ULTRASONIC-ASSISTED EXTRACTION OF CELLULOSE FROM SUGARCANE BAGASSE

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ULTRASONIC-ASSISTED EXTRACTION OF CELLULOSE FROM SUGARCANE BAGASSE

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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

Faculty of Engineering and Green Technology

Universiti Tunku Abdul Rahman

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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 "**ULTRASONIC-ASSISTED EXTRACTION OF CELLULOSE FROM SUGARCANE BAGASSE**" was prepared by **PHUANG KE SHONG** 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

The large amount of lignocellulosic biomass have contributed to the environmental issues. Therefore, it is essential to recycle them and convert it into more useful products via environmental friendly technology. In this research, sugarcane bagasse (SCB) is used as the feedstock for cellulose extraction since it can be easily found worldwide with about 50 %(w/w) of cellulose content. In addition, ultrasonic-assisted alkaline extraction was used as the green technology to obtain the cellulose with vital consideration to reduce the chemical and energy usage compared to current conventional extraction technology. The SCB was autoclaved with distilled water before undergo ultrasonic extraction in alkali medium. The treatments were carried out by manipulating three different processing parameters, which are ultrasonic amplitude of 20 %, 30 % and 40 %, temperature at 70 ^{o}C, 80 ^{o}C and 90 ^{o}C and concentration of potassium hydroxide solution vary from 0.25 M to 1.25 M, with interval of 0.25 M. Approximate 56.58 %(w/w) to 83.22 %(w/w) of cellulose has been successfully extracted from SCB samples. It was found that SCB treated at ultrasonic amplitude of 30 %, 80 ^{o}C and 1.25 M KOH resulted the highest amount of cellulose. This treated SCB sample was then further converted into carboxymethyl cellulose (CMC) through alkalization with 17.5 %(w/v) NaOH and etherification process by addition of sodium monochloroacetate (SMCA). The synthesis process gave CMC with degree of substitution of 0.3624. A low DS was obtained as the experiment is not carried out in optimum condition. Meanwhile, a film is successfully produced, further assure the properties of produced CMC. The characteristics of each of the treated SCB and CMC were analysed by Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and High Pressure Liquid Chromatography (HPLC). The testing further verified that the properties of cellulose and CMC extracted from SCB are significantly promising for large scale production.

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LIST OF SYMBOLS / ABBREVIATIONS

AGU	Anhydroglucopyranose units
CMC	Carboxymethyl cellulose
DS	Degree of Substitution
DSC	Differential Scanning Calorimetry
FTIR	Fourier Transform Infrared Spectroscopy
HPLC	High Pressure Liquid Chromatography
HCl	Hydrochloric acid
KBr	Potassium bromide
KOH	Potassium hydroxide
NaCl	Sodium chloride
NaOH	Sodium hydroxide
SCB	Sugarcane bagasse
SEM	Scanning electron microscopy
SMCA	Sodium monochloroacetate
H_2SO_4	Sulphuric acid
XRD	X-ray Powder Diffraction

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Lately, agricultural residues have been generated greatly from various industries which leads to environmental challenges. The agricultural residues include sugarcane bagasse, maize stalk, sisal, oil cakes, rice husk and coconut husk. These residue is also known as lignocellulosic biomass. Therefore, recycling of this residue is essential to reduce the pollution to the environment, on the same time extracting useful products such as lignin and cellulose, which turn the wastes into profit (Adebisi et al., 2017).

Some of these lignocellulosic biomass serve as feedstock material for conversion into biogas, steam and power generation. Generally, lignocellulosic biomass comprises the major component of cellulose, hemicellulose and lignin. Sugarcane bagasse (SCB) is an agricultural waste which is available globally and inexpensive since the yield is continuously supplied without running out. In Brazil, the supply of SCB can be up to 186 million tons per year (Karp et al., 2013).

SCB is the major byproduct produced in sugarcane industry. SCB is the cane fibrous residue which can be obtained through the crushing of sugarcane to extract the juice. The growth region and surrounding condition of the sugarcane will affect its constituents (Parameswaran, 2009). Generally, approximate 41-55 %(w/w) of SCB is cellulose with 20.0-27.5 %(w/w) of hemicellulose, 18.0-26.3 %(w/w) of lignin and about 7 %(w/w) of other inorganic materials (Mokhena et al., 2018). All these biopolymers are strongly intermeshed and chemically bonded (Pérez et al., 2002).

The current properties of lignocellulosic biomass is resistant to enzymatic attack. Besides that, the strong crystalline arrangement of cellulose and the resistance of lignin to microbial attack have create difficulty to the hydrolysis process (Rocha et al., 2012). Thus, treatment is needed to disrupt and solubilise hemicellulose and lignin from the lignocellulosic biomass, thereby enhance the efficiency in extracting cellulose from SCB for further usage in other application. Several types of treatment can be carried out which includes physicochemical, chemical, mechanical, hydrothermal and enzymatic treatment. Steam explosion is one of the common method for physicochemical treatment while mechanical treatment normally utilized ultrasonic or microwave system. The most common treatment is chemical treatment which uses acid, alkali and organic solvent. On the other hand, hot water and wet oxidation are examples for hydrothermal treatment. Along the treatment process, the structure of the SCB, lignin and hemicellulose is broken down and solubilised (Karp et al., 2013). As the result, cellulose is extracted from the lignocellulosic material, SCB. The yield of cellulose obtained is dependent on the condition of different process parameters. An effective treatment will limits the formation of degrade products and by-products, while carry out the treatment at lower capital and operating cost. It is advisable to employ the treatment process which is environmentally friendly and low energy usage, on the same time, able to extract the adequate amount of cellulose.

Cellulose is a long chain homopolymer composed of β-1,4 glycosidic bonds linked D-glucose subunits. Hydrogen bonds and van der Waals forces link the long chain together (Karp et al., 2013). It is a natural polysaccharide that exist within cell coats of plants. Cellulose consists of both amorphous and crystalline region. Moreover, cellulose is a very stable insoluble compound, biodegradable, non-toxic and have high thermal stability and tensile strength, which make it widely used in various application (Quesada Cabrera et al., 2011). It is mainly used as raw material in the form of cement composite in construction and for chemical conversions (Klemm, Schmauder and Heinze, 2005). The main cellulose derivatives are cellulose ether and cellulose ester which frequently used in pharmaceutical and cosmetic industries. Ethyl cellulose (EC), hydroxypropyl cellulose (HPC) and carboxymethyl cellulose (CMC) are the examples for cellulose ether. Meanwhile, cellulose ester includes cellulose acetate (CA), cellulose acetate phthalate (CAP) and cellulose acetate trimelitate (CAT) (Shokri and Adibkia, 2013).

The wide range application of cellulose in textile, paper, pulp, paints, oil drilling fluids, food and beverage industry are likely to drive the market demand of cellulose in the coming years (Grand View Research, 2018). The production of cellulose is approximate to be 10^{11} to 10^{12} tons each year (Heinze, El Seoud and Koschella, 2018). Meanwhile, according to Global Market Insights (2018), it is expected the market for derivatives of cellulose, CMC to exceed USD 1.7 billion in the year 2024. An increasing demand of CMC with high purity in food, pharmaceuticals, oil and gas application are the factors that boost the market demand by 2024.

In this project, cellulose will further convert to its derivative, carboxymethyl cellulose (CMC) for wider application usage. The production of CMC involves two major reactions, which is alkalization and etherification. The extracted cellulose will undergo alkalization with sodium hydroxide solution, followed by etherification using sodium monochloroacetate (SMCA) to produce CMC (Huang et al., 2017). It is a hydrophilic polysaccharide which is soluble in water. In addition, CMC possesses low toxic, biocompatible and biodegradable properties (Siritientong and Aramwit, 2015). It can function as thickener, water binder, emulsifier, film former, gelling agent and additive in various industrial sectors especially oil drilling and petrochemical (Wertz, Mercier and Bédué, 2010).

1.2 Problem Statement

Sugarcane bagasse is one of the abundant agricultural byproduct available in Malaysia (Kadir and Maasom, 2013). Such a large amount of the agricultural byproduct can lead to safety hazards and environmental problem such as contamination of land and water sources. Therefore, to resolve this issue, it is crucial to recycle SCB into value added product instead of discarding them. One of the usable product that can be extracted out from SCB is cellulose. Cellulose is embedded with lignin, pectin, hemicellulose and other carbohydrate polymer (Abdel-Halim, 2013). Lignin, pectin and hemicellulose have to be removed to obtain pure cellulose. Presence of lignin will decrease biomass digestibility (Chang and Holtzapple, 2000). Removal of pectin and lignin can be

carried out through dewaxing and delignification process which require substantial amount of chemicals and energy to obtain high quality cellulose. The monomer of cellulose is glucose. Cellulose has a polymer chain length of 10,000 glucose units and is linear in structure (Chen, 2014). Besides that, the morphology of cellulose can be combination of crystalline and amorphous region. They are rigid and less reactive to reaction.

In recent years, chemical treatment have been practised widely in industry as compared to other treatments. Acid hydrolysis treatment causes the disruption of lignocellulosic structure, whereby hemicellulose is solubilised. The common acid used in the treatment includes phosphoric acid, sulphuric acid, hydrochloric acid and acetic acid at various level of concentration (Supranto et al., 2015). However, the use of concentrated acid could leads to the corrosion of equipment. Meanwhile, some of the organic acid have high flammability which would result in high pressure solvents (Balan, 2014). Dilute acid will be more favour in assisting the extraction of cellulose since it tend to remove large amount of hemicellulose (Yang and Wyman, 2008).

On the other hand, alkaline treatment can effectively remove lignin through disruption of the structural linkage, dissolve the hemicellulose and maximize the cellulose content (Karp et al., 2013). Sodium hydroxide, potassium hydroxide, lime and ammonia are example of alkali employed in this process. However, there is still the presence of minor hemicellulose in the mixture. Further treatment is needed to remove the hemicellulose (Bian et al., 2012). Besides that, large amount of water is needed especially when utilized alkaline hydrogen peroxide and lime in the treatment (Balan, 2014).

The steam explosion and liquid hot water treatment is one of the technology that can be used to extract cellulose. However, there need to be conducted in high temperature and pressure, thus require high control on operating parameters. Meanwhile, wet oxidation conducted at high temperature assists the cleavage of hemicellulose and lignin. The treatment process is exothermic and requires oxygen gas supply (Tarherzadeh and Karimi, 2008). On the other hand, ultrasonic treatment utilizes ultrasound wave to induce cavitation phenomena on the cell wall, causing the solubilisation of lignin and hemicellulose (Sun et al, 2004). Ultrasonic treatment

requires shorter extraction time and smaller amount of solvent as compared to other treatment (Anna and Zdenka, 2010). Ultrasonic treatment has been widely practised in laboratory scale based. However, researcher have found some difficulties to achieve extraction in industrial scale (Vilkhu et al., 2008).

Aside from this common treatment which utilized the usage of large amount of chemicals to extract cellulose, there is a need to develop a treatment process which require a mild reaction conditions and less energy demand with a higher yield of extracted cellulose. Therefore, this research will study on the use of alkali with the assist of ultrasonic homogenizer to increase the extraction efficiency.

1.3 Aims and Objectives

The objectives of this research is to:

i. prepare and characterize the sugarcane bagasse.

ii. investigate the effect of ultrasonic-assisted extraction process to the yield of cellulose by varying the temperature, ultrasonic amplitude and alkali concentration.

iii. characterize extracted cellulose with DSC, FTIR and HPLC.

iv. convert extracted cellulose to carboxymethyl cellulose.

1.4 Scope of Study

The scope of study are listed as follows:

i. Study the use of renewable lignocellulosic biomass, sugarcane bagasse as the feedstock for cellulose extraction process. The characteristics and availability of sugarcane in Malaysia is studied.

ii. Study the preparation of sugarcane bagasse powder process. The sugarcane bagasse will need to be dried, cut and crush into smaller size powder for cellulose extraction.

iii. Study the treatment method required in assisting the extraction of cellulose from the sugarcane bagasse. Ultrasonic-assisted treatment in potassium hydroxide solution is used in the cellulose extraction process.

iv. Study the structure of the treated sugarcane bagasse. FTIR, DSC and HPLC characterization test will be carry out for treated sugarcane bagasse to determine its properties.

v. Study and analyse the yield of extracted cellulose. The obtained cellulose will further undergo chemical process to produce carboxymethyl cellulose.

vi. Study the properties of carboxymethyl cellulose produced from cellulose extracted from sugarcane bagasse. The properties include degree of substitution and production of film from synthesised carboxymethyl cellulose.

CHAPTER 2

LITERATURE REVIEW

2.1 Feedstock for Cellulose Production

Cellulose is one of the most abundant polymer available worldwide. It is a common organic compound which serve as feedstock for the production of pulp, fibrous chemical and cellulose derivatives for various application (Chen, 2014). The production of cellulose is approximate 10^{11} to 10^{12} tons per annual (Heinze, El Seoud and Koschella, 2018). The commercial source of cellulose are mainly comes from wood pulp which consists of 40-50 %(w/w) cellulose and cotton linters with 90 %(w/w) cellulose content.

The cellulose in wood pulp is obtained through the treatment of the wood plant. Pulping process is employed to separate lignin, hemicellulose and other substances from the wood either by mechanical or chemical means. On the other hand, cotton linter is the short fiber of seed hairs attached around the cotton seed. It is relatively curly with cylindrical shape and has thick wall. The good accessibility of cotton linter to chemical reagents have make it a high reactivity material as compared to cotton staple fiber. High purity of cellulose content can be obtained from cotton linter through bleaching process (Heinze, El Seoud and Koschella, 2018). However, environmental drawback such as pesticide usage during cotton cultivation have make the dependent of production of cellulose to cotton linter decreases (Olsson and Westman, 2013).

In view of the arising environmental problem with the resulting adverse effect due to increase of residue waste, an alternative source for the production of cellulose is studied and developed. Recently, lignocellulosic biomass as a renewable source has been used in extracting cellulose. It has become the alternative feedstock since it mainly consists of cellulose, hemicellulose and lignin. The main source of lignocellulosic biomass comes from agricultural waste such as sugarcane bagasse, wheat straw, maize stalk, corn cob, coconut husk, nut shell and empty fruit brunch. Other biomass can be derived from food wastes, forest residues, municipal and industrial wastes (Lee, Hamid and Zain, 2014). Table 2.1 shows the content of cellulose, hemicellulose and lignin in some of the agriculture waste.

Table 2.1: Content of Cellulose, Hemicellulose and Lignin in Agriculture Waste.

Agriculture waste	Cellulose %(w/w)	Hemicellulose %(w/w)	Lignin %(w/w)	Source
Corn cob	45	35	15	(Sun and Cheng, 2002)
Coconut husk	20-25	3-12	35-45	(Cabral et al., 2016)
Palm kernel cake	35.7	30.3	15.6	(Shibata et al., 2008)
Sugarcane bagasse	40-50	25-35	18-24	(Mandal and Chakrabarty, 2011)
Wheat straw	35-45	20-30	15	(del Río et al., 2012)

The high fixed carbon content in corn cob make the removal of lignin to be difficult (Shariff et al., 2016). The fixed carbon is the residue of combustible biomass after the ash and volatile matter have been eliminated. The aromatic ring structure with various branches have cause lignin in corn cob only degrades at temperature higher

than 900 ^{o}C (Satimanont, Luengnaruemitchai and Wongkasemjit, 2012). This will lead to presence of lignin in cellulose of corn cob. Meanwhile, the extraction of cellulose from palm kernel cake involves several processes, from oil extraction, pretreatment, bleaching to delignification process. More chemicals are involved in the extraction process, such as hexane used to extract oil, sodium hydroxide, sodium chlorite to delignify wood and acetic acid to hydrolyse hemicellulose. The usage of sodium chlorite and acetic acid have to be optimum as they are toxic and hazardous (Bono et al., 2009). The high usage of chemical will increase the operational cost. On the other hand, wheat straw have high ash content of 6-12 weight percent. Therefore the inorganic compounds within wheat straw will react with each other at high temperatures (NL Agency, 2013). Besides that, it has high carbon to nitrogen content which result in a low biodegradability. The separation of hemicellulose and lignin from cellulose will take a longer time.

Sugarcane bagasse (SCB) is a renewable source and can be obtained in Malaysia even though the cultivation of sugarcane is relatively small, with annual production of 5714 tons of sugarcane in 2016 (Quandl, 2018). SCB is the fiber residue obtained from the crushing of sugarcane in sugar milling process. Basically, fresh sugarcane is composed of 43-52 %(w/w) of fiber, with moisture content of 45-50 %(w/w) at wet basis and 2-6 %(w/w) soluble solids. Meanwhile, composition of cellulose inside SCB is approximate 26.6-54.3 %(w/w), with 14.3–24.45 %(w/w) of lignin and 22.3-29.7 %(w/w) hemicellulose (Katyal, Thambimuthu and Valix, 2003). It has served as raw material for the generation of steam and electricity in boiler. The heating value of approximate 7738 ± 100 kJ/kg enables bagasse to act as alternative fuel within the boiler by burning as a pile, thus increases the combustion efficiency (Barroso et al., 2003). Moreover, it is cheap and has low pollution to the environment.

2.2 Characteristics of Sugarcane Bagasse

The growth of sugarcane is commonly found in tropical and subtropical countries. The world largest sugarcane producer is Brazil with production of 659 million tons of sugarcane per year (Carvalho, 2016). In addition, sugarcane has play an important role in producing billions of gallons of fuel in Brazil, making them energy independent country. Sugarcane bagasse is the left over cane stalk residue from the crushing of the sugarcane for juice extraction. The two main components of the sugarcane stalk are inner pitch surrounded by outer rind. (Mokhena et al., 2018).

Nowadays, the use of sugarcane bagasse as feedstock for the production of chemicals, fuels and additives has been increased. Furthermore, it can also be used in making biodegradable products, such as paper, plates and toilet paper. It is one of the lignocellulosic biomass with abundant supply. Hence, it has become the alternate renewable source to compensate with the depletion of petroleum and fossil fuel resources. Every lignocellulosic biomass comprises of three main biopolymers, which are cellulose, hemicellulose with lignin as shown in Figure 2.1. The exact composition of the biopolymers are vary depending on the source and type of biomass.

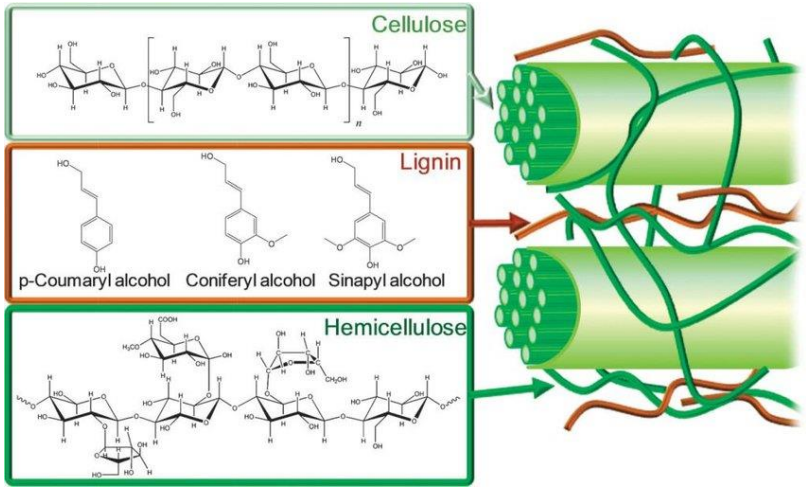

Figure 2.1: Structure of Lignocellulosic Biomass with Cellulose, Hemicellulose and Lignin (Alonso, Wettstein and Dumesic, 2012).

Hemicellulose is a polysaccharide with much lower molecular weight as compared to cellulose. It composed of different monomers that may vary according to its source, mainly from hardwood and softwood. Besides that, there is only small amount of crystalline region presence in hemicellulose (Chen, 2014). The large portion of amorphose structure in hemicellulose make it easily hydrolysed by hemicellulase enzyme, hot dilute acid or cold 5 %(w/v) NaOH solution. It forms chemical bonds with lignin and hydrogen bond with cellulose microfibrils (Chen, 2014; O'Hara, 2011). Hemicellulose normally concentrated in primary and secondary layer of plant cell wall (Saleh, 2014). For SCB, the hemicellulose mainly composed of xylan polysaccharides with glucoronic acid and arabinose as the side groups (Brienzo et al., 2016). Meanwhile, hemicellulose film have oxygen permeability property which make it favourable material for production of food packaging. (Hansen and Plackett, 2008).

Lignin is a complex structure of highly branched phenolic polymer with high molecular weight (Pérez et al., 2002). It is formed from three precursor alcohols which are p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol as shown in Figure 2.2 (Karp et al., 2013). Respective alcohol can further derived to phenylpropane olignol units, which are p-hydroxylphenyl (H), guaiacyl (G) and syringyl (S) which linked randomly through bonding of hydroxyl and carbonyl structure (Chen, 2014). The study from Doherty et.al. (2017) shows that SCB consists lignin rich in p-hydroxylphenyl unit. Lignin which binds covalently to adjacent cellulose fibers provide strength to the cell wall rigidity and resistance against pests, diseases and oxidative stress. It is an amorphous heteropolymer, optically inactive, insoluble in water and poly-aromatic in nature.

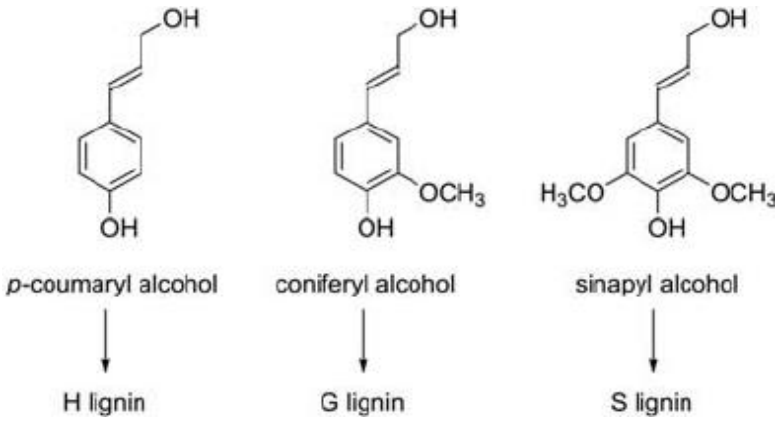

Figure 2.2: Structure Unit of Lignin (Stark, Yelle and Agarwal, 2015).

Cellulose is the main constituent of the lingocellulosic plant cell wall with about 30-50 %(w/w) of content in lignocellulosic biomass. It is a linear polymer comprises of D-anhydroglucopyranose (AGU) unit linked together by β-1,4-glycosidic bonds. There are three hydroxyl groups attached to AGU, with secondary OH on C2 and C3 position and a primary OH on C6 as shown in Figure 2.3 (Olsson and Westman, 2013). Meanwhile, the number of AGU unit within the chain can be expressed as the degree of polymerization. Normally, SCB will have the degree of polymerization between 800 to 1900 (O'Hara, 2011). These polysaccharide chains are packed together in microfibrils by hydrogen bonds. Meanwhile, these microfibrils attached to each other by hemicelluloses and other polymer, with lignin as cover (Taherzadeh and Karimi, 2008). The reactivity of cellulose can be affected by its amorphous and crystalline structure. The high crystallinity of cellulose has make it insoluble in most of the common solvent (Viera et al., 2007).

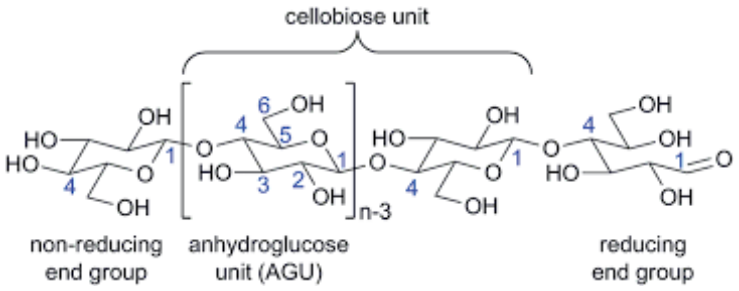

Figure 2.3: Structure of Cellulose (Credou and Berthelot, 2014).

2.3 Cellulose Production Process

Every lignocellulosic biomass mainly composes of cellulose, hemicellulose and lignin. Cellulose is the main component in lignocellulosic biomass which consist of mostly crystalline and some amorphous structure, embedded in composite structure, mainly composed of lignin and hemicellulose. It can be found in the rigid cell wall of the plant and is associated with hydrogen bonding, making it resist to destruct or degrade in organic solvent (Singh and Singh, 2012). The commercialized cellulose production are mainly from wood pulp and cotton linter.

The cellulose in wood pulp can be obtained through pulping process shown in Figure 2.4. The bark has to be removed from the wood, which then cut into chips. Firstly, it will be fed into digester for alteration of its structure into individual fibers. The digester is favoured to operate in continuous mode since it requires lesser capital investment as compared to batch mode. In the process, sodium sulphide is used to dissolve lignin structure in the presence of heat. However, there is still presence of small amount of lignin and short chain carbohydrate within the structure. A further treatment is required to purify the structure and obtained a pure cellulose. Then, the wood pulp is mixed with chlorine to get rid of the remaining lignin. It is a continuous operation at low pH and short treatment time. After that, the chlorinated pulp proceeds to caustic soda extraction to remove any of the chlorinated lignin and short chain carbohydrate. Finally, the wood cellulose will undergo bleaching process to obtain a whiter cellulose since wood pulp is brownish. Sulphur dioxide can be soured on the cellulose to kill any residual of bleach (Wayman, 1958).

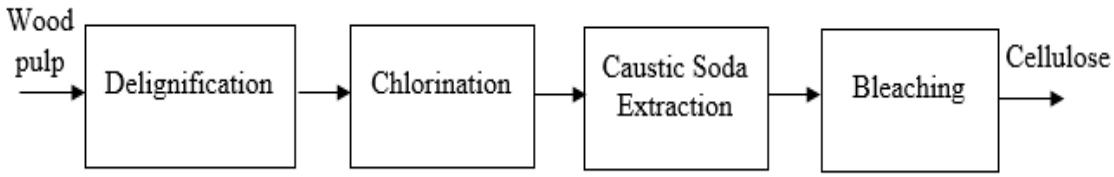

Figure 2.4: Production of Cellulose from Wood Pulp (Wayman, 1958).

Meanwhile, cotton ball is harvested and separated to cotton seed and linter fuzz through grinning process. The cotton seed and linter fuzz is further processed in oil mill to produce vegetable oil. During the process, linter is being separated out before oil is pressed. The cotton linter obtained need to be purified to obtained pure cellulose. Firstly, it will undergo bale opening to remove any contamination and impurities such as sand, stone and pectin. Then, caustic soda is added in the digestion process to solubilised pectin, protein and saponify fats and waxes. The cotton linter is then go through bleaching process to obtain high purity cellulose (Heinze, El Seoud and Koschella, 2018).

2.4 Pretreatment Technology in Cellulose Extraction

2.4.1 Autoclaving

Pretreatment of biomass is studied to remove the impurities such as ash and wax before it is treated for cellulose extraction process. One of the pretreatment is by autoclaving, whereby it works with the combination of pressure, steam, temperature and time (Avinash, 2018). Autoclave is a sterilizer that operates at high pressure, thereby increase the heat content in autoclave and enable the steam to reach high temperature (Judelson, 2004). In the extraction of cellulose from SCB, a high temperature will induce the breakage of intramolecular hydrogen bonds of the impurities component, which is lignin and hemicellulose, thereby solubilised the unwanted component. The common temperature used in autoclave is 121 ^{o}C.

The autoclave provides sterilization and disinfection function. The high temperature helps to remove contamination and kill microorganism, spore and bacteria by dehydrating the cell (Avinash, 2018). Autoclave is also used in assist the determination of carbohydrate and lignin content in rice hull (Martín et al., 2007). On the other hand, combination of alkaline pretreatment with autoclave at 121 ^{o}C for 40 minutes is used to solubilise the lignin and hemicellulose content in coconut husk (Eduardo et al., 2016). Meanwhile, Wheat straw or wood chips undergo autoclaving process at 121 ^{o}C for 60 minutes to eliminate the germinated spores within the composition (Kuijk et al., 2016).

2.4.2 Soxhlet Extraction

Soxhlet extraction is used in removing wax, oil and break the chain of hemicellulose and lignin. It is a continuous extraction whereby the mixture is repeatedly washed with organic solvent under reflux in a special glassware. There is supply of inert gas in the setup to prevent oxidation during extraction (Ain and Sukri, 2012).

The setup of soxhlet extractor consists of thimble equipped with condenser on top and connected to conical flask at the bottom. The solid mixture wrapped with small cloth is placed inside the thimble, in which solvent will pass through the area. A hot plate is placed at the bottom of the flask to maintain the temperature of solvent at desired temperature, ensuring the flow of solvent vapour housing the solid mixture. Meanwhile, solvent vapour that rises up through distillation arm will be cool down by condenser and drip back to the extraction chamber (Ain and Sukri, 2012). The solvent will gradually filled up the extraction chamber, resulting in the removal of solvent through siphon side arms.

The cycle is generally repeated for 6 hours to remove the unwanted component such as wax, lignin and hemicellulose inside SCB with chloroform/ethanol (2:1, v/v) as organic solvent (Liu et al, 2006). On the other hand, cornstalk undergo 24 hours of soxhlet extraction in toluene/ethanol (2:1, v/v) to isolate hemicellulose from the structure (Shui et al., 2017). Besides that, hexane is used to separate the lipids and hydrocarbon compound in wheat straw through 6 hours of soxhlet extraction (Naik et al., 2010). The extraction time is varied according to the type of material being extracted.

The common organic solvents used in soxhlet extraction are ethanol, isopropanol, hexane and combination of toluene with ethanol. The solvent used is based on the polarity of the extracted material. Ethanol and isopropanol are polar solvent, thus it should be used to extract materials that are less likely soluble in water. Meanwhile, hexane and toluene are non-polar solvent. They can be used to extract wax which is non polar, similar properties as them without harming the safety of environment. Besides that, hexane is relative easy to be removed from the solid and only require low energy usage due to its low sensible of heat. In addition, it does not cause great harmful to human skin even expose with long usage time (Anderson, 2018). Soxhlet extraction is normally used as treatment because it gives a good and even contact between biomass and solvent, enhancing the transfer equilibrium. Furthermore, the equipment is inexpensive and the temperature of the process can be maintained, thereby assisting the extraction process (Luque and García-Ayuso, 1998).

2.4.3 Steam Explosion

Steam explosion is one of the physicochemical treatment used to treat lignocellulosic biomass. This treatment process is carried out at high pressure vary from 0.69 MPa to 4.83 MPa and temperature range of 160 ^{o}C to 260 ^{o}C. (Karp et al., 2013). A sudden reduce in pressure will result in the explosive decompression of biomass, subsequently breakdown the lignocellulosic structure. Meanwhile, the high temperature will disrupt and degrade the hemicellulose and lignin structure within the agriculture biomass.

The factors such as residence time, temperature and pressure of treatment will affect the steam explosion treatment. A high temperature and short residence time will assist in hemicellulose solubilisation (Kumar et al., 2009). The studies of Saelee et al. (2014) on steam explosion treatment of SCB was carried out at temperature of 195 ^{o}C for 15 minutes. It has shown that the initial 44.5 %(w/w) of cellulose content within SCB have increase to 65.7 %(w/w) after treatment with steam explosion. On the other hand, the hemicellulose and lignin content have decreases after treatment. It was found out that the hemicellulose have a larger reduction as compared to lignin since lignin only undergoes partial hydrolysis and decomposition. The strong interaction of cellulose fibers with polyphenolic compounds resulting in a lower reduction of lignin (Saelee et al., 2014).

Steam explosion only needs a lower capital investment and create less environmental impact to the environment. The common chemicals used in the treatment are ethanol and 1-2 %(w/v) of sodium hydroxide solution. The quantity requires are little, thus less hazardous (Avellar and Glasser, 1998). Meanwhile, there are treatment that does not require any input of chemical. However, there is some limitation such as the disruption of lignin is incomplete and only small portion of xylan fraction is removed. In addition, the current reactor available is in batch design, thus prohibiting treatment in large volume (Jacquet et al., 2015).

2.4.4 Liquid Hot Water Treatment

Liquid hot water is cooked under high pressure which allow the penetration of water into the lignocellulosic biomass. As a result, the hot liquid water percolating through the lignocellulosic biomass causing solubilisation of hemicellulose and lignin under high temperature that subsequently leach out the biopolymer (Taherzadeh and Karimi, 2008). Inside the reactor, the operation mode of liquid hot water treatment can be either in concurrent, countercurrent or flow through configuration (Mosier et al., 2005). It is environmental friendly hydrothermal treatment since no chemical is required in this process. In addition, it has high pentosan recovery and less formation of undesired product due to the high removal of hemicellulose in oligomers form. Generally, this treatment mainly removes hemicellulose and small amount of lignin (Taherzadeh and Karimi, 2008).

