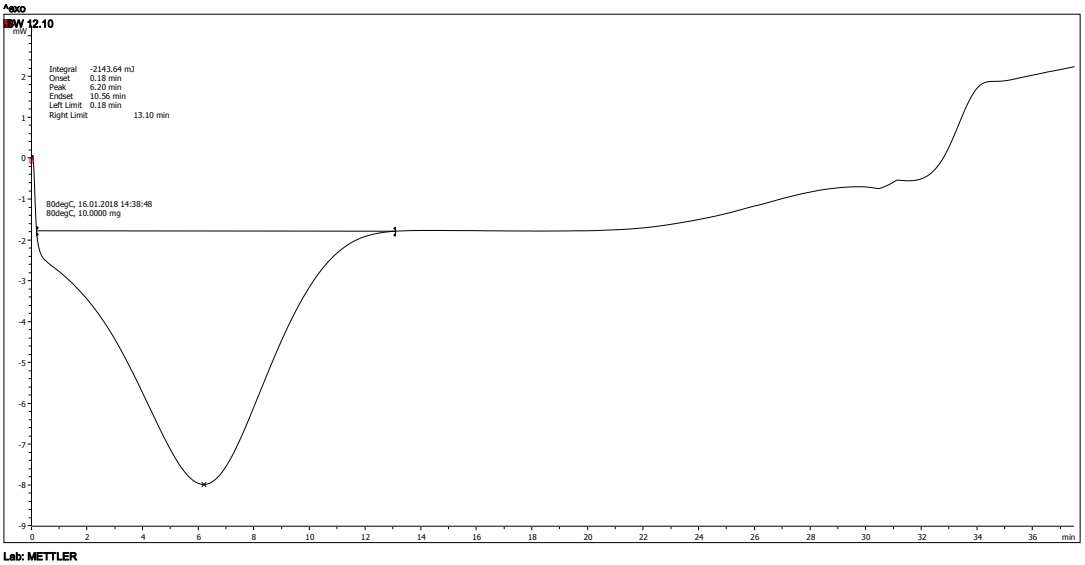

Figure M.3: DSC Thermogram of Treated SCB at 80 °C

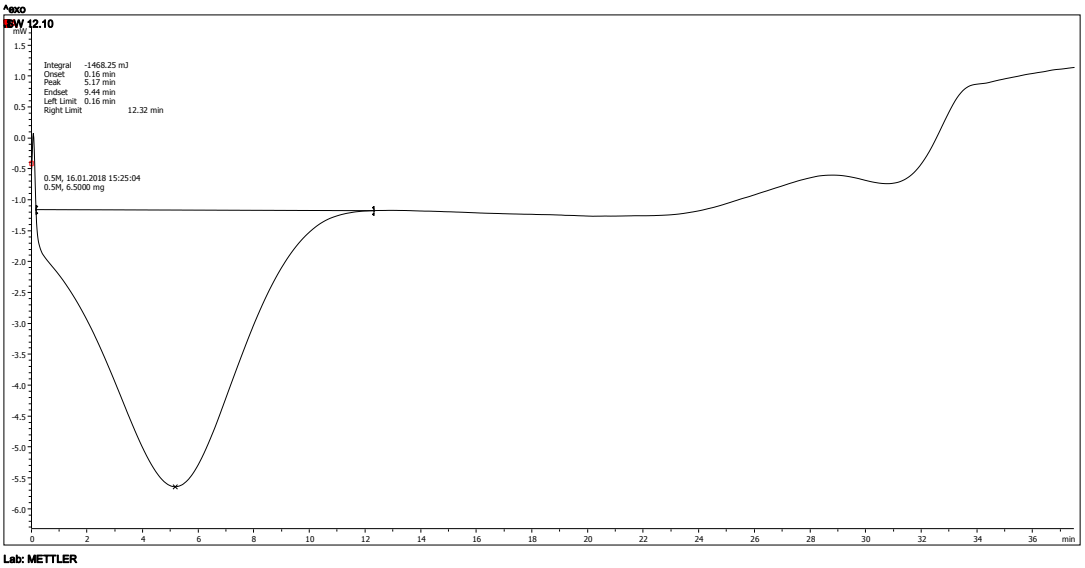

Figure M.4: DSC Thermogram of Treated SCB at 0.5 M

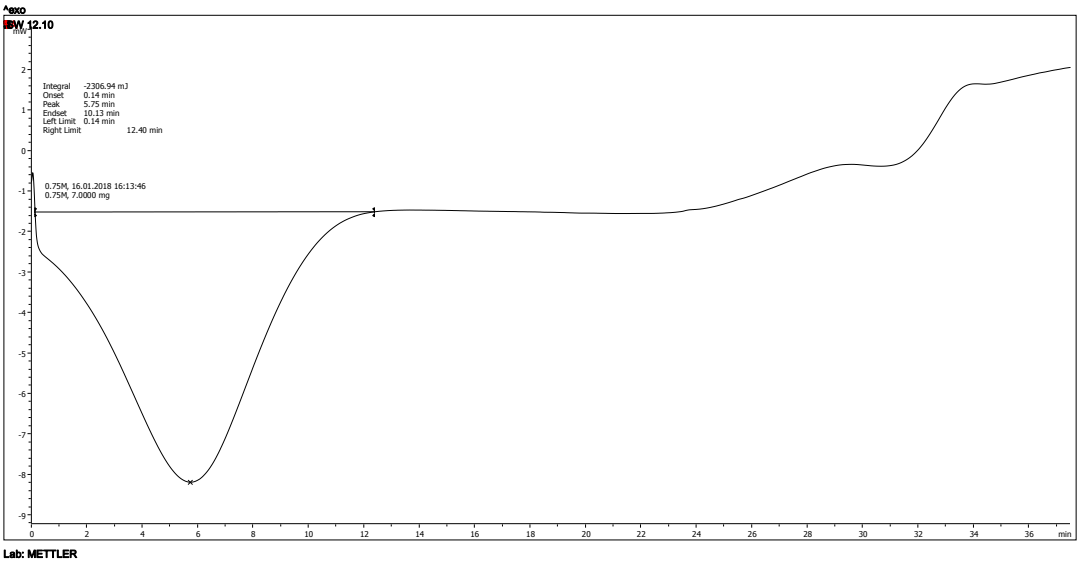

Figure M.5: DSC Thermogram of Treated SCB at 0.75 M

