SYNTHESIS OF BACTERIAL CELLULOSE BY A*cetobacter xylinum* sp. USING WATERMELON RIND WASTE FOR BIOCOMPOSITE APPLICATION

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ABSTRACT

Cellulose was the most abundant polymer or polysaccharide that presents as the structural component of the primary cell wall of green plants but also signify for microbial extracellular polymer. The production of cellulose by microorganism such as *Acetobacter xylinum* sp. was most favored by researchers because the cellulose that produced was extremely pure and had a higher degree of polymerization and crystallinity than plant cellulose. The production of bacterial cellulose was expected to fulfill the high demand of cellulose in the industry. This study was focusing more on the effect of temperature and pH in the synthesis of bacterial cellulose by *Acetobacter xylinum* sp. using watermelon rind juice. The value of temperature and pH that being investigated was varied from 28 °C to 32 °C and from pH 4 to pH 8 respectively. The concentration for the watermelon rind juice was fixed at 7 g/L and the culture medium was incubated at fixed condition of 120 rpm. Differ from previous studies, this study use watermelon rind waste as the high potential carbon source replacing the pure carbon sources as the substrate for the synthesis of bacterial cellulose. The results data obtained shows that the optimum condition for the *Acetobacter xylinum* to produce the highest yield was at temperature 30 °C and pH 6 where the amount was 8.3439 g. The FT-IR analysis proves that the gelatinous membrane that produced from the experiment is cellulose. It can be shown by the appearance of absorbance peak for the C-C bonding, C-O bonding, C-OH bonding and C-O-C bonding after FT-IR analysis. In conclusion, from the data presented in this paper shows that watermelon rind waste has a high potential as the carbon source for the synthesis of bacterial cellulose and it is possible to carry out a mass production of bacterial cellulose.

ABSTRAK

Selulosa adalah polimer atau polisakarida yang paling banyak terhasil dimana ianya bertindak sebagai salah satu komponen dalam struktur dinding sel primer tanaman hijau dan juga polimer yang dihasilkan oleh mikroorganisma secara ekstraselular. Penghasilan selulosa oleh mikroorganisma seperti *Acetobacter xylinum* sp. menjadi pilihan para penyelidik kerana selulosa yang terhasil adalah sangat tulen dan mempunyai darjah kepolimeran dan kristaliniti yang lebih tinggi daripada selulosa tumbuhan. Penghasilan selulosa bakteria diharapkan dapat memenuhi permintaan yang tinggi terhadap selulosa di industri. Kajian ini lebih fokus kepada kesan suhu dan pH dalam proses sintesis selulosa oleh *Acetobacter xylinum* sp. yang menggunakan jus kulit buah tembikai. Variasi suhu dan pH yang dikaji masing-masing adalah dari suhu 28 °C sehingga 32 °C dan dari pH 4 sehingga pH 8. Kepekatan jus kulit buah tembikai adalah tetap iaitu 7 g/L dan medium kultur akan disimpan di dalam inkubator dengan kelajuan yang ditetapkan iaitu 120 rpm. Berbeza dari kajian sebelumnya, kajian ini menggunakan sisa kulit buah tembikai sebagai sumber karbon yang berpotensi tinggi menggantikan sumber karbon tulen sebagai substrat dalam sintesis selulosa bakteria. Data keputusan yang diperolehi menunjukkan bahawa keadaan optimum bagi *Acetobacter xylinum* untuk menghasilkan jumlah selulosa yang tertinggi adalah pada suhu 30 °C dan pH 6 dengan jumlah 8.3439 g. Analisis FT-IR membuktikan bahawa membran agar-agar yang terhasil daripada eksperimen adalah selulosa. Hal ini dapat dibuktikan dengan kemunculan puncak absorbansi bagi ikatan C-C, ikatan C-O, ikatan C-OH dan ikatan C-O-C selepas analisis FT-IR. Sebagai kesimpulannya, data yang diperolehi ini menunjukkan bahawa sisa kulit buah tembikai mempunyai potensi yang tinggi sebagai sumber karbon untuk sintesis selulosa dan mempunyai kebarangkalian untuk dihasilkan secara besar-besaran.