The high temperature of water enable the breakage of bonding in lignocellulosic biomass, thereby eliminates the need for size reduction of the lignocellulosic biomass before the treatment (Mosier et al., 2005). From the studies of Allen et al. (1996), SCB is completely immersed within the liquid hot water at temperature of 190 to 230 ^{o}C. All hemicellulose with more than 60 % of the acid-insoluble lignin is solubilised in the water medium. The residence time of the process can be reduced by adding the hot liquid water into the reactor instead of preheat the cool water inside the reactor. This treatment enables a better pH control which help to reduce the formation of inhibitors (Maurya, Singla and Negi, 2015). However, the large water consumption in the treatment is a major issue that need to be considered.

2.4.5 Wet Oxidation

Wet oxidation is suitable to be used as treatment for lignocellulosic biomass with high lignin content. The important parameters in this treatment are temperature, oxygen pressure and reaction time. Typically, the biomass will be treated with water and air or oxygen at temperature higher than 120 ^{o}C with reaction time of 10 to 20 minutes. Besides that, the air pressure inside the reaction vessel is maintained at 12 bar (Brodeur

et al., 2011). Initially, the wet oxidation process will form acid from hydrolytic process. The increases of acid concentration will cause the breakdown of hemicellulose into lower molecular weight fragment (Mcginnis, Wilson and Mullen, 1983).

The studies of Martín, Klinke and Thomsen (2007) on wet oxidation treatment of SCB has shown that the optimum amount of cellulose is obtained at temperature of 195 ^{o}C and residence time of 15 minutes in alkali medium with the addition of sodium carbonate (Na_2CO_3). The treatment is carried out in alkaline condition, obtaining 70 %(w/w) of cellulose content. On the other hand, the initial 31.1 %(w/w) of hemicellulose and 11.4 %(w/w) of lignin has reduced to 4.1 %(w/w) and 9.5 %(w/w) respectively. The phenolic derivatives unit inside lignin are reactive to the wet oxidation condition. Thus, the high temperature and long residence time with alkaline medium facilitate the solubilisation of lignin. The alkaline medium also helps to reduce the formation of toxic by-product such as furaldehydes. Meanwhile, the wet oxidation at same temperature and residence time but in acidic medium will gives low hemicellulose fraction as well, on the same time a lower cellulose content since acid has destruct some of the cellulose, forming by-product such as carboxylic acid.

Besides that, wet oxidation of rice husk at temperature of 195 ^{o}C and residence time of 10 minutes with addition of 1 g sodium carbonate (Na_2CO_3) has extracted 68.6 %(w/w) of cellulose. The content of lignin and hemicellulose has reduced drastically under the high pressure and temperature condition (Banerjee et al., 2009). Furthermore, the cellulose content in wheat straw which undergo wet oxidation at 170 ^{o}C and residence time of 10 minutes in alkali medium has increases from 38 %(w/w) to 74.1 %(w/w). The amount of hemicellulose has reduced half due to its branched structure which make it unstable during wet oxidation (Bjerre et al., 1996). The drawback of this treatment is it requires to operate at high temperature and pressure, leading to high utility and maintenance cost. Besides that, cost of oxygen will need to be taken into consideration for this treatment (Martín, Klinke and Thomsen, 2007).

2.4.6 Acid Hydrolysis Treatment

The chemical treatment used to treat the lignocellulosic biomass includes acid hydrolysis, alkali hydrolysis and organosolv treatment. The acid hydrolysis treatment can be performed in concentrated or diluted form to extract the cellulose. The most common acid used is sulphuric acid, while the other type of acids used are nitric acid, hydrochloric acid, phosphoric acid and acetic acid. This treatment could carried out at temperature range of 130 ^{o}C to 210 ^{o}C, with 0.2 %(w/w) to 2.5 %(w/w) of acid (Brodeur et al., 2011).

The use of dilute acid is more favourable to be used in industry scale since it will form less furfural and hydroxymethylfurfural. Besides that, it can avoid the hydrolysis of hemicellulose into monomers which can subsequently degrades to furfural (Brienzo et.al., 2016). Meanwhile, concentrated acid treatment is the contrary of dilute acid treatment as it will form the inhibiting compounds such as furfural and phenolic acid. The high concentration of acid will cause corrosion problem since it is toxic and hazardous chemical which need to be handle with care. On the other hand, dilute acid helps to reduce the occurrence of corrosion problem. Nowadays, the two common dilute acid treatment process available can be carried out either at high temperature with a short residence time or low temperature with longer retention time (Maurya, Singla and Negi, 2015).

The research conducted by Canilha et al. (2011) shows 59.3 %(w/w) of cellulose, 3.7 %(w/w) of hemicellulose and 33.8 %(w/w) of lignin in 41.7 %(w/w) of solubilised SCB when immersed in 2.5 M of dilute sulphuric acid at temperature of 150 ^{o}C and residence time of 30 minutes. A high reaction rate can be achieved through dilute acid treatment. It was found that hemicellulose solubilise more rapidly as compared to lignin. This phenomena happens because dilute acid can easily hydrolyse acetylated glucuronoarabinoxylan, which present within hemicellulose. Meanwhile, the lignin still available in a large proportion due to the condensation reactions which prevent the solubilisation of lignin (Candido, Godoy and Gonçalves, 2012). The structure of cellulose does not break down since it has high crystallinity. Therefore, the final product consists of cellulose and lignin will need to undergo further reaction to obtain pure cellulose (Neureiter et al., 2002). Similar result obtained when corn cobs

undergo acid treatment using 5 %(w/v) of sulphuric acid. The amount of cellulose has increased from 42.75 %(w/w) to 55.37 %(w/w) while 45 %(w/w) of hemicellulose has decreased to 29.11 %(w/w) as the reaction time increases from 10 to 40 minutes. The amount of lignin being removed is very low (Ogunbayo, Olanipekun and Babatunde, 2016).

2.4.7 Alkaline Hydrolysis Treatment

Alkaline hydrolysis is the most common treatment used in extraction of cellulose because less degradation of cellulose occurs (Karp et al., 2013). It commonly uses sodium hydroxide, potassium hydroxide, ammonium hydroxide and calcium hydroxide as the treatment agent. It can be carried out at lower temperature and higher alkali concentration with a longer treatment time (Mosier et al., 2005). Alkali treatment can alter and degrade ester and glycosidic side chains of lignin without disrupt the other components, assist the removal of acetyl and uronic acid present in hemicellulose (Brodeur et al., 2011).

The common alkali used will be sodium hydroxide, NaOH since it has relatively high alkalinity which can assist the fractionation of lignocellulosic biomass (Bensah and Mensah, 2013). Dilute NaOH helps to loosen and separate the bonds and linkage between the lignin, thereby disrupt the lignin structure, providing a bigger internal surface area which will promotes the intake of water for further disruption of lignin. This treatment is preferable since it can be carried out in milder condition, solubilise the lignin without affecting other component (Brienzo et.al., 2016). The remaining hemicellulose and cellulose can be further separated at room temperature.

In the alkaline treatment developed by Henderson, Champagne and Tudoret (2003), the extraction of cellulose from SCB can achieved up to 70 %(w/w) by using 0.5 N potassium hydroxide, KOH at temperature of 70 ^{o}C with duration of 1 hour. The process involves the soaking of SCB in the alkaline solution medium and mix it for a period of time. On the other hand, coconut husk which undergo 5 %(w/v) NaOH alkali treatment shows the increases of cellulose content from 24.7 %(w/w) to 55.17 %(w/w).

The alkali treatment also leads to reduction on its lignin content from 40.1 %(w/w) to 29.91 %(w/w) (Cabral et al., 2016). When wheat straw is pretreated with 1.5 %(w/v) NaOH, around 60 %(w/w) of lignin content is solubilised and removed (Sun, Lawther and Banks, 1995).

Recently, treatment with lime has draws the attention of researcher since it only requires low cost and easily recovered as compared to other alkaline treatment. Besides that, it can be operated under low temperature and pressure (Chang, Nagwani and Holtzapple, 1998). Kim (2004) has shown that the lime treatment of corn stover at temperature range of 25 ^{o}C to 55 ^{o}C is able to solubilise lignin and hemicellulose without affecting the cellulose content of material, hence increase the crystallinity of corn stover. Despite the advantage of lime treatment, there are some drawbacks as compared to other alkali reagent. Lime is slightly insoluble in water, thus making the dissolving rate to be lower and more water is needed in the treatment to overcome the low solubility (Bensah and Mensah, 2013).

2.4.8 Organosolv Treatment

Organosolv treatment utilize organic solvent, with or without acid catalyst to extract cellulose from lignocellulosic biomass by breaking the internal network of hemicellulose and lignin. Methanol, acetone and ethylene glycol are the common solvent used in this treatment process (Kumar et al., 2009). The acid catalyst such as hydrochloric acid, phosphoric acid and formic acid are added to enhance the degradation of lignin. Moreover, it enables the treatment to be carried out at temperature below 180 ^{o}C. Low acid concentration will assure the maximum degrade of lignin with high cellulose content. This treatment assist the extraction of cellulose while lignin and hemicellulose are separated out from biomass (Zhao, Cheng and Liu, 2009).

Generally, the lignocellulosic biomass is treated with organic solvent at temperature range of 100-250 ^{o}C depending on the presence of catalyst. Then, lignin is removed and hemicellulose is solubilised along the treatment process. After the

treatment, organic solvent will be drained out of the reactor. Since the organic solvent is expensive, recovery process is needed to recover the solvent, which on the same time causes consumption of energy. The organic solvent has to be evaporated and condensed before recycle back to the reactor (Zhao, Cheng and Liu, 2009). However, the disadvantage of this treatment is that organic solvent has relatively high volatility that limit the treatment to be conducted in a tight or non-explosive medium.

From the study of Area, Felissia and Vallejos (2009), SCB undergo alkali pretreatment using NaOH followed by organosolv treatment of ethanol with sulphuric acid as catalyst. Under optimum condition at temperature of 160 ^{o}C and residence time of 120 minutes, the lignin presence is the lowest. Basically, the crystallinity of treated SCB is increased as more amorphous lignin and hemicellulose is removed during the treatment. Besides that, acetone-based organosolv treatment with acetone-water 50:50 %(w/w) conducted on wheat straw at 205 ^{o}C has shown a high cellulose recovery of 93 %(w/w), with 79 %(w/w) of lignin is being solubilised (Huijgen, Reith and den Uil, 2010). In the research of Fialho (2015), corn cob in ethanol:water, 50:50 %(w/w) gave 79 %(w/w) of lignin removal and high amount of hemicellulose is solubilised at 190 ^{o}C. It is favourable to use low molecular weight alcohol such as methanol and ethanol as the solvent since it has lower boiling point which can be recovered by simple distillation with relative lower energy. However, the treatment with ethanol is safer since it is less hazard compared to methanol (Mesa et al., 2011).

2.4.9 Ultrasonic-assisted Treatment

Ultrasonic-assisted treatment is a mechanical treatment which helps to separate the component of lignocellulosic biomass and extract mainly the low molecular weight components. The sonication will induce cavitation, forming bubbles which will grow, oscillate and split at critical pressure. The collapse of the cavitation bubbles causes generation of shock wave which carry energy, thereby assist the components of lignocellulosic biomass to be removed and extracted out (Segneanu et al., 2013). Lignin and hemicellulose are removed out through homolysis of lignin-carbohydrate bonds during sonication process (Ur Rehman et al., 2013). The range of frequency of

ultrasound is between 20 kHz to 1 GHz. Different frequency will gives different vibration which propagate at various speed (Gonzalez-Fernandez et.al, 2015). Usually, the ultrasonic process will be assisted with physical or chemical treatment for the extraction of cellulose.

Liu et al. (2006) had come out with a research whereby extracting cellulose by treating the SCB with chlorite followed by ultrasonic irradiation process. The composition of SCB before pretreatment has 43.6 %(w/w) of cellulose. In the treatment, sodium chlorite is an alkali added for the removal of hemicellulose from SCB. Then, SCB undergo ultrasonic process in the presence of 10 %(w/v) potassium hydroxide, KOH at temperature of 23 ^{o}C for 16 hours. The treatment has extracted 57.2 %(w/w) of cellulose. The increases of cellulose amount is due to the presence of alkali which disrupted the cell wall of SCB and solubilised hemicellulose that present at outer surface of SCB.

On the other hand, ultrasound-assisted ammonia treatment have been carried out by Ramadoss and Muthukumar (2014). SCB is added with 10 %(w/v) liquid ammonia at 80 ^{o}C and undergo ultrasonic for 45 minutes. The untreated SCB with 38 %(w/w) of cellulose has increase to 56.1 %(w/w) after the treatment. Ammonia is a volatile chemical that can break the complex bonds in SCB and degrade molecular structure of lignin. This treatment gives the benefit of no by-product formation and can be carried out at moderate temperature. Besides that, the ultrasonic treatment of wheat straw with 0.5 M KOH for 35 minutes has solubilised 50 %(w/w) of the lignin, extracting a higher amount of cellulose (Sun and Tomkinson, 2002).

The acceleration of extraction of cellulose during ultrasonic treatment can be achieved due to the mechanical and chemical effects of ultrasound process. The extraction efficiency is depending on the frequency, temperature and duration of ultrasonic process. The frequency of ultrasound at 10 to 100 kHz is ideal in assisting the degradation and breakage of lignocellulosic biomass (Ur Rehman et al., 2013). Meanwhile, the temperature of ultrasonic process is dependent on the type of lignocellulosic biomass. The temperature changes between 20 to 60 ^{o}C will not affect the dynamics of cavitation formed. However, a large uplift of temperature will result

in adverse effect of the ultrasonic treatment process, since more volatile components are formed in the cavity (Prabhu, Gogate and Pandit, 2004). Lastly, different lignocellulosic biomass has an ideal duration for ultrasonic treatment. The increase of duration without exceeding the limit helps to increase the delignification of lignocellulosic biomass, thus increase the extraction of cellulose (Ur Rehman et al., 2013).

2.5 Production of Carboxymethyl Cellulose (CMC)

The low solubility of treated cellulose in water or organic solvents has reduces its usage in some of the industrial process. The way to overcome this problem is by further converting cellulose into its derivative such as carboxymethyl cellulose (CMC) which is soluble in water due to the presence of hydroxyl and carboxylic groups (Golbaghi, Khamforoush and Hatami, 2017). It involves a simple and low cost production process. The process flow for production of CMC is shown in Figure 2.5 which involves two main reaction steps which are alkalization and etherification.

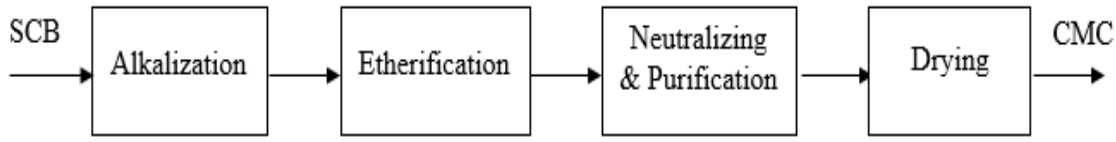

Figure 2.5: Process Flow for Production of CMC (Shui et al., 2017).

CMC is a linear long chain polysaccharide that is biodegradable and non-toxic. The physical and chemical properties of CMC are determined by their molecular weight, the number of carboxymethyl group per anhydroglucose unit (AGU), which is also known as degree of substitution (DS) and clustering of carboxyl substituent's in each polymer chains (Singh and Singh, 2012). The DS will affect CMC solubility, shearing stability and its rheological behavior (Bono et.al., 2009). A high DS can be achieved by uplift the concentration of NaOH to 30 %(w/v), whereby causing CMC has more resistant to degradation. It has extensive usage in various industries such as

in pharmaceutical, oil drilling, paint, paper, textile, cosmetics, detergent and food industries. Table 2.2 shows the application and function of CMC in each of the industries.

Table 2.2: Application of CMC in Various Industry.

Industry	Application	Function	Reference
Detergent	Laundry	Anti-dirt agent	(Singh and Singh, 2012)
Food	Ice cream	Thickener	(Lavanya et al., 2011)
Oil drilling	Ingredient of drilling mud	-Viscosity modifier -Water retention agent	(Lavanya et al., 2011)
Pharmaceutical	Drug formulation	Gelling agent	(Shokri and Adibkia, 2013)
Textile	Printing paste	Thickener	(Fijan et al., 2009)

During the production of CMC, it will firstly undergo alkalization as shown in Equation 2.1 by mixing the cellulose powder with isopropanol at different sodium hydroxide (NaOH) concentration. Isopropanol serves as the function to provide accessibility of etherifying agent to the reaction centers of cellulose chain. The reaction efficiency is high when using low polarity solvent such as isopropanol (Toğrul and Arslan, 2003). The mixture is then place in water bath and undergo stirring process to alkalize the cellulose. Sodium hydroxide is able to swollen the cellulose chain, thereby providing the space for the substitution of sodium carboxymethyl group in cellulose units (Alizadeh, Mousavi and Labbafi, 2017).

After that, different amount of sodium monochloroacetate (SMCA) is added and mechanically stirred to initiate the etherification process as shown in Equation 2.2. A larger amount of SMCA can result in increasing of CMC yield (Huang et al., 2017).

SMCA is the reagent that substitutes hydroxyl group at C2, C3 and C6 with sodium carboxymethyl group (Alizadeh, Mousavi and Labbafi, 2017).The solid residue formed is filtered and neutralized with acetic acid to remove the leftover NaOH in the mixture. Next, the CMC formed is being washed with ethanol and filtered to obtain a pure product. The residual of alcohol is removed by drying in an oven at temperature range of 60 to 80 ^{o}C. The purified CMC will be in white or cream color, tasteless and odorless. Equation 2.2 also shows the presence of NaCl as by-product during the production of CMC.

$$R_{Cell}OH + NaOH \rightarrow R_{Cell}OH.NaOH \quad (2.1)$$

$$R_{Cell}OH.NaOH + ClCH_2COONa \rightarrow R_{Cell}OCH_2COONa + NaCl + H_2O \quad (2.2)$$

Where,

$R_{Cell}OH$	= Cellulose chain
$R_{Cell}OH.NaOH$	= Alkali cellulose
$ClCH_2COONa$	= Sodium monochloroacetate
$R_{Cell}OCH_2COONa$	= Carboxymethyl cellulose
NaCl	= Sodium chloride

CHAPTER 3

METHODOLOGY

3.1 Research Flow Chart

The feedstock used to extract cellulose in this study is sugarcane bagasse (SCB). Figure 3.1 presented the research flow chart. At first, SCB was collected and prepared in powder form for subsequent treatment. Autoclave and reflux pretreatment were performed and pretreatment resulted higher cellulose content was selected. Then, pretreated SCB further undergo alkaline ultrasonic-assisted extraction by varying the ultrasonic amplitude, temperature and concentration of potassium hydroxide solution. The sample with highest amount of cellulose was further converted to carboxymethyl cellulose (CMC) through alkalization and etherification process. Characterization of SCB, treated SCB and CMC were carried out to determine its properties.

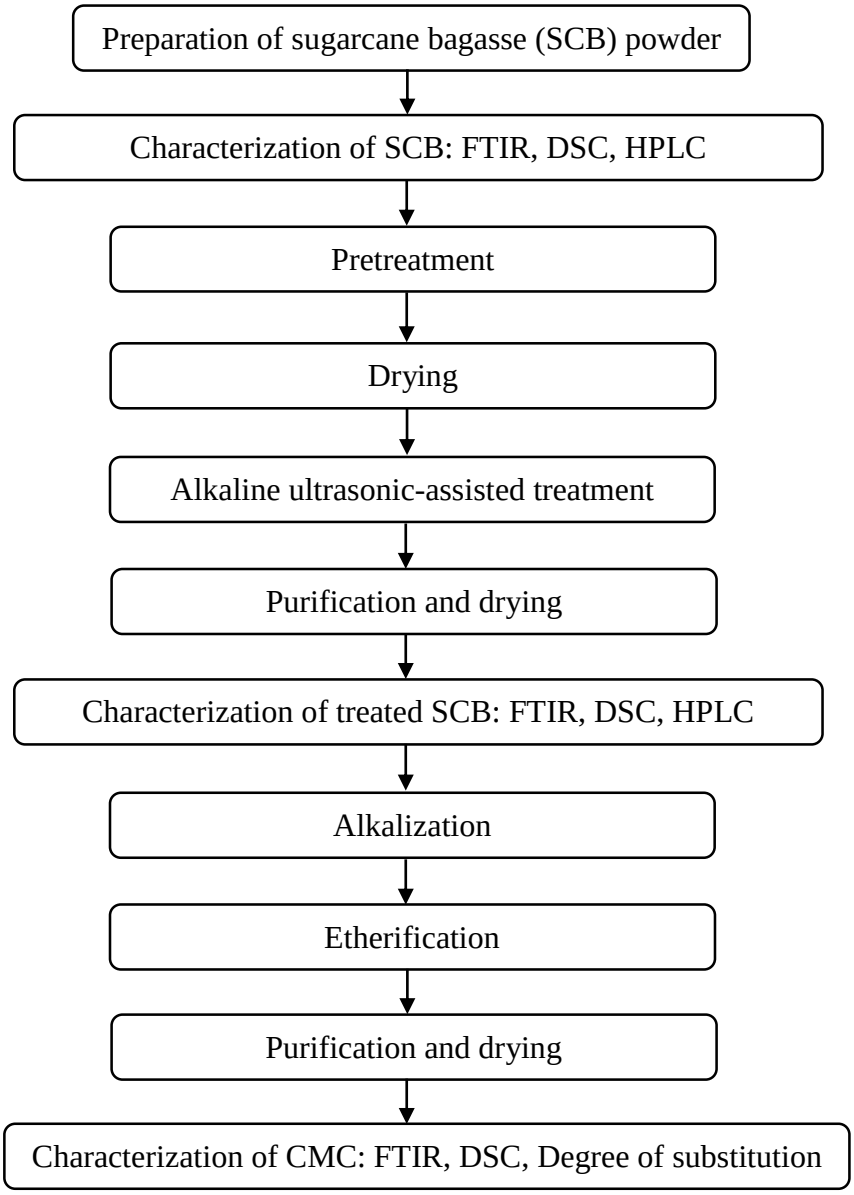

Figure 3.1: Research Flow Chart.

3.2 Material and Chemicals

Table 3.1: Chemical Used in the Research.

Chemical	Source	Purity
Ethanol	HmbG Chemicals, UK	95-98 %
Glycerol	R&M Chemicals, UK	99.8 %
Hydrochloric acid	Qrec, Johor	37 %
Methanol	Merck, UK	99.9 %
Nitric acid	Merck, UK	70 %
Potassium hydroxide	R&M Chemicals, UK	-
Sodium hydroxide	R&M Chemicals, UK	-
Sodium monochloroacetate (SMCA)	Merck, UK	98 %
Sulphuric acid	R&M Chemicals, UK	95-98 %
Isopropanol	Parchem, Singapore	99.5 %

3.3 Preparation of Sugarcane Bagasse Powder

The sugarcane bagasse (SCB) was collected from Kampar, Perak and cleaned before cut into small pieces. It was then dried in an oven overnight at 60 ^{o}C. The mass of the SCB was weighed and drying process continues until achieved a constant mass. Then, the dried SCB was ground and crushed to powder using a crusher and a mortar. After that, the grounded powder will be filtered to 18 mesh size. The SCB powder obtained will be stored in an air-tight container to preserve in dry condition.

3.4 Pretreatment of SCB

Approximate 10 g of dried SCB powder were undergo pretreatment to remove wax and small amount of lignin and hemicellulose by refluxing in 500 ml of 95-98 %(v/v) ethanol at 80 ^{o}C for 2 hours. Figure 3.2 shows the set-up of the refluxing process. Then, it was washed with distilled water and dried in the oven at 60 ^{o}C for 16 hours.

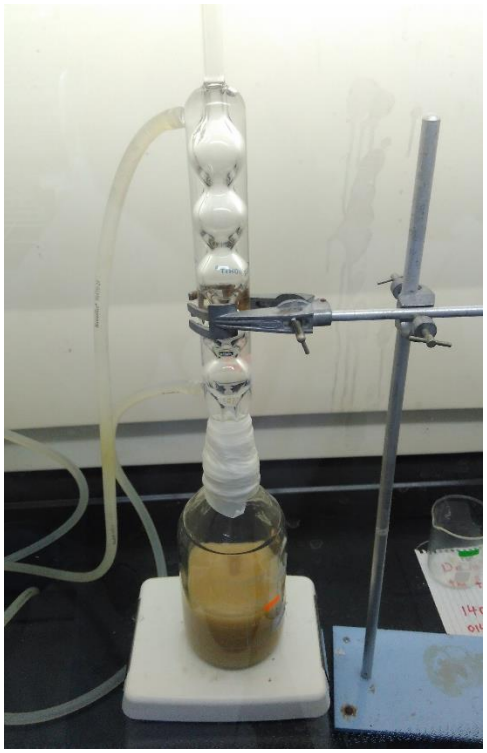

Figure 3.2: Reflux Pretreatment Setup.

On the other hand, another pretreatment was studied to determine the ideal pretreatment for extraction of cellulose from SCB. 10 g of the dried and ground SCB were added into Scott bottle with 200 ml of distilled water. Then, it was placed inside autoclave machine and treated for 30 minutes at 120 ^{o}C (Abo-State et al., 2013). Next, the treated SCB was filtered and the precipitate was dried in the oven at 60 ^{o}C for 16 hours.

3.5 Treatment for Extraction of Cellulose from SCB

Ultrasonic-assisted alkali treatment as shown in Figure 3.3 was used to extract the cellulose from SCB. Firstly, 5 g of pretreated SCB was added into beaker containing 150 ml of potassium hydroxide (KOH) solution. The duration of ultrasonic treatment was fixed to 30 minutes. Meanwhile, the manipulated parameters in this research are the amplitude of ultrasonic homogenizer, treatment temperature and concentration of potassium hydroxide. For each 10 minutes interval, the beaker was taken out from the ultrasonic homogenizer and heated with a heating plate to its desired temperature with the purpose to overcome the inconsistency of the temperature during the treatment.

There were 11 sets of experiments conducted by varying one of the manipulated parameter while the other two parameters were fixed. First investigated parameter was ultrasonic amplitude at 20 %, 30 % and 40 % as shown in Table 3.2 while the treatment temperature and KOH concentration were fixed at 80 ^{o}C and 0.75 M respectively. Meanwhile, Table 3.3 shows the treatment temperature was being manipulated at 70 ^{o}C, 80 ^{o}C and 90 ^{o}C, with ultrasonic amplitude of 30 % and 0.75 M of KOH were fixed along the process. Concentration of potassium hydroxide solution was manipulated within the range of 0.25 M to 1.25 M, with interval of 0.25 M as tabulated in Table 3.4. Ultrasonic amplitude of 30 % with temperature of 80 ^{o}C were constant when concentration of KOH was varied.

Figure 3.3: Ultrasonic Homogenizer Equipment Setup.

Table 3.2: Treatment at Various Amplitudes by Using Ultrasonic Homogenizer.

Experiment No.	SCB (g)	Temperature (°C)	Concentration of potassium hydroxide (M)	Amplitude (%)
1	5	80	0.75	20
2	5	80	0.75	30
3	5	80	0.75	40

Table 3.3: Treatment at Various Temperatures by Using Ultrasonic Homogenizer.

Experiment No.	SCB (g)	Temperature (°C)	Concentration of potassium hydroxide (M)	Amplitude (%)
4	5	70	0.75	30
5	5	80	0.75	30
6	5	90	0.75	30

Table 3.4: Treatment at Various Potassium Hydroxide Solution Concentration.

Experiment No.	SCB (g)	Temperature (°C)	Concentration of potassium hydroxide (M)	Amplitude (%)
7	5	80	0.25	30
8	5	80	0.5	30
9	5	80	0.75	30
10	5	80	1.0	30
11	5	80	1.25	30

After the treatment process, the mixture was stirred at 900 rpm for 10 minutes. Then, it was washed continuously for 3 times with 200 ml distilled water to remove the potassium hydroxide and soluble extractives products. After that, the residue was dried in the oven at 60 °C for 16 hours. The dried treated SCB was weighted and the yield of treated SCB was calculated using Equation 3.1.

$$Yield\ of\ treated\ SCB\ (\%) = \frac{weight\ of\ treated\ SCB\ (g)}{weight\ of\ SCB\ used\ (g)}\ x\ 100\ \% \qquad (3.1)$$

Characterization tests including FTIR, HPLC and DSC were carried out to study the properties of treated SCB.

3.6 Synthesis of CMC from Cellulose

Firstly, 5 g of cellulose powder was added with 100 ml of isopropanol into Scott bottle. Then, it undergoes alkalization process by addition of 10 ml of 17.5 %(w/v) NaOH drop wise and let it stirred for 1 hour at ambient temperature. Next, 6 g of SMCA was added into the mixture with continuous stirring for 2 hours in water bath with temperature maintaining at 50 ^{o}C. The mixture obtained is then filtered and purified with hot ethanol for 3 times to remove undesired product. After that, the mixture was filtered with 200 ml of methanol. The obtained CMC was dried in an oven at 60 ^{o}C (Bono et al., 2009). After that, it was weighted and the yield of CMC was calculated using Equation 3.2.

$$Yield\ of\ CMC\ (\%) = \frac{weight\ of\ dried\ CMC\ (g)}{weight\ of\ cellulose\ used\ (g)}\ x\ 100\ \% \qquad (3.2)$$

3.7 Characterization of SCB, treated SCB and CMC

3.7.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR serves as the function in determining the functional groups changes within a biopolymer substance (Alizadeh, Mousavi and Labbafi, 2017). In the research, FTIR spectrophotometer helps to identify functional group presence in SCB by mixing 1 % of dried ground sample with potassium bromide (KBr) to produce pellets (Zhang et al., 2013). Then, each spectrum with step size of 4 cm^{-1} was collected for 28 scans.

The recorded infra spectra was in the wavelength range of 400 to 4000 cm^{-1} (Singh and Singh, 2012). Samples that tested using FTIR includes grounded SCB, treated SCB and CMC.

3.7.2 Differential Scanning Calorimeter (DSC) Analysis

DSC is a type of thermal analysis that identify the changes of heat capacity of SCB with temperature. This analysis allows the determination of melting and phase change transition of lignocellulosic biomass. About 3 to 10 mg of SCB powder were sealed in an aluminium crucible covered with lid at atmospheric pressure and flushed with ultra-pure dry nitrogen at flow rate of 10 ml/min. The sample was then heated from 25 to 500 ^{o}C at heating rate of 10 ^{o}C/min. The scale for temperature and energy used in DSC equipment were calibrated using iridium (Filho et al., 2007). This analysis was also performed for treated SCB and CMC.

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

HPLC helps in determining the amount of simple sugar units in solution (Käuper et al., 1998). The analysis started by hydrolysing 300 mg of treated SCB with 3 ml of 72 %(v/v) sulphuric acid in a 30 ^{o}C water bath maintaining at 30 ^{o}C for 1 hour. Continuous stirring is keep constant along the hydrolysing process. After that, mixture was diluted with 84 ml of deionised water. Then, the mixture was autoclaved at 121 ^{o}C for 1 hour (Nuno and Carvalho, 2009). The mixture was neutralised with 2 ml of 1 M of sodium hydroxide solution at low temperature for complete precipitation of H_2SO_4. The neutralized solution was centrifuged for 15 minutes. Then, the supernatant is diluted with distilled water before filter with syringe filter. The obtained solution was analysed by using HPLC that equipped with ion exchange, Bio-Rad Organic Acid (H^+ form) column which operates at 60 ^{o}C. The concentration of H_2SO_4, 0.005 M was used as mobile solution with pump flow rate of 0.6 ml/min (Heinze and Pfeiffer, 1999).

3.7.4 Film Preparation

Firstly, 1 g of CMC is added into 50 ml of distilled water at 70 ^{o}C until it is fully solubilised in it. Then, 0.5 ml of glycerol is added as a plasticizer and stirred for 10 minutes at 70 ^{o}C. The film solution is then cooled down to ambient temperature and cast onto a petri dishes. Film solution is then evaporated at room temperature for 36 hours and the film is obtained (Tufan et al., 2016).

3.7.5 Determination of Degree of Substitution (DS) of CMC

Degree of substitution is the measurement on amount of hydroxyl group in cellulose which replaced by carboxymethyl group of SMCA. Firstly, 1 g of CMC was mixed with 50 ml of 95 %(v/v) ethanol. Then, 5 ml of 2 M nitric acid was added into the mixture and agitated for 10 minutes. Next, the mixture was heated for 5 minutes and stirred further for 15 minutes. The mixture was then left to settle. It was filtered and washed with 100 ml of hot ethanol for 3 times. After that, the precipitate was washed with 50 ml of methanol. The final product was dried in an oven at 105 ^{o}C for 3 hours. After drying, about 0.5 g of CMC was added into 100 ml of water in a 250 ml Erlenmeyer flask and stirred. Then, 25 ml of 0.3 M NaOH was added and boiled for 20 minutes. Finally, the mixture was titrated with 0.3 M of HCl by using phenolphthalein as the indicator (Tufan et al., 2016). HCl was added until the indicator colour changes from pink to colourless. The amount of HCl solution used was recorded. Equation 3.3 and 3.4 show the calculation for degree of substitution.

$$Degree\ of\ Substitution = \frac{0.162\ x\ A}{1 - 0.058\ x\ A} \tag{3.3}$$

$$A = \frac{BC - DE}{F} \tag{3.4}$$

Where, B = Volume of NaOH added in ml,

C = Concentration of NaOH added,

D = Volume of HCl consumed in ml,

E = Concentration of HCl added,

F = Weight of sample used in g.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 SCB Characterization

Sugarcane bagasse is the feedstock used for extraction of cellulose. The characteristics of SCB were determined through fourier-transform infrared (FTIR) spectrophotometer, differential scanning calorimetry (DSC) and high performance liquid chromatography (HPLC). FTIR testing is used to determine the functional group of SCB as shown in Figure 4.1.