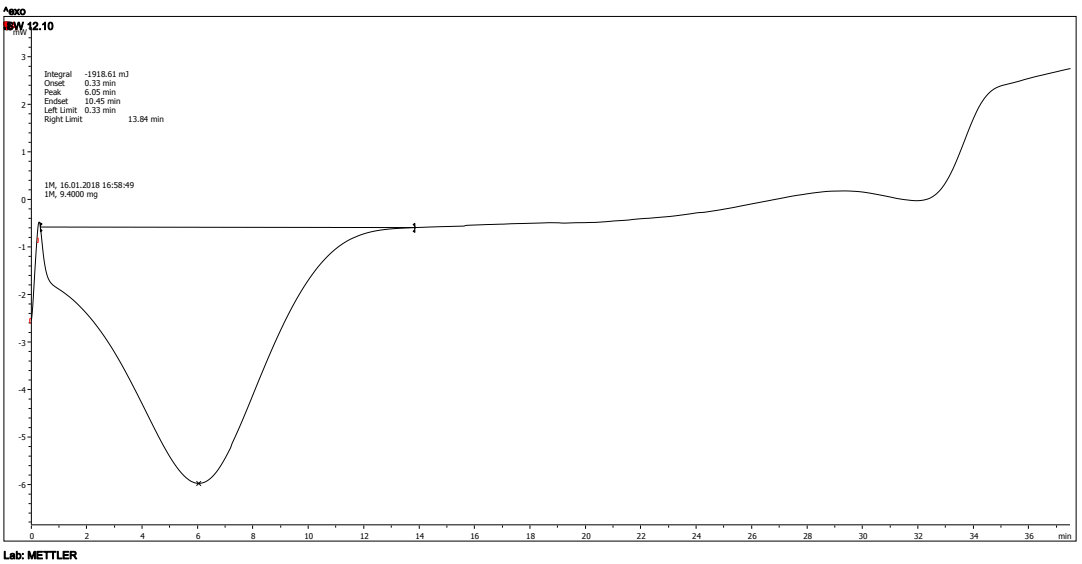

Figure M.6: DSC Thermogram of Treated SCB at 1 M

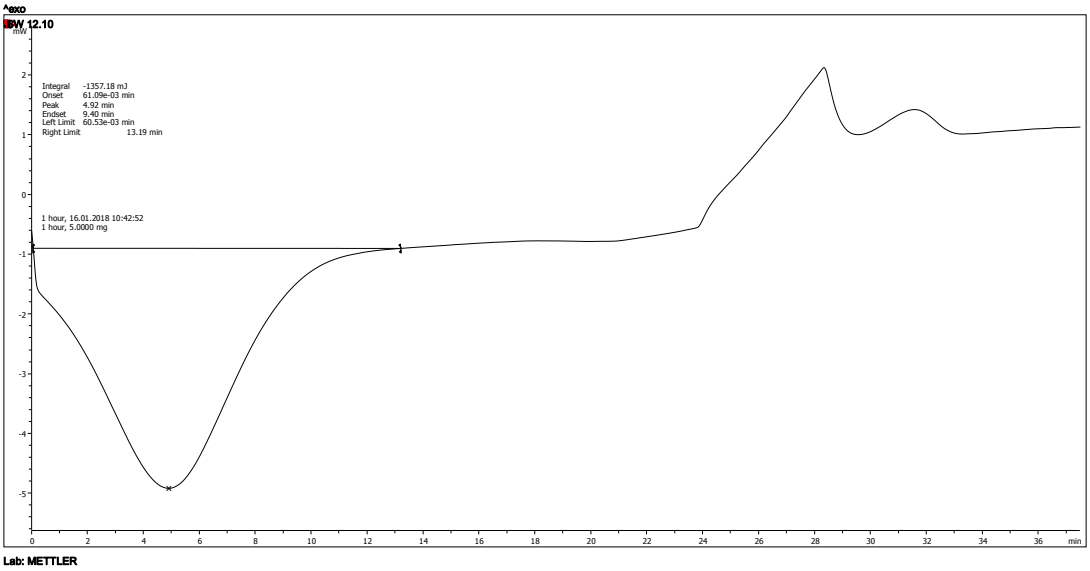

Figure M.7: DSC Thermogram of Treated SCB at 1 hour

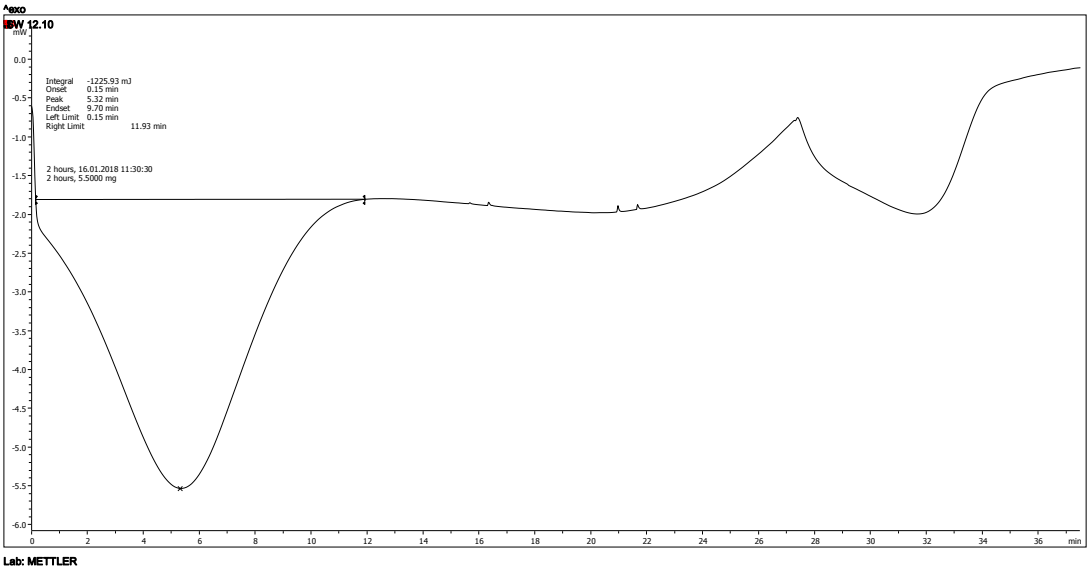

Figure M.8: DSC Thermogram of Treated SCB at 2 hours