TABLE OF CONTENTS

CHAPTER	SUBJECT	PAGE
	DECLARATION	ii
	DEDICATION	vi
	ACKNOWLEDGEMENT	vii
	ABSTRACT	viii
	ABSTRAK	ix
	TABLE OF CONTENTS	x
	LIST OF TABLE	xiii
	LIST OF FIGURES	xiv
	LIST OF NOMENCLATURE	xv
1	**INTRODUCTION**	
	1.1 Background	1
	1.2 Problem statement	3
	1.3 Objective of the study	3
	1.4 Scopes of the study	3
2	**LITERATURE REVIEW**	
	2.1 Bacterial Cellulose	4
	2.1.1 Introduction	4
	2.1.2 Advantages and Disadvantages	4
	2.1.3 Application	5
	2.1.4 Bacterial Cellulose Biosynthesis	6
	2.2 Strain Bacteria	7
	2.3 Medium Condition	8

	2.4	Watermelon Rind Waste	8
	2.5	Fourier Transform InfraRed Spectroscopy	10
	2.5.1	Introduction	10
	2.5.2	Advantages and Disadvantages	10
	2.5.3	Bacterial Cellulose Wavelength Region	11
3	**METHODOLOGY**		
	3.1	Introduction	15
	3.2	Material and Apparatus	15
	3.2.1	Blender	16
	3.2.2	Microbiological Incubator	17
	3.2.3	Stackable Incubator Shaker	17
	3.2.4	Fourier Transform InfraRed Spectroscopy	18
	3.3	Experimental Procedure	18
	3.3.1	Preparation of Acetobacter xylinum sp. Culture Broth	18
	3.3.2	Preparation of Watermelon Rind Juice	20
	3.3.3	Synthesis of Bacterial Cellulose	22
	3.3.4	Bacterial Cellulose Analysis	24
4	**RESULT AND DISCUSSION**		
	4.1	Introduction	25
	4.2	Bacterial Cellulose Analysis	25
	4.3	Synthesis of Bacterial Cellulose	28
	4.3.1	Effect of Temperature	28
	4.3.2	Effect of Medium pH	29
5	**CONCLUSIONS**		
	5.1	Conclusion	31
	5.2	Recommendation	32
	REFERENCES		33

APPENDIX A 37

APPENDIX B 40

APPENDIX C 43

LIST OF TABLES

TABLE	TITLE	PAGE
2.1	Composition of Compound Material in Watermelon	9
2.2	Table of Characteristic IR Absorptions	12

LIST OF FIGURES

FIGURES	TITLE	PAGE
2.1	Simplified Model for the Biosynthetic Pathway of Cellulose	7
2.2	Characteristic wavelength regions (in wavenumbers, cm-1) for different vibrations	10
3.1	Watermelon Rind	16
3.2	Blender	16
3.3	Microbiological Incubator	17
3.4	Stackable Incubator Shaker	17
3.5	Fourier Transform InfraRed Spectroscopy	18
3.6	Preparation of *Acetobacter xylinum sp.* Culture Broth	19
3.7	Preparation of Watermelon Rind Extract	21
3.8	Synthesis of Bacterial Cellulose	23
3.9	Bacterial Cellulose Analysis	24
4.1	Molecular Structure of Cellulose	26
4.2	FTIR Spectra for Bacterial Cellulose	27
4.3	The Effect of Temperature toward Bacterial Cellulose Yield	29
4.4	The Effect of pH toward Bacterial Cellulose Yield	30

LIST OF NOMENCLATURE

β	Beta
FTIR	Fourier Transform InfraRed
°C	degree C
B	Broad
BC	bacterial cellulose
C	Carbon
C_3	carbon 3
C_6	carbon 6
cm	Centimetre
DNS	Dinitrosalicylic
g	Gram
g/L	gram per litre
H	Hydrogen
He	Helium
Ne	Neon
IR	Infrared
iu	international unit
M	Medium
MARDI	Malaysian Agricultural Research and Development Institute
mg	Milligram
MgSO4.7H2O	Magnesium Sulphate Heptahydrate
mL	Millilitre
N	Narrow
N	Nitrogen
Na_2HPO_4	Disodium Hydrogen Phosphate
NaOH	sodium hydroxide

nm	nanometre
O	oxygen
pH	potentiometric hydrogen ion concentration
ppm	part per million
rpm	rotation per minute
S	strong
Sh	sharp
Si	silicon
UV-VIS	ultra violet visible
V	variable

CHAPTER 1

INTRODUCTION

1.1 Background

Cellulose is the most abundant polymer on earth and the major component of plant cell wall. Cellulose is the major component of wood and cotton that has been the major resources for all cellulose products such as paper, textiles, construction materials, cardboard, as well as such cellulose derivatives as cellophane, rayon, and cellulose acetate. Bacterial cellulose is an extracellular polymer that produced by microorganism. Bacterial cellulose (biocellulose or microbial cellulose) is widely used in the industry such as health food industry, the making of audio component, wound care product and in the production of paper product. It is the most abundant bio-polymer on earth with 180 billion tons per year produced in nature (Englehardt, 1995). Bacterial cellulose has been preferred than plant cellulose because of its unique characteristic including good mechanical strength, high water absorption capacity, high crystallinity, ultra-fine and highly pure fiber network structure. Advantages of using a bacterial system for production of cellulose is that the bacterium grows rapidly under controlled conditions and produces cellulose from a variety of carbon sources including glucose, ethanol, sucrose, and glycerol. Although synthesis of an extracellular gelatinous mat by A. xylinum was reported for the first time in 1886 by A. J. Brown, BC attracted more attention in the second half of the 20th century (Bielecki, Krystynowicz, MariannaTurkiewicz, & Kalinowska, 2000).