The large absorption band shown at 3432 cm^{-1} indicates the axial deformation of O-H group in SCB due to presence of carbohydrates and lignin (Mothé and De Miranda, 2009). The peak at 2928 cm^{-1} is characterised as symmetrical stretching of C-H group. At 2367 cm^{-1}, the band is attributed to asymmetric stretching of CO_2. On the other hand, the band at 1723 cm^{-1} belongs to stretching vibration of carbonyl bond C=O of hemicellulose. The carbonyl stretching of C=C bond with aromatic ring of lignin produces a peak at 1628 cm^{-1} (Viera et al., 2007). These bands are commonly found in lignin structure. The small band at 1440 cm^{-1} is associated to CH_2 symmetric bending of lignin (Liu et al., 2006). Meanwhile, the band at 1375 cm^{-1} is in connection with deformation of C-H in polysaccharides (J. X. Sun, Xu, et al., 2004). The peak at 1247 cm^{-1} originates from C-O stretching of aryl group in lignin (Le Troedec et al., 2008). Moreover, the C-O-C asymmetric stretching due to pyranose ring skeletal vibration of cellulose and hemicellulose is seen at 1150 cm^{-1} (Yang et al., 2007). The band intensity at 1053 cm^{-1} arises from O-H symmetric stretching of primary alcohol. The region at 910 cm^{-1} represents β-glycosidic linkage between glucose in cellulose.

It is contributed by the deformation of glycosidic C-H bonds and OH bending (Liu et al., 2006). There are many weak peaks presence in between 880 and 600 cm^{-1} which refers to the aromatic structure of the components (Varma and Mondal, 2016). The absorption band at 828 cm^{-1} is corresponds to the aromatic C-H deformations (J. X. Sun, Xu, et al., 2004).

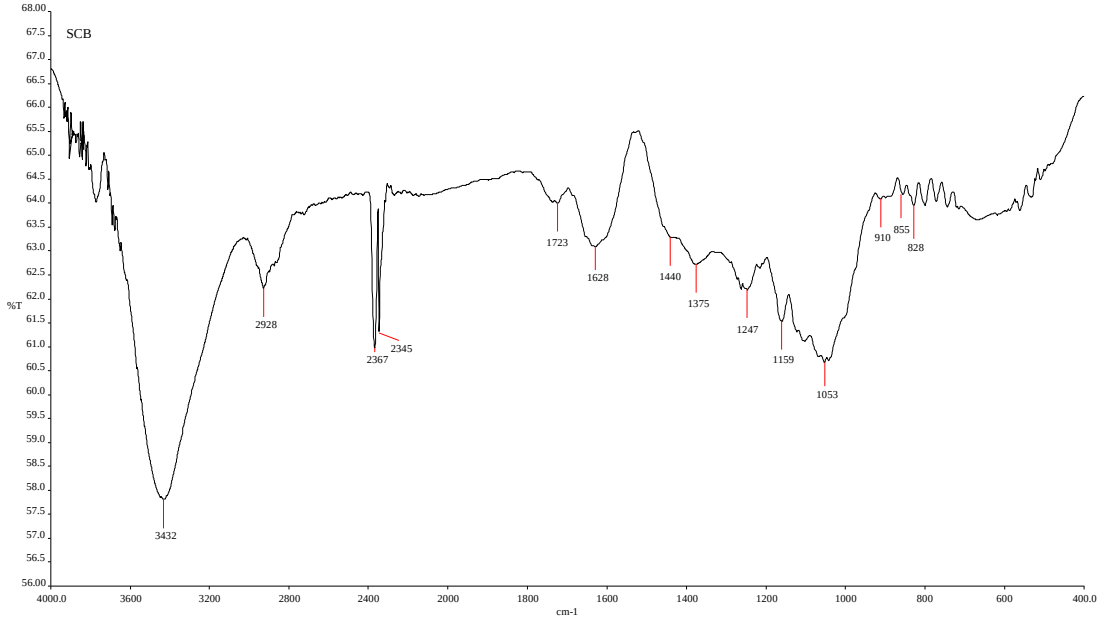

Figure 4.1: FTIR Spectra of Raw Sugarcane Bagasse.

DSC is used to determine the thermal degradation behaviour of lignin, hemicellulose and cellulose in SCB. It was being conducted at heating rate of 10 ^{o}C/min with nitrogen gas flow rate of 10 ml/min. The specific enthalpy of SCB observed in Figure 4.2 is -373.76 J/g with temperature peak of 57.11 ^{o}C. This endothermic peak indicates the removal of moisture due to heating of the sample. The elimination of moisture normally occurred at temperature below 100 ^{o}C (Mandal and Chakrabarty, 2011). The enthalpy of SCB was represented by the integral area of the first endothermic peak, which is -373.76 J as presented in Figure 4.2. The high enthalpy is attributed to the large amount of amorphous region in SCB.

Meanwhile, the second endothermic peak presents in 344.80 ^{o}C refers to the decomposition of cellulose in SCB (Ramajo-escalera et al., 2006). It was within the cellulose thermal decomposition range of 300-375 ^{o}C for most of the organic biomass

product (Chen, 2014). An exothermic peak present at 324 ºC was corresponded to the charring process of lignin due to its aromatic structure (Yang et al., 2007).

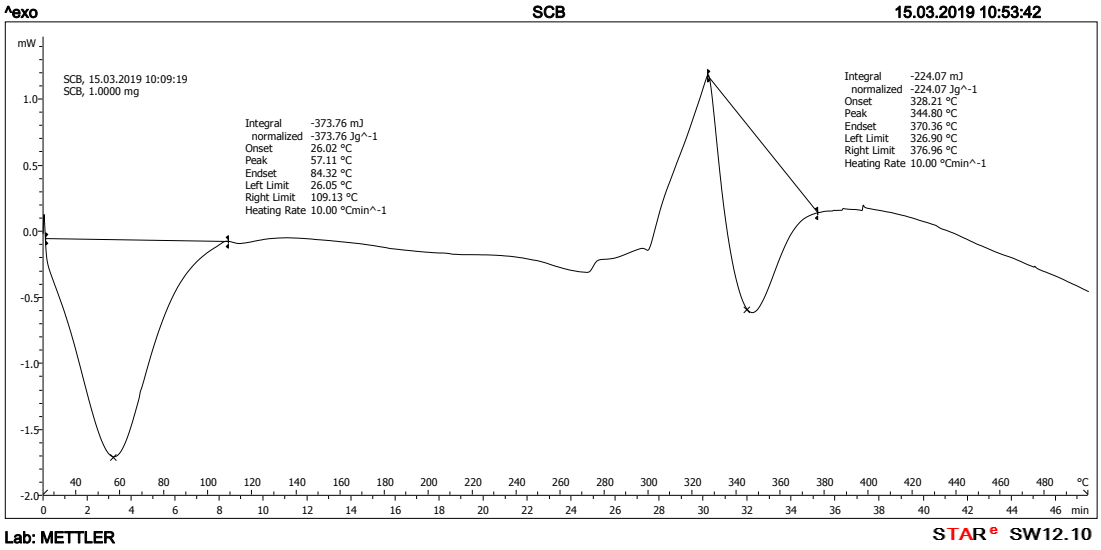

Figure 4.2: DSC Thermograph of SCB.

HPLC helps to carry out the qualitative analysis and quantitative determination of monosaccharide content such as xylose, dextrose, glucose, galactose and arabinose in SCB (Lavarack, Griffin and Rodman, 2002). The prepared dextrose, xylose and arabinose solution at various concentration of 0.2 g/L, 0.4 g/L, 0.6 g/L, 0.8 g/L and 1.0 g/L were injected into HPLC to determine its retention time and HPLC area as shown in Appendix C, D and E respectively. From Appendix C, the dextrose retention time is within 10.004 minutes to 10.012 minutes. As for xylose, the area peak is prominent at retention time of 10.628 minutes to 10.634 minutes as presented in Appendix D. The retention time of arabinose shown in Appendix E is in the range from 11.376 minutes to 11.898 minutes. On the other hand, Appendix C, D and E also show the preparation of the calibration curve for dextrose, xylose and arabinose. The sugar content can be detected through refractive index detector.

Since cellulose is a polysaccharide which insoluble in water, it needs to be converted into dextrose through acid hydrolysis process at high temperature of 121 ºC for 1 hour (Öhgren et al., 2007). Cellulose composition could be calculated through analysis in HPLC column based on the dextrose content. Meanwhile, xylose is the

dominant monosaccharide component presence in hemicellulose of SCB (Brienzo et al., 2016). Besides that, hemicellulose also composed of arabinose which bonded to the backbone of xylose (Lavarack, Griffin and Rodman, 2002). As for lignin, it can be in soluble or insoluble form. Since HPLC can only analyse and detect the solubilised reducing sugar, the soluble lignin is estimated based on the xylose and arabinose content.

In Figure 4.3, the dextrose peak presents at 10.008 minutes gives an area of 169801. The retention time is within the range of the standard retention time of dextrose obtained in Appendix C. From calculation in Appendix F, the dextrose content in SCB is 41.01 %(w/w), which means there is 0.1230 g of dextrose component in 0.3 g of SCB. Besides that, 17.65 %(w/w) of xylose at retention time of 10.636 minutes is observed and small amount of arabinose about 5.56 %(w/w) which hardly be seen in Figure 4.3 is detected at retention time of 11.443 minutes. The amount of cellulose present in the SCB used in this research is 41.01 %(w/w), within the range of 40 to 50 %(w/w) of cellulose present in SCB that is being used by Mandal and Chakrabarty (2011) in their research. Moreover, Table 4.1 reveals the total carbohydrate content for SCB is 64.22 %(w/w), matches the SCB carbohydrate content of 60 to 80 %(w/w) as studied by Ramadoss and Muthukumar (2014).

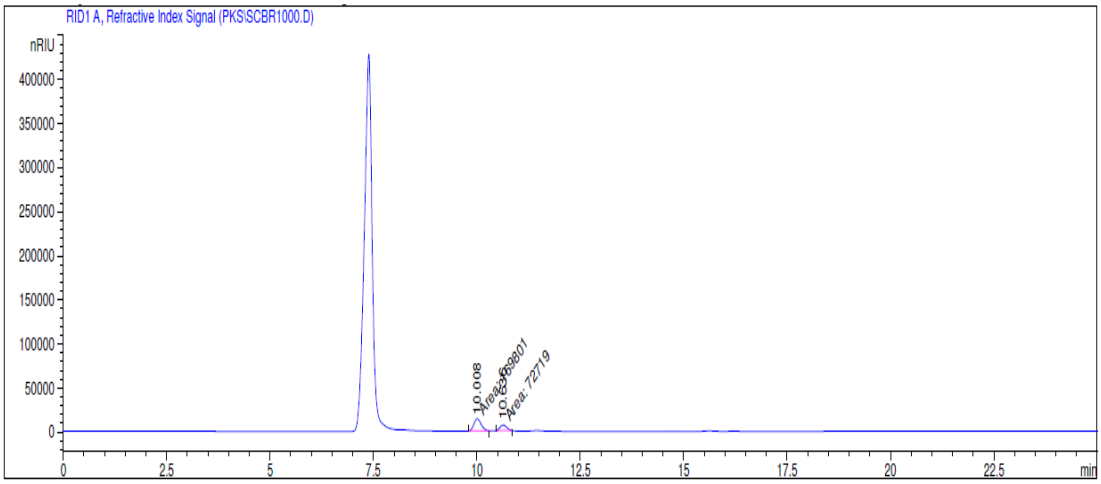

Figure 4.3: HPLC Chromatogram of SCB.

Table 4.1: Carbohydrate Content of SCB Based on HPLC Analysis.

	Dextrose %(w/w)	Xylose %(w/w)	Arabinose %(w/w)	Total carbohydrate %(w/w)
SCB	41.01	17.65	5.56	64.22

4.2 Comparison between Autoclave and Reflux Pretreatment

The SCB was first pretreated with autoclave or reflux to remove traces amount of minerals, impurities and wax within the structure before undergoing ultrasonic treatment. According to Table 4.2, the yield of pretreated SCB is higher in autoclave treatment, which is 94.52 %(w/w) as compared to SCB treated in reflux.

Table 4.2: Yield of Pretreated SCB.

Pretreatment	Mass of SCB before pretreatment (g)	Mass of SCB after pretreatment (g)	Yield of pretreated SCB %(w/w)
Autoclave	10.0005	9.4524	94.52
Reflux	10.0000	8.8529	88.53

FTIR spectra in Figure 4.4 shows that the peaks presence for autoclave and reflux pretreatment were similar to SCB. There is still presence of peak at 1253 cm^{-1}, small intensity of absorption band at wavelength of 1438 cm^{-1} and 1730 cm^{-1}, indicating the pretreatment does not fully solubilised hemicellulose and lignin in SCB. The assignment of absorption band for both pretreatment are summarised in Table 4.3.

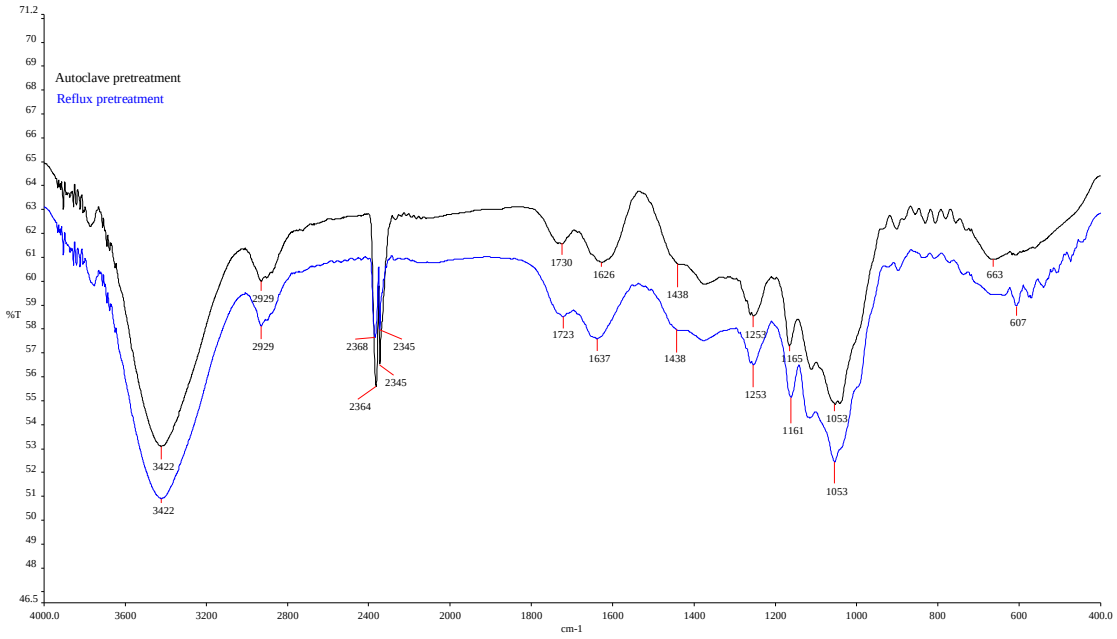

Figure 4.4: FTIR Spectra of SCB Pretreated with Autoclave and Reflux.

Table 4.3: Assignment of Absorption Band in Autoclave and Reflux SCB (Liu et al., 2006; Viera et al., 2007).

Wavenumber (cm^{-1})		Vibration
Autoclave	Reflux	
3422	3422	axial deformation of O-H group
2929	2929	symmetrical stretching of C-H group
2364	2368	asymmetric stretching of CO_2
1730	1723	stretching of C=O
1636	1637	carbonyl stretching of C=C bond with aromatic ring
1438	1438	CH_2 symmetric bending of lignin
1253	1253	C-O stretching of aryl group
1165	1161	C-O-C asymmetric stretching
1053	1053	C-O symmetric stretching of primary alcohol
663	607	C-OH out of plane bending

Since the treatment of SCB with autoclave and reflux did not fully remove the hemicellulose and lignin content in SCB, it further undergoes ultrasonic treatment to enhance the extraction of cellulose. During ultrasonic treatment, the ultrasonic amplitude of 30 %, treatment temperature of 80 ^{o}C with KOH concentration of 0.75 M were fixed along the treatment. Table 4.4 shows the yield of treated SCB with reflux pretreatment and ultrasonic-assisted alkali treatment is slightly higher compared to autoclave pretreatment with ultrasonic-assisted alkali treatment. FTIR, DSC and HPLC analysis are carried out to determine the exact composition of treated SCB.

Table 4.4: Yield of treated SCB after ultrasonic-assisted alkali treatment.

Pretreat method	Mass of SCB before ultrasonic treatment (g)	Mass of SCB after ultrasonic treatment (g)	Yield of treated SCB %(w/w)
Autoclave	5.0012	2.3171	46.33
Reflux	5.0014	2.4557	49.10

FTIR analysis in Figure 4.5 has shown a similar absorption band for both treatment method. It can be seen that the peak at 1723 cm^{-1} and 1247 cm^{-1} present in SCB have disappeared in treated SCB samples, indicating removal of lignin and hemicellulose during the ultrasonic treatment (Viera et al., 2007). On the other hand, a small intensity of absorption band at 1435 cm^{-1} is still presence after refluxed treatment, revealing that there is still presence of insoluble lignin after reflux treatment. The vibration band at 1105 cm^{-1} is due to the C-O-C glycol ether band stretching (Corrales et al., 2012). It is also refers to crystalline cellulose (Zhang et al., 2013). The other absorption band presence is similar as SCB, which can be found in Table 4.5.

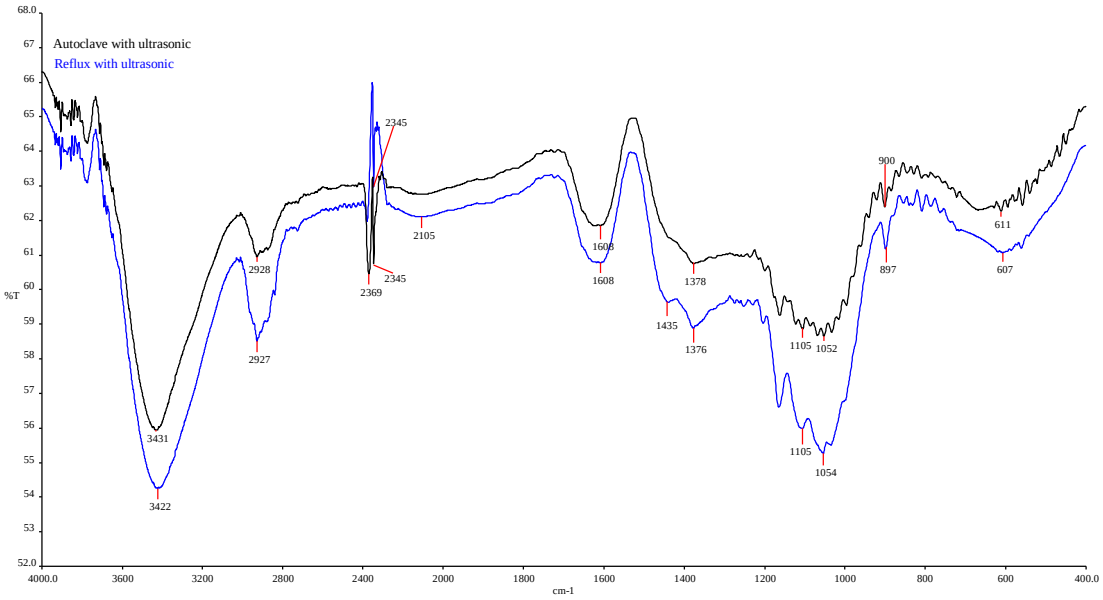

Figure 4.5: FTIR Spectra of Autoclave or Reflux SCB with Ultrasonic Treatment.

Table 4.5: Assignment of Absorption Band in SCB, Autoclave or Reflux SCB with Ultrasonic Treatment (Liu et al., 2006; Viera et al., 2007).

Wavenumber (cm^{-1})			Vibration
SCB	Autoclave	Reflux	
3432	3431	3422	axial deformation of O-H group
2928	2928	2927	symmetrical stretching of C-H group
2367	2345	2345	asymmetric stretching of CO_2
1723	-	-	stretching vibration of C=O
1628	1608	1608	carbonyl stretching of C=C bond with aromatic ring
1440	-	1435	CH_2 symmetric bending of lignin
1375	1378	1376	C-H deformation
1247	-	-	C-O stretching of aryl group
-	1105	1105	C-O-C stretching
1053	1052	1054	C-O symmetric stretching of primary alcohol
910	900	897	β-glycosidic linkage
-	611	607	C-OH out of plane bending

From the DSC graph in Figure 4.6, the SCB sample which undergo autoclave treatment with ultrasonic extraction has peak temperature of 68.72 ^{o}C with specific enthalpy of -204.34 J/g. On the other hand, the peak temperature for SCB sample with reflux and ultrasonic treatment is 77.54 ^{o}C. The endotherm temperature for water desorption process in reflux treatment is higher, portraying the increase of amorphous content in SCB (Lee, Wahit and Othman, 2015). Besides that, the specific enthalpy of reflux treatment sample is -242.43 J/g, higher than the value obtained from autoclave treatment sample. The larger area of endothermic peak denotes presence of larger fraction of amorphous cellulose. The water absorption capability in cellulose is dependent on the availability of the free hydroxyl groups. Thus, water absorption occurs in amorphous region of cellulose, disregard the presence of free hydroxyl group on the surface of the crystallites (Bertran and Dale, 1986).

The second endothermic peak is observed for autoclave treatment sample. From Table 4.6, the endothermic peak temperature at 352.56 ^{o}C is associated with the thermal degradation of cellulose attributed by the breakage of glycosidic bond (Lee, Wahit and Othman, 2015). The second endothermic peak indicates the presence of crystalline cellulose in the sample (Poletto, 2016). Meanwhile, the second peak is insignificant for reflux treatment sample mainly due to the large portion of amorphous cellulose in the treated sample as shown in the first endothermic peak area.

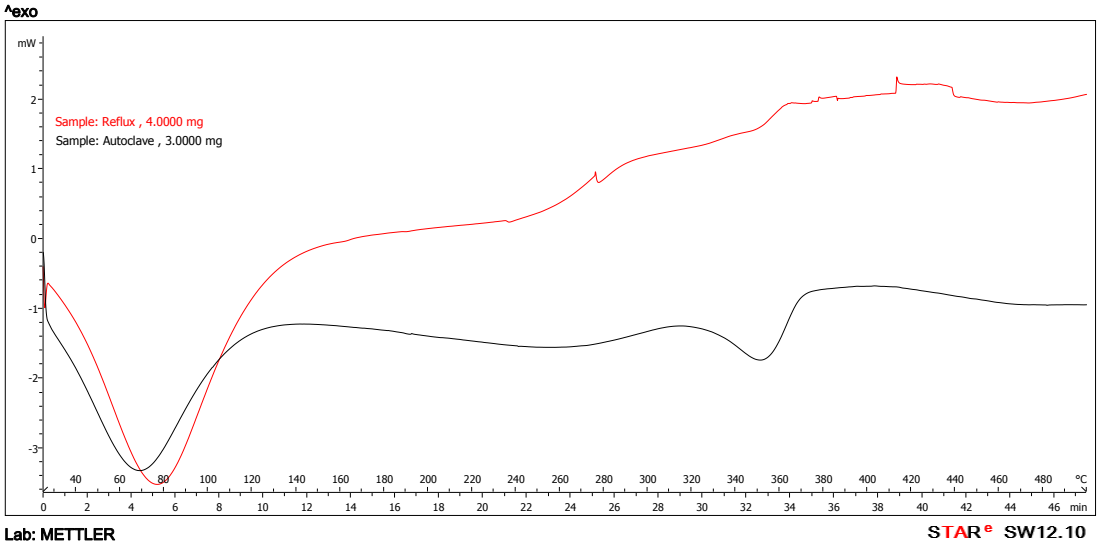

Figure 4.6: DSC Thermograph of Autoclave or Reflux SCB with Ultrasonic Treatment.

Table 4.6: DSC Thermograms of Autoclave or Reflux SCB with Ultrasonic Treatment.

Treatment method	1st Endothermic peak			2nd Endothermic peak		
	T_o, °C	T_p, °C	Δh, J/g	T_o, °C	T_p, °C	Δh, J/g
Autoclave	31.04	68.72	-204.34	325.99	352.56	-41.88
Reflux	35.84	77.54	-242.43	-	-	-

T_o - Onset temperature, T_p - Peak temperature, Δh - Specific enthalpy

After SCB undergoes autoclave or reflux pretreatment with ultrasonic treatment, it was clearly seen that there is a drastic increase in cellulose content as compared to the raw SCB. The cellulose content is 41.01 %(w/w) in untreated SCB. When SCB undergoes autoclave or reflux with ultrasonic treatment, cellulose content of 75.83 %(w/w) and 72.86 %(w/w), respectively are obtained. Autoclave operates at high temperature and pressure can help to remove the impurities and decrease lignin content in SCB (Giraud, Fonty and Besle, 1997). On the other hand, SCB refluxed with ethanol aids in extracting the wax found on the surface of SCB, thereby enhance the extraction of cellulose (Qi et al., 2016). Table 4.7 presented autoclaved SCB with ultrasonic treatment has higher yield in term of cellulose as compared to refluxed SCB with ultrasonic treatment while the large amount of arabinose in refluxed SCB portrayed the presence of highly branching xylan chains in SCB (Sun et al., 2004). This comparison concluded that autoclave pretreatment is more effective in assisting ultrasonic treatment to extract cellulose from SCB.

Table 4.7: Carbohydrate Content of Treated SCB Based on HPLC Analysis.

Treatment Method	Dextrose %(w/w)	Xylose %(w/w)	Arabinose %(w/w)	Total carbohydrate %(w/w)
Autoclave + ultrasonic-assisted alkaline treatment	75.83	12.97	4.08	92.89
Reflux + ultrasonic-assisted alkaline treatment	72.86	15.89	7.20	95.94

FTIR spectra for both autoclave and reflux pretreatment followed by ultrasonic-assisted alkali treatment have shown the disappearing of peak for carbonyl bond C=O of hemicellulose and C-O stretching of aryl group in lignin. However there is still small intensity of absorption band for CH_2 symmetric bending of lignin exist in refluxed SCB. Besides, DSC thermograph of sample with autoclave pretreatment and further ultrasonic-assisted alkali treatment indicates larger portion of crystalline cellulose. Autoclave followed with ultrasonic treatment also provide a higher amount of extracted cellulose as observed in HPLC analysis, whereby the pretreatment medium required in autoclave is water, cheaper as compared to reflux treatment which require ethanol as the solvent. Thus, autoclave is chosen as the pretreatment process for SCB before it undergo ultrasonic extraction process.

4.3 Effect of Ultrasonic Amplitude on Pretreated SCB

The ultrasonic homogenizer is operated at frequency of 20 kHz and power of 500 W. The frequency is within the range of 10 to 100 kHz recommended by Gogate, Sutkar and Pandit (2011) for the breakage of biomass bonding and degradation of biopolymer. Based on Table 4.8, the yield of treated SCB at ultrasonic amplitude of 40 %, temperature of 80 ^{o}C and KOH concentration of 0.75 M is the highest, 53.95 %(w/w).

Table 4.8: Yield of Treated SCB at Various Ultrasonic Amplitude.

Amplitude (%)	Mass of SCB before treatment (g)	Mass of SCB after treatment (g)	Yield of treated SCB %(w/w)
20	5.0008	2.3893	47.78
30	5.0000	2.3870	47.74
40	5.0004	2.6975	53.95

Figure 4.7 shows a similar FTIR spectra peak of 3 samples treated at ultrasonic amplitude of 20 %, 30 % and 40 %. However, for sample treated at ultrasonic amplitude of 20 % and 40 %, the absorption band at 1607 cm^{-1} and 1600 cm^{-1} reveal the C=C stretching of aromatic ring in lignin (Kline et al., 2010). Meanwhile, the peak

of 1625 cm^{-1} exists in sample treated in ultrasonic amplitude of 30 % refers to O-H bond of cellulose structure (Golbaghi, Khamforoush and Hatami, 2007). Table 4.9 summarizes the functional group and type of vibration for each band presence in the FTIR spectra.

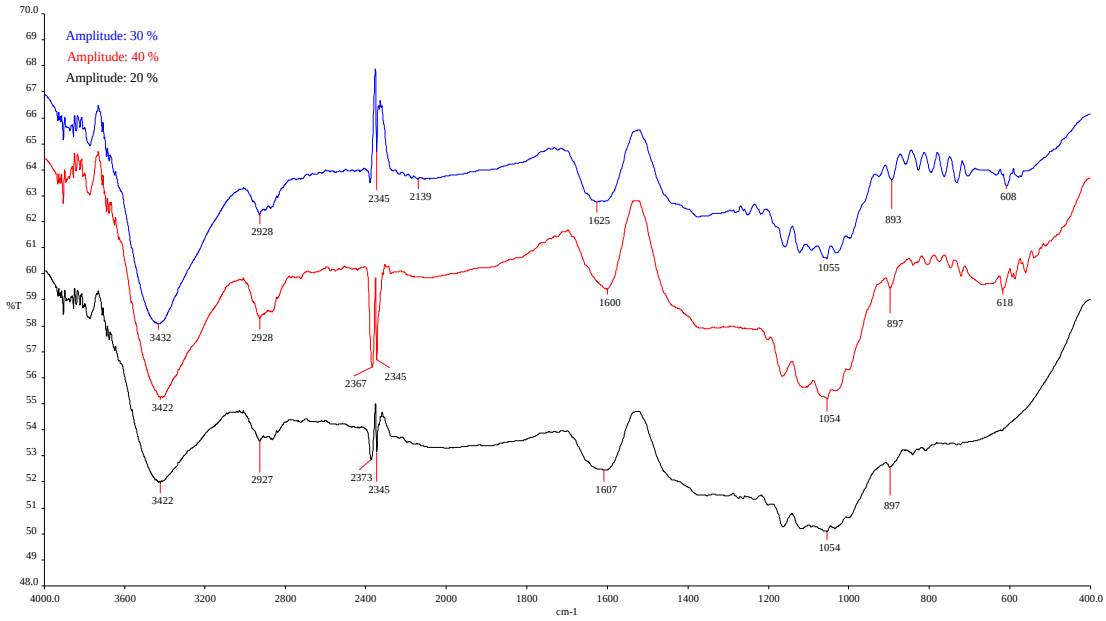

Figure 4.7: FTIR Spectra of Treated SCB at Various Ultrasonic Amplitude.

Table 4.9: Assignment of Absorption Band in Treated SCB at Various Ultrasonic Amplitude (Liu et al., 2006; Viera et al., 2007).

Amplitude			Functional group	Type of vibration
20 %	30 %	40 %		
3422	3432	3422	O-H	Stretching
2927	2928	2928	C-H	Symmetric stretching
2373	2345	2367	CO_2	Asymmetric stretching
1607	-	1600	C=C	Symmetric stretching
-	1625	-	O-H	Bending
1054	1055	1054	C-O	Symmetric stretching
897	898	897	-	β-glycosidic linkage

Figure 4.8 demonstrates the DSC thermographs of 3 samples treated with ultrasonic amplitude varies from 20 %, 30 % to 40 %. In Table 4.10, it is observed that the sample treated with ultrasonic amplitude of 20 % has the highest peak temperature of 76.37 ^{o}C and specific enthalpy of -317.91 J/g. This phenomena indicates the amorphous cellulose within the treated sample is the highest as compared to sample treated at ultrasonic amplitude of 30 % and 40 % (Lee, Wahit and Othman, 2015). A higher amount of heat of dehydration is required to dehydrate the water which is absorbed by amorphous cellulose. The endothermic dehydration peak is largely dependent on the humidity and temperature of the sample (Bertran and Dale, 1986). Thus, all the samples are stored in the desiccator before DSC analysis is conducted.

The second endothermic peak shows that the sample treated with 30 % of ultrasonic amplitude has the highest onset temperature of 327.59 ^{o}C with narrow width of endotherm. This indicates treatment at ultrasonic amplitude of 30 % assists in the rearrangement of cellulose into a more compact structure, producing a more crystallized cellulose (Mandal and Chakrabarty, 2011).