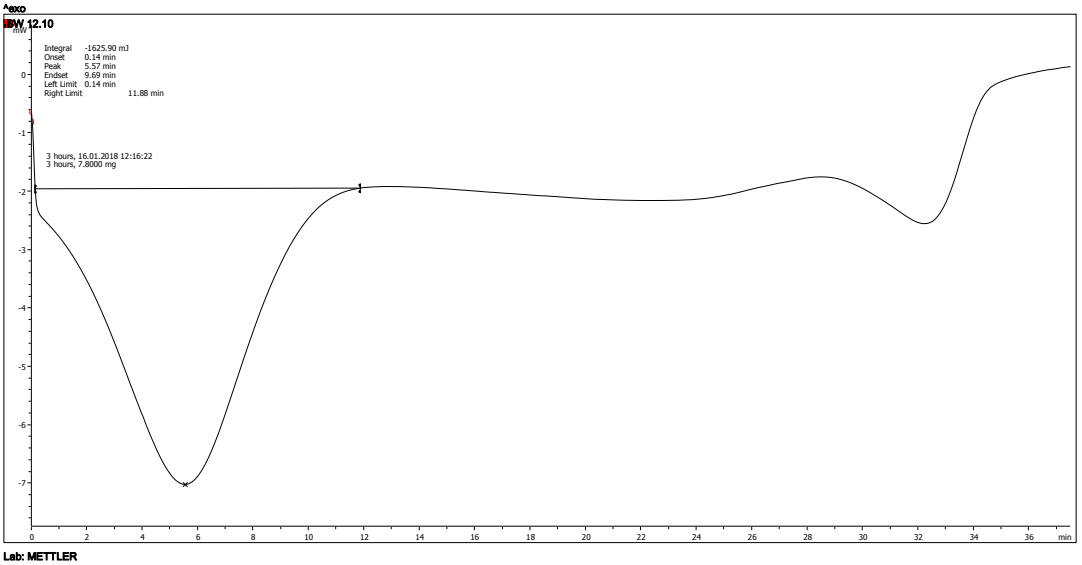

Figure M.9: DSC Thermogram of Treated SCB at 3 hours

APPENDIX N: TGA Thermographs of SCB and Treated SCB

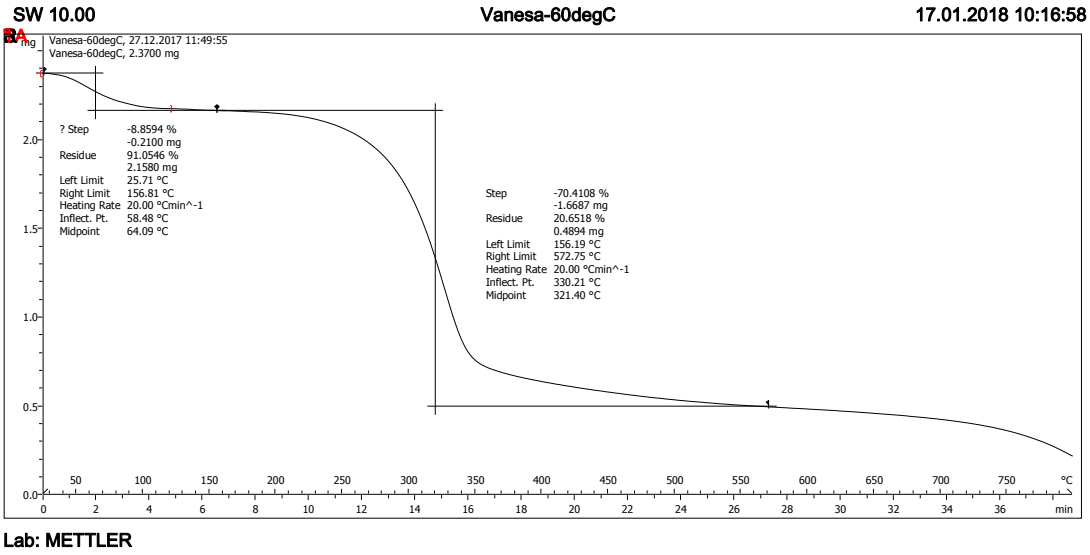

Figure N.1: TGA Thermogram of Treated SCB at 60 °C

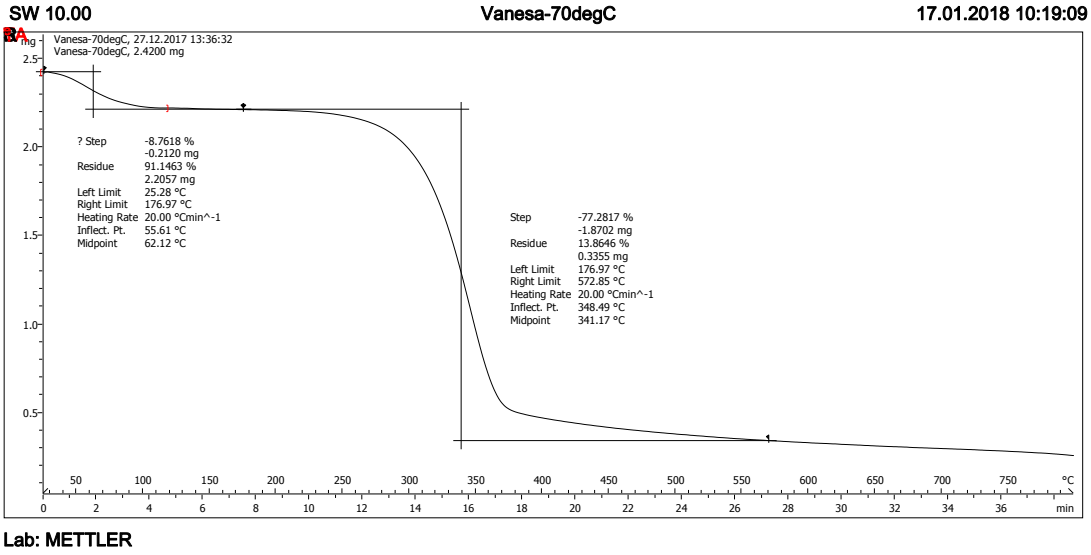