The bacteria that can produce a pure and high yield of bacterial cellulose are *Acetobacter xylinum* sp. This bacterium is an acetic acid bacterium that can be found in yeast fermentation of sugar. Intensive studies on BC synthesis, using A. xylinum as a

model bacterium, were started by Hestrin et al. (1947, 1954), who proved that resting and lyophilized Acetobacter cells synthesized cellulose in the presence of glucose and oxygen (Bielecki, Krystynowicz, MariannaTurkiewicz, & Kalinowska, 2000). There are also other bacteria that can produce bacterial with genus *Achromobacter, Aerobacter, Agrobacterium, Alcaligenes, Pseudomonas, Rhizobium, Sarcina, and Zoogloea* (Bielecki, Krystynowicz, MariannaTurkiewicz, & Kalinowska, 2000). All of this bacteria produced bacterial cellulose in different form. *Acetobacter* produced cellulose in the form of extracellular pellicle composed of ribbons while *Achromobacter, Aerobacter, Alcaligenes* produce cellulose in fibrils form, *Agrobacterium* and *Rhizobium* produces cellulose in the form of short fibril, *Pseudomonas* produce bacterial cellulose with no distinct fibril, *Sarcina* produce an amorphous cellulose and *Zoogloea* produce cellulose in not well defined form.

Bacterial cellulose is an extracellular polymer that produced from monosaccharides or simple sugar such as glucose, xylose and glucose that act as a substrate or other carbon source such as ethanol and glycerol. Some of the previous study use substrate that is purely glucose while some other use substrate taken from fruit juice such as orange juice, mango juice, apple juice, pineapple juice and sugarcane juice that contain highly amount of glucose. BC is still expensive compared with other popular commercial organic products of the usage of pure glucose as a substrate (Shoda & Sugano, 2005). Rather than pure glucose, wastes that contain glucose usually in small amount also can be use as a substrate such as corn steep liquor, fruit waste, fruit skin or stale milk. Using waste as a potential substrate is not only can reduce the bulk wastes that produce from food and beverage but it can also promote a low cost substrate for the bacterial cellulose production.

The bacterium *A. xylinum* produces pure cellulose where a single cell may polymerize up to 200,000 glucose residues per second into β-1,4-glucan chains (Saxena, Dandekar, & Jr, 2000). This polymerization process involved a number of reactions using an enzyme to convert the glucose into cellulose and required energy. The process includes the synthesis of uridine diphosphoglucose (UDPGlc), which is the cellulose precursor, followed by glucose polymerization into the β-1,4-glucan chain, and nascent chain association into characteristic ribbon-like structure, formed by hundreds or even

thousands of individual cellulose chains (Bielecki, Krystynowicz, MariannaTurkiewicz, & Kalinowska, 2000).

1.2 Problem Statement

Biocomposite is a material formed by a matrix of starch or resin and reinforced by a natural fiber usually derived from cellulose. In order to fabricate and enhance the properties of the biocomposite, cellulose is used due to its fiber structure and the biodegradable characteristic. However, most cellulose is obtained from the plant cell wall and it is difficult to purify the cellulose from lignin and hemicellulose. Bacterial cellulose is used as an alternative instead of plant cellulose in order to produce high purity cellulose and to reduce the forest depletion. Watermelon rind is used in this research as a raw material of carbon source in order to reduce the bulk watermelon rind wastes that produced from food and beverage industries.

1.3 Research Objective

The objective for this research is to investigate the effect of temperature and pH in the production of bacterial cellulose by *Acetobacter xylinum* sp. using watermelon rind juice.

1.4 Scope of Study

The scopes of this study are as follows:

i) To analyze the bacterial cellulose detection using Fourier Transform Infrared Spectroscopy (FTIR).

ii) To investigate the effect of temperature of the medium that varied between 28°C to 32°C.

iii) To study the effect of pH of the medium that varied between pH 4 to pH 8.