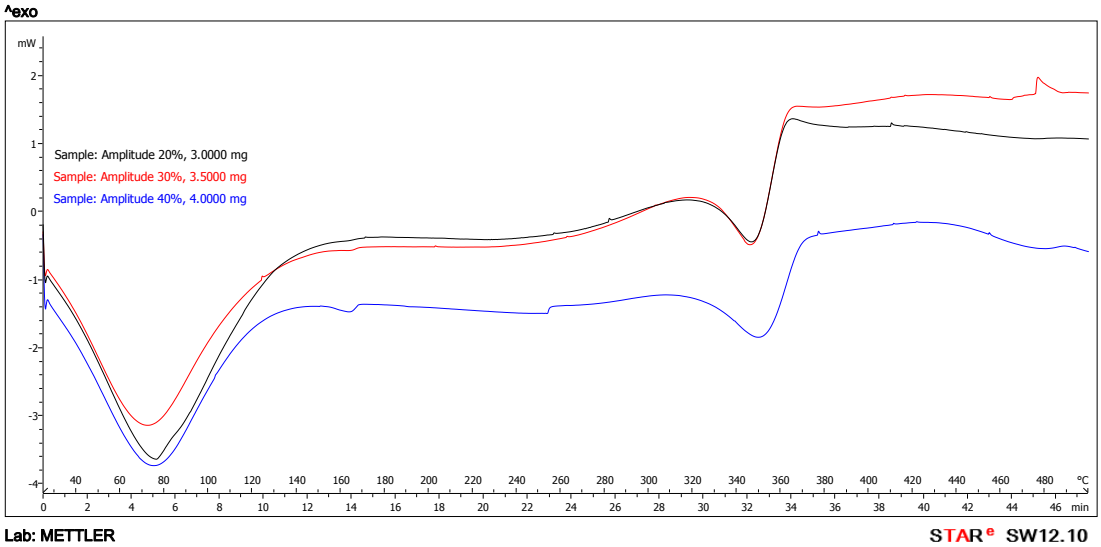

Figure 4.8: DSC Thermograph of Treated SCB at Various Ultrasonic Amplitude.

Table 4.10: DSC Thermograms of Treated SCB at Various Ultrasonic Amplitude.

Ultrasonic amplitude, %	1st Endothermic peak			2nd Endothermic peak		
	T_o, °C	T_p, °C	Δh, J/g	T_o, °C	T_p, °C	Δh, J/g
20	32.60	76.37	-317.91	326.44	348.31	-56.97
30	31.05	72.72	-228.11	327.59	347.80	-52.51
40	31.60	75.20	-191.11	322.65	351.38	-51.41

T_o - Onset temperature, T_p - Peak temperature, Δh - Specific enthalpy

The ultrasonic amplitude that being used in this research can only be adjusted from 20 % to 40 %. According to the calculation demonstrated in Appendix H, the extracted cellulose at low (20 %) and medium (30 %) ultrasonic amplitude are increasing, from 70.44 %(w/w) to 73.92 %(w/w). However, from Table 4.11, the SCB treated at 40 % ultrasonic amplitude only extract 56.58 %(w/w) of cellulose, which is 1.5263 g out of the total treated SCB of 2.6975 g. The reason for reduction of extracted cellulose at high ultrasonic amplitude might caused by the adverse cavitation effects. This phenomena happened as high ultrasonic amplitude will generate larger amount of cavitational bubbles at the tip of the ultrasonic transducer, which will hinder the energy transfer to the solvent medium containing SCB (Ramadoss and Muthukumar, 2016). Therefore, less amount of cellulose is being extracted. The arabinose present in the treated SCB is relative small, unable to observe through the peak in the HPLC chromatogram. It can be explained that location of arabinose in the branches of macromolecules have make it easier to be hydrolysed during the alkaline ultrasonic treatment process (Martín, Klinke and Thomsen, 2007).

Table 4.11: Carbohydrate Content of Treated SCB at Various Ultrasonic Amplitude Based on HPLC Analysis.

Ultrasonic amplitude (%)	Dextrose %(w/w)	Xylose %(w/w)	Arabinose %(w/w)	Total carbohydrate %(w/w)
20	70.44	10.46	1.02	82.52
30	73.92	9.78	3.98	87.68
40	56.58	9.98	2.05	68.61

The extraction of cellulose is happened at different ultrasonic amplitude, thereby the FTIR spectra for each treatment is similar. In summary, the SCB treated at ultrasonic amplitude of 30 % estimating better thermal stability with larger amount of cellulose detected by DSC while treatment conducted at 30 % of ultrasonic amplitude has successfully extracted high amount of cellulose as well. Therefore, treatment at ultrasonic amplitude of 30 % has been selected to study for following extraction parameters.

4.4 Effect of Temperature on Pretreated SCB

The temperature of treatment is varied from 70 ^{o}C to 90 ^{o}C, with the interval of 10 ^{o}C during the ultrasonic extraction process. In this case, ultrasonic amplitude was fixed at 30 % with KOH concentration of 0.75 M. It is shown in Table 4.12 that the yield of SCB is the lowest when the sample is treated at 90 ^{o}C.

Table 4.12: Yield of Treated SCB at Various Temperature.

Temperature (^{o}C)	Mass of SCB before treatment (g)	Mass of SCB after treatment (g)	Yield of treated SCB %(w/w)
70	5.0009	2.5236	50.46
80	5.0012	2.4291	48.57
90	5.0006	2.3033	46.06

Figure 4.9 shows the FTIR spectra of 3 samples treated at different temperature of 70 ^{o}C, 80 ^{o}C and 90 ^{o}C. The only difference observed in this three treated samples is C=C aromatic stretching in lignin at 1600 cm^{-1} that detected in sample treated at 90 ^{o}C. Other than that, similar spectra peak is observed for the three treated samples as presented in Table 4.13. No peak at range of 1511-1515 cm^{-1} and 1323-1327 cm^{-1} are detected in samples, indicating the absence of C=C of aromatic ring and C=O bond of syringyl unit in lignin (Kline et al., 2010).

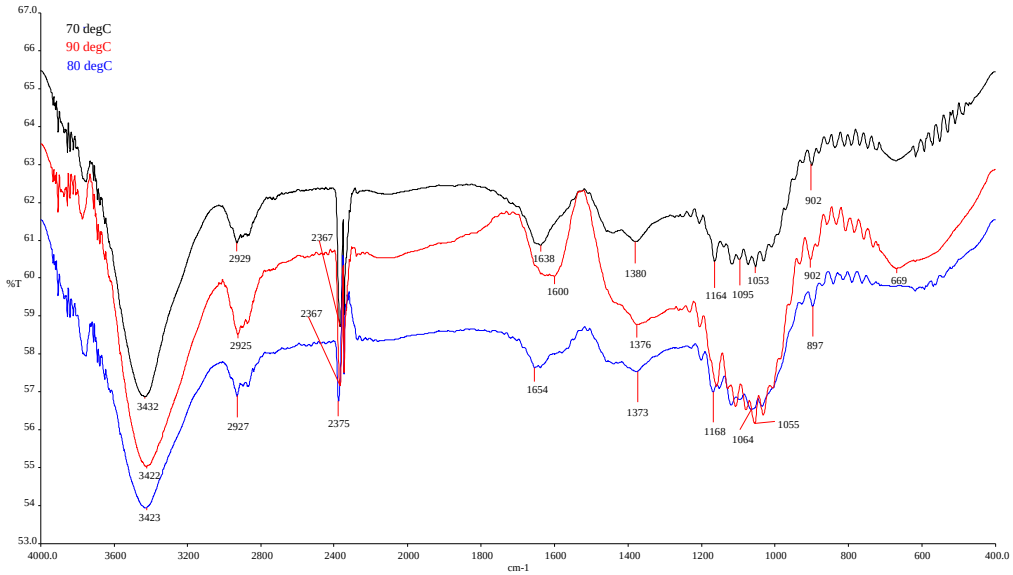

Figure 4.9: FTIR Spectra of Treated SCB at Various Temperature.

Table 4.13: Assignment of Absorption Band in Treated SCB at Various Temperature (Liu et al., 2006; Viera et al., 2007).

Temperature			Functional group	Type of vibration
70 °C	80 °C	90 °C		
3432	3423	3422	O-H	Stretching
2929	2927	2925	C-H	Symmetric stretching
2367	2375	2367	CO_2	Asymmetric stretching
1638	1634	-	O-H	Bending
1380	1373	1376	C-H	In the plane bending
1164	1168	-	C-O-C	Antisymmetric stretching
1053	1064	1055	C-O	Symmetric stretching
902	897	902	-	β-glycosidic linkage

According to the DSC analysis as presented in Figure 4.10, at different temperature, it is observed that the peak temperature of the first endothermic curve is decreasing from 70 ^{o}C to 90 ^{o}C. The SCB sample treated at temperature of 90 ^{o}C consist of the lowest peak temperature at 72.75 ^{o}C with specific enthalpy of -189.08 J/g. This indicates more crystalline component is present within this sample, less heat is needed to dehydrate the moisture (Poletto, 2016).

Meanwhile, the sample treated at 70 ^{o}C gives a broader and lower peak temperature of 347.39 ^{o}C at second endothermic peak. The specific enthalpy needed to degrade the cellulose is lowest, -27.01 J/g as observed in Table 4.14, due to less crystallize cellulose is present within the sample (Poletto, 2016).

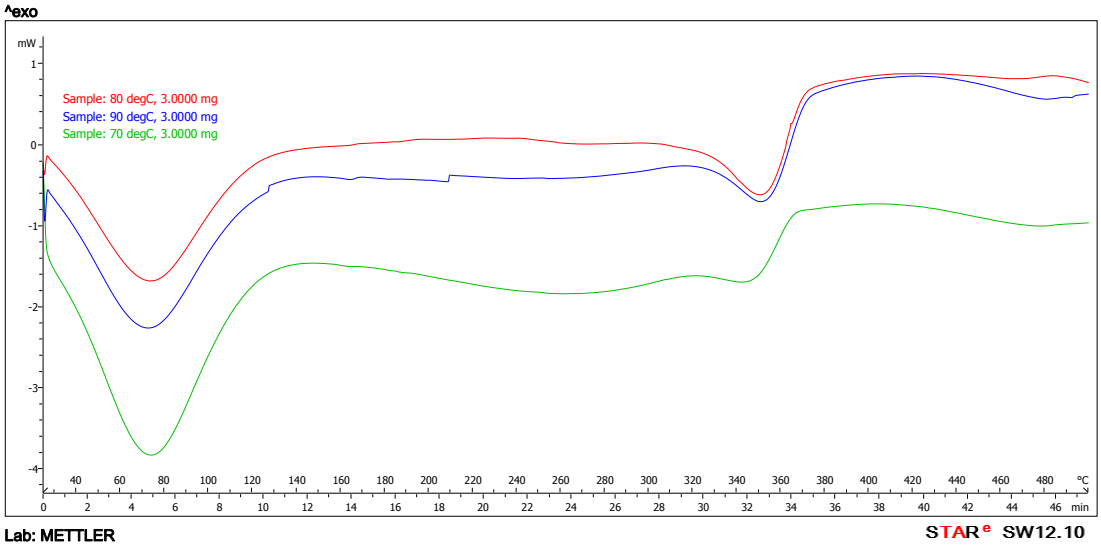

Figure 4.10: DSC Thermograph of Treated SCB at Various Temperature.

Table 4.14: DSC Thermograms of Treated SCB at Various Temperature.

Temperature, ^{o}C	1st Endothermic peak			2nd Endothermic peak		
	T_o, ^{o}C	T_p, ^{o}C	Δh, J/g	T_o, ^{o}C	T_p, ^{o}C	Δh, J/g
70	34.16	74.03	-238.64	319.52	347.39	-27.01
80	31.12	73.94	-166.41	321.99	351.96	-66.85
90	30.81	72.75	-189.08	324.63	353.12	-53.89

T_o - Onset temperature, T_p - Peak temperature, Δh - Specific enthalpy

The calculations based on HPLC analysis in Appendix I show that the extracted cellulose at temperature of 70 ºC, 80 ºC and 90 ºC are 63.75 %(w/w), 70.31 %(w/w) and 73.59 %(w/w) respectively. The dextrose found for this three samples in HPLC chromatogram are at retention time of 10.002 minutes to 10.007 minutes, similar to the retention time obtained for standard dextrose. The increase of temperature is able to disrupt and break down the solute and biomass matrix interaction such as Van der Waals force, hydrogen bonding and covalent bonding present in between hemicellulose and lignin of SCB (Ramadoss and Muthukumar, 2016). Thus, from Table 4.15, it can be found that the xylose content has been reduced from 17.65 %(w/w) before treatment to weight composition below 10 %. In addition, the high temperature with the presence of alkali enhance the reaction rate thus assist higher cellulose extraction. Hence, maximum extraction of cellulose is obtained when SCB treated at 90 ºC as depicted in Table 4.15.

Table 4.15: Carbohydrate Content of Treated SCB at Various Temperature Based on HPLC Analysis.

Temperature (ºC)	Dextrose %(w/w)	Xylose %(w/w)	Arabinose %(w/w)	Total carbohydrate %(w/w)
70	63.75	9.46	1.71	74.92
80	70.31	9.52	3.48	83.31
90	73.59	9.72	2.11	85.42

4.5 Effect of Potassium Hydroxide Concentration on Pretreated SCB

From Table 4.16, the yield of treated SCB decrease as the concentration of potassium hydroxide concentration increase. This may due to the alkaline KOH that assist the disruption of covalent bond between lignocellulosic components in SCB, thereby hydrolyse hemicellulose and lignin present in SCB into soluble products (Castañón-Rodríguez et al., 2015). The research was carried out at ultrasonic amplitude of 30 % and temperature of 80 ºC.

Table 4.16: Yield of Treated SCB at Various Potassium Hydroxide Concentration.

KOH concentration (M)	Mass of SCB before treatment (g)	Mass of SCB after treatment (g)	Yield of treated SCB %(w/w)
0.25	5.0002	2.8360	56.72
0.50	5.0012	2.5520	51.03
0.75	5.0011	2.3928	47.85
1.0	5.0003	2.2787	45.57
1.25	5.0004	2.1768	43.53

The FTIR spectra of 5 samples treated at KOH concentration of 0.25 M, 0.50 M, 0.75 M, 1.0 M and 1.25 M are presented in Figure 4.11. The FTIR spectra for this five samples have shown a similar spectra peak, excluding the C=C aromatic stretching in lignin which present at 1599 cm^{-1} in SCB treated at 0.25 M of KOH. When SCB treated at low alkali concentration, the lignin bonding is not fully break, causing the presence of lignin in the sample. Table 4.17 provides an overview of the functional group and type of vibration for each band presence in the FTIR spectra.

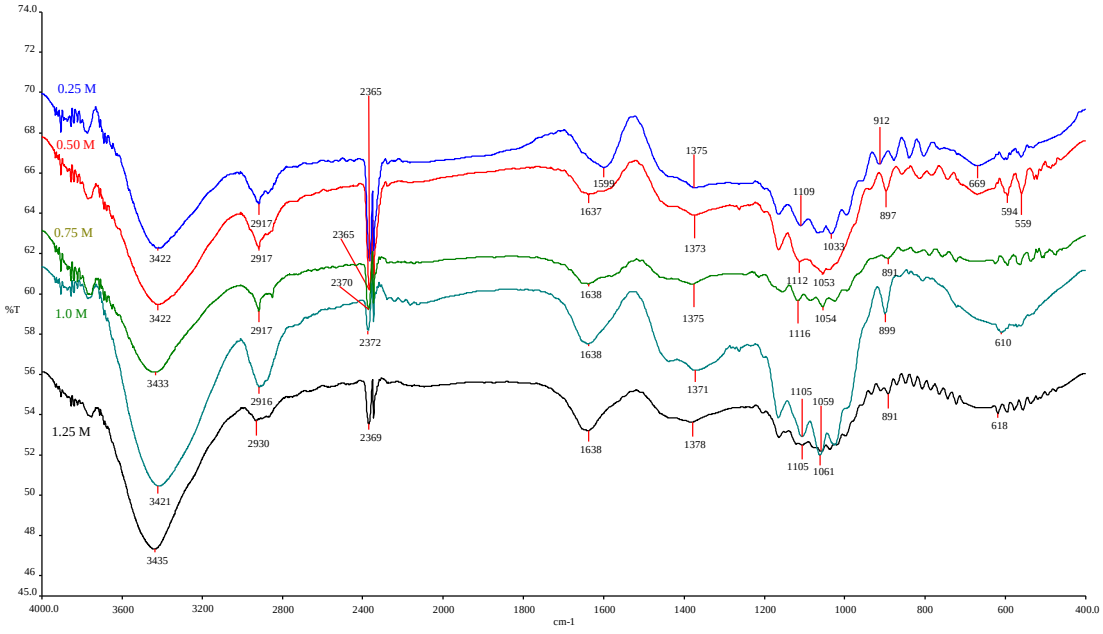

Figure 4.11: FTIR Spectra of Treated SCB at Various KOH Concentration.

Table 4.17: Assignment of Absorption Band in Treated SCB at Various KOH Concentration (Liu et al., 2006; Viera et al., 2007).

KOH Concentration					Functional group	Type of vibration
0.25 M	0.50 M	0.75 M	1.0 M	1.25 M		
3422	3422	3433	3421	3435	O-H	Stretching
2917	2917	2917	2916	2930	C-H	Symmetric stretching
2365	2365	2370	2372	2360	CO_2	Asymmetric stretching
-	1637	1638	1638	1628	O-H	Bending
1375	1373	1375	1371	1378	C-H	In the plane bending
1109	1112	1116	1105	1105	C-O-C	Stretching
1053	1053	1054	1055	1059	C-O	Symmetric stretching
912	897	891	899	891	-	β-glycosidic linkage
660	-	-	610	618	C-OH	Out of plane bending

The SCB sample with treatment at 1.25 M of sodium hydroxide treatment gives the lowest peak temperature of 66.11 ^{o}C and low specific enthalpy, -165.71 J/g as indicated in Table 4.18. Thus, it has high cellulose crystallinity content as compared to other sample treated with lower concentration of sodium hydroxide solution. The alkali treatment assists the removal of lignin by causing swelling of the biomass. This swelling will increase the internal surface area of SCB, on the same time weaken and break the bond linkage of lignin with other component such as cellulose and hemicellulose (Bussemaker and Zhang, 2013). Therefore, it probably leads to the high amount of crystallize cellulose within the sample.

For second endotherm peak, the high peak temperature, 356.38 ^{o}C is observed in sample treated with the highest sodium hydroxide concentration as shown in Figure

4.12. Alkali treatment has aid in breaking inter and intramolecular bonds and cross linking between lignin, hemicellulose with cellulose, resulted solubilisation of lignin and rearrangement of molecular chain (Lee, Wahit and Othman, 2015). More crystallize cellulose is present, which then being reoriented into a more compact structure (Mandal and Chakrabarty, 2011). The arrangement of molecule is being packed together, ushering a higher crystallize melting temperature since crystalline cellulose will increase the thermal stability of SCB. Hence, more energy is needed to degrade the cellulose, resulting a higher specific enthalpy, -83.34 J/g.

Table 4.18: DSC Thermograms of Treated SCB at Various KOH Concentration.

KOH concentration, M	1st Endothermic peak			2nd Endothermic peak		
	T_o, °C	T_p, °C	Δh, J/g	T_o, °C	T_p, °C	Δh, J/g
0.25	38.33	78.69	-333.96	324.98	346.69	-19.19
0.50	34.29	74.69	-249.99	325.62	350.09	-16.79
0.75	29.72	71.13	-223.70	325.21	354.01	-55.24
1.0	32.04	73.39	-210.01	325.58	353.72	-69.14
1.25	29.49	66.11	-165.71	327.77	356.38	-83.34

T_o - Onset temperature, T_p - Peak temperature, Δh - Specific enthalpy

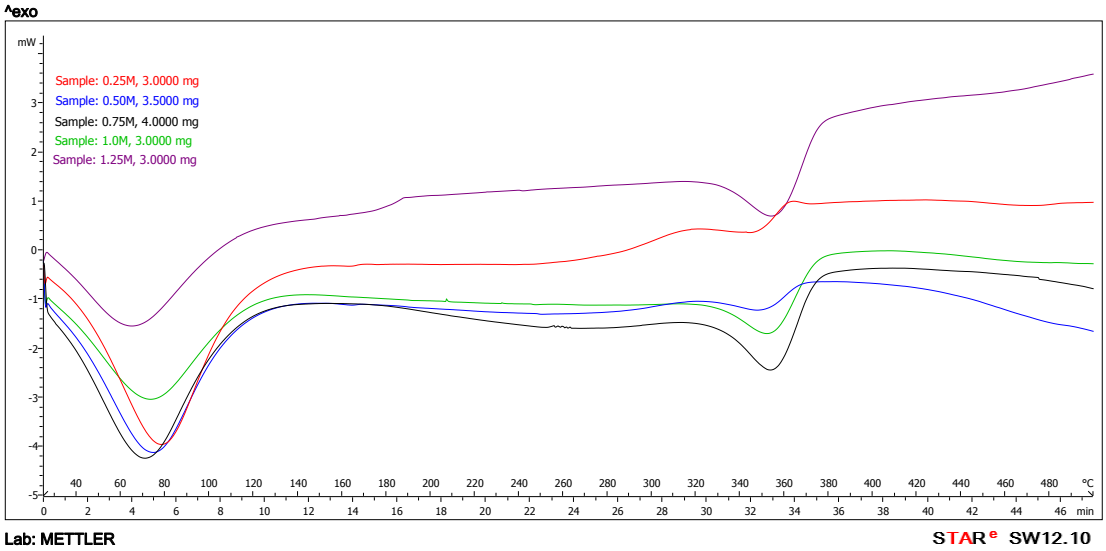

Figure 4.12: DSC Thermograph of Treated SCB at Various KOH Concentration.

HPLC analysis is used to determine the amount of cellulose being extracted when SCB treated at different KOH concentration. It is observed that the extraction of cellulose in SCB is improved progressively when undergo ultrasonic treatment at higher KOH concentration as depicted in Table 4.19. Calculation in Appendix J presents 83.22 %(w/w) of cellulose is extracted when treated with 1.25 M of KOH. Alkali treatment assists the degradation and cleavage of ester and glycosidic side chains, causing the swelling of lignin and hemicellulose, which then being released into the alkaline solution, thereby enhance the separation and extraction of cellulose (Liu et al., 2006). Meanwhile, the ultrasonic irradiation mechanically disrupt the cell wall, increases the accessibility of alkali to SCB, thus enhances the mass transfer of component in SCB, in which accelerate the cellulose extraction process (Sun et al., 2004). Hence, it is observed that 65.9 %(w/w) of xylose and 76.1 %(w/w) of arabinose removal are achieved when treated with 1.25 M of KOH. Since hemicelluloses are present on the outer surface of SCB cell wall, thus enable hemicellulose to solubilise easily in alkaline solution (Liu et al., 2006). When conducting the experiment, it is found that the alkaline solution containing SCB after treatment was turned to viscous state, indicating the solubilisation of hemicellulose since it is relative affinity to water (Chen, 2014). In addition, treatment with alkali reduces the possibility of sugar degradation in SCB (Velmurugan and Muthukumar, 2012). Thus, it can be found the total carbohydrate content in treated SCB is relatively higher, at the range of 85.65 %(w/w) to 90.58 %(w/w).

Table 4.19: Carbohydrate Content of Treated SCB at Various KOH Concentration Based on HPLC Analysis.

KOH concentration (M)	Dextrose %(w/w)	Xylose %(w/w)	Arabinose %(w/w)	Total carbohydrate %(w/w)
0.25	65.82	17.52	3.61	86.96
0.50	69.03	12.06	4.55	85.65
0.75	73.43	9.97	2.26	85.66
1.0	76.00	8.04	1.70	85.74
1.25	83.22	6.02	1.33	90.58

Treatment of SCB with 1.25 M of KOH is selected since FTIR spectra for all SCB treated at different concentration of KOH are similar. However, the sample which gives the highest amount of cellulose and shows great extraction of cellulose with least hemicellulose and lignin content is SCB which undergo treatment at 1.25 M of KOH.

4.6 Analysis of Cellulose Extraction from Treated SCB

The manipulation variables investigated in this research are ultrasonic amplitude, temperature and concentration of KOH. When one of the parameter is being manipulated, the other two parameters will be in constant. In the midst of manipulating the three parameters, there are three sets of data with same parameters are being repeated, which is SCB treated at ultrasonic amplitude of 30 %, temperature of 80 ^{o}C and 0.75 M of KOH. The carbohydrate content of the treated SCB can be found in Table 4.20.

Table 4.20: Carbohydrate Content of Treated SCB at 80 ^{o}C, 30 % Ultrasonic Amplitude and 0.75 M KOH Based on HPLC Analysis.

Experiment No	Dextrose %(w/w)	Xylose %(w/w)	Arabinose %(w/w)	Total carbohydrate %(w/w)
2	73.92	9.78	3.98	87.68
5	70.31	9.52	3.48	83.31
9	73.43	9.97	2.26	85.66

Based on the data collected from these three sets of experiments, a standard error bar is plotted in Figure 4.13 to estimate the range of deviation for each collected data from the mean value. A smaller standard error of mean values indicates the data collected has higher accuracy. By referring to Figure 4.13, the standard error of mean for dextrose is small. The deviation range is in control, without largely affect the accuracy of the amount of cellulose extraction. A tiny error bar is observed for the xylose and arabinose component exist in treated SCB. This means that the dissolution

of xylose and arabinose were very concise in each of the treatment. Smaller standard error of mean indicate the mean value of xylose and arabinose content are relatively precise and accurate.

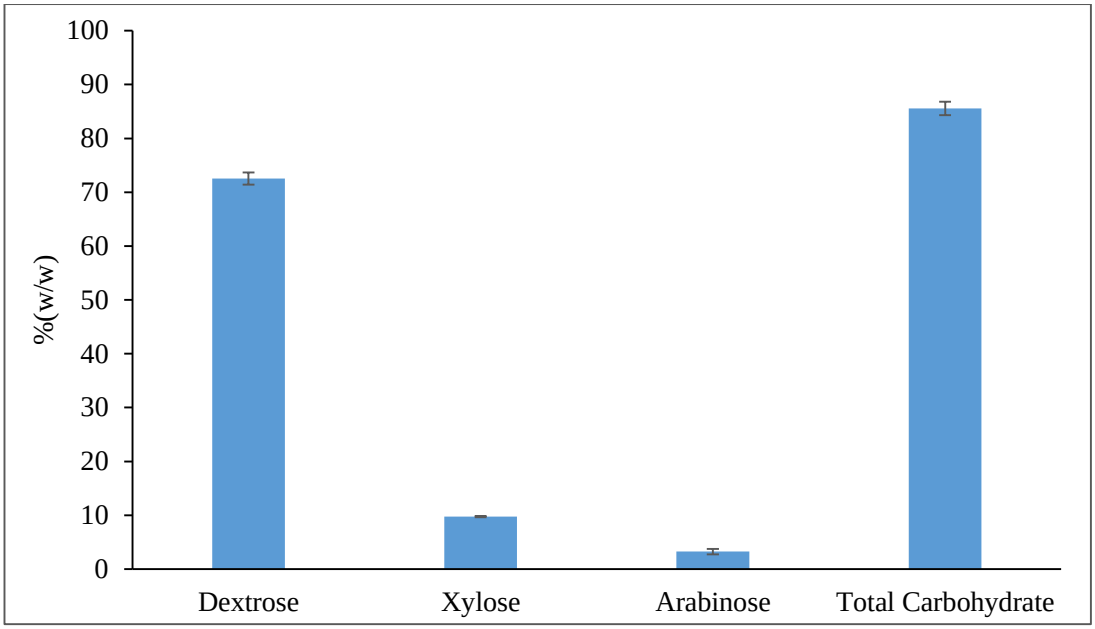

Figure 4.13: Error Bar of Carbohydrate Content of Treated SCB at 80 ºC, 30 % Ultrasonic Amplitude and 0.75 M KOH.

This standard error of mean is then used to compute confidence interval for determination of possible interval probability in which the mean will fall (McHugh, 2008). 95 % of confidence interval is used in this research. Based on Table 4.21, the 95 % confidence interval for dextrose is 2.2158 %(w/w), implying there is 95 % probability whereby the data obtained is within the calculated confidence interval. From the calculation, it is known that the confidence interval for cellulose extraction is within the range of 70.3375 %(w/w) to 74.7692 %(w/w). In this research, the cellulose extracted are 73.92 %(w/w), 70.31 %(w/w) and 73.43 %(w/w), whereby one of the cellulose extracted was less than the interval limit. The reason for the difference in cellulose extraction may be attributed to the inconsistency of temperature supplied during the ultrasonic treatment. Since the sample needs to be heated up every 10 minutes, heat might dissipated to the environment when moving the sample from hot plate to the ultrasonic homogenizer box. Meanwhile, xylose, arabinose and total carbohydrate content in treated SCB were in close agreement with the value predicted

in 95 % confidence interval. Due to limitation of time and budget, the other samples with different parameters were conducted for one time. From the analysis of this three replicates, it can be considered the yield of extracted cellulose and dissolution of hemicellulose and lignin were accurate without large deviation.

Table 4.21: Analysis of Standard Error of Mean and Confidence Interval of Carbohydrate Content in Treated SCB at 80 ^{o}C, 30 % Ultrasonic Amplitude and 0.75 M KOH.

	Mean	Standard deviation	Standard error of mean	95 % Confidence interval, %(w/w)	Lower bound, %(w/w)	Upper Bound, %(w/w)
Dextrose	72.5533	1.9582	1.1306	2.2158	70.3375	74.7692
Xylose	9.7567	0.2259	0.1304	0.2556	9.5010	10.0123
Arabinose	3.2400	0.8848	0.5108	1.0012	2.2388	4.2412
Total carbohydrate	85.5500	2.1871	1.2627	2.4749	83.0751	88.0249

4.7 Comparison of Cellulose Extraction with Commercialised Process

Cellulose is the main component in lignocellulosic biomass which consist of mostly crystalline and some amorphous structure, embedded in composite structure, mainly consist of lignin and hemicellulose. It can be found in the rigid cell wall of the plant and is associated with hydrogen bonding, making it resist to destruction or degradation in most of the organic solvent (Singh and Singh, 2012). Therefore, several consecutive steps as shown in Figure 4.14 are required in assisting the extraction of cellulose in industry.

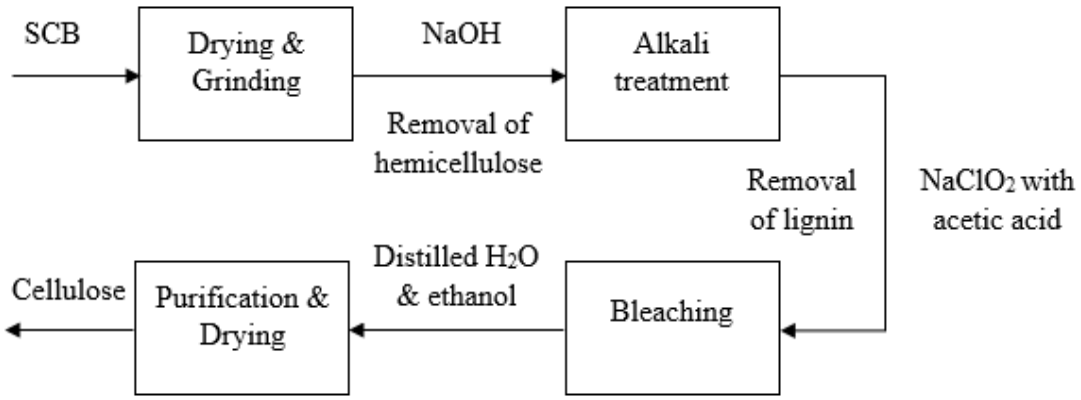

Figure 4.14: Process Flow for Extraction of Cellulose in Industry (Huang et al., 2017).

Firstly, the juice in sugarcane is being extracted out, leaving SCB as the fibrous residue. The lignocellulosic biomass, SCB obtained will need to be cut into small pieces and dried in oven at temperature range of 60 to 80 ^{o}C (Huang et al., 2017). Then, the dried SCB is grounded into powder and filtered using a mesh sieve. The SCB powder will then undergo treatment process to remove hemicellulose and lignin, thereby extract out the cellulose. The hemicellulose content can be solubilised and removed by cooking the SCB in the presence of alkali medium, mainly sodium hydroxide. After that, the mixture is bleached with bleaching agent such as hydrogen peroxide (H_2O_2) or sodium chlorite ($NaClO_2$) in acidic solution to whiten the cellulosic fibers and help in complete removal of lignin (Huang et al., 2017). However, the decomposition of $NaClO_2$ will emit chlorine dioxide, a strong oxidation gas. Therefore, the rate of evolution of chlorine dioxide needs to be controlled by manipulate the bleaching bath temperature or its pH by addition of weak acid (Abdel-Halim, 2014). Hence, acetic acid is presence within the bleaching agent used. Further, the mixture will be filtered and washed with distilled water and ethanol to remove impurities and any breakdown products (Sun, et al., 2004). Lastly, the extracted cellulose is dried and ready to be used.