Figure N.2: TGA Thermogram of Treated SCB at 70 °C

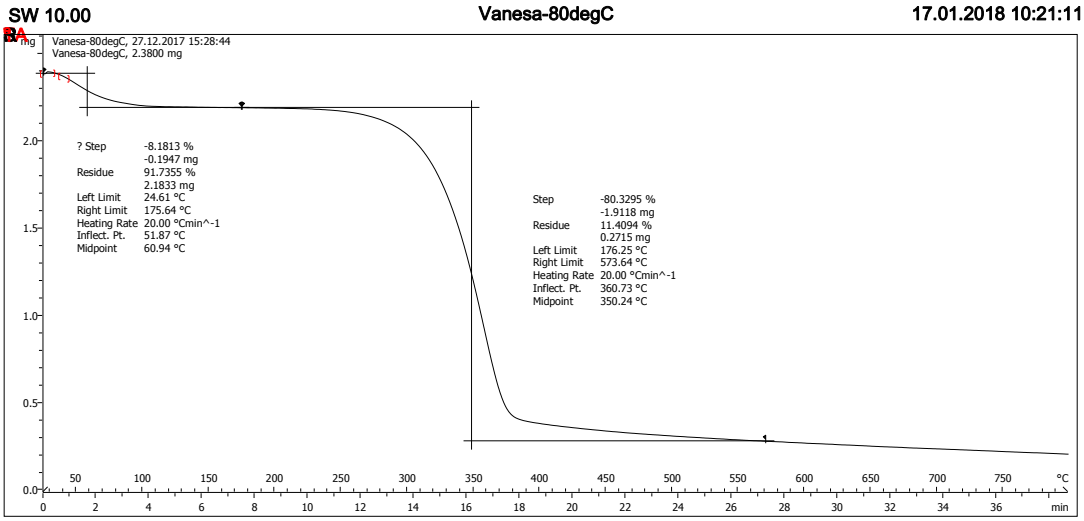

Figure N.3: TGA Thermogram of Treated SCB at 80 °C

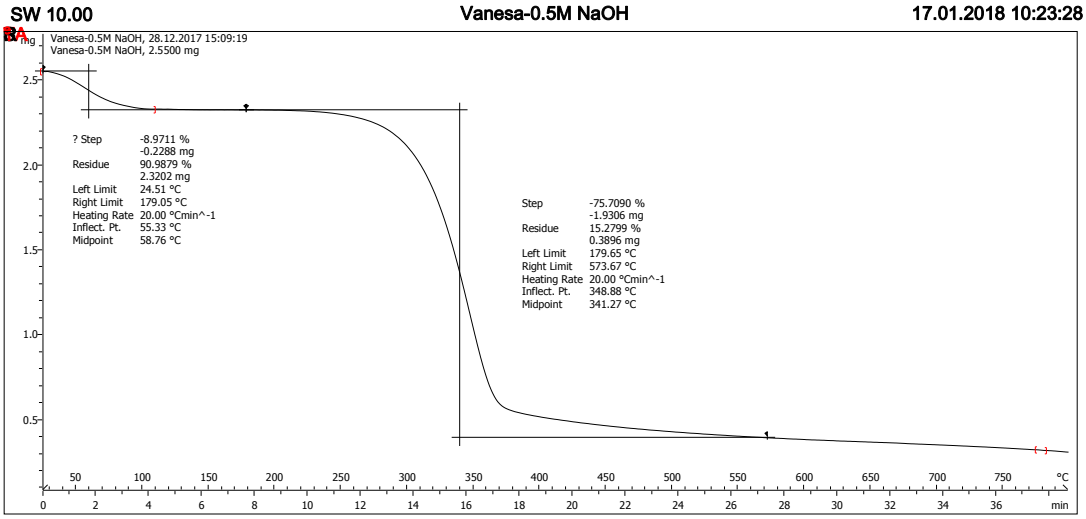

Figure N.4: TGA Thermogram of Treated SCB at 0.5 M

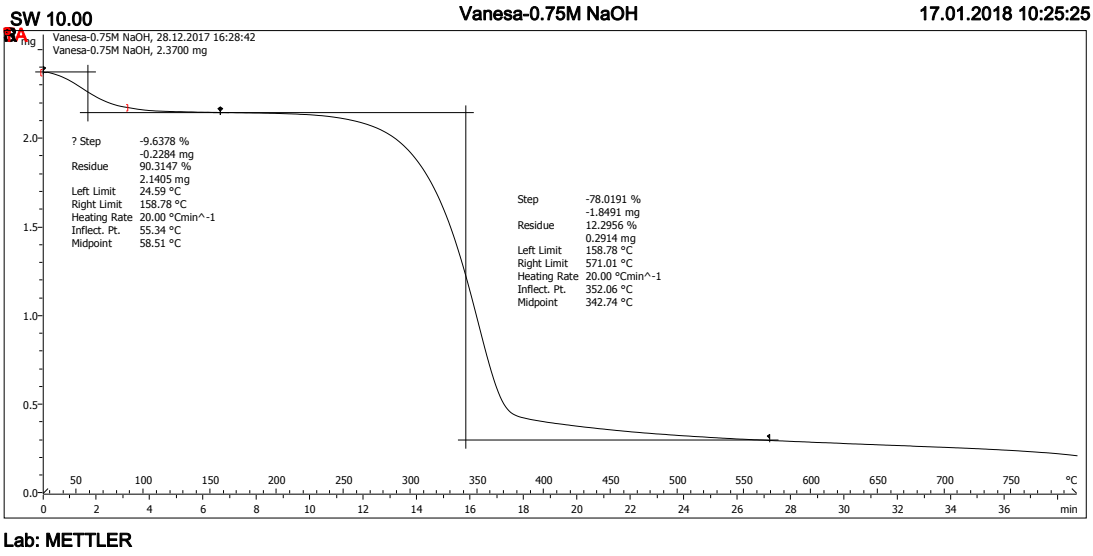