CHAPTER 2

LITERATURE REVIEW

2.1 Bacterial Cellulose

2.1.1 Introduction

Cellulose is the most abundant polymer that is present as the structural component of the primary cell wall of green plants. Other than cellulose that comes from plant cell, there is certain strain of bacteria that can produce cellulose extracellularly in the form of fibril that attached to the bacterial cell (Young, Sang, Jung, Yu, & Yu, 1998). There are four different pathways in forming the cellulose biopolymer. The first pathway is by the isolation of cellulose from plant. This pathway needs another separation process step to remove lignin and hemicellulose. The second pathway is the synthesis of cellulose by microorganism *Acetobacter xylinum sp.* The third and the fourth method are by the first enzymatic in-vitro synthesis starting from cellobiosyl fluoride and the first chemosynthesis from glucose by ring opening polymerization of benzylated and pivaloylated derivatives (Klemm, Schumann, Udhart, & Marsch, 2001).

2.1.2 Advantages and Disadvantages

Bacterial cellulose (BC) belongs to specific products of primary metabolism and mainly as a protective coating, whereas plant cellulose (PC) plays a structural role in plant (Bielecki, Krystynowicz, MariannaTurkiewicz, & Kalinowska, 2000). Bacterial cellulose (BC) produced by bacteria has a unique physical and chemical properties differ

than cellulose that produce from plant in the form of its size, crystallinity and purity. Furthermore, bacterial cellulose has a high purity where it is free from lignin, hemicelluloses and waxy aromatic substance than plant cellulose that usually associated with these materials where the removal is very difficult (Son, Kim, Kim, Kim, Kim, & Lee, 2003). Besides that, bacterial cellulose has high crystallinity, high water absorption capacity, and high mechanical strength in wet state, ultrafine network structure, mouldability in situ and availability in an initial wet state (Klemm, Schumann, Udhart, & Marsch, 2001). Bacterial cellulose can virtually grown in any shape such as a film or mats if using a static (Surma-Slusarska, Presler, & Danielewicz, 2008) and a fibrous suspension, irregular masses, pellets or spheres (Krystynowicz, Czaja, Wiktorowska-Jezierska, Goncalvez-Miskiewicz, Turkiewicz, & Bielecki, 2002).

Although bacterial cellulose has a unique characteristic than the plant cellulose, it also has disadvantages that need to be encounter whereby the price for the substrate which is sugar is very expensive but the yeild of the process is low. One of the alternative that can overcome this problem is by using the fruit waste such as fruit peel, kernel and dregs. The example of fruit waste that can be utilizes as substrates to produce cellulose are the mango peel, apple peel, orange peel, pineapple core, watermelon peel and other fruit wastes. Besides, lactose which has a lower price also potential to be used as a substrate for cellulose production the method of using static culture being the barrier to produce cellulose in large production.. Nowadays, many researches attempt to develope new method to produce cellulose in large scale production . The most popular method is by using shaking culture. The equipment that suitable for this method are airlift bioreactor, rotating biological contactor and membrane reactor.

2.1.3 Application

Bacterial cellulose has a wide application in industry especially in medical sector. Bacterial cellulose is fully utilize in the making of an artificial blood vessels for microsurgery (Klemm, Schumann, Udhart, & Marsch, 2001). Bacterial cellulose also used in wound dressing such as XCell Wound Dressing and Cell Antimicrobial Wound Dressing. Another application in medical sector is in tissue scaffolding (Zhang & Lim,

2008) for soft tissue replacement and bladder neck suspension. Bacterial cellulose is also popular in the application of papermaking (Surma-Slusarska, Presler, & Danielewicz, 2008). Most of paper production used cellulose pulp from plant and thus gives a problem on forest depletion. Many researches has been conducted on producing paper from bacterial cellulose and as a result, there is an improvement of the paper's strength properties and protect the surface of paper (Surma-Slusarska, Presler, & Danielewicz, 2008). Bacterial cellulose also been used as the matrix for electronic paper. In food and beverage industry, the bacterial cellulose is used as ingredients such as nata de coco and diet food.