Meanwhile, in current research, the extraction process is modified by addition of ultrasonic treatment with alkali which shown in Figure 4.15 to enhance the extraction process. Before that, SCB mixed in distilled water has undergo autoclave pretreatment to remove impurities and wax present in SCB (Giraud, Fonty and Besle, 1997). Then, SCB undergo ultrasonic treatment by utilising KOH as the alkali medium. The mechanical vibration generated by ultrasonic homogenizer creates pressure wave which induced the formation of cavitation bubbles. The collapse of bubble during compression of the pressure wave causes generation of shock wave which provides adequate shear force to break the bonding of SCB, enhance mass transfer and thereby facilitate cellulose extraction. Furthermore, alkali medium aids in solubilised hemicellulose component by cleavage of glycosidic linkage (Bussemaker and Zhang, 2013). In the case of lignin, the ether linkage is susceptible to hydroxyl attack when treated with alkali. Lastly, treated SCB is washed and purified with distilled water and ethanol. The modified extraction process with combination of ultrasonic and alkali solvent requires lesser use of chemical as compared to the commercialised cellulose extraction process. This method is more economical saving and has lesser impact to environment (Bussemaker and Zhang, 2013).

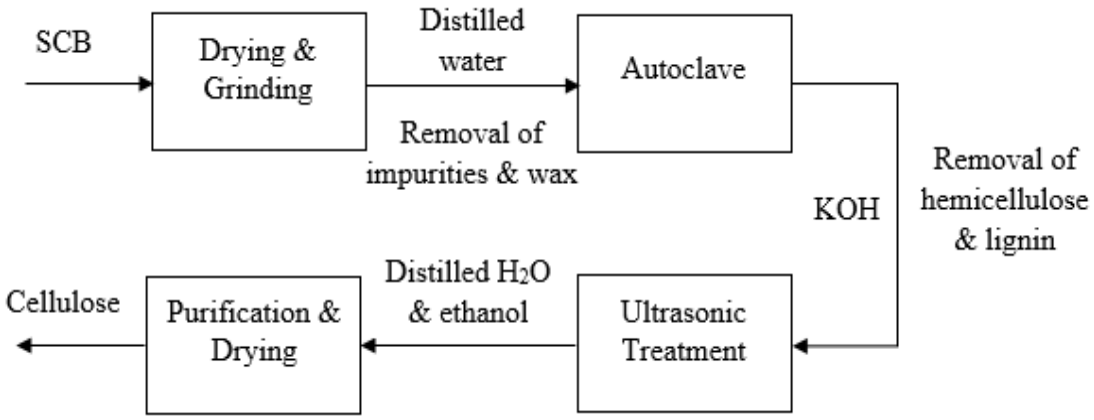

Figure 4.15: Process Flow for Modified Cellulose Extraction Process.

4.8 CMC Characterization

Based on experimental results discussed before, SCB was treated at ultrasonic amplitude of 30 %, temperature of 80 ^{o}C and 1.25 M of KOH to produce cellulose for synthesis of carboxymethyl cellulose (CMC). Since cellulose is insoluble in most of the solvent, further production to CMC actually increases the applicability of cellulose (Viera et al., 2007). The synthesis process gave CMC yield of 165.45 %(w/w) as tabulated in Table K.1 in Appendix K. The high yield is resulted from the reaction of cellulose with sodium monochloroacetic acid, whereby the hydroxyl group of cellulose is substituted with carboxymethyl group (Tasaso, 2015). Figure 4.16 displays the appearance of CMC which is light brownish in colour.

Figure 4.16: Appearance of CMC.

FTIR spectra of CMC compared with SCB is presented in Figure 4.17. It is found that there are similar absorption bands between CMC and SCB. For example, hydroxyl group of OH stretching is present at wavenumber of 3432 cm^{-1}, C-H aliphatic group at peak of 2928 cm^{-1} and 2930 cm^{-1}, CO_2 at absorption band of 2367 cm^{-1}, hydrocarbon group of CH_2 scissoring at 1425 cm^{-1} to 1440 cm^{-1} and β-glycosidic bond at 895 cm^{-1} to 910 cm^{-1} (Corrales et al., 2012) . Meanwhile, C=O bond in hemicellulose at peak of 1723 cm^{-1}, C=C bond and C-O bond of lignin at absorption band of 1628 cm^{-1} and 1247 cm^{-1} in SCB are absent in CMC, portraying most of the hemicellulose and lignin were removed during treatment (Golbaghi, Khamforoush and Hatami, 2007). The presence of new peak at 1608 cm^{-1} verify the occurrence of methylation

reaction in extracted cellulose. It is the evidence on which the hydroxyl group in cellulose is being substituted with carboxymethyl, COONa group (Bono et al., 2009). Comparison of the absorption band between CMC and SCB were tabulated in Table 4.22.

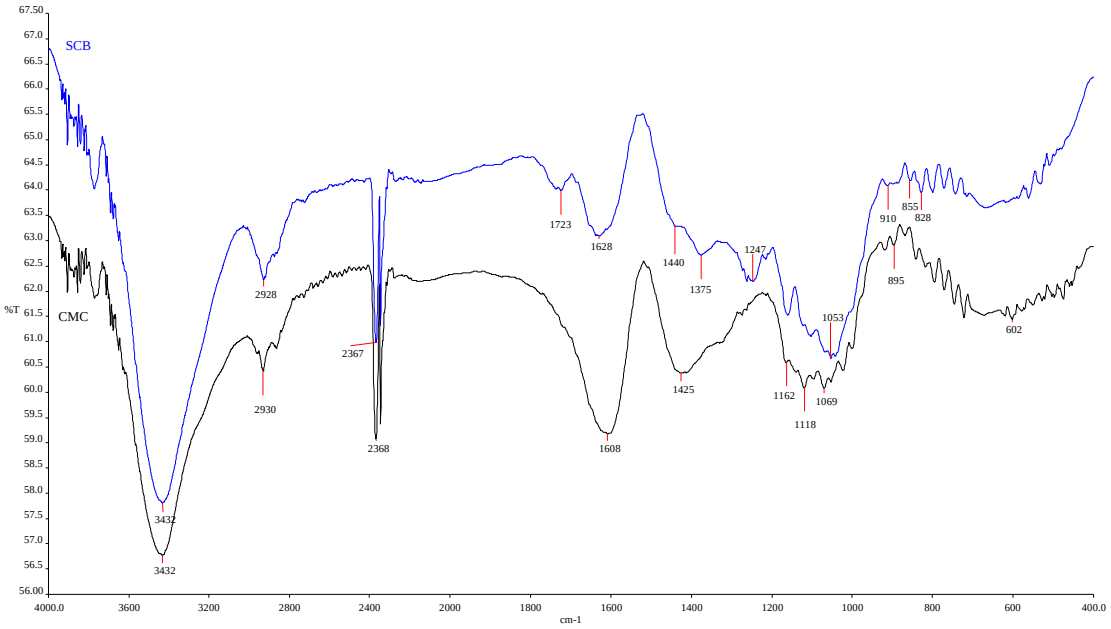

Figure 4.17: FTIR Spectra of SCB and CMC.

Table 4.22: Assignment of Absorption Band in CMC and SCB (Viera et al., 2007).

Wavenumber (cm^{-1})		Vibration
SCB	CMC	
3432	3432	axial deformation of O-H group
2928	2930	symmetrical stretching of C-H group
2367	2368	asymmetric stretching of CO_2
1723	-	stretching vibration of C=O
1628	-	carbonyl stretching of C=C bond with aromatic ring
-	1608	stretching vibration of COO^- group
1440	1425	CH_2 symmetric bending of lignin
1375	-	C-H deformation
1247	-	C-O stretching of aryl group
1053	1069	C-O symmetric stretching of primary alcohol
910	895	β-glycosidic linkage

DSC analysis in Figure 4.18 demonstrates the endothermic peak of CMC is broader and requires more heat of dehydration as compared to SCB. CMC has peak temperature at 80.34 ^{o}C, comparatively higher than peak temperature of SCB at 57.11 ^{o}C. On the other hand, the specific enthalpy for CMC is - 382.98 J/g while SCB has specific enthalpy of -373.76 J/g. The presence of this endothermic peak in CMC was likely due to the interaction between water and hydroxyl groups in cellulose which were not being substituted (Viera et al., 2007). Moreover, the high temperature peak and specific enthalpy indicates CMC is less crystalline as compared to SCB (Bertran and Dale, 1986). Reduction of crystallinity in CMC was mainly influenced by the alkalization of 17.5 %(w/v) of NaOH which causes the cleavage of hydrogen bond of cellulose (Singh and Singh, 2012).

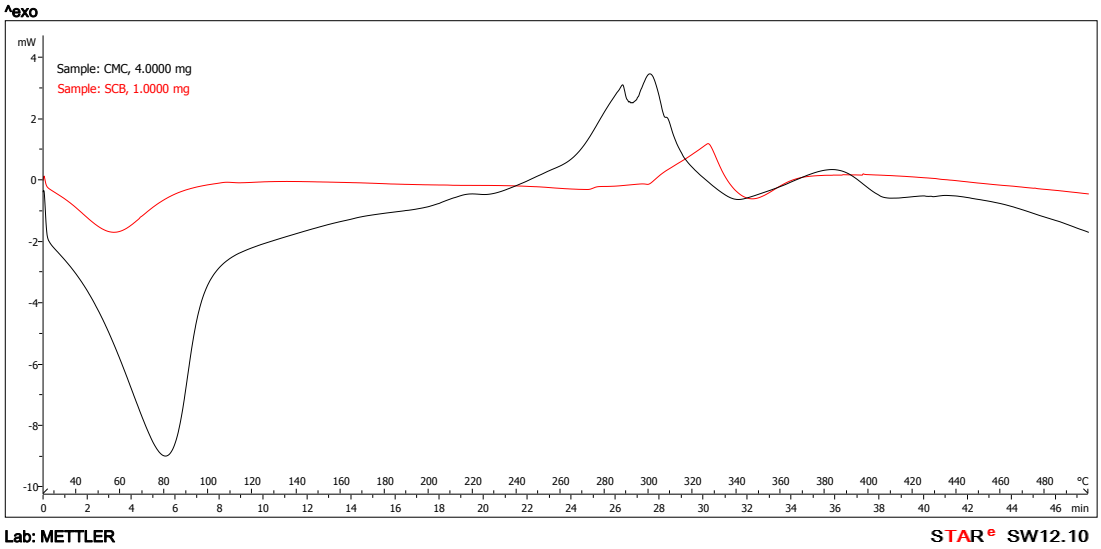

Figure 4.18: DSC thermograph of SCB and CMC.

Degree of substitution is a very important indicator used to determine the extent on which the substitution of carboxymethyl group in cellulose structure of C2, C3 and C6 are being carried out. It is identified through potentiometric titration whereby the sample was titrated with 0.3 M of HCl until solution turns colourless (Bono et al., 2009). From Appendix L, the degree of substitution (DS) calculated is 0.3624. The average DS for cellulose which undergo alkalization followed by carboxymethylation process with SMCA was in the range of 0.4-1.3 (Reuben and Conner, 1983). When DS is below 0.4, CMC produced is swellable but insoluble (Waring and Parsons,

2001). The reason of low DS obtained in this research was due to the reaction not being carried out in optimum condition. The temperature, time of reaction, different concentration of NaOH and amount of SMCA used will affect the value of DS. According to research conducted by Alizadeh, Mousavi and Labbafi (2017), DS of 0.78 was obtained for CMC produced from cellulose extracted from SCB when NaOH concentration of 30 %(w/v) was used. An optimum NaOH concentration will avoid the predomnating of side reaction, which reduce DS due to formation of sodium glycolates. Meanwhile, the carboxymethylation process was carried out at 55 ^{o}C for 3 hours. Longer time provides more time for carboxymethylation reaction, result in higher DS (Toğrul and Arslan, 2003). Besides, the accessibility of etherification agent to cellulose chain is influenced by the solvent used (Zhao et al., 2003). The substitution of carboxymethyl group can be further enhanced by using mixture of solvent as reaction medium at appropriate ratio (Pitaloka et al., 2017).

The properties of CMC produced can be further identified by producing a film as shown in Figure 4.19. Glycerol was added in film production which serve as plasticizing agent to overcome brittleness. This film is used for material that are non-toxic, biocompatible and non-allergenic (Tufan et al., 2016). In summary, cellulose extracted from SCB using ultrasound-assisted alkali treatment is promising as feedstock to produce CMC for industries application including oil-drilling industries.

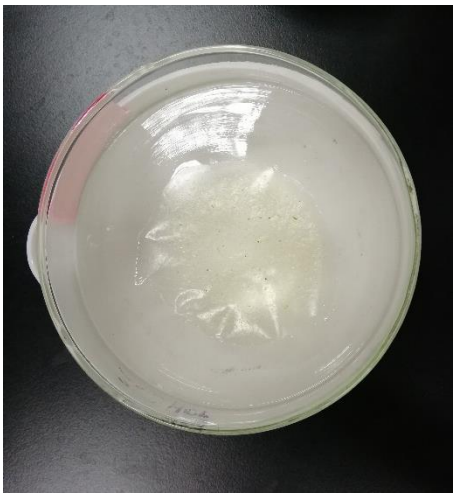

Figure 4.19: Film Production from Synthesize CMC.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, the extraction of cellulose upon autoclaved pretreatment with ultrasonic-assisted treatment was successfully been carried out. FTIR spectra has shown the disappearance or decrease in intensity of absorption band for hemicellulose and lignin, portraying the treatment process have reduced and solubilised the hemicellulose and lignin component in SCB. The thermal stability of cellulose extracted is estimated through DSC testing. On the other hand, the two step treatments have extracted approximate 56.58 %(w/w) to 83.22 %(w/w) of cellulose from SCB samples. The cellulose, hemicellulose and lignin content in treated SCB were determined by using HPLC.

There are three operating parameters being manipulated along this research study, which are ultrasonic amplitude, temperature and KOH concentration. It was observed that highest amount of cellulose, 83.22 %(w/w) was extracted when SCB treated at optimum condition of 30 % ultrasonic amplitude, 80 ^{o}C and 1.25 M KOH. This result portrayed the successful pretreatment of autoclave which help in removal of soluble impurities and lignin in SCB. Meanwhile, the ultrasonic-assisted treatment in alkali medium aids in breaking the bonding of hemicellulose and lignin, enhance mass transfer and thereby facilitate cellulose extraction. As for DSC thermographs, it has the lowest peak temperature at the first endothermic peak with the highest peak temperature at second endothermic peak, estimating the presence of cellulose content in the treated sample.

Besides that, CMC has been synthesized from the treated SCB with the highest cellulose content to further enhance its application. It was found that the degree of substitution of carboxyl group on cellulose is 0.3624 through potentiometric titration. FTIR spectra has confirmed the presence of carboxyl group at absorption wavelength of 1608 cm^{-1}. In addition, the production of film has given supporting evidence for the properties of produced CMC where it is widely used in various industries due to its solubility properties.

In current research, SCB is used as the feedstock since it has high availability with promising cellulose content about 50 %(w/w). The combination of ultrasonic treatment with alkali medium is an effective modified method which can be used in extracting higher amount of cellulose to accommodate its application in various industries. Mild alkali treatment result in lower degradation and formation of side products. Meanwhile, the ultrasonic treatment is more economic saving since the treatment requires less chemical usage and shorter extraction time.

5.2 Recommendation

SCB as the feedstock consists of relative higher cellulose content as compared to other lignocellulosic biomass. This cellulose can be used in textile, paper, paints, cosmetic and pharmaceutical industry. In this research, the treatment of SCB with autoclave and ultrasonic-assisted treatment has successful extracted large amount of cellulose. However, there are still places for improvement after conducting this experiment. Firstly, in this research, the temperature of the SCB treatment medium is unstable since hot plate is not able to place within the ultrasonic homogenizer protector box. The temperature of treated medium will fluctuate within 5 ^{o}C. Therefore, when manipulating the temperature of SCB in alkali medium, it is recommended to use jacketed beaker to maintain its temperature during the ultrasonic treatment process.

Besides that, a further study on the type of solvent used during the ultrasonic treatment can be conducted since different chemicals will affect the viscosity of the SCB mixture solution, which in turn the ultrasonic effect on the solution. Other than

that, the reaction time and ultrasonic duty cycle can be studied as it can bring different effect on the amount of cellulose extracted.

In addition, X-ray diffraction (XRD) and scanning electron microscopy (SEM) characterization can be used to further determine the properties of the treated SCB. XRD can be carried out to determine the exact amount of crystalline and amorphous component in SCB constituents before and after the treatment process. This will aid in determine the amount of cellulose being extracted since it is crystalline in nature as compared to hemicellulose and lignin. On the other hand, SEM helps to analyse the structure change within SCB and treated SCB, whether there is removal or damage on hemicellulose and lignin after undergo autoclave and ultrasonic treatment.

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APPENDICES

APPENDIX A: FTIR Spectra of Treated SCB

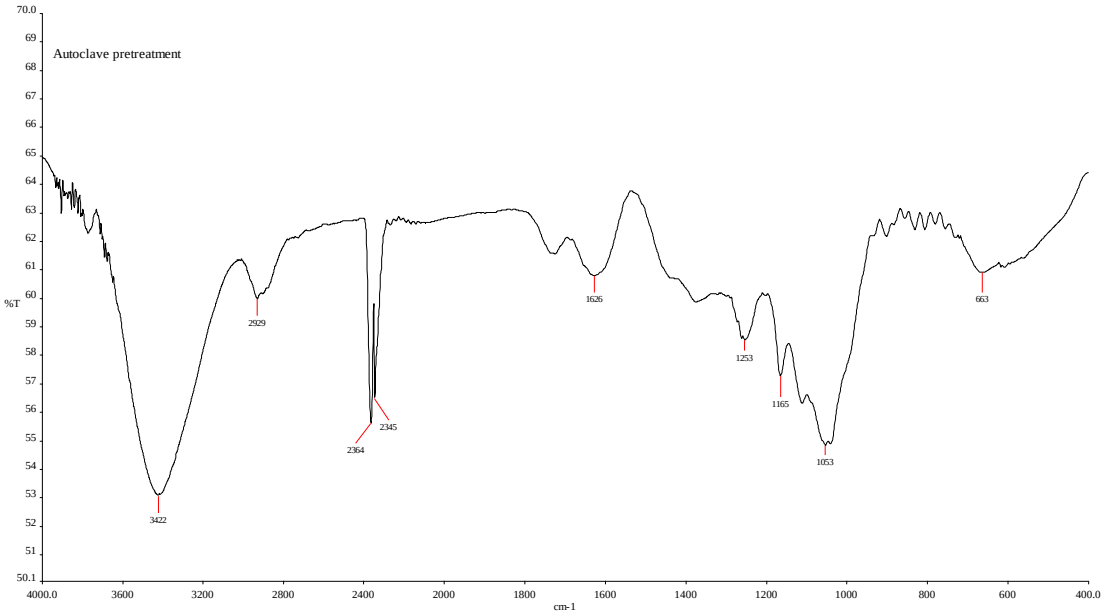

Figure A.1: FTIR Spectra of Autoclave Pretreatment SCB.

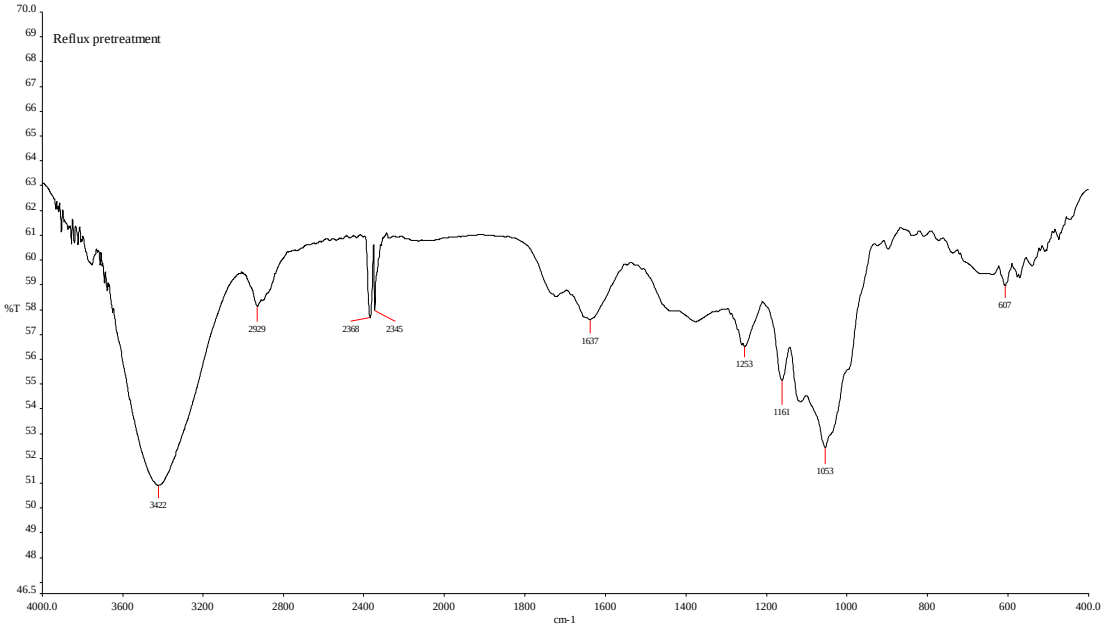

Figure A.2: FTIR spectra of Reflux Pretreatment SCB.

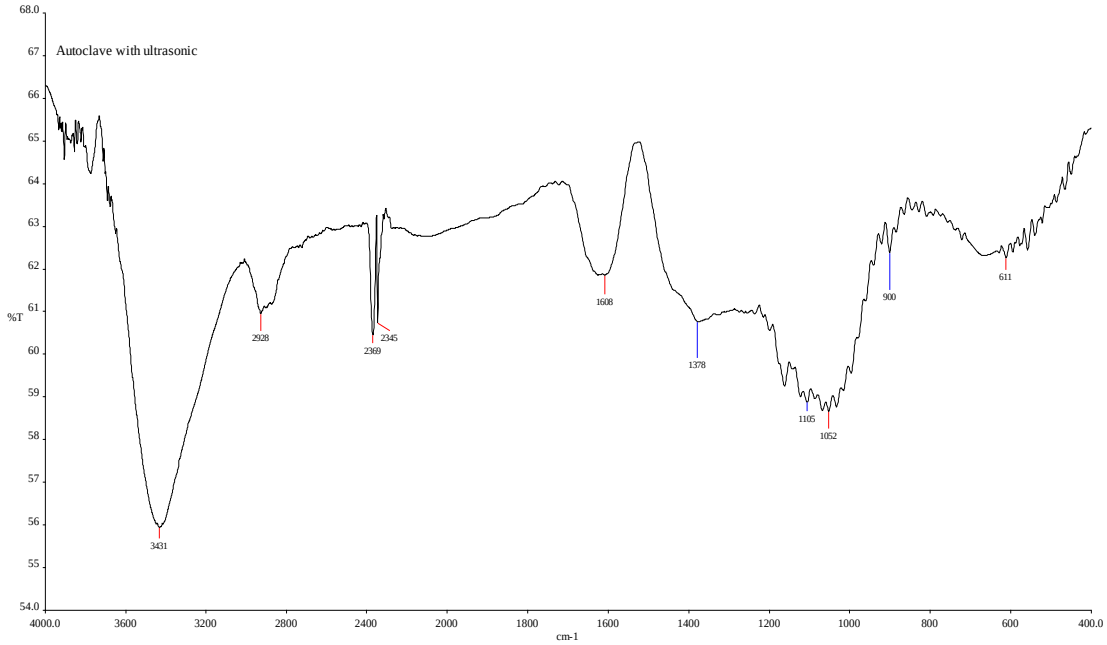

Figure A.3: FTIR Spectra of Autoclaved SCB with Ultrasonic Treatment.

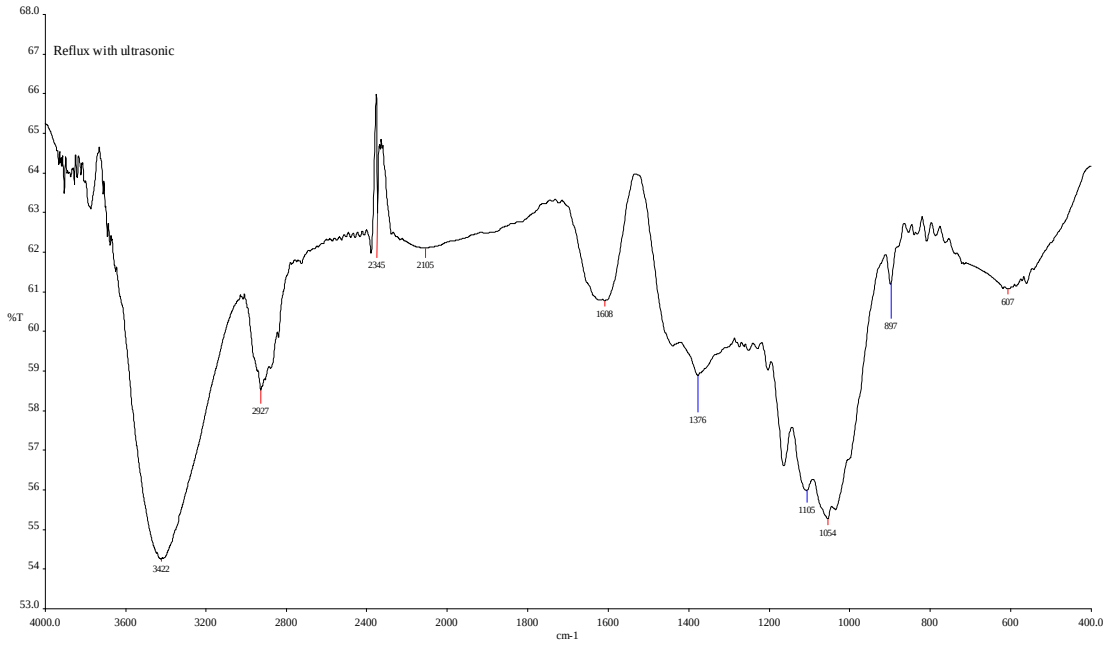

Figure A.4: FTIR Spectra of Refluxed SCB with Ultrasonic Treatment.

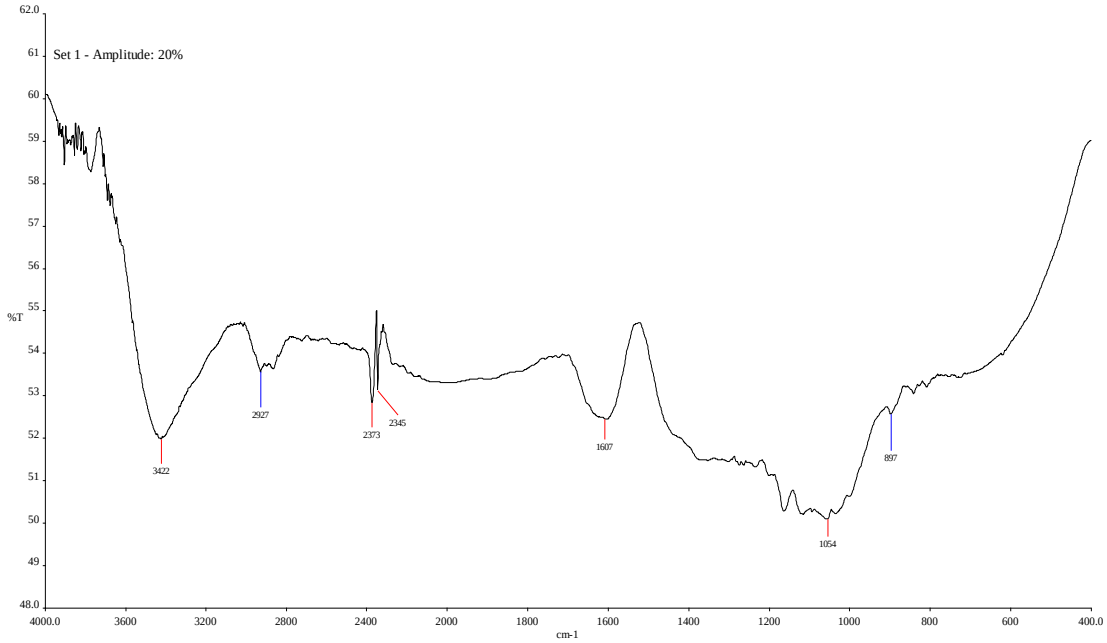

Figure A.5: FTIR Spectra of Treated SCB at Ultrasonic Amplitude of 20 %.

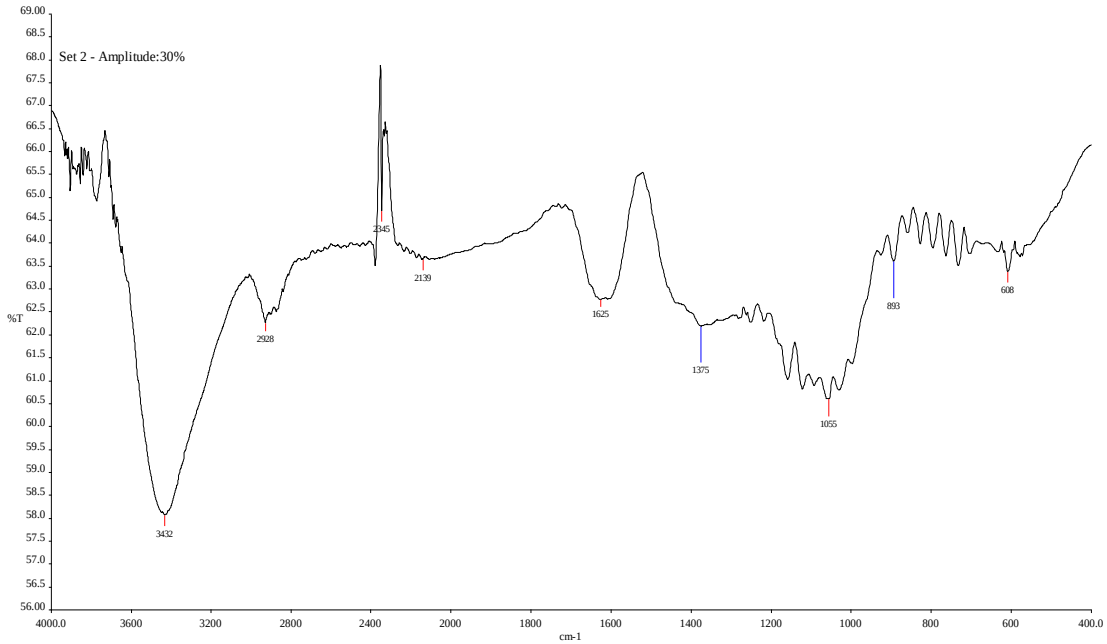

Figure A.6: FTIR Spectra of Treated SCB at Ultrasonic Amplitude of 30 %.

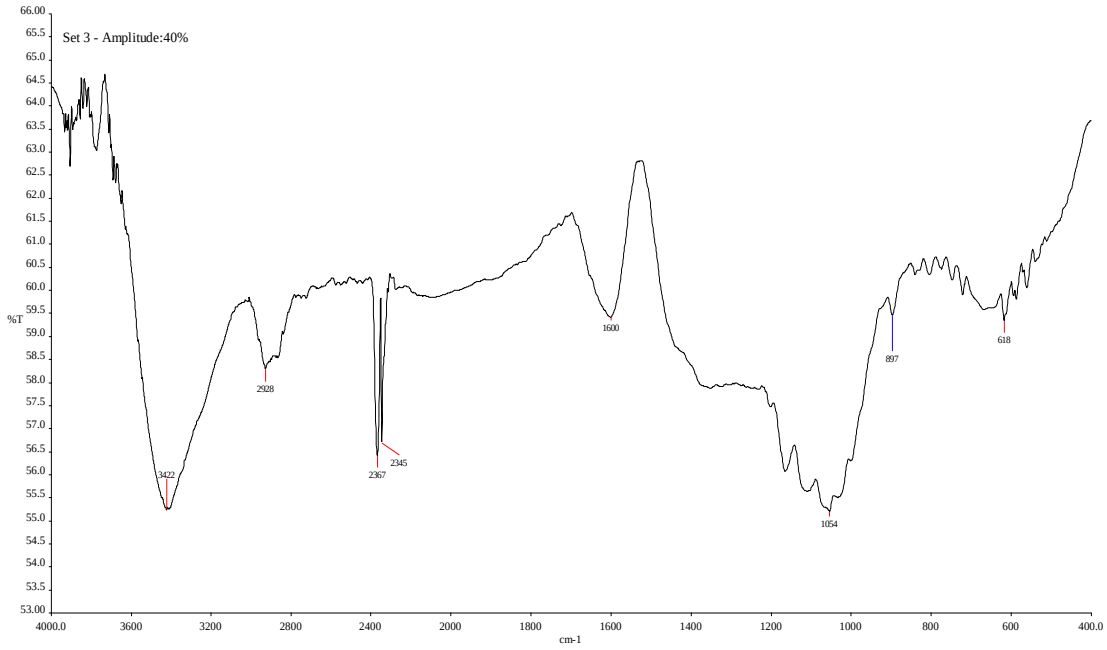

Figure A.7: FTIR Spectra of Treated SCB at Ultrasonic Amplitude of 40 %.