Figure N.5: TGA Thermogram of Treated SCB at 0.75 M

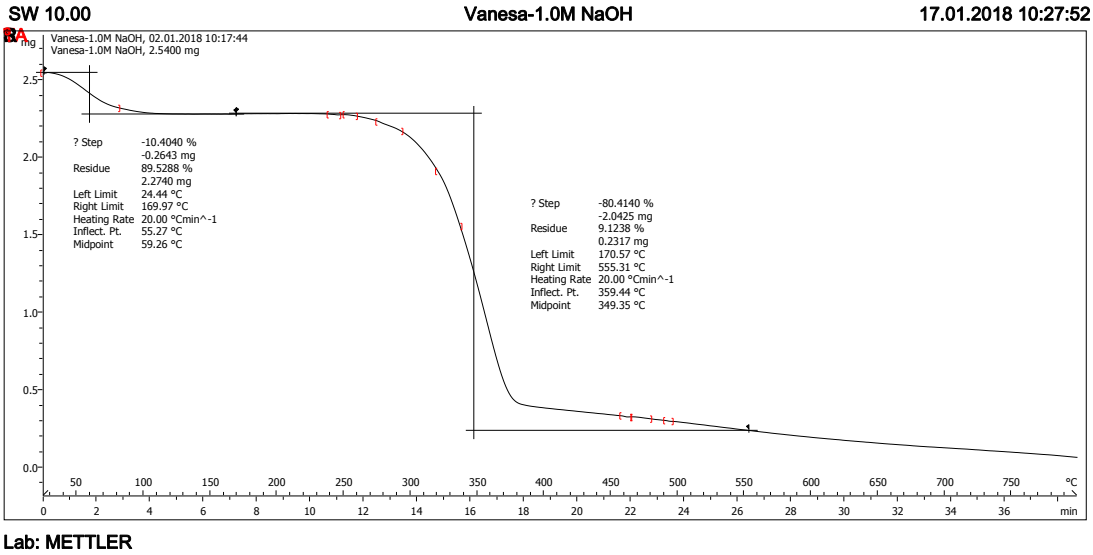

Figure N.6: TGA Thermogram of Treated SCB at 1 M

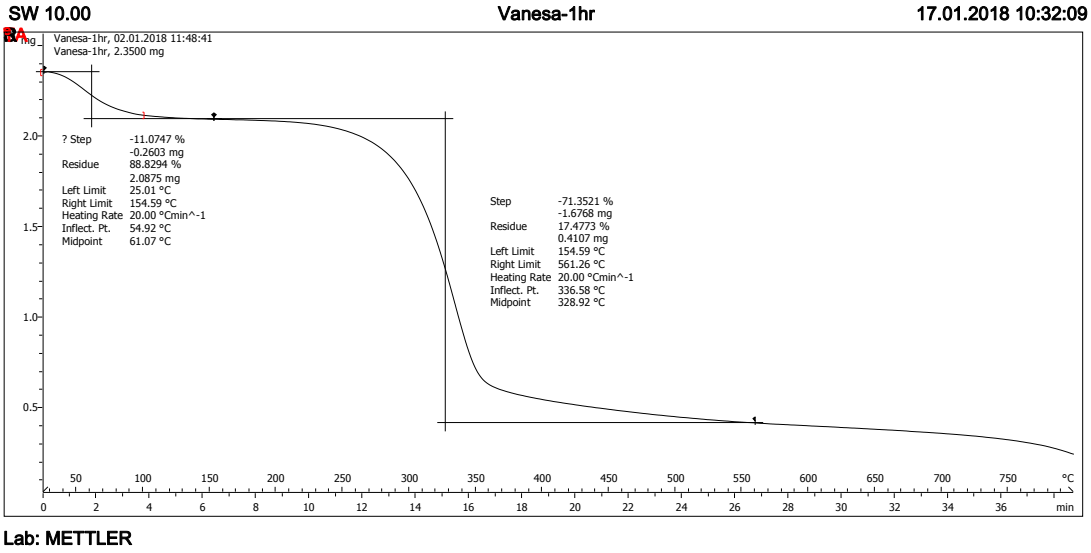

Figure N.7: TGA Thermogram of Treated SCB at 1 hour

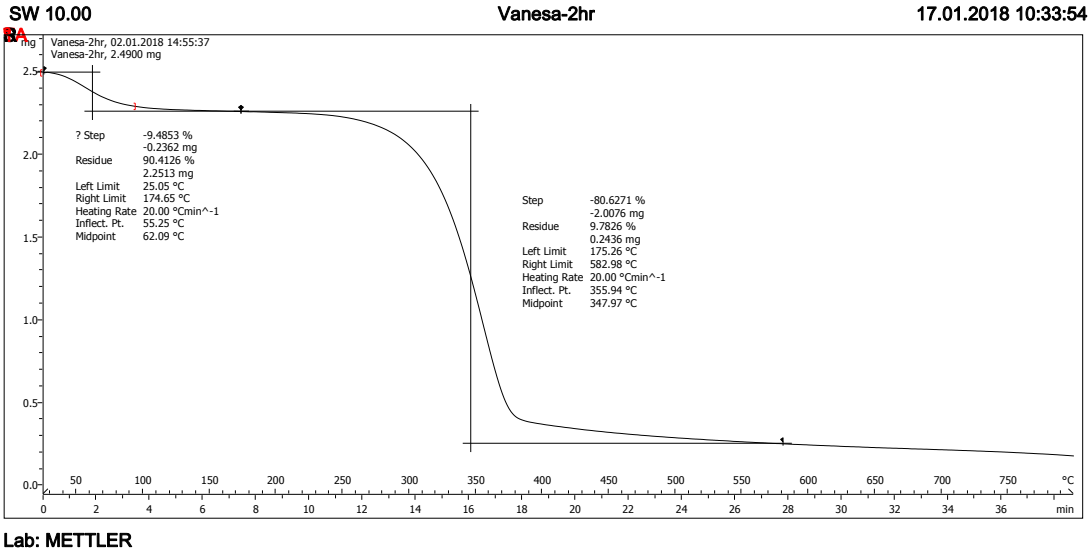

Figure N.8: TGA Thermogram of Treated SCB at 2 hours