2.1.4 Bacterial Cellulose Biosynthesis

Cellulose produced by Acetobacter strain was found to be chemically pure, free of lignin and hemicellulose and to have different properties from wood derived cellulose and recently, taking advantages of its properties, bacterial cellulose has been applied to practical uses (Masaoka, Ohe, & Sakota, 1993). *A. xylinum* has been applied as a model for the basic and applied studies on cellulose because of its ability to produce relatively high levels of polymer from wide range of carbon and nitrogen sources. The mechanism of converting the glucose to cellulose by *Acetobacter xylinum* is cellulose biosynthetic pathways. Synthesis of bacterial cellulose is a precisely and specifically regulated multi-step process, involving a large number of both individual enzymes and complexes of catalytic and regulatory proteins, whose supramolecular structure has not yet, be well defined (Bielecki, Krystynowicz, MariannaTurkiewicz, & Kalinowska, 2000). The synthesis of cellulose in *A. xylinum* and any other cellulose-producing organism including plant follows two intermediate steps: (*i*) formation of β-1,4-glucan chain with polymerized of glucose units, and (*i*) assembly and crystallization of cellulose chain (Chawla, Bajaj, Survase, & Singhal, 2008). The overall mechanism for cellulose biosynthetic pathway is illustrated in Figure 2.1.

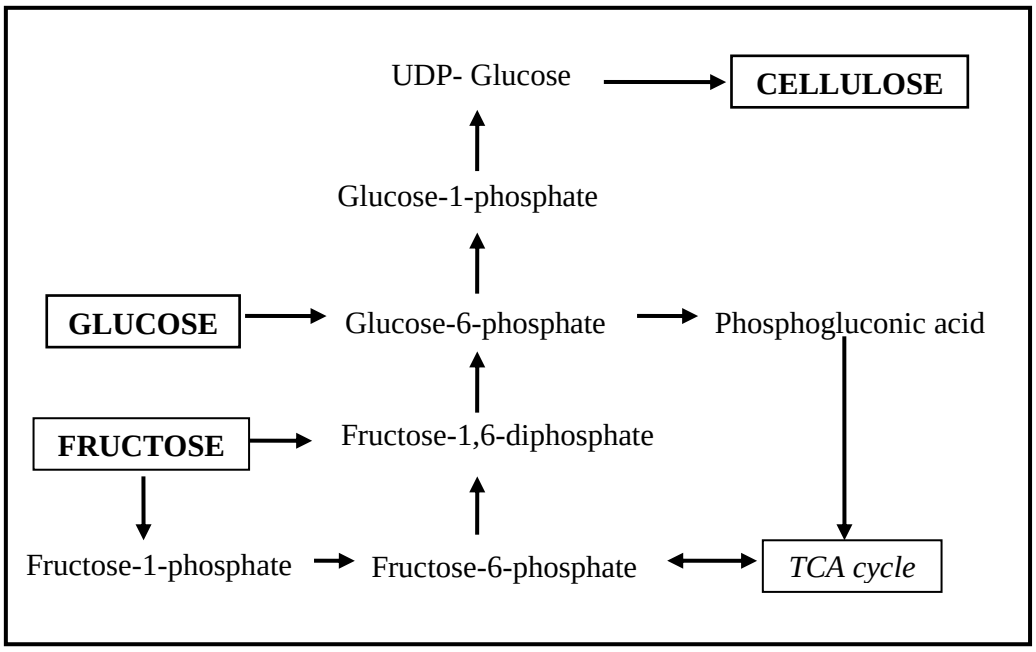

Figure 2.1: Simplified Model for the Biosynthetic Pathway of Cellulose (Yoshinaga, Tonouchi, & Watanabe, 1997)

2.2 Strain Bacteria

Bacteria that can produce highest cellulose amount than other bacteria are *Acetobacter xylinum* sp. *Acetobacter xylinum* sp. is a gram-negative bacterium that synthesizes cellulose in the present of glucose. *Acetobacter xylinum* sp. usually can be found on the wall of bioreactor for the production of ethanol from yeast fermentation of sugars and plant carbohydrates. They also can be isolated from the nectar of flowers, damaged fruit, fresh apple cider and unpasteurized beer which has not been filtering sterilized. *Acetobacter xylinum* sp. synthesizes cellulose by fully utilizing the monosaccharides of carbohydrate or simple sugar such as glucose, fructose, sucrose or lactose. There are several step involved in the cellulose formation starting from the aggregation of glucan chain that consist of approximately 6 – 8 are elongated from the complex. The formations of microfibril occur by assembling this sub elementary fibril and tighten the microfibril in order to form an interwoven ribbon. The matrix of interwoven ribbon constitutes the bacterial cellulose membrane or pellicle and the *Acetobacter xylinum* cell is distributed throughout the network of the cellulose ribbons. (Klemm, Schumann, Udhart, & Marsch, 2001).