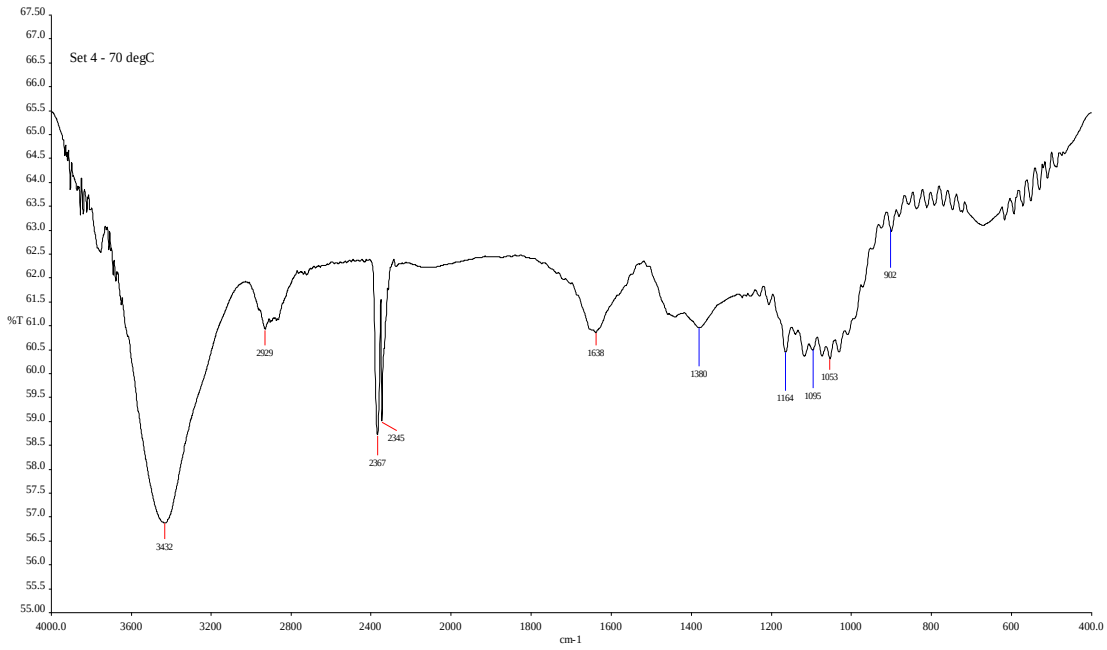

Figure A.8: FTIR Spectra of Treated SCB at Temperature of 70 ^{o}C.

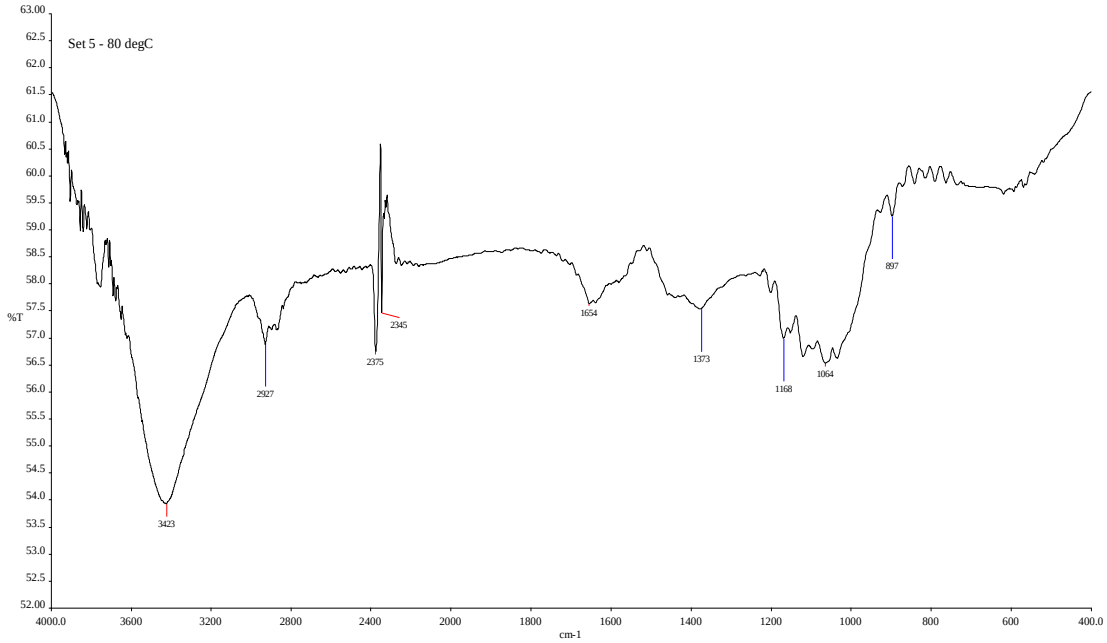

Figure A.9: FTIR Spectra of Treated SCB at Temperature of 80 ºC.

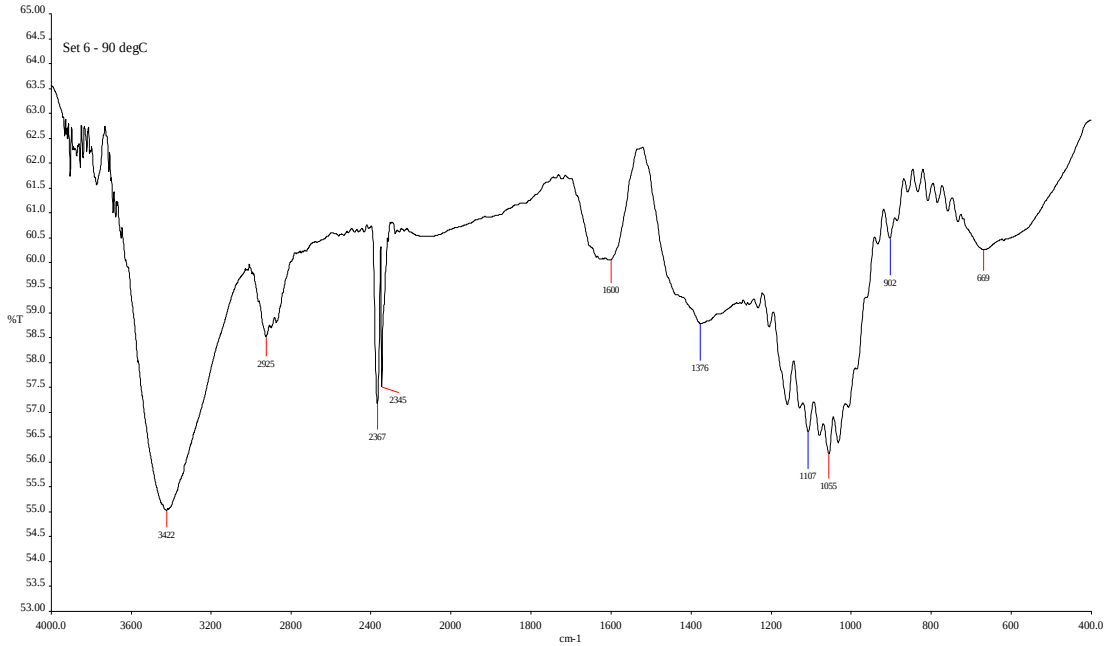

Figure A.10: FTIR Spectra of Treated SCB at Temperature of 90 ºC.

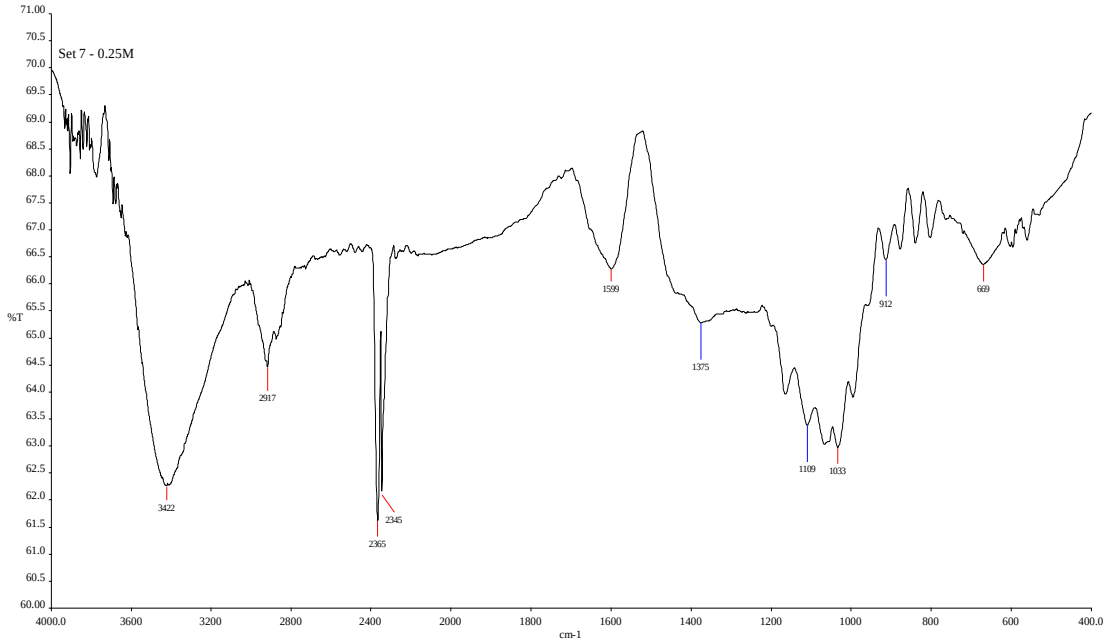

Figure A.11: FTIR Spectra of Treated SCB at KOH Concentration of 0.25 M.

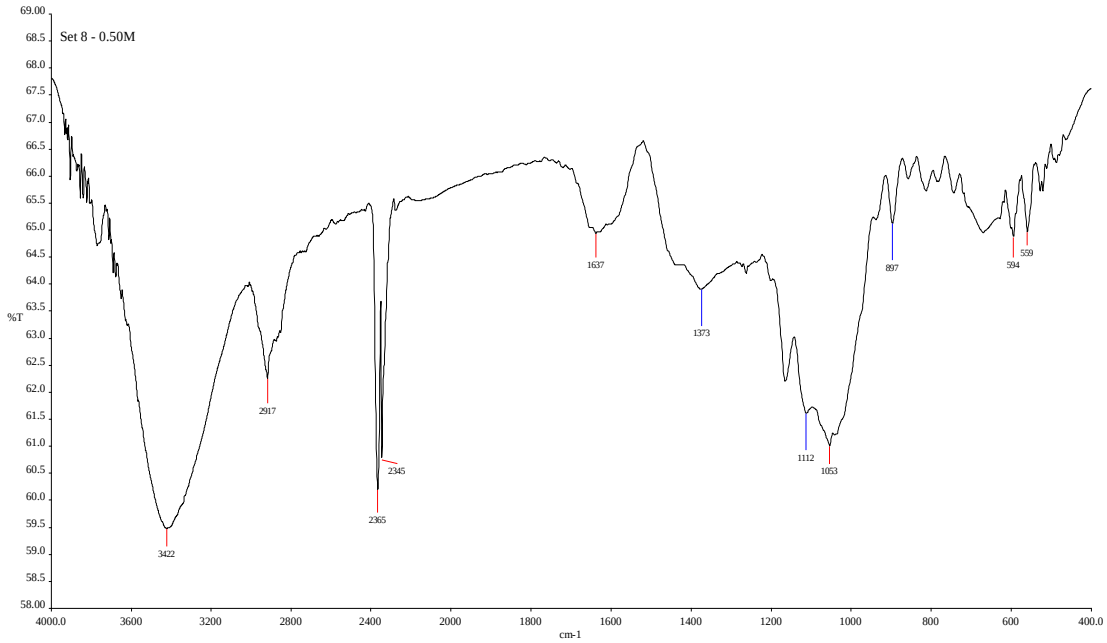

Figure A.12: FTIR Spectra of Treated SCB at KOH Concentration of 0.50 M.

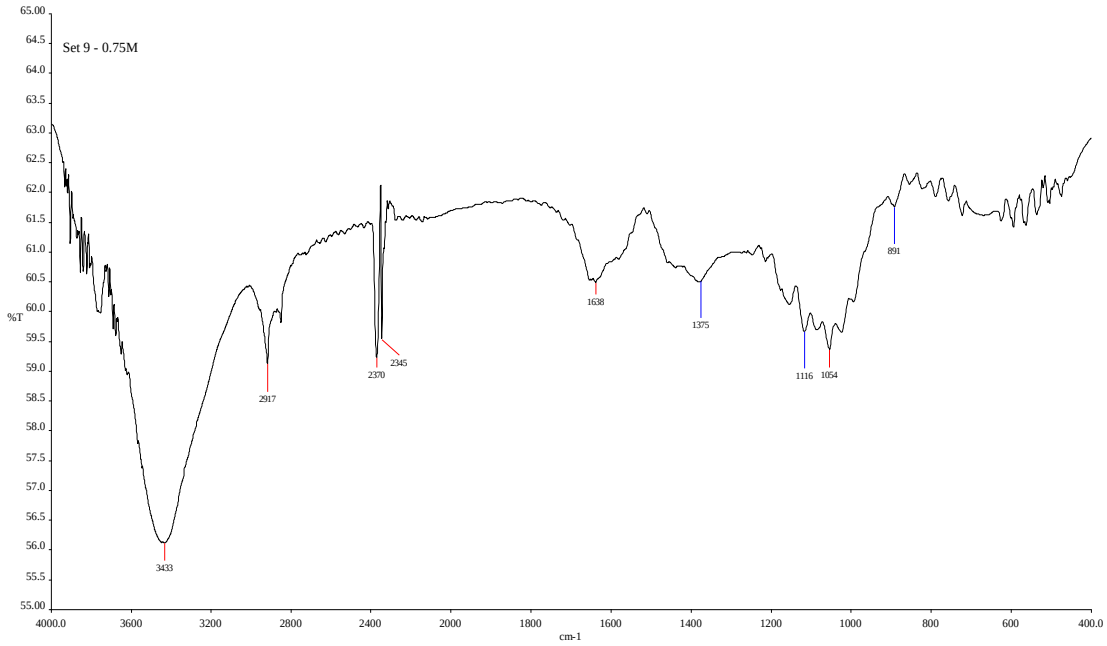

Figure A.13: FTIR Spectra of Treated SCB at KOH Concentration of 0.75 M.

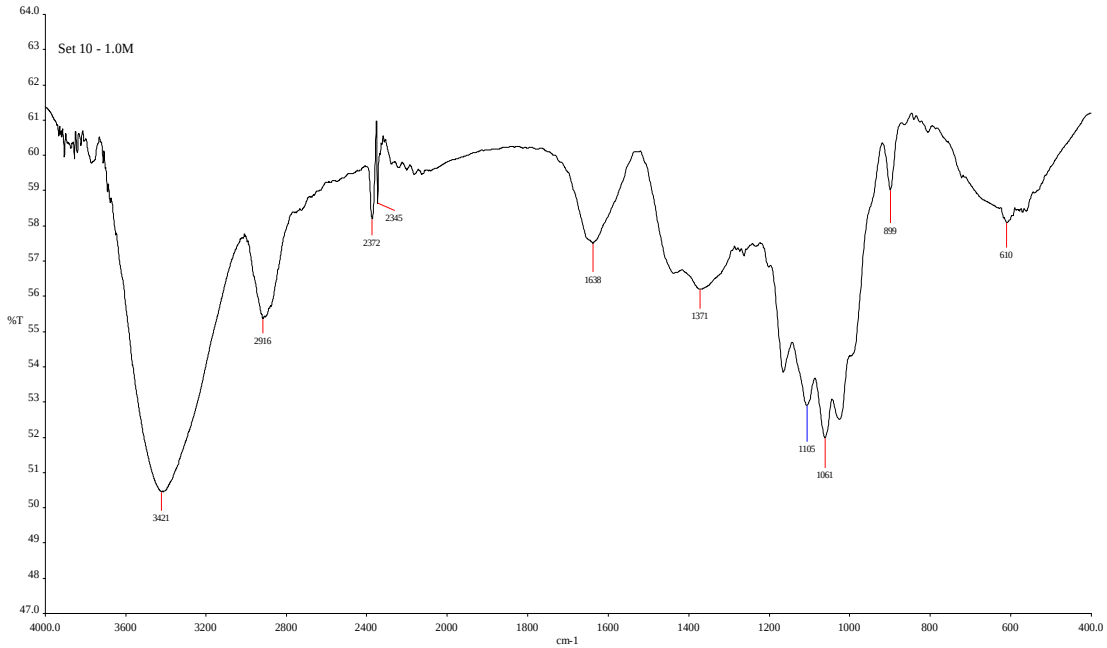

Figure A.14: FTIR Spectra of Treated SCB at KOH Concentration of 1.0 M.

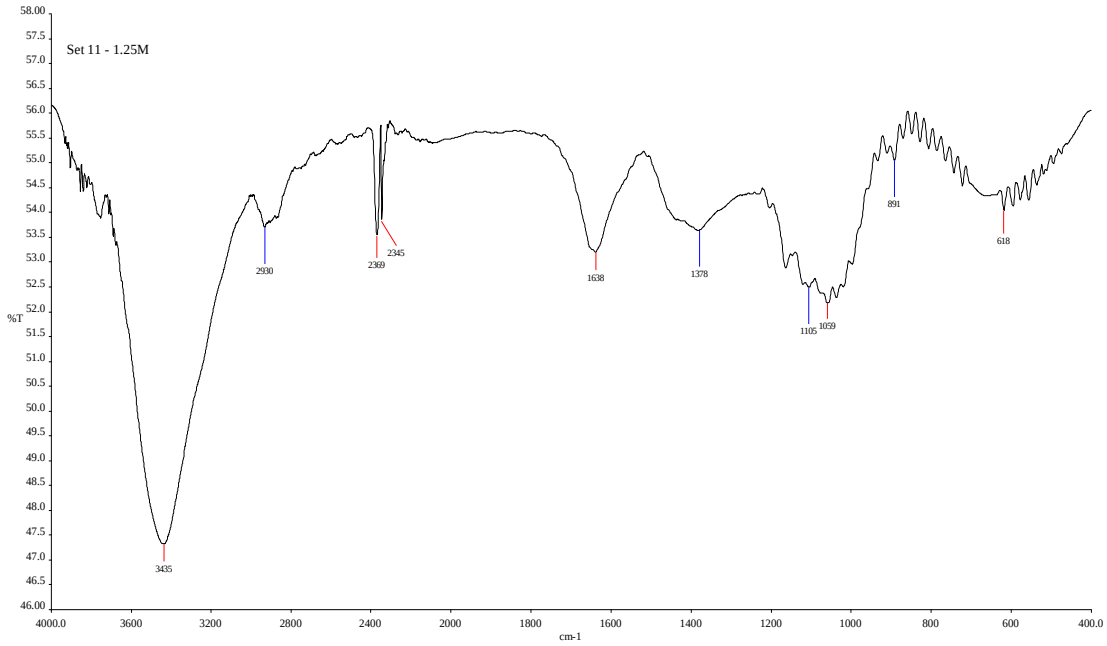

Figure A.15: FTIR Spectra of Treated SCB at KOH Concentration of 1.25 M.

APPENDIX B: DSC Thermograph of Treated SCB

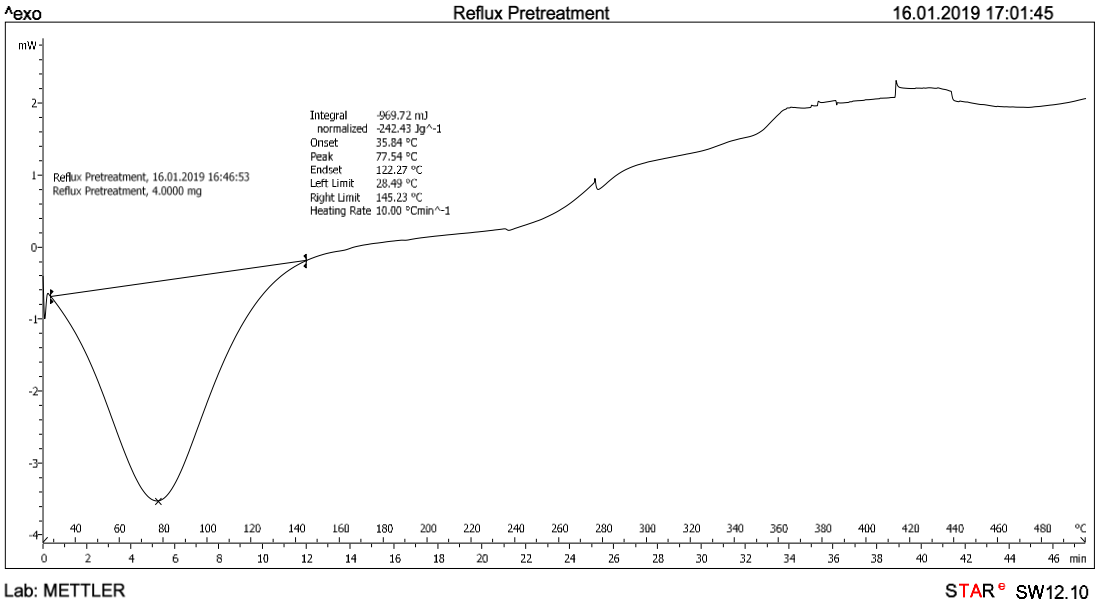

Figure B.1: DSC Thermograph of Refluxed SCB with Ultrasonic Treatment.

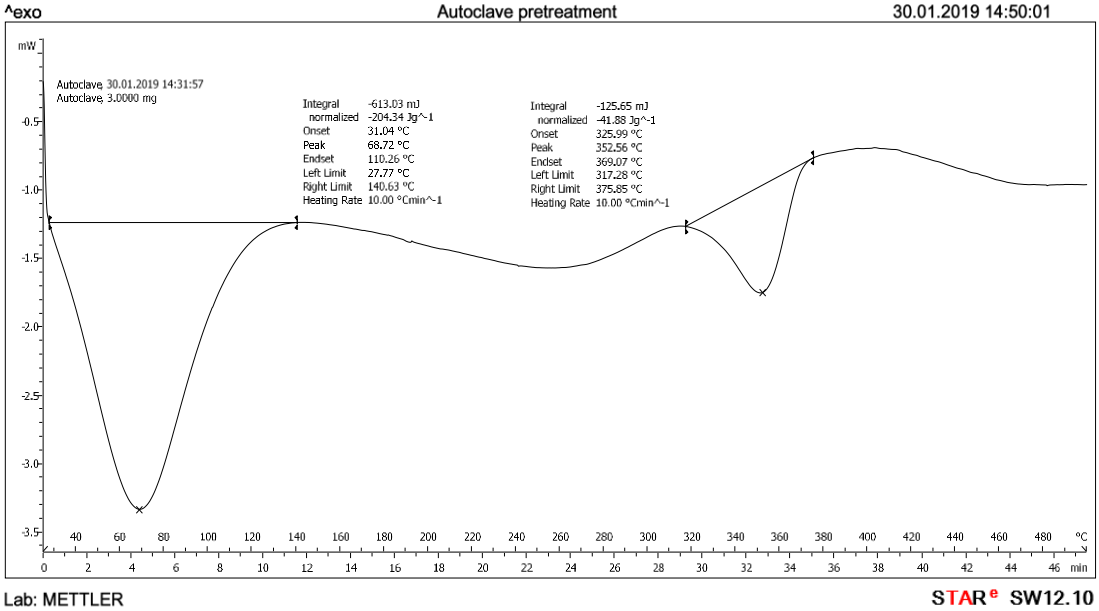

Figure B.2: DSC Thermograph of Autoclaved SCB with Ultrasonic Treatment.

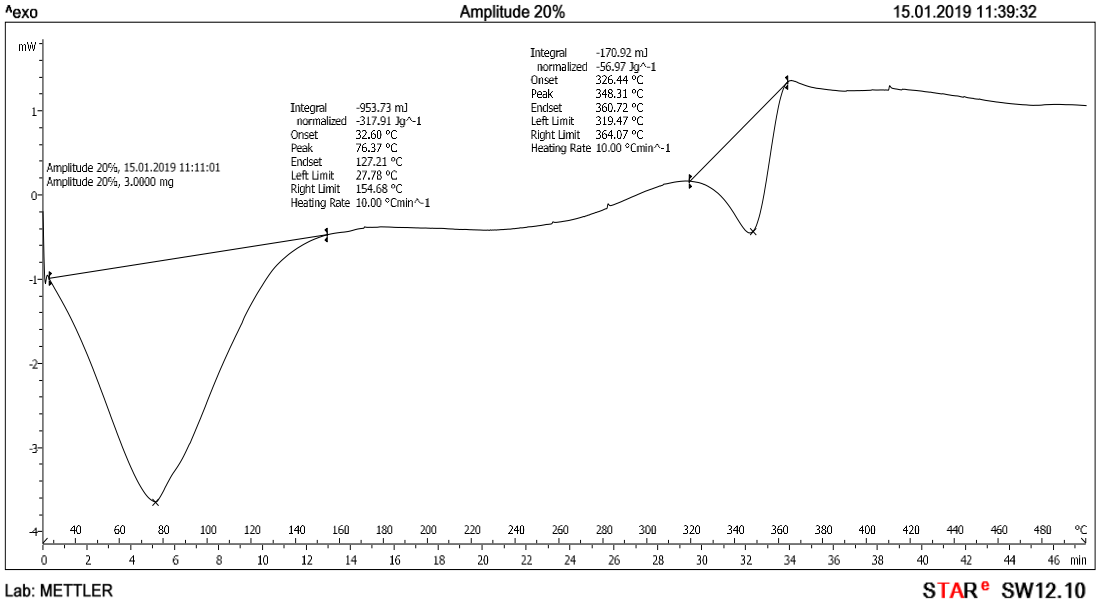

Figure B.3: DSC Thermograph of Treated SCB at Ultrasonic Amplitude of 20 %.

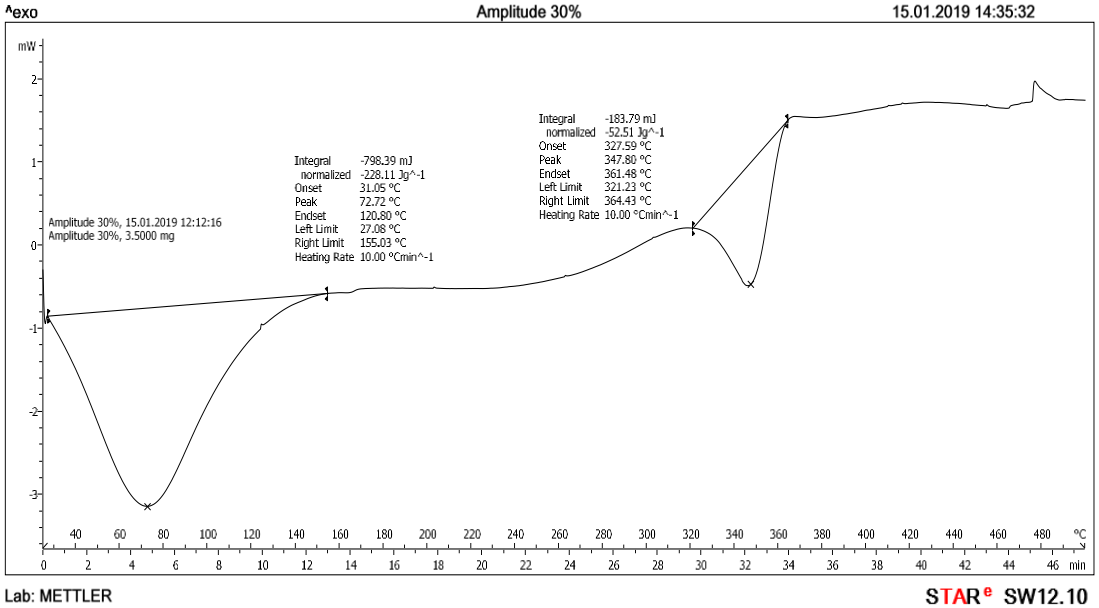

Figure B.4: DSC Thermograph of Treated SCB at Ultrasonic Amplitude of 30 %.

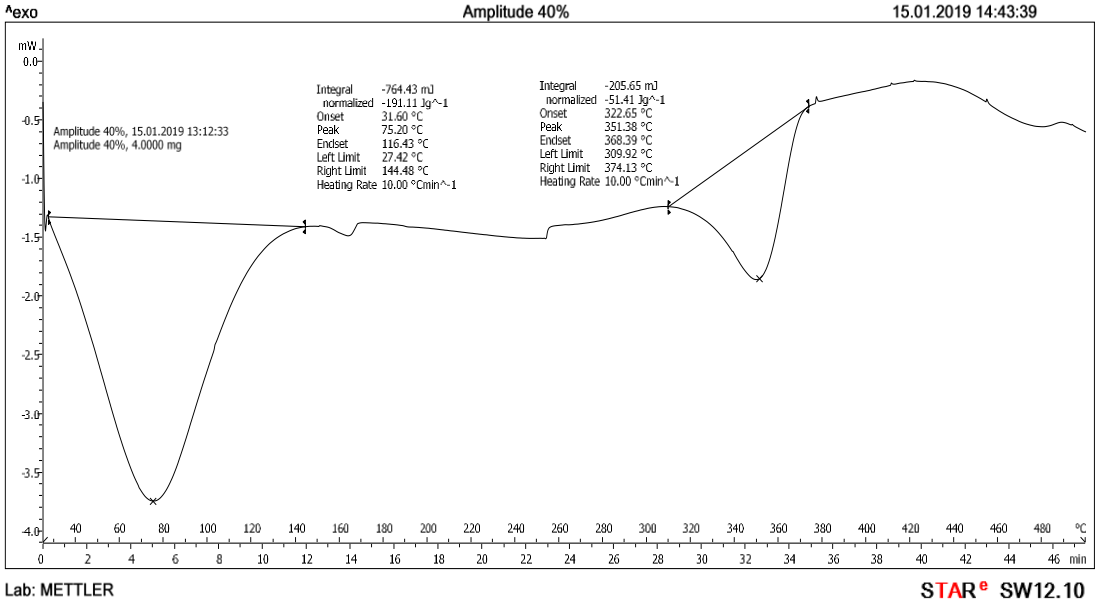

Figure B.5: DSC Thermograph of Treated SCB at Ultrasonic Amplitude of 40 %.

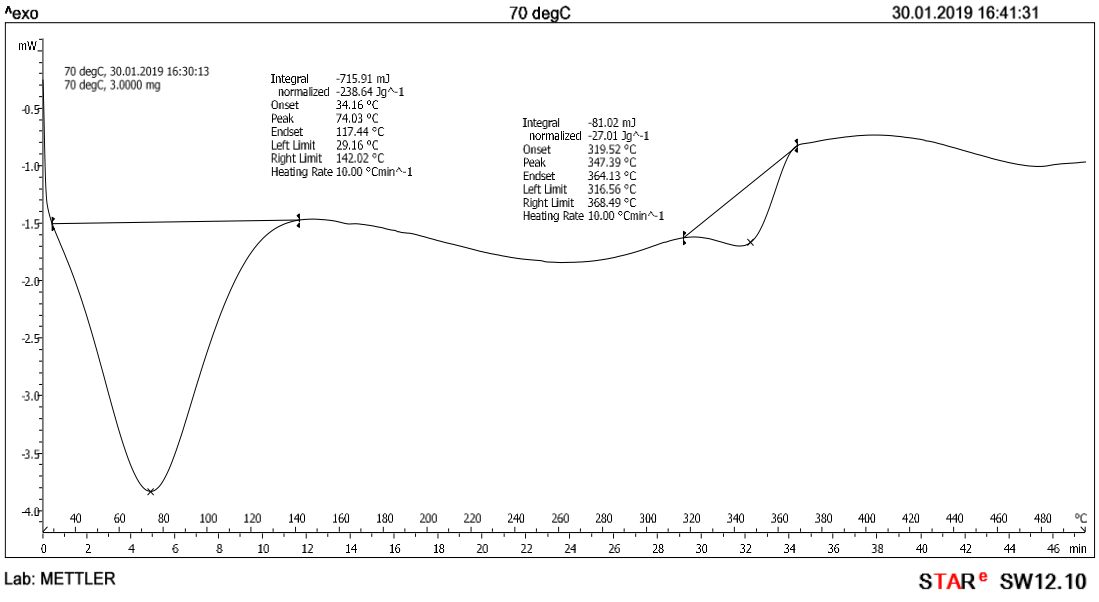

Figure B.6: DSC Thermograph of Treated SCB at Temperature of 70 ^{o}C.