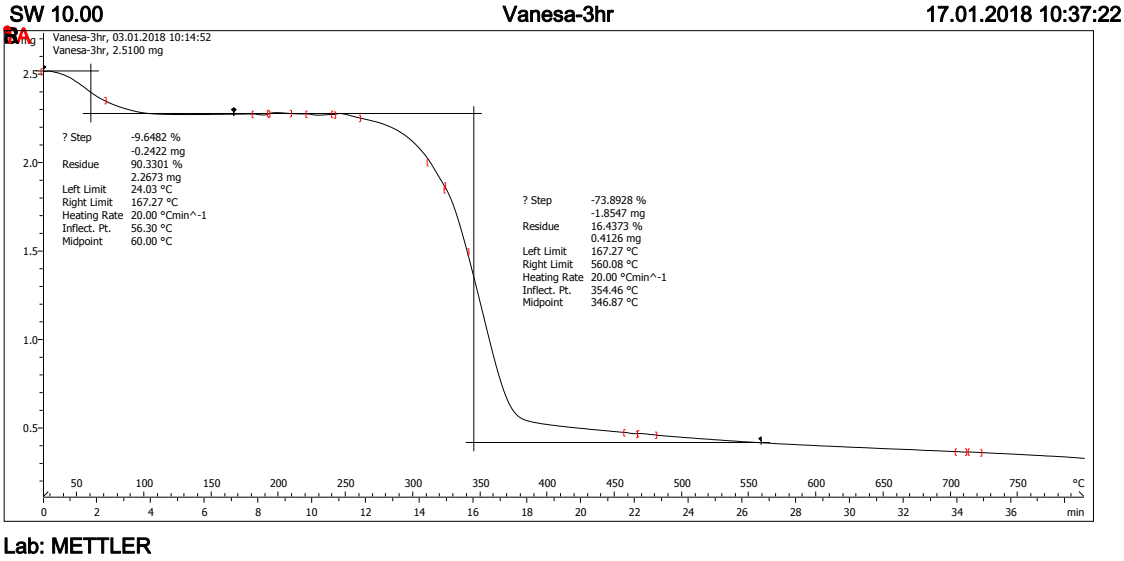

Figure N.9: TGA Thermogram of Treated SCB at 3 hours

APPENDIX O: Sample Calculation to Determine Degree of Substitution and Viscosity of CMC

To calculate degree of substitution, using the Equation (3.2),

$$B = 0.1 \times \frac{b}{G}$$

$$B = 0.1 \times \frac{9.9}{0.35}$$

$$B = 2.83$$

Substituting the above result into Equation (3.3):

$$Degree\ of\ substitution\ (DS) = \frac{0.162 \times B}{1 - 0.080 \times B}$$

$$DS = \frac{0.162 \times 2.83}{1 - 0.080 \times 2.83}$$

The degree of substitution will be $DS = 0.59$

To calculate the viscosity by using Equation (3.4),

$$\eta = \rho k t$$

$$\eta = \left(2.17 \frac{kg}{m^3}\right) \times \left(0.00978 \frac{m^2}{s}\right) \times (129s)$$

$$\eta = 2.74\ cP$$

Where η= Viscosity of the solution

ρ = density of the solution

t = flow time