2.3 Medium Condition

Fermentation process by *Acetobacter xylinum* using carbon source as a substrate is one of the methods to produce cellulose. The condition of the culture medium such as temperature, pH, static/agitation condition, carbon source concentration, nitrogen source concentration and nutrien concentration, must be control in order to produce higher yield of glucose. One of important parameter that must be control is temperature of the culture medium. The temperature must be control because it is associated with the energy supplied to the bacteria to grow and for the conversion of glucose to cellulose. Most of the previous study stated that the maximal cellulose production was observed between 28 °C to 30 °C (Chawla, Bajaj, Survase, & Singhal, 2008). This is due to the energy that supplied is enough for the bacterial growth and cellulose biosynthetic pathway that required energy for the conversion of glucose to cellulose (Bielecki, Krystynowicz, MariannaTurkiewicz, & Kalinowska, 2000). Other important parameter that must be control is the pH of the medium. Every bacteria has their own pH condition in order for them to grow as well as *Acetobacter xylinum*. *Acetobacter xylinum* is an acetic acid bacteria that need an acidic condition for growth. (Yamada, et al., 1999). The previous study done by Chawla, Bajaj, Survase, & Singhal, (2008) stated that the optimum pH of the culture medium for bacterial cellulose production is in range of 4.0 and 6.0. During the fermentation, the pH of the medium change throughout the process. Besides the conversion of glucose to cellulose, *Acetobacter* xylinum also convert glucose to gluconic acid. The accumulation and consumption of gluconic acid contribute to the changes of the pH medium (Hwang, Yang, Hwang, Pyun, & Kim, 1999).

2.4 Watermelon Rind Waste

Watermelon is an important crop that grows in a warmer region where it is utilized for the production of juice, nectar and fruit cocktail while the major by-product rind is utilized for the products like pickle, preserve, pectin and other product (Wani, Kaur, Ahmed, & Sogi, 2007). According to the Table 2.1, watermelon consists of 68% of flesh, 30% of rind and 2% of seed kernel. The seed is approximately consisting of 42% kernel and 58% of hull while 4.36% of the rind is peel and the other is inside whitish

portion (Campbell, 2006). The rind is higher in percent fresh weight, dietary fiber, and potassium but lower in total sugar than the flesh (Veazie & Penelope, 2002). Watermelon rind juice is one of the unconventional media indentified that can promote a low cost substrate for the production of bacterial cellulose. Although the amount of sugars in the watermelon rind is much lower than the total sugar in the watermelon flesh, it still can act as a carbon source for *Acetobacter xylinum* to produce bacterial cellulose. The cost of collecting the watermelon rind waste is much lower than buying the pure glucose medium for the cellulose production.

Table 2.1: Composition of Compound Material in Watermelon (Campbell, 2006)

Part Of Watermelon	Compound Material	Amount
Flesh (68 %)	Water	92.6 g
	Protein	0.5 g
	Fat	0.2 g
	Total Carbohydrate	6.4 g
	Fibre	0.3 g
	Ash	0.3 g
	Calcium	0.7 mg
	Vitamin A	590 international unit (iu)
	Thiamine	0.03 mg
	Riboflavin	0.03 mg
	Niacin	0.2 mg
	Ascorbic Acid	7 mg
Seed Kernel (2 %)	Crude Protein	35.7%
	Crude Oil	50.1%
	Crude Fibre	4.83%
	Total Ash	3.60%
	Nitrogen Free Extract	5.81%
Rind (30 %)	Moisture	93.8%
	Nitrogen	0.1%
	Ash	0.49%
	Sugars	2.1%

2.5 Fourier Transform InfraRed Spectroscopy

2.5.1 Introduction

Fourier Transform Infrared Spectroscopy (FT-IR) is one of the equipment used to identify a chemical or a mixture of chemical compound either organic or inorganic in the form of solid, liquid or gasses. The spectra obtained by FT-IR provide information about the presence of specific molecular structure or type of chemical bonding (functional group). The term Fourier Transform Infrared Spectroscopy (FT-IR) refers to a fairly recent development in the manner in which the data is collected and converted from an interference pattern to a spectrum. Some of the common application of FT-IR is identification of unknown organic or inorganic, mixture of microscopic compound, detection or characterization of organic and some inorganic additive in polymers at level as low as few percent, characterization of changes in chemical structure of organic materials as result of polymer cure, sterilization, heat treatment, plasma treatment and else (EAGLABS Fourier Transform InfraRed Spectroscopy (FT-IR) Services, 2009).