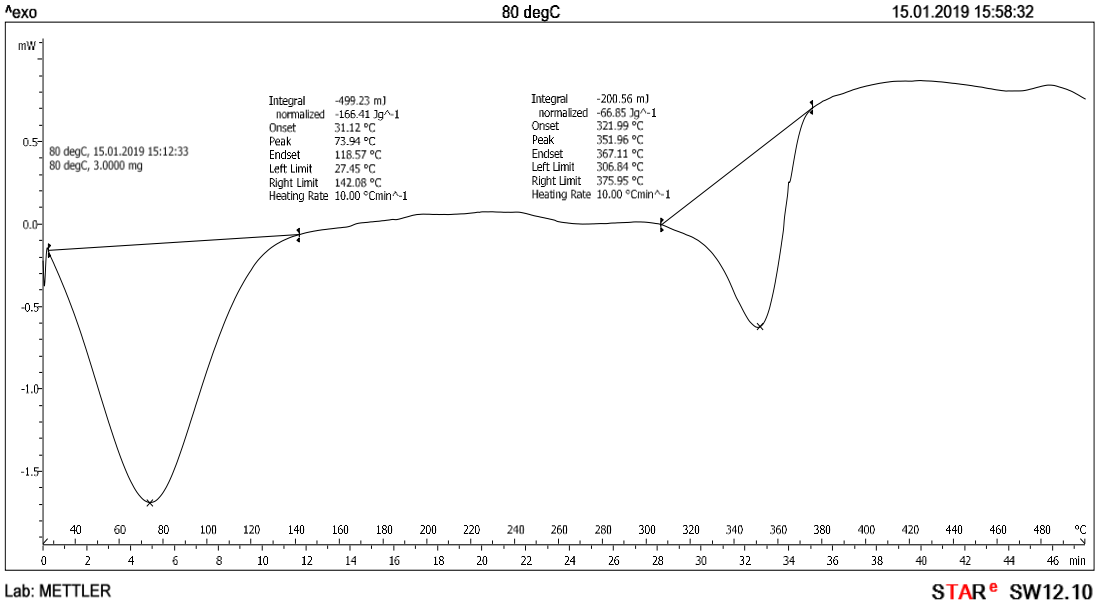

Figure B.7: DSC Thermograph of Treated SCB at Temperature of 80 ^{o}C.

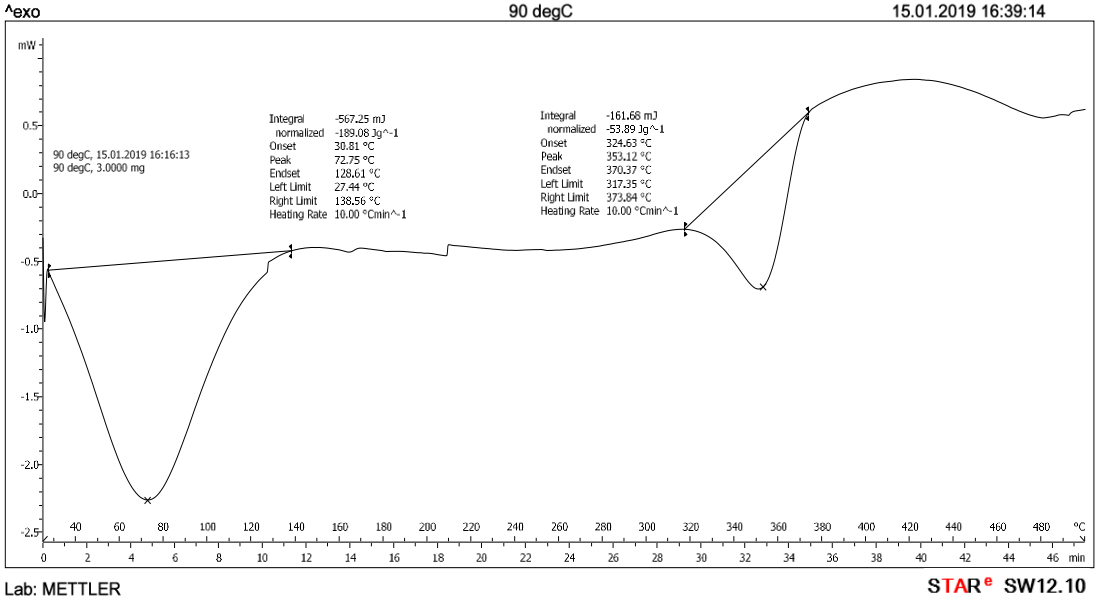

Figure B.8: DSC Thermograph of Treated SCB at Temperature of 90 ^{o}C.

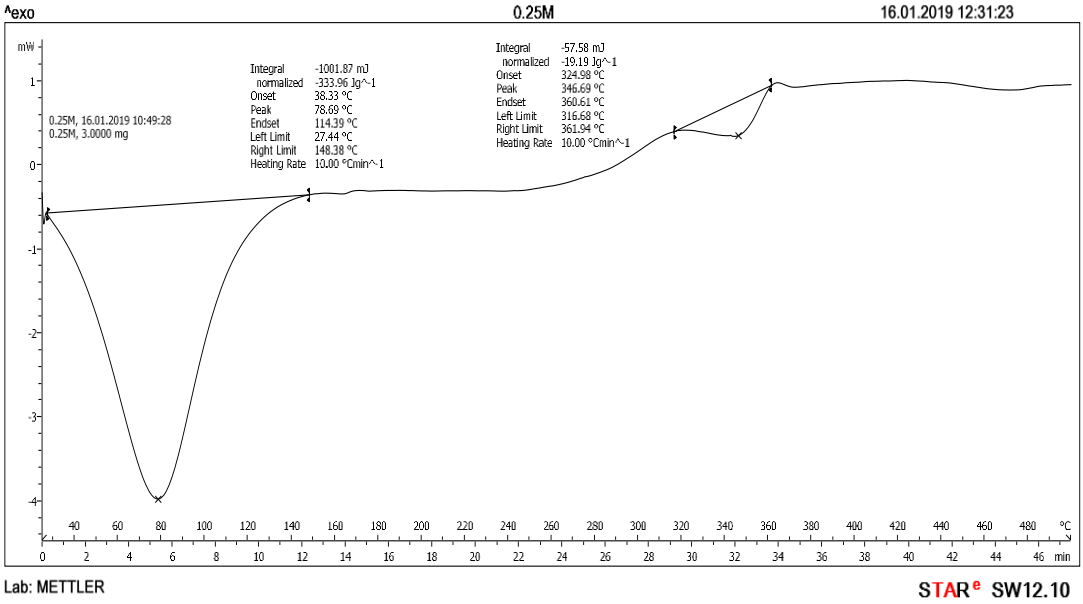

Figure B.9: DSC Thermograph of Treated SCB at KOH Concentration of 0.25 M.

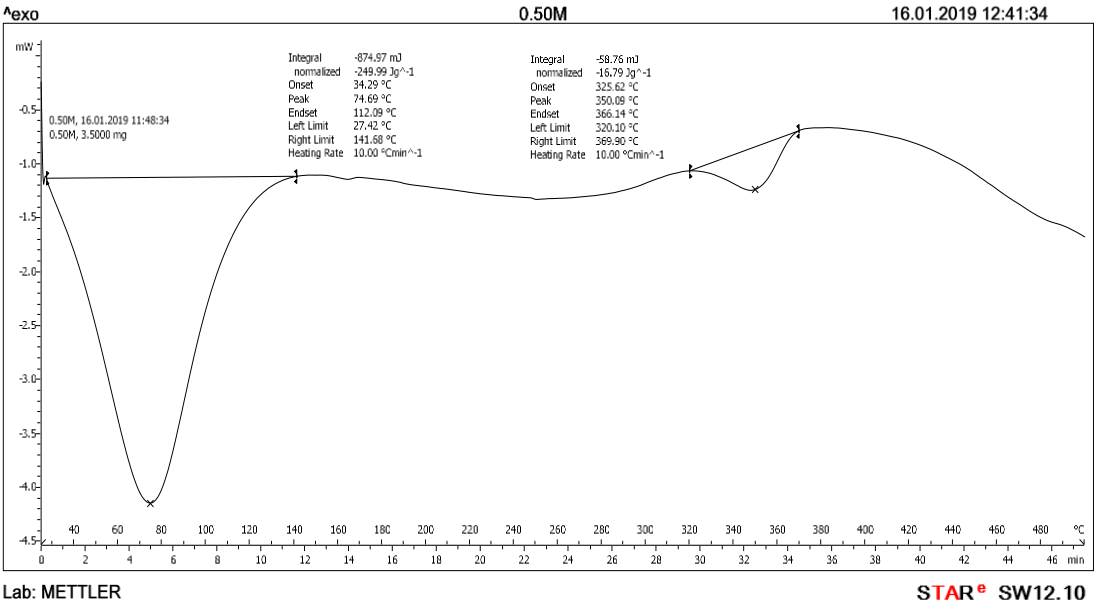

Figure B.10: DSC Thermograph of Treated SCB at KOH Concentration of 0.50 M.

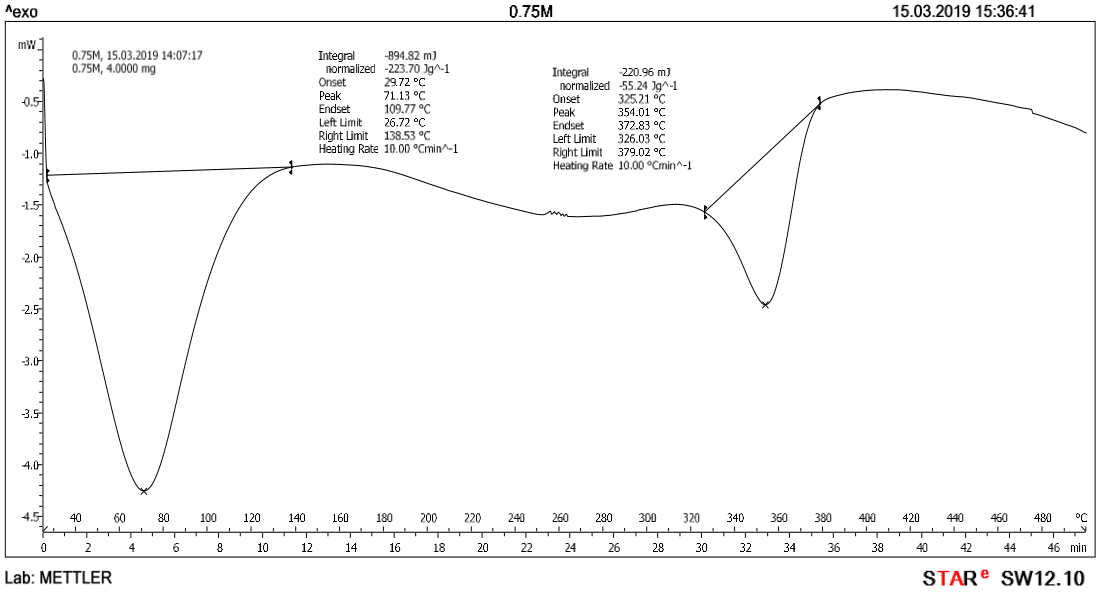

Figure B.11: DSC Thermograph of Treated SCB at KOH Concentration of 0.75 M.

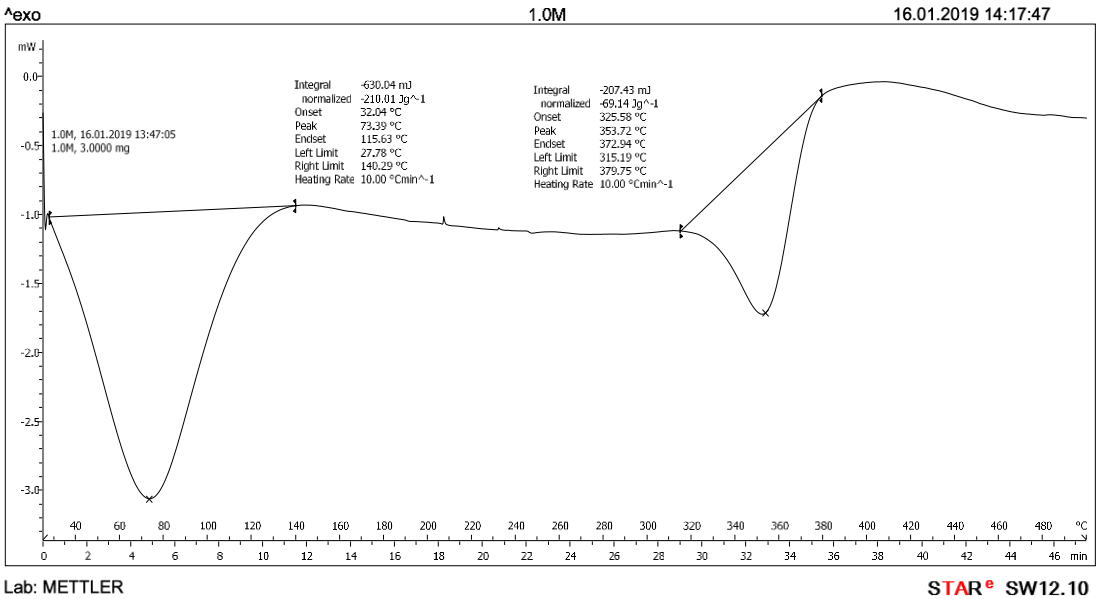

Figure B.12: DSC Thermograph of Treated SCB at KOH Concentration of 1.0 M.

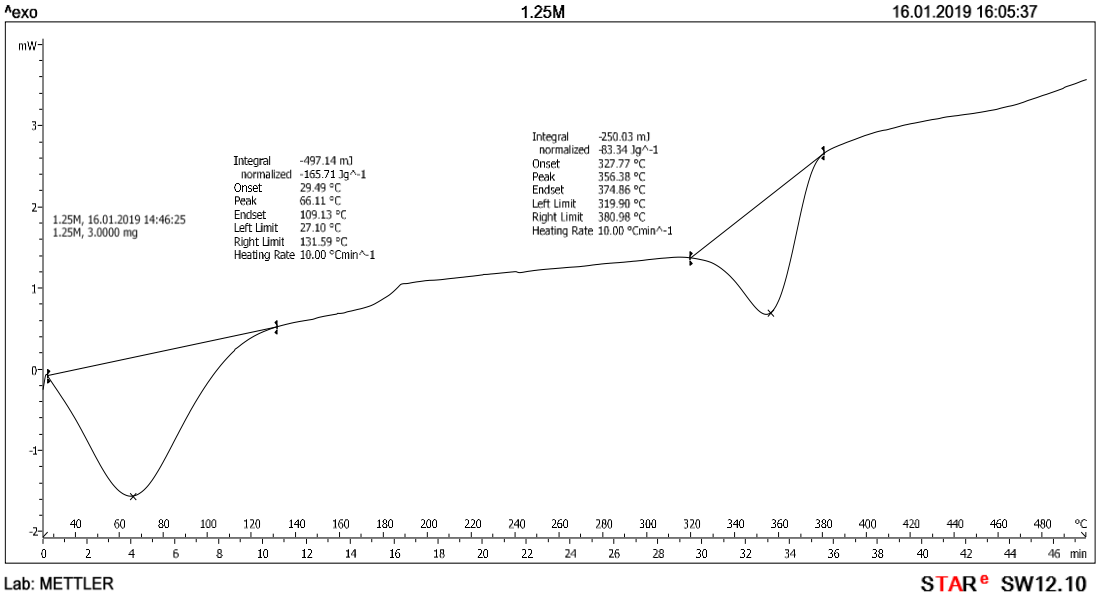

Figure B.13: DSC Thermograph of Treated SCB at KOH Concentration of 1.25 M.

APPENDIX C: HPLC Calibration Curve for Dextrose

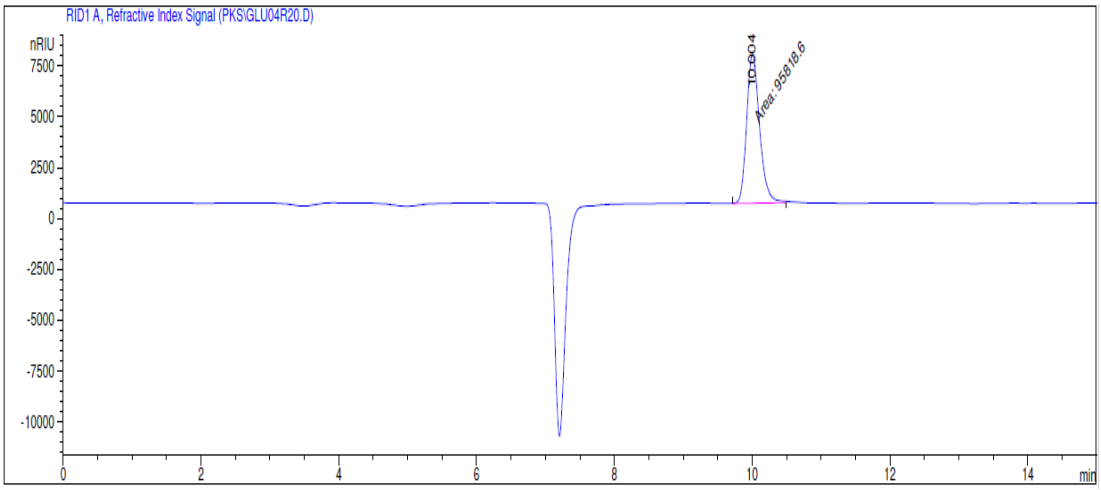

Figure C.1: HPLC Analysis for Dextrose at 0.4 g/L.

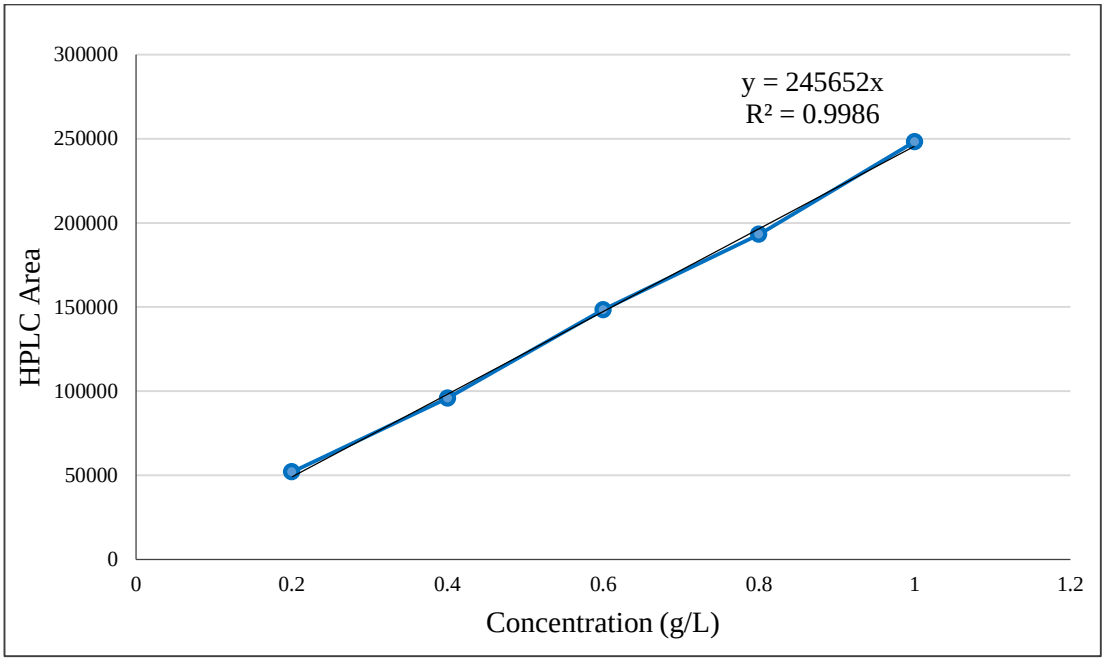

Figure C.2: Dextrose Calibration Curve.

APPENDIX D: HPLC Calibration Curve for Xylose

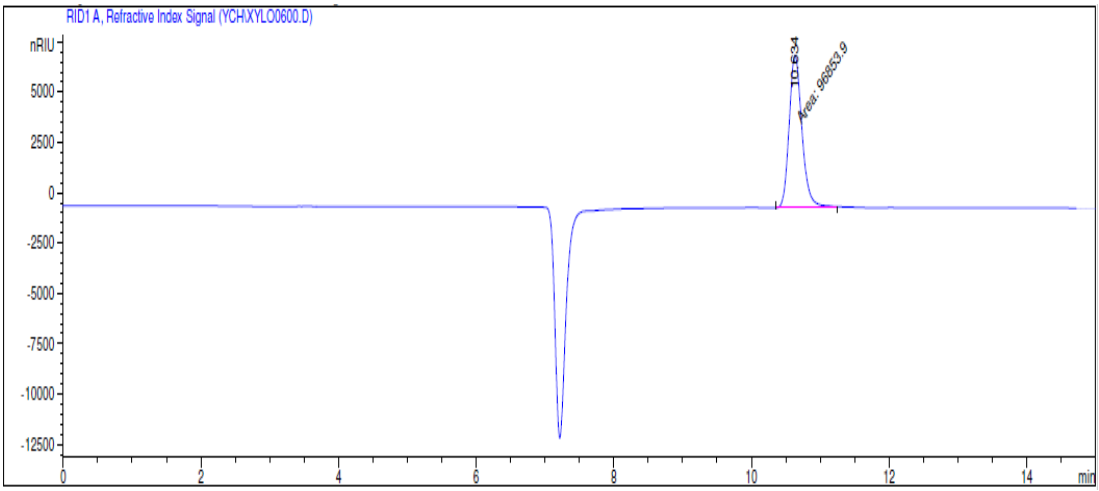

Figure D.1: HPLC Analysis for Xylose at 0.4 g/L.

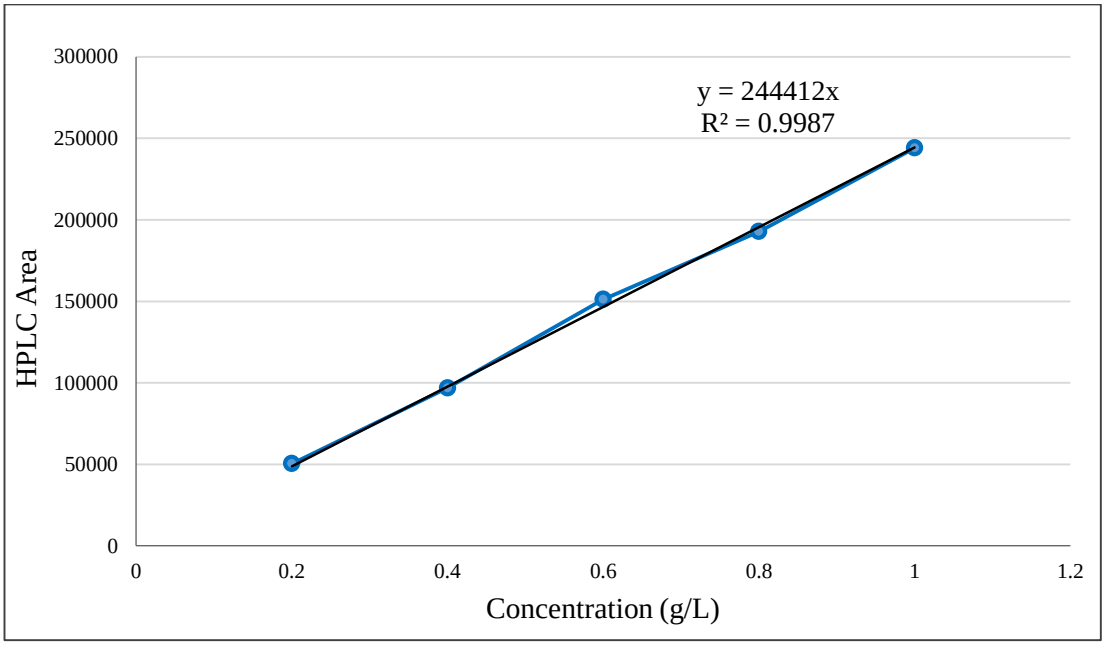

Figure D.2: Xylose Calibration Curve.

APPENDIX E: HPLC Calibration Curve for Arabinose

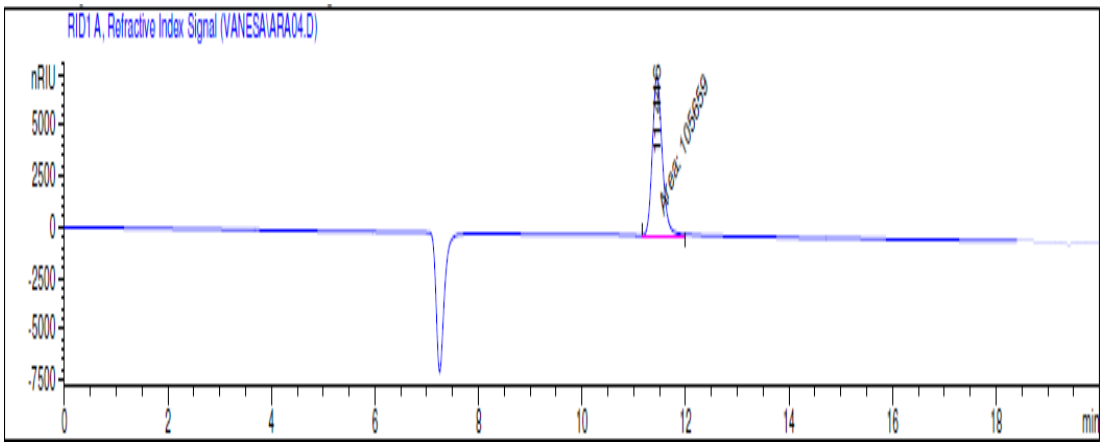

Figure E.1: HPLC Analysis for Arabinose at 0.4 g/L.

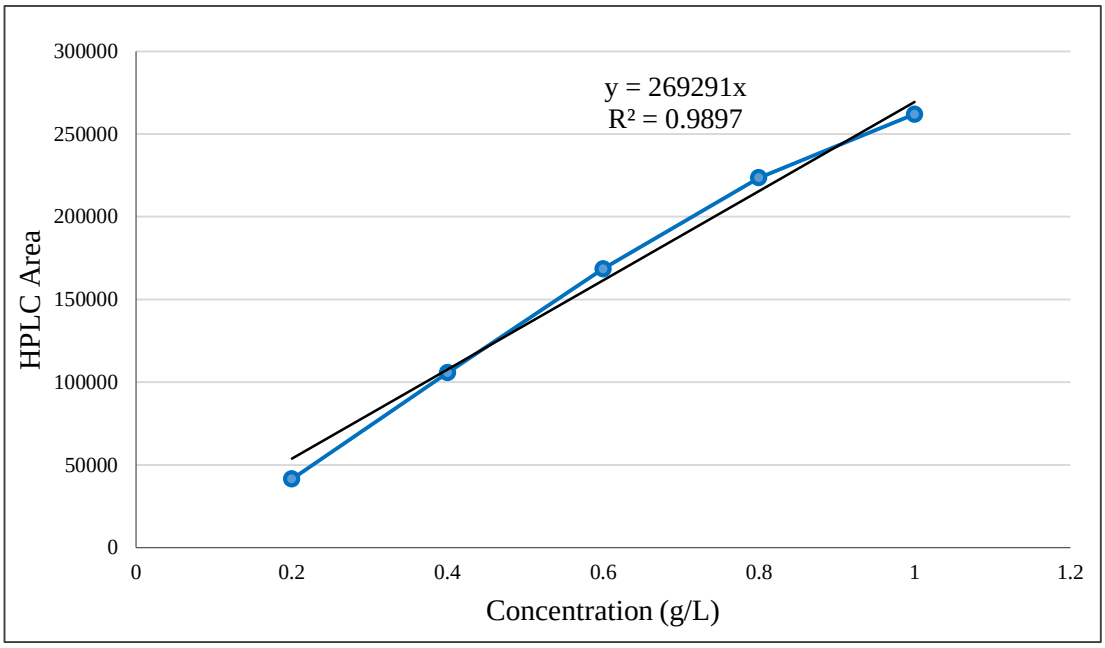

Figure E.2: Arabinose Calibration Curve.

APPENDIX F: Calculation of Carbohydrate Content of Sugarcane Bagasse in HPLC

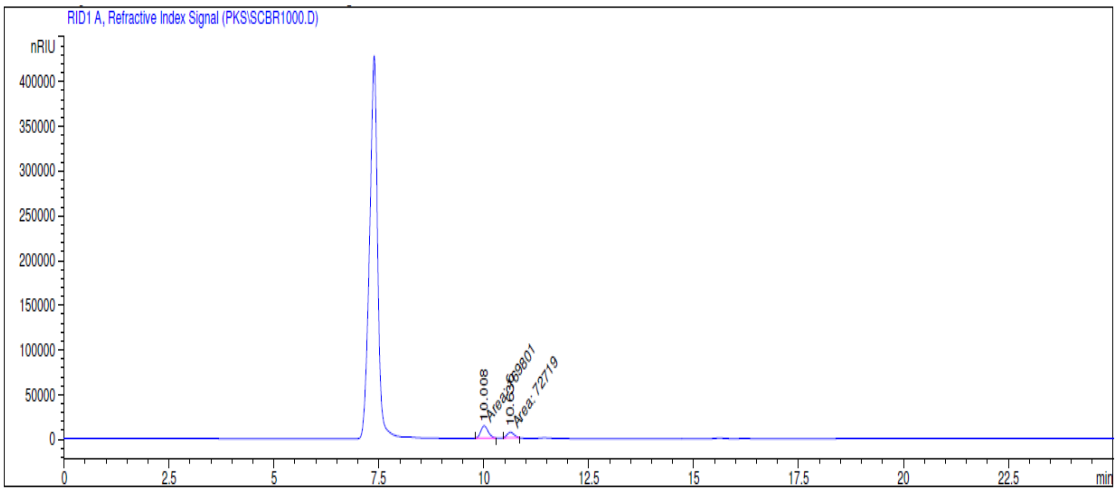

Figure F.1: HPLC Chromatogram of Sugarcane Bagasse.

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

$y = 245652x$

HPLC area obtained at 10.008 minutes = 169801

$169801 = 245652x$

$x = 0.6912$ g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [0.6912 g/L] [10 mL]

$M_1 = 1.3825$ g/L

Dextrose content = concentration (M_1) x total volume

= 1.3825 g/L x 0.089 L

= 0.1230 g

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

$y = 244412x$

HPLC area obtained at 10.636 minutes = 72719

$72719 = 244412x$

$x = 0.2975$ g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [0.2975 g/L] [10 mL]

M_1 = 0.5951 g/L

Xylose content = concentration (M_1) x total volume

= 0.5951 g/L x 0.089 L

= 0.0530 g

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

y = 269291x

HPLC area obtained at 11.443 minutes = 25247.1

25247.1 = 269291x

x = 0.0938 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [0.0938 g/L] [10 mL]

M_1 = 0.1875 g/L

Arabinose content = concentration (M_1) x total volume

= 0.1875 g/L x 0.089 L

= 0.0167 g

(IV) Mass of estimated cellulose content

= [0.1230 g / 0.3 g x 100 %] x 0.3 g

= 0.1230 g

(V) Total carbohydrates content in weight percentage

= [0.1230 g + 0.0530 g + 0.0167 g] / 0.3 g x 100 %

= 64.23 wt %

(VI) Mass of estimated carbohydrate content

= 64.23 wt % x 0.3 g

= 0.1927 g

APPENDIX G: HPLC Analysis of Autoclaved and Refluxed SCB with Ultrasonic Treatment

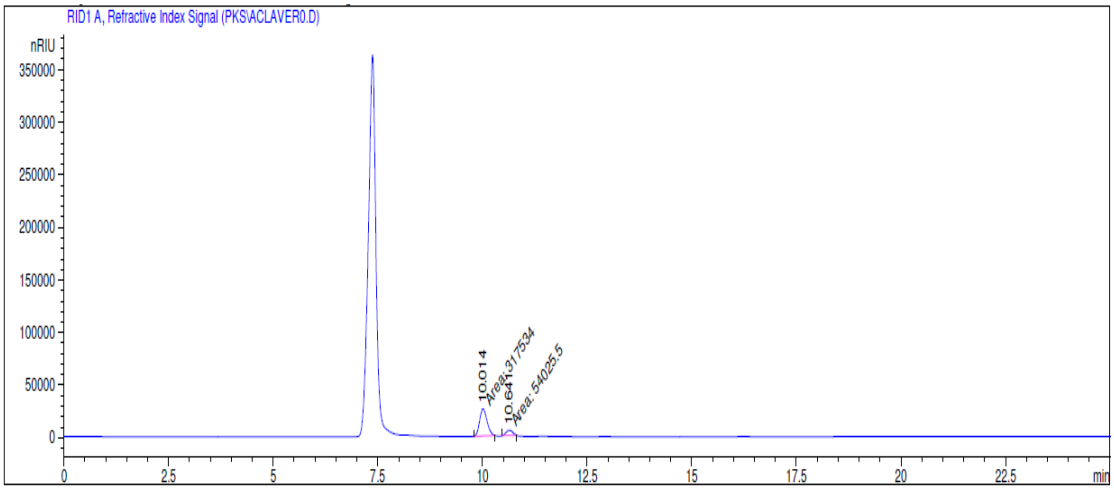

Figure G.1: HPLC Chromatogram of Autoclaved SCB with Ultrasonic Treatment.