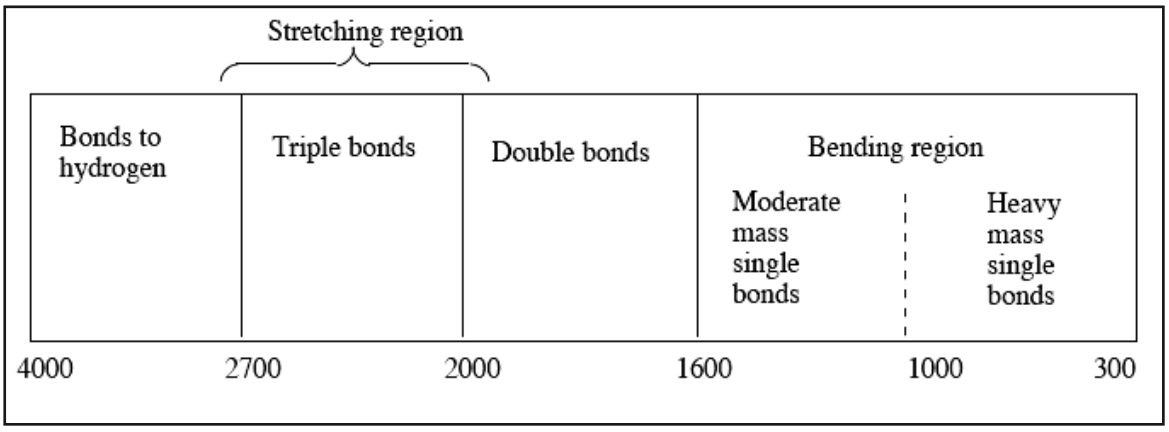

Figure 2.2: Characteristic wavelength regions (in wavenumbers, cm-1) for different vibrations (Modern Techniques in Chemistry: Infrared Spectroscopy, 2005)

2.5.2 Advantages and Disadvantages

Every equipment design has its own advantages and disadvantages. One of the advantages of Fourier Transform InfraRed Spectroscopy (FT-IR) is the measurement is

faster than other equipment because the frequencies are measured simultaneously the sensitivity if FT-IR is improving. The detector employs are much more sensitive, the optical throughput is much higher which result in much lower noise levels and the fast scans enables the co-addition of several scans in order to reduce the random measurement noise to any desired level. There are also very little possibility of mechanical breakdown because the moving mirror in the inferometer is the only part in this equipment that continuously moving. Besides that, this equipment can self-calibrate while using the HeNe laser as an internal wavelength calibration standard. Beside advantages, FT-IR also has several disadvantages where the minimum analysis area is approximately 15 micron. Besides that, the library data for the inorganic compound is limited and quantitative information only available when calibration samples are used.

2.5.3 Bacterial Cellulose Wavelength Region

One of the methods to analyze bacterial cellulose is using Fourier Transform Infrared Spectroscopy (FT-IR). FT-IR analyzes cellulose using the chemical bonding that present in the polymer. One of the important bonding in cellulose polymer is the β-1,4-glycosocidic linkage where this bonding connecting the carbohydrate monomer into a polymer with the C-O-C bonding notation. This chemical bonding will appear at the wavenumber near 1160 cm-1 and 900 cm-1 (Sun, et al., 2008). Other chemical bonding that present in cellulose are C-O stretching and C-C stretching. Strong peak that appear at 1060 cm-1 and 1030 cm-1 are the indicative of C-O stretching at C3, C-C stretching and C-O stretching at C6 (Sun, et al., 2008). The absorption peak of carbonyl groups (C=O) with intramolecular hydrogen bonds is also found at around 1650 cm-1 (Guo, Chen, Chen, Men, & Hwang, 2008). A small peak that appears at 672 cm^{-1} and 711 cm^{-1} correspond to the out-of-plane bending of C-O-H (Sun, et al., 2008). Besides that, there are also chemical bonding between carbon and hydrogen (C-H bonding). This bonding appears between 1430 cm^{-1} to 1290 cm^{-1} wavenumber (Glagovich, 2007). The band that appears at 1635 cm^{-1} – 1640 cm^{-1} has been attributed to the absorbed water bending vibrations that present in cellulose (Cao & Tan, 2004).

All the absorbance stated above are associated with the chemical bonding that present in cellulose polymer. Some of the absorbance peak will change either decrease or shifted to greater or lower wavenumber when the cellulose structure is changing. The absorbance band also can become narrower or wider when there is a change in the cellulose molecular structure. This statement was proven by researchers including Sun, et al (2008), Cao & Tan (2004) and Oh, et al., (2005). The previous studies were investigate about the crystalline structure of cellulose after being treated with enzyme, diluted acid, sodium hydroxide and carbon dioxide. The result shown that the absorbance peak of wavenumber is either decreased or shifted to higher value or to lower value because of the change or rearrangement of the cellulose structure. When cellulose is treated with the enzyme, diluted acid, sodium hydroxide and carbon dioxide, some of the chemical bond on the surface of cellulose in is broken down in the reaction and exposing the hidden internal chemical bond, for example the effect of acid first was on the surface and amourphous zone, the hydrogen bonds was broken and more bond types C-OH, C-O-C, and C-C were exposed, thereby the stretching absorbancy increased (Sun, et al., 2008). Table 2.2 shows the characteristic of infrared absorption acording to the functional group.