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

y = 245652x

HPLC area obtained at 10.014 minutes = 317534

317534 = 245652x

x = 1.2926 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [1.2926 g/L] [10 mL]

M_1 = 2.5852 g/L

Dextrose content = concentration (M_1) x total volume

= 2.5852 g/L x 0.088 L

= 0.2275 g

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

y = 244412x

HPLC area obtained at 10.641 minutes = 54025.5

54025.5 = 244412x

x = 0.2210 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [0.2210 g/L] [10 mL]

M_1 = 0.4421 g/L

Xylose content = concentration (M_1) x total volume

= 0.4421 g/L x 0.088 L

= 0.0389 g

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

y = 269291x

HPLC area obtained at 11.446 minutes = 18746.8

18746.8 = 269291x

x = 0.0696 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [0.0696 g/L] [10 mL]

M_1 = 0.1392 g/L

Arabinose content = concentration (M_1) x total volume

= 0.1392 g/L x 0.088 L

= 0.0122 g

(IV) Mass of estimated cellulose content

= [0.2275 g / 0.3 g x 100 %] x 2.3171 g

= 1.7571 g

(V) Total carbohydrates content in weight percentage

= [0.2275 g + 0.0389 g + 0.0122 g] / 0.3 g x 100 %

= 92.89 wt %

(VI) Mass of estimated carbohydrate content

= 92.89 wt % x 2.3171 g

= 2.1523 g

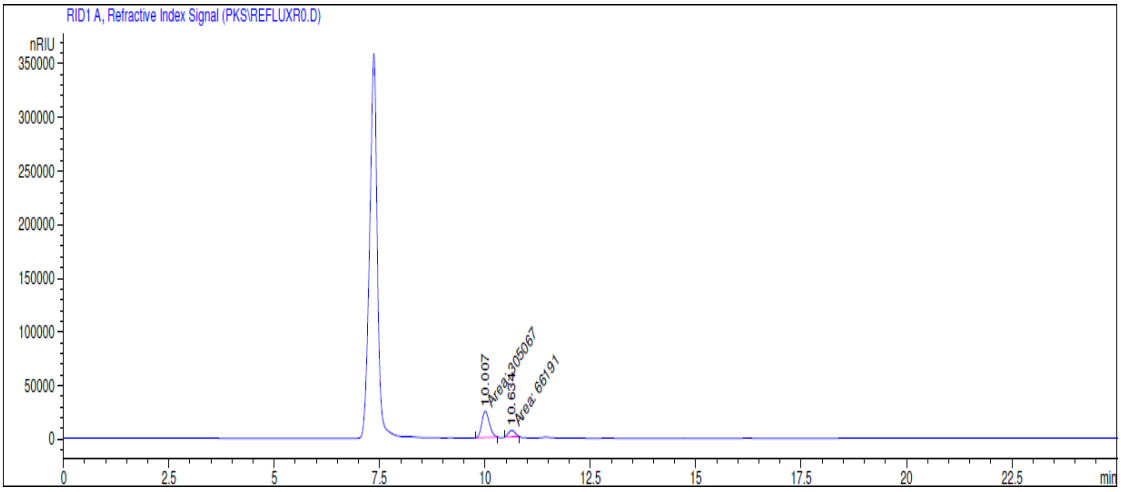

Figure G.2: HPLC Chromatogram of Refluxed SCB with Ultrasonic Treatment.

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

y = 245652x

HPLC area obtained at 10.007 minutes = 305067

305067 = 245652x

x = 1.2419 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [1.2419 g/L] [10 mL]

M_1 = 2.4837 g/L

Dextrose content = concentration (M_1) x total volume

= 2.4837 g/L x 0.088 L

= 0.2186 g

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

y = 244412x

HPLC area obtained at 10.634 minutes = 66191

66191 = 244412x

x = 0.2708 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [0.2708 g/L] [10 mL]

M_1 = 0.5416 g/L

Xylose content = concentration (M_1) x total volume

= 0.5416 g/L x 0.088 L

= 0.0477 g

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

y = 269291x

HPLC area obtained at 11.441 minutes = 33043.6

33043.6 = 269291x

x = 0.1227 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [0.1227 g/L] [10 mL]

M_1 = 0.2454 g/L

Arabinose content = concentration (M_1) x total volume

= 0.2454 g/L x 0.088 L

= 0.0216 g

(IV) Mass of estimated cellulose content

= [0.2186 g / 0.3 g x 100 %] x 2.4557 g

= 1.7891 g

(V) Total carbohydrates content in weight percentage

= [0.2186 g + 0.0477 g + 0.0216 g] / 0.3 g x 100 %

= 95.94 wt %

(VI) Mass of estimated carbohydrate content

= 95.94 wt % x 2.4557 g

= 2.3560 g

APPENDIX H: HPLC Analysis of Treated SCB at Different Ultrasonic Amplitude

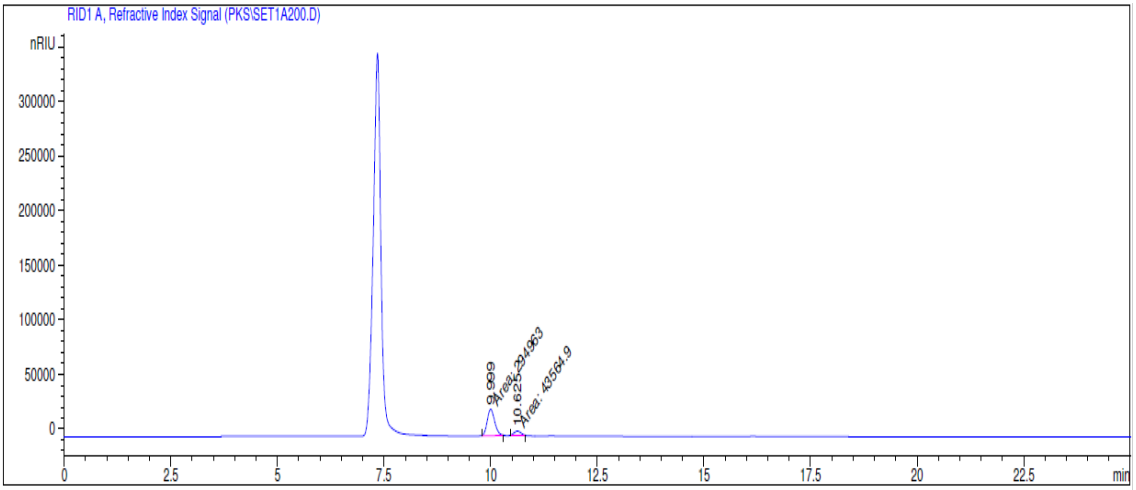

Figure H.1: HPLC Chromatogram of Treated SCB at Ultrasonic Amplitude of 20 %.

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

y = 245652x

HPLC area obtained at 9.999 minutes = 294963

294963 = 245652x

x = 1.2007 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [1.2007 g/L] [10 mL]

M_1 = 2.4015 g/L

Dextrose content = concentration (M_1) x total volume

= 2.4015 g/L x 0.088 L

= 0.2113 g

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

y = 244412x

HPLC area obtained at 10.625 minutes = 43564.9

43564.9 = 244412x

x = 0.1782 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [0.1782 g/L] [10 mL]

M_1 = 0.3565 g/L

Xylose content = concentration (M_1) x total volume

= 0.3565 g/L x 0.088 L

= 0.0314 g

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

y = 269291x

HPLC area obtained at 11.422 minutes = 7415.25

7415.25 = 269291x

x = 0.0275 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [0.0275 g/L] [10 mL]

M_1 = 0.0551 g/L

Arabinose content = concentration (M_1) x total volume

= 0.0551 g/L x 0.088 L

= 0.0048 g

(IV) Mass of estimated cellulose content

= [0.2113 g / 0.3 g x 100 %] x 2.3893 g

= 1.6831 g

(V) Total carbohydrates content in weight percentage

= [0.2113 g + 0.0314 g + 0.0048 g] / 0.3 g x 100 %

= 82.52 wt %

(VI) Mass of estimated carbohydrate content

= 82.52 wt % x 2.3893 g

= 1.9715 g

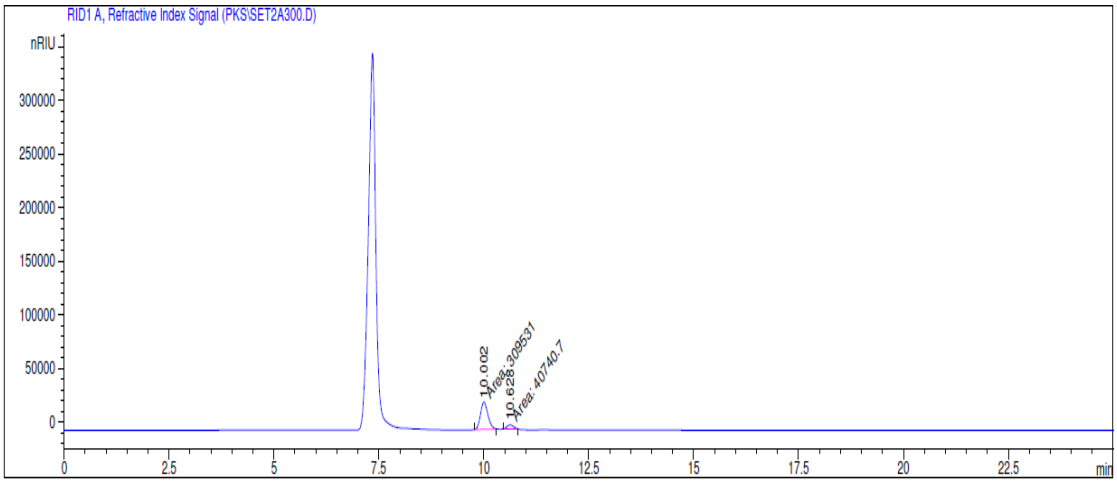

Figure H.2: HPLC Chromatogram of Treated SCB at Ultrasonic Amplitude of 30 %.

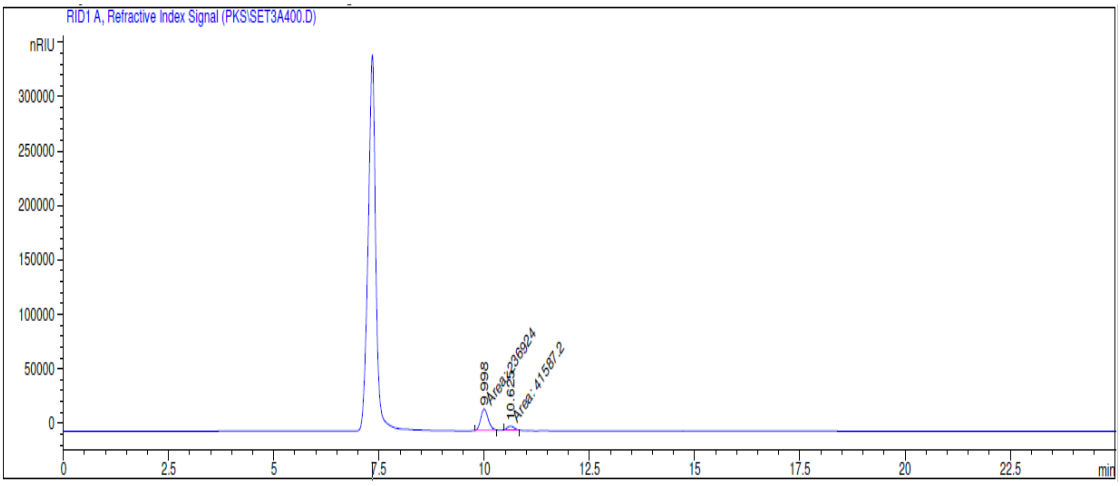

Figure H.3: HPLC Chromatogram of Treated SCB at Ultrasonic Amplitude of 40 %.

Table H.1: Summary of Total Carbohydrate Content in Treated SCB at Different Ultrasonic Amplitude.

Ultrasonic amplitude		20 %	30 %	40 %
Dextrose	Retention time, (min)	9.999	10.002	9.998
	Mass, (g)	0.2113	0.2217	0.1697
Xylose	Retention time, (min)	10.625	10.628	10.625
	Mass, (g)	0.0314	0.0293	0.0300
Arabinose	Retention time, (min)	11.422	11.425	11.418
	Mass, (g)	0.0049	0.0119	0.0006
Mass of total carbohydrate, (g)		1.9715	2.0930	1.8508
Mass of estimated cellulose, (g)		1.6831	1.7645	1.5263

APPENDIX I: HPLC Analysis of Treated SCB at Different Temperature

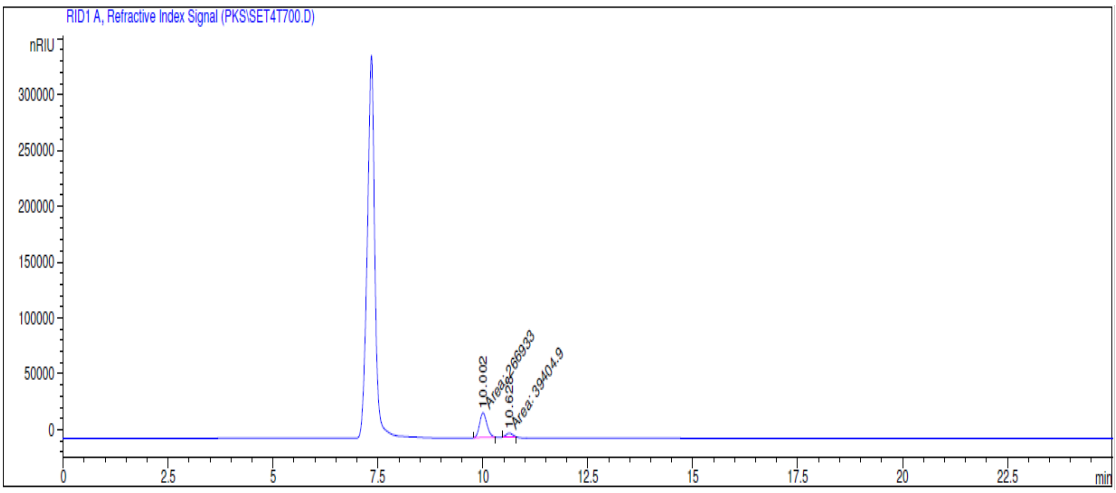

Figure I.1: HPLC Chromatogram of Treated SCB at Temperature of 70 ºC.

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

y = 245652x

HPLC area obtained at 10.002 minutes = 266933 g/L

y = 245652x

266933 = 245652x

x = 1.0866 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [1.0866 g/L] [10 mL]

M_1 = 2.1733 g/L

Dextrose content = concentration (M_1) x total volume

= 2.1733 g/L x 0.088 L

= 0.1912 g

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

y = 244412x

HPLC area obtained at 10.628 minutes = 39404.9

39404.9 = 244412x

x = 0.1612 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [0.1612 g/L] [10 mL]

M_1 = 0.3225 g/L

Xylose content = concentration (M_1) x total volume

= 0.3225 g/L x 0.088 L

= 0.0284 g

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

y = 269291x

HPLC area obtained at 11.425 minutes = 7867.73

7867.73 = 269291x

x = 0.0292 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [0.0292 g/L] [10 mL]

M_1 = 0.0584 g/L

Arabinose content = concentration (M_1) x total volume

= 0.0584 g/L x 0.088 L

= 0.0051 g

(IV) Mass of estimated cellulose content

= [0.1912 g / 0.3 g x 100 %] x 2.5236 g

= 1.6088 g

(V) Total carbohydrates content in weight percentage

= [0.1912 g + 0.0284 g + 0.0051 g] / 0.3 g x 100 %

= 74.92 wt %

(VI) Mass of estimated carbohydrate content

= 74.92 wt % x 2.5236 g

= 1.8907 g

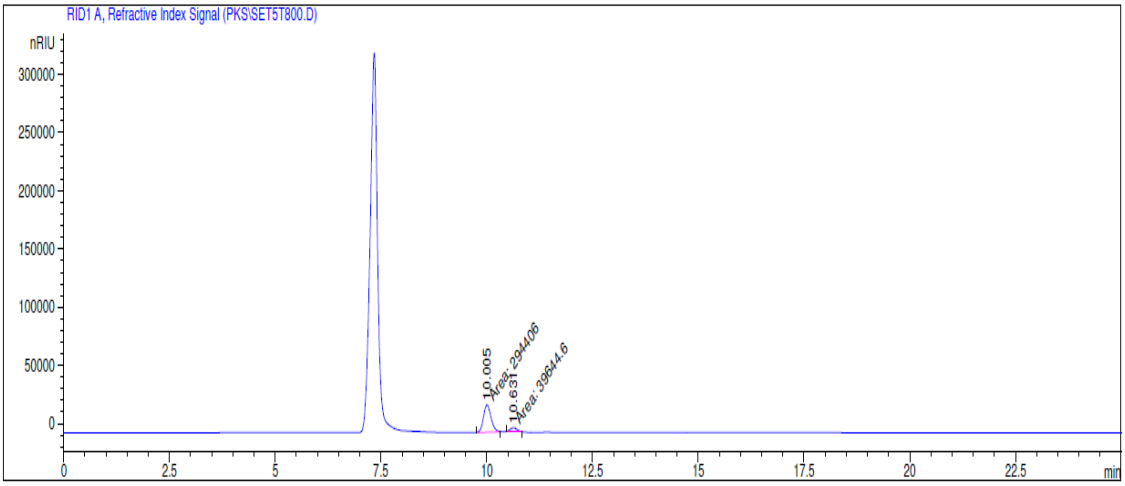

Figure I.2: HPLC Chromatogram of Treated SCB at Temperature of 80 ^{o}C.

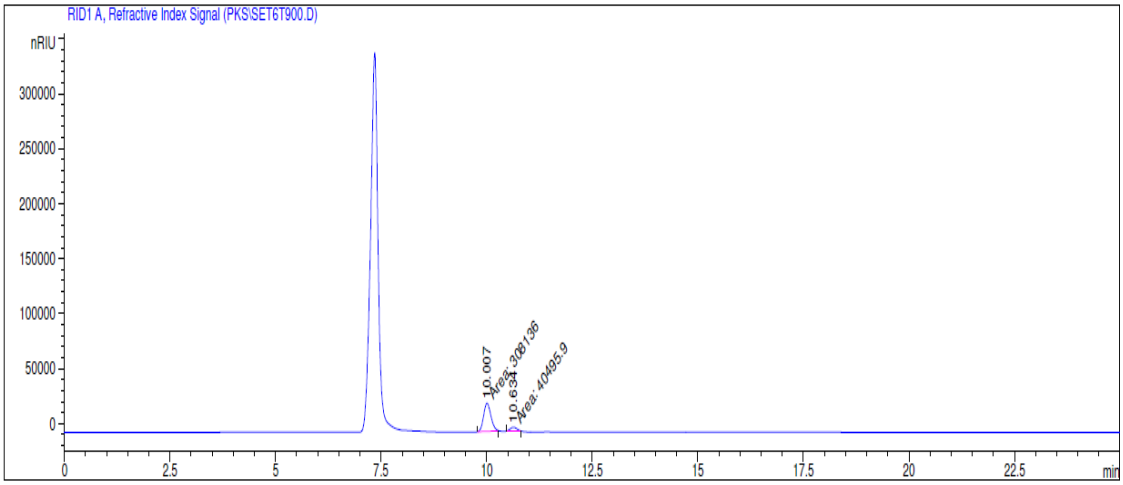

Figure I.3: HPLC Chromatogram of Treated SCB at Temperature of 90 ^{o}C.

Table I.1: Summary of Total Carbohydrate Content in Treated SCB at Different Temperature.

Temperature		70 ^{o}C	80 ^{o}C	90 ^{o}C
Dextrose	Retention time, (min)	10.002	10.005	10.007
	Mass, (g)	0.1912	0.2109	0.2208
Xylose	Retention time, (min)	10.628	10.631	10.634
	Mass, (g)	0.0284	0.0286	0.0292
Arabinose	Retention time, (min)	11.425	11.421	11.427
	Mass, (g)	0.0051	0.0105	0.0063
Mass of total carbohydrate, (g)		1.8907	2.0237	1.9674
Mass of estimated cellulose, (g)		1.6088	1.7079	1.6950

APPENDIX J: HPLC Analysis of Treated SCB at Different KOH Concentration

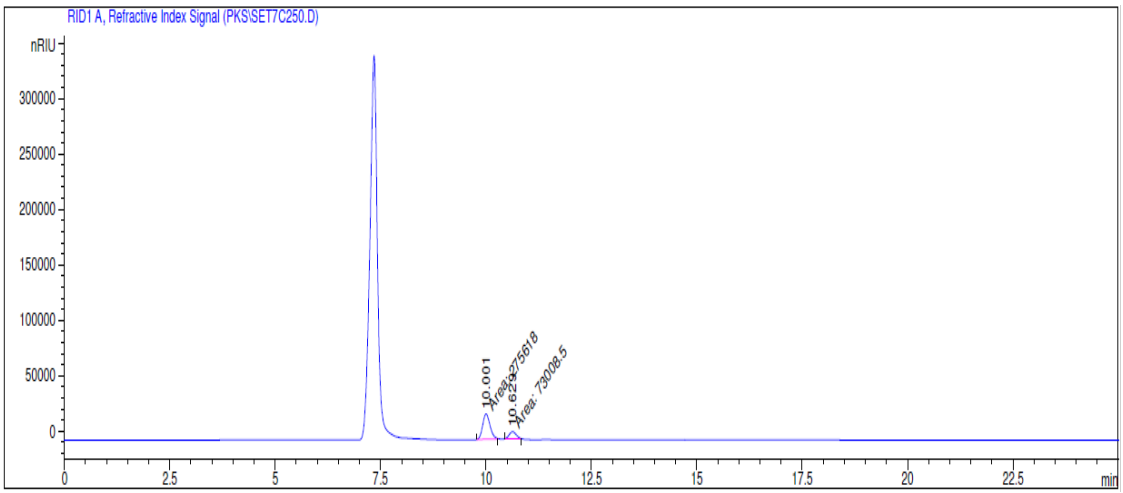

Figure J.1: HPLC Chromatogram of Treated SCB at KOH Concentration of 0.25 M.

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

y = 245652x

HPLC area obtained at 10.001 minutes = 275618

275618 = 245652x

x = 1.1220 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [1.1220 g/L] [10 mL]

M_1 = 2.2440 g/L

Dextrose content = concentration (M_1) x total volume

= 2.2440 g/L x 0.088 L

= 0.1975 g

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

y = 244412x

HPLC area obtained at 10.629 minutes = 73008.5 g/L

73008.5 = 244412x

x = 0.2987 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [0.2987 g/L] [10 mL]

M_1 = 0.5974 g/L

Xylose content = concentration (M_1) x total volume

= 0.5974 g/L x 0.088 L

= 0.0526 g

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

y = 269291x

HPLC area obtained at 11.426 minutes = 16585

16585 = 269291x

x = 0.0616 g/L

$M_1V_1 = M_2V_2$

M_1 [5 mL] = [0.0616 g/L] [10 mL]

M_1 = 0.1232 g/L

Arabinose content = concentration (M_1) x total volume

= 0.1232 g/L x 0.088 L

= 0.0108 g

(IV) Mass of estimated cellulose content

= [0.1975 g / 0.3 g x 100 %] x 2.8360 g

= 1.8668 g

(V) Total carbohydrates content in weight percentage

= [0.1975 g + 0.0526 g + 0.0108 g] / 0.3 g x 100 %

= 86.96 wt %

(VI) Mass of estimated carbohydrate content

= 86.96 wt % x 2.8360 g

= 2.4662 g

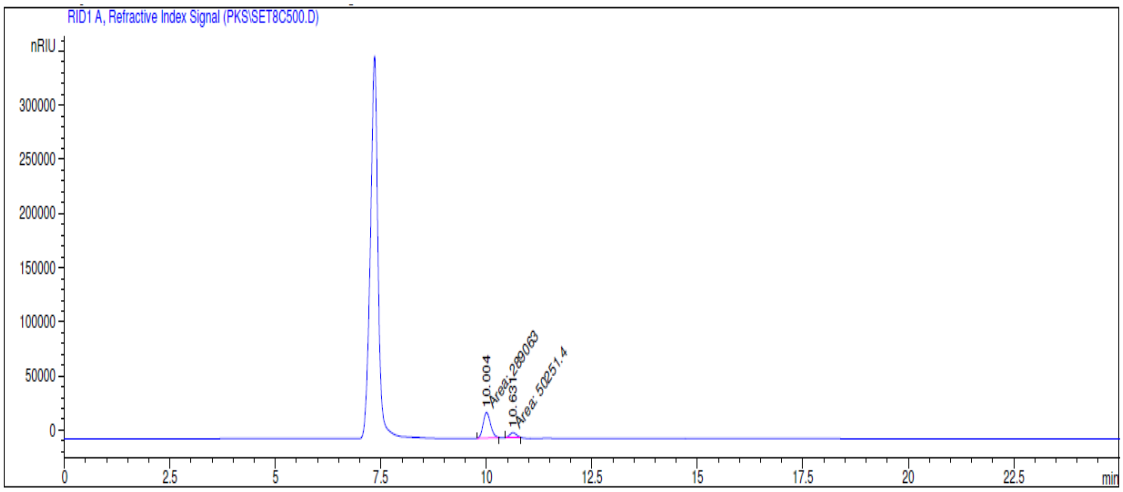

Figure J.2: HPLC Chromatogram of Treated SCB at KOH Concentration of 0.50 M.

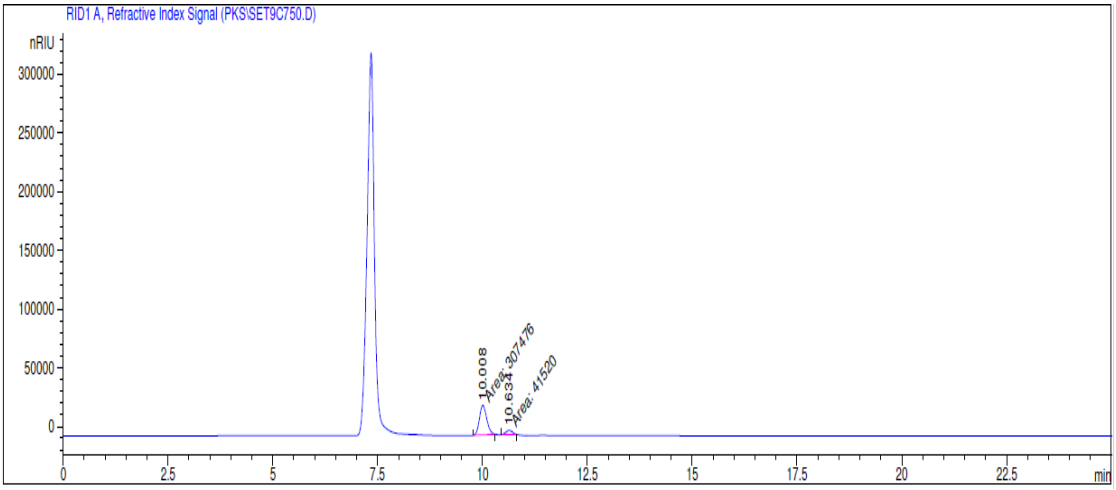

Figure J.3: HPLC Chromatogram of Treated SCB at KOH Concentration of 0.75 M.

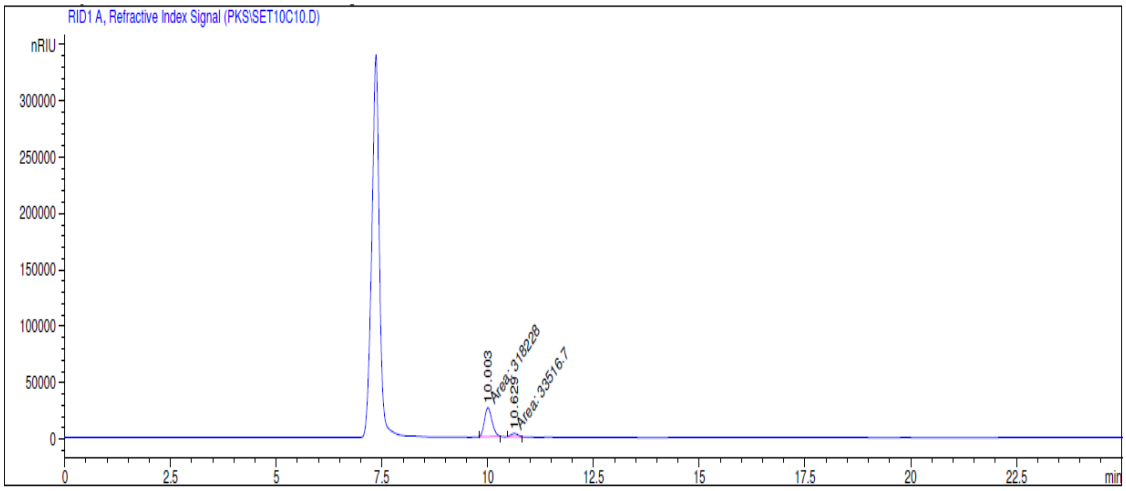

Figure J.4: HPLC Chromatogram of Treated SCB at KOH Concentration of 1.0 M.

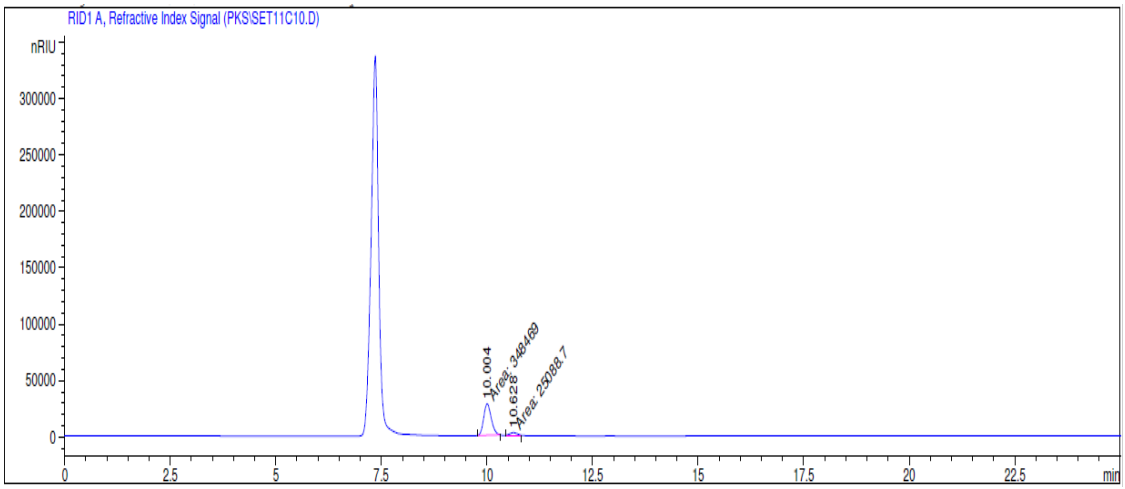

Figure J.5: HPLC Chromatogram of Treated SCB at KOH Concentration of 1.25 M.

Table J.1: Summary of Total Carbohydrate Content in Treated SCB at Different KOH Concentration.

KOH concentration		0.25 M	0.50 M	0.75 M	1.0 M	1.25 M
Dextrose	Retention time, (min)	10.001	10.004	10.008	10.003	10.004
	Mass, (g)	0.1975	0.2071	0.2203	0.2280	0.2497
Xylose	Retention time, (min)	10.629	10.631	10.634	10.629	10.628
	Mass, (g)	0.0526	0.0362	0.0299	0.0241	0.0181
Arabinose	Retention time, (min)	11.426	11.433	11.437	11.433	11.435
	Mass, (g)	0.0108	0.0137	0.0068	0.0051	0.0040
Mass of total carbohydrate, (g)		2.4662	2.1858	2.0497	1.9539	1.9717
Mass of estimated cellulose, (g)		1.8668	1.7618	1.7571	1.7318	1.8116

APPENDIX K: Yield of CMC

Table K.1: Yield of CMC.

Mass of extracted cellulose (g)	Mass of CMC, (g)	Yield of CMC, %(w/w)
5.0034	8.2779	165.45

APPENDIX L: Calculation of Degree of Substitution

From Equation 3.4,

$$A = \frac{BC - DE}{F}$$

$$= \frac{25\ (0.3) - 21.7(0.3)}{0.5}$$

$$= 1.98$$

Equation 3.3 is used to calculate degree of substitution by substituting the calculated A value.

$$\text{Degree of Substitution} = \frac{0.162\ x\ A}{1 - 0.058\ x\ A}$$

$$= \frac{0.162\ x\ 1.98}{1 - 0.058\ x\ 1.98}$$

$$= 0.3624$$