Table 2.2: Table of Characteristic IR Absorptions (Glagovich, 2007)

Functional Group	**Molecular Motion**	**Wavenumber (cm^{-1})**
alkanes	C-H stretch	2950-2800
	CH_2 bend	~1465
	CH_3 bend	~1375
	CH_2 bend (4 or more)	~720
alkenes	=CH stretch	3100-3010
	C=C stretch (isolated)	1690-1630
	C=C stretch (conjugated)	1640-1610
	C-H in-plane bend	1430-1290
	C-H bend (monosubstituted)	~990 & ~910
	C-H bend (disubstituted - E)	~970
	C-H bend (disubstituted - 1,1)	~890
	C-H bend (disubstituted - Z)	~700
	C-H bend (trisubstituted)	~815

Alkynes	acetylenic C-H stretch	~3300
	C,C triple bond stretch	~2150
	acetylenic C-H bend	650-600
aromatics	C-H stretch	3020-3000
	C=C stretch	~1600 & ~1475
	C-H bend (mono)	770-730 & 715-685
	C-H bend (ortho)	770-735
	C-H bend (meta)	~880 & ~780 & ~690
	C-H bend (para)	850-800
alcohols	O-H stretch	~3650 or 3400-3300
	C-O stretch	1260-1000
ethers	C-O-C stretch (dialkyl)	1300-1000
	C-O-C stretch (diaryl)	~1250 & ~1120
aldehydes	C-H aldehyde stretch	~2850 & ~2750
	C=O stretch	~1725
ketones	C=O stretch	~1715
	C-C stretch	1300-1100
carboxylic acids	O-H stretch	3400-2400
	C=O stretch	1730-1700
	C-O stretch	1320-1210
	O-H bend	1440-1400
esters	C=O stretch	1750-1735
	C-C(O)-C stretch (acetates)	1260-1230
	C-C(O)-C stretch (all others)	1210-1160
acid chlorides	C=O stretch	1810-1775
	C-Cl stretch	730-550
anhydrides	C=O stretch	1830-1800&1775-1740
	C-O stretch	1300-900

amines	N-H stretch (1 per N-H bond)	3500-3300
	N-H bend	1640-1500
	C-N Stretch (alkyl)	1200-1025
	C-N Stretch (aryl)	1360-1250
	N-H bend (oop)	~800
amides	N-H stretch	3500-3180
	C=O stretch	1680-1630
	N-H bend	1640-1550
	N-H bend (1^{o})	1570-1515
alkyl halides	C-F stretch	1400-1000
	C-Cl stretch	785-540
	C-Br stretch	650-510
	C-I stretch	600-485
nitriles	C,N triple bond stretch	~2250
isocyanates	-N=C=O stretch	~2270
isothiocyanates	-N=C=S stretch	~2125
imines	R_2C=N-R stretch	1690-1640
nitro groups	-NO_2 (aliphatic)	1600-1530&1390-1300
	-NO_2 (aromatic)	1550-1490&1355-1315
mercaptans	S-H stretch	~2550
sulfoxides	S=O stretch	~1050
sulfones	S=O stretch	~1300 & ~1150
sulfonates	S=O stretch	~1350 & ~11750
	S-O stretch	1000-750
phosphines	P-H stretch	2320-2270
	PH bend	1090-810
phosphine oxides	P=O	1210-1140

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter presents the detail procedure for the synthesis of bacterial cellulose by *Acetobacter xylinum* sp using watermelon rind waste. The first step was the preparation of the *Acetobacter xylinum* bacterium and the preparation of watermelon rind juice. The synthesis of bacterial cellulose process takes place after the preparation of the culture medium and the last step was to analyze the cellulose produced when pH and temperature varied.

3.2 Material and Apparatus

The raw materials used in this study were *Acetobacter xylinum* sp. and watermelon rind juice. The chemical used for the preparation of the culture medium such as yeast extract, peptone, magnesium sulfate heptahydrate, disodium hydrogen phosphate and citric acid were purchased from Merck Sdn Bhd. The main apparatus used in this experiment were blender, incubator, stackable incubator shaker and Fourier Transform Infrared Spectroscopy (FTIR).

The bacterium that used in this experiment was *Acetobacter xylinum* sp. This bacterium was taken from Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor.