PRODUCTION OF BACTERIAL CELLULOSE: EFFECT OF DIFFERENT MEDIUMS AND MODES OF OPERATION

MUHAMMAD SYAFIQ BIN KHIRRUDIN

CHEMICAL ENGINEERING (BIOTECHNOLOGY)

UNIVERSITI MALAYSIA PAHANG

PRODUCTION OF BACTERIAL CELLULOSE: EFFECT OF DIFFERENT MEDIUMS AND MODES OF OPERATION

MUHAMMAD SYAFIQ BIN KHIRRUDIN

Thesis submitted in fulfillment of the requirements

for the award of the degree of

Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical Engineering and Natural Resources

UNIVERSITY MALAYSIA PAHANG

JANUARY 2012

SUPERVISOR'S DECLARATIONS

"I hereby declare that I have read this thesis and in my opinion this thesis has fulfilled the qualities and requirements for the award of Degree of Bachelor of Chemical Engineering (Biotechnology)

Signature : …….

Name of Supervisor : En. Junaidi Bin Zakaria

Date : 20 January 2012

STUDENTS'S DECLARATION

I declared that this thesis entitled "Production of Bacterial Cellulose: Effect of Different Mediums & Modes of Operation" is the result of my own research except as cited in references. The Thesis has not been accepted for any degree and not concurrently submitted in candidature of any other degree.

Signature : …….

Name : Muhammad Syafiq Bin Khirrudin

Date : 20 January 2012

To my beloved parents, brother and sister

ACKNOWLEDGEMENTS

The highest gratitude is for Allah for giving me the strength and blesses me with abundance of people around me that have been so helpful. There have been so many people that have been involved in this project directly or indirectly. I might have no chance to thank of all them, but surely I hope that their assistant would be rewarded by Allah.

Firstly, I would like to thank my supervisors, Mr Junaidi Bin Zakaria, which has guided me along of this research. Without her continued support and interest, this thesis would not have been the same as presented here.

Besides that, I would also like to thank my friends and team mates in this research, which have been so supportive and helpful to me. Without their assistant and support, I believe I would not have the courage to finish this thesis within the time.

I am also indebted to all teaching engineers and people at FKKSA's laboratory. With their help and assistant in my experiment, I had managed to finish my experiment although there were problem and obstacle. Last but not least, I would like to thank to my parents for supporting me all the times throughout my studies in University Malaysia Pahang (UMP)

ABSTRACT

Bacterial cellulose, an exopolysaccharide produced by A*cetobacter Xylinum s.p*, has unique structural and mechanical properties and is highly pure as compared to plant cellulose. This objective of this study was to assess the effect of different medium (Hestrin Schramm medium, banana peel, coconut water, and hibiscus waste) on microbial decomposition using three categories of operation mode (Batch, fed-batch and repeated batch).Production of bacterial cellulose in this experiment include the production of inoculum using the fresh coconut water and it was conduct under optimum temperature and pH which is 30^{o} C and 5.5 respectively. From the result, banana peel gives the highest yield in fed batch mode of operation, followed by coconut water, hibiscus waste and Hestrin Schramm medium. The high production efficiency of banana peel was due to the high purity of carbon source in banana peel and the continuously feeding of the fermentation medium using the fed batch mode. These results show that, the consumption of carbon source is not the only factor that effects the efficiency production of bacterial cellulose. The operation mode might play another important factor in the production of bacterial cellulose. From the FTIR analysis, the bacterial cellulose produce appears to have very good crystallinity characteristics similar to the microcrystalline cellulose (MCC). SEM analysis concludes that the ribbon network is produce under the surface of bacterial cellulose.

ABSTRAK

Bakteria selulosa merupakan sejenis polisakarida yang dihasilkan daripada metabolism bakteria *Acetobacter Xylinum* .Bakteria selulosa memiliki struktur yang unik, ciri mekanikal yang teguh dan sangat asli jika dibandingkan dengan selulosa dari pokok. Objektif kajian ini adalah untuk mengkaji kesan medium yang berbeza ke atas pertumbuhan selulosa yang dihasilkan dari metabolism bakteria *Acetobacter Xylinum.* Medium yang digunakan adalah Hestrin Schramm, kulit pisang, air kelapa tua dan sisa bunga raya.Selain itu, objektif kajian ini juga adalah untuk mengkaji teknik fermentasi yang terbaik dalam penghasilan selulosa. Teknik fermentasi yang digunakan adalah fermentasi secara statik ,fermentasi secara berterusan dan juga fermentasi secara berulang. Penghasilan bakteria selulosa dalam kajian ini termasuk penyediaan inokulum dengan menggunakan air kelapa sebagai medium fermentasi. Inokulum dan fermentasi bakteria selulosa dijalankan pada suhu dan pH optimum iaitu 30 ^{0}C dan pH 5.5. Dari hasil kajian, medium kulit pisang dalam teknik fermentasi berterusan menghasilkan kadar hasil selulosa yang paling tinggi diikuti medium dari air kelapa tua, sisa bunga raya dan Hestrin Schramm medium. Ini adalah kerana kandungan karbon yang tinggi dalam kulit pisang membolehkan selulosa dihasilkan dalam kuantiti yang banyak jika dibandingkan dengan medium yang lain. Dari hasil kajian ini juga membuktikan, teknik fermentasi juga mempengaruhi kadar penghasilan selulosa. Daripada analisis menggunakan FTIR, selulosa yang terbentuk mempunyai ciri kristal yang sama dengan MCC. Keputusan dari SEM menunjukkan terdapat jalur seperti ribbon pada permukaan bakteria selulosa.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURE

LIST OF SYMBOLS

%	Percentage
^{0}C	Degree Celcius
g	Gram
mL	Milliliter

LIST OF ABBREVIATIONS

BC	Bacterial cellulose
CO_2	Carbon Dioxide
FTIR	Fourier Transform Infrared Spectroscopy
KOH	Potassium Hydroxide
K_2CO_3	Potassium Carbonate
MARDI	Malaysian Agricultural Research and Development Institute
MCC	Microcrystalline cellulose
NaOH	Sodium Hydroxide
Na_2CO_3	Sodium Carbonate
SEM	Scanning Electron Microscope
sp.	species
et al.	An others

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Cellulose is the earth's major biopolymer and it is a tremendous economic importance globally (Delmer and Amor, 1995). Cellulose is the major constituent of cotton and wood. Together, cotton and wood are 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. The production of cellulose nowadays was very limited because we are using the plant to get this material, so it will affect the earth and also will affect the global warming condition (Frenchen, 2000). Preservation of forest resources is essential to prevent global warming because the increasing in CO_2 concentration can be stopped only by the absorption of CO_2 by plants and trees (Park *et al*., 1996). However, the use of trees for the production of paper and construction materials has continuously depleted forest resources (Nobles *et* al., 2001).

Bacterial cellulose is the only alternative for plant cellulose because bacteria produce bacterial cellulose in a few days, while trees need more than 30 years to realize full growth. In this respect, bacterial cellulose is one of the key material for preventing global warming and preservation of the nature (Steven, 2004).Several general of bacterium were used to produce the cellulose include *Sarcina,*

Agrobacterium, Rhizobium, and *Acetobacter* (Barbara *et al*., 2008) However, only *Acetobacter Xylinum* is only species known to be capable of producing cellulose (Brown, 1993). This organism and its product were first identified and characterized over a century ago, although both are very common in the production of vinegar industry. *Acetobacter Xylinum* is a gram negative bacterium and is unique in its prolific synthesis of cellulose (Brown, 1994). *Acetobacter* is the model system for study of the enzymes and genes involved in cellulose biosynthesis and also known as a new biopolymer. It has several unique properties such as high purity and ultrafine or fiber network, very high hydrophilic, good mechanical strength, and outstanding shape and retention and high crystanility. The optimal temperature for growth is between 25^{o} C to 30^{o} C and the pH optimum about 5.4 to 6.3 (Vershuren, 1999).

To date, the process for production of bacterial cellulose commonly in the static cultivation method, with pellicles of bacterial cellulose formed on the surface of the static culture. However, this requires a large area in which to place the culture vessel and is impractical for large scale bacterial cellulose production (Okiyama *et al.*, 1992).Therefore, an economical mass production system based on shaking culture condition is necessary. Bacterial cellulose produced by *Acetobacter Xylinum* has several unique properties include high polymerization, high purity and have a quality fiber network. One of the famous applications of bacterial cellulose is in the production of nata de Coco in Philippines which was the famous diet for Philippines dessert (Yoshinaga, 1997).

Fed-batch, repeated and batch culture are the mode using in this research. These three different modes are used to optimize the production of bacterial cellulose. Batch culture is a static where fermentation medium is added to a culture containing the substrate consisting of a carbon source, an energy source and nutrients. The cells grow using these and at some time later, one of these is exhausted. The carbon source that use in this research is glucose. If the carbon and energy source runs out, then the biomass stop growing and the remaining excess

nutrients are not up taken (Benziman, 1991). If the nutrient runs out first, then the remaining excess carbon and energy source may continue to be utilized .Thus further products may be formed. Since there are no nutrients remaining, there can be no further biomass (Tang *et al*., 2009).The second mode that to be optimized is repeated batch. Repeated batch is where the fermentation medium are added to culture the medium in a schedule time recycle, so the production of biomass is growth depend on the fermentation medium and it will give the effect to the concentration of biomass. In the repeated batch, the fermentation medium is added step by step until it arrive the optimum stage where the bacteria will produce high yield of concentration of bacterial cellulose (Park *et al*., 2001). The fed-batch mode of operation is where the fermentation medium is added to the culture continuously. This is to maximize the use of fermentation medium by bacteria to get the high production of bacterial cellulose (Okiyama, 2004). The composition in the fermentation medium also effects the production of cellulose. In adequate nutrient in fermentation medium will lead to the less productivity of bacterial cellulose. According to Brown *et al*. (1993), the carbon sources are very important to enhance the bacterial cellulose productivity. The carbon sources usually derived from the glucose or carbohydrate contents in the medium. If the fermentation medium possess a high number of glucose or carbon content, it will stimulate the bacteria to enhance the productivity.

1.2 PROBLEM STATEMENT

Until recently, much effort has been put on developing process for the production of bacterial cellulose. The amount of biomass obtain in the reactor is comparatively low and therefore exist a need for an improved process for the production of bacterial cellulose. For the previous report, the production of bacterial cellulose was very low in the agitated culture (Tsuchida *et al.*, 1997).So in this study, the method of static culture is used to see the production of bacterial cellulose. A wide variety of modes were found to have ability to produce the high concentration of biomass in the production of bacterial cellulose. However, the

problem exists was to select the mode that not only can produce the bacterial cellulose but at the same time must have the highest biomass and substrate consumption (Okiyama *et al.*, 2003). Furthermore, an economical source is essential to have successful operation of the process. The main reason of this study is because most the production of cellulose is based on the plant cellulose. When the plant is cut everyday just to get the cellulose, it will give the effect to the global warming (French, 2007). This study will provide other alternative in production of cellulose. The last problem about the cellulose is in the plant cellulose itself. The production of plant cellulose will take a lot of time (Park *et al*., 2000). For example, the plant or tree will take about several months to grow, instead of this matter the production of bacterial cellulose will only take about a week for the production of cellulose.

1.3 RESEARCH OBJECTIVE

The objectives of this research is to determine the production of bacterial cellulose by *Acetobacter Xylinum s.p* in a static culture using different mediums and modes of operation which are batch,fed batch and the repeated batch using glucose as the carbon source

1.4 SCOPE OF STUDY

1.4.1 To determine modes of operation that can produce highest yield of bacterial cellulose which are batch, fed batch or repeated batch by using the glucose as a carbon source

1.4.2 To determine the medium that can produce high yield of bacterial cellulose

1.4.3 To study the characteristic bacterial cellulose using the FTIR

1.4.4 To characterize the morphology of the BC produced by using Scanning Electron Microscope

1.5 SIGNIFICANCE OF THE RESEARCH

There are several significant of this study that can be review from previous research paper. The most important thing that the production of bacterial cellulose can reduce the global warming in the earth (Brown, 1989). This is very obvious effect due to the reason that decreases of dependability to the plant cellulose. Besides that, the purpose of this study is, to increase the production of bacterial cellulose in large scale. In this study, the production of bacterial cellulose is conduct in three different modes. One of the modes will give the high yield of production and this mode will be used to increase the production of bacterial cellulose in future. In fact, the significant of this study is to decrease the dependability to the plant cellulose (Brown, 1989). This study is the most appropriate alternative that can be implementing in production of bacterial cellulose.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Cellulose is the most abundant organic polymer in nature, where it plays the important rule in the plant cell (Delmer and Amor, 1995). Plant is used widely in the production of paper and textile industries. It is also important to know that the plant also play a very important role in the preventing the global warming from becoming worst. So the scientist and researcher have come with a new alternative to get new cellulose. The bacterial cellulose is the one of the alternative that was used widely in this world. The first man who study and discovered about the bacterial cellulose was Adrian Brown in 1886 (Brown, 1986). Bacterial cellulose is extremely pure and exhibit high degree of polymerization. It is known that some bacteria will produce the cellulose by their metabolism system. In addition, there are some strains of the non-photosynthetic organism *Acetobacter* which have the ability to synthesize high quality cellulose (Brown *et al*., 1992).Bacterial cellulose has the good and quality cellulose which make them very useful.

2.2 APPLICATION OF BACTERIAL CELLULOSE

2.2.1 Food Application

Cellulose that has a high purity in chemical properties has been used in processed foods as thickening and stabilizing agent (Iguchi, 2005). The first use of

bacterial cellulose in food industry was in the production of nata de coco in the Philippines (Bhudiono, 2005). The gel-like properties of bacterial cellulose, combined with its complete indigestibility in the human intestinal tract, made this an attractive food base (Rosidi, 1999) .In 1970s, mevinolin which is the important metabolite in *Monascus sp*, has been identified and shown to inhibit the production of cholesterol (Westland *et al*., 1990). Nata is one of the bacterial cellulose products. Nata is prepared from the metabolism of *Acetobacter Xylinum* is a popular food in the Philippines and Asia countries. It also use widely in food processing because it contain a soft texture and high fiber. The combination of functional characteristics of monacolin K and bacterial cellulose in Monascus – nata complex have potential novel functional food stuff (Yamamoto, 2006). In 1992s, bacterial cellulose has been use for production of diet stuff that been introduce in the drink. *Acetobacter* was grown along with the yeast and the tea extract and sugar. This is consumed as a kombucha, or Manchurian tea for improved health needs (Stephens, 1990)

2.2.2 Pharmaceutical and medical application

Bacterial cellulose contains high tensile strength, high porosity and microfibrillar structure (Brown, 1999). For example, the chronic wounds such as venous and diabetic ulcer are very difficult to heal. The treatment for the chronic wounds involves a lot of materials. As bacterial cellulose contain of highly porous material, it allow the potential transfer antibiotics or other medicine into the wound (Takagi, 1993). It was report by Bielecki to satisfy the requirements of modern wound dressing material. Bacterial cellulose has a very high water holding capacity (Ichimura *et al*., 1998). This characteristic made the bacterial cellulose is very effective to heal the ulcers and wounds. Brazilian company Biofil has investigate the unique characteristics that can heal the wound and they produce two product which is Bioprocess and Gengflix that acts as a dressing material for extensive wounds (Fontana *et al*., 1990, 1991). A bacterial cellulose produce by Xylos Corp (USA) namely as Prima Cel^{TM} has been applied in clinical test for heal the ulcer and wounds (Risberg *et al*., 2006). Tissue engineered blood vessel (TEBV) represent an

alternative for overcome reconstructive problem that have a link with the vascular disease by providing small caliber vascular grafts and bacterial cellulose exhibits the properties that can effectively use as a scaffolds for tissue engineering blood vessels (Helenius *et al*., 2006)

2.2.3 Other application

The unique physical and mechanical properties of bacterial cellulose such as high reflectivity, flexibility , light mass and ease of portability has been apply in the electronic paper display (Shah *et al*., 2005). The bacterial cellulose fragmented has very good prospects in papermaking which are very suitable for bank note and also the bible paper (Iguchi *et al*., 2000). Bacterial cellulose also has been applied in the mineral and oil industry. The new research from the University of Michigan reported by Westland in 2009, there are pattern inventions that have a relation to the use of bacterial cellulose in hydraulic fracturing of geological formations at selected levels of wells drilled for recovery of hydrocarbon. Addition of cellulose microfibrils obtained by acid hydrolysis of cellulose fibers at low concentration to polymer gels and films has shown the significant change in the strength and mechanical properties (Laszkiewicz, 1876). Based on the tensile strength, the bacterial cellulose has a great potential to be used as a packaging material in food packaging where the continuous moisture removal and minimal oxygen transmission properties play an important role (Jonas and Farah, 1995). The unique dimensional stability of bacterial cellulose give a potential in making the sound transducing membrane which can maintains the high sonic velocity over a many frequencies ranges. Thus, the Sony Corporation in Japan has taken this advantage to develop the first audio speaker diaphragms using bacterial cellulose (Ichimura *et al*, 1988)

2.3 BACTERIUM STRAIN

Among the bacteria, one of the most advances types of purple bacteria is the common vinegar bacteria that are *Acetobacter Xylinum* (Brown, 1986). The *Acetobacter* is a non-photosynthetic organism that can use the glucose, sugar, and glycerol and turn it to the pure cellulose (Brown *et al*., 1976). A typical single cell of *Acetobacter Xylinum* can convert about 108 glucose molecules per hours to cellulose (Brown *et al*., 1989). A single cell of *Acetobacter Xylinum* has a linear row of pores from which glucan chain polymer aggregates are spun. *Acetobacter Xylinum* have a great absorptivity strength constitute two of many novel features of bacterial cellulose (Brown, 1989; White and Brown, 1989; Brown, 1992; Brown; 1994). The optimal temperature for growth of *Acetobacter Xylinum* between 25^{O} C to 30^{O}C and the pH optimum is about 5.4 to 6.3 (Verschuren, 1999). *Acetobacter Xylinum* has been applied as a model of microorganism for basic and applied in the production of bacterial cellulose (Park *et al*., 2003). It is commonly has been used in the study because it possess the ability to produce high level of polymer from a wide range of carbon and nitrogen sources (Delmer *et al*., 1991, Weigend *et al*., 2007). It is a gram negative that have a rod shape bacteria that produce the cellulose in the form of interwoven extracellular ribbons as part of primary metabolite. This bacterium grows and produces cellulose from a wide range variety of substrate. The Table 2.1 shows the different strain that produce the bacterial cellulose.

Table 2.1: Strain for production of bacterial cellulose

Microorganism	Carbon Sources	Supplement	Culture Time	Yield (g/L)
A. xylinum BRC 5	Glucose	Ethanol, Oxygen	50h	15.30
G. hansenii PJK (KCTC 10505 BP)	Glucose	Oxygen	48 h	1.72
G. hansenii PJK (KCTC 10505 BP)	Glucose	Ethanol	72 h	2.50
Acetobacter sp. V6	Glucose	Ethanol	8 day	4.16
Acetobacter sp. A9	Glucose	Ethanol	8 day	15.20
A. xylinum BPR 2001	Molasses	None	8 day	7.820
A. xylinum BPR 2001	Fructose	Agar oxygen	72 h	14.10
A. xylinum BPR 2001	Fructose	Agar	56 h	12.00
Acetobacter xylinum ssp.sucrofermentans BPR2001	Fructose	Oxygen	52 h	10.40
Acetobacter xylinum ssp.sucrofermentans BPR2001	Fructose	Agar oxygen	44 h	8.70
Acetobacter xylinum E25	Glucose	No	7 day	3.50
G. xylinus strain (K3)	Mannitol	Green tea	7 day	3.34
Gluconacetobacter xylinus IFO 13773	Glucose	Lignosulphonate	7 day	10.10
Acetobacter xylinum NUST4.1	Glucose	Sodium alginate	5 day	6.00
Gluconacetobacter xylinus IFO 13773	Molasses	No	7 day	5.76
Gluconacetobacter sp. RKYs+	Glycerol	No	144 h	5.63

Sources: Son *et al.* (2001)

2.4 BACTERIAL CELLULOSE BIOSYNTHETIC PATHWAY

Synthesis of bacterial cellulose involves multiple step process and a large number of enzymes. The process includes the formation of uridine disphoglucose (UDPGlc) which is the precursor in the cellulose formation (Brown *et al.*, 1987). Then, its follow by glucose polymerization into the β-1-4 glucan chain and a chain is form like a ribbon structure of cellulose chain. Its form in a hundred or even a thousand of individual cellulose chain (Delmer *et al*., 1995). In *Acetobacter Xylinum,* cellulose synthesis is tightly connected with the catabolic process of oxidation and use approximately 10% of energy derived from the catabolic reactions (Amor *et al*., 1995). *Acetobacter Xylinum* converts various carbon compounds such as hexoses, glycerol, glucose and molasses into cellulose (Bielecki *et al*., 2005). Figure 2.1 shows the biochemical pathway for cellulose synthesis by *Acetobacter Xylinum*.

The synthesis of cellulose in *Acetobacter Xylinum* or any other cellulose follows two steps. First is formation of β-1-4 glucan chain polymerization of glucose units and the second is assembly and crystallization of cellulose chain (Lin *et al*., 1985). The rate of polymerization is limited by the rate of assembly and crystallization. Cellulose synthase catalyzes the cellulose biosynthesis by polymerizing the glucose units into the β-1-4 glucan chain (Valla *et al*., 1989). The formation of cellulose fibrils is shown in Figure 2.2.

Two hypotheses for this mechanism in *Acetobacter Xylinum* have been reported. The first hypothesis assumes that the polymerization of the β-1-4 glucan chain does not involve a lipid intermediate (Delmer *et al*., 2000). The glucose residue were added to the nonreducing end of the polysaccharide and those reducing ends were nascent polymer chains situated away from the cells (Yamanaka *et al*., 1993). The second hypothesis states that the polymerization of β-1-4 glucan involves a lipid intermediate (Benziman *et al*., 1989). The involve of

lipid intermediate in the synthesis of acetan which is a soluble polysaccharide has been proven. The polymer synthesis produce no lipid component (Klemm, 2003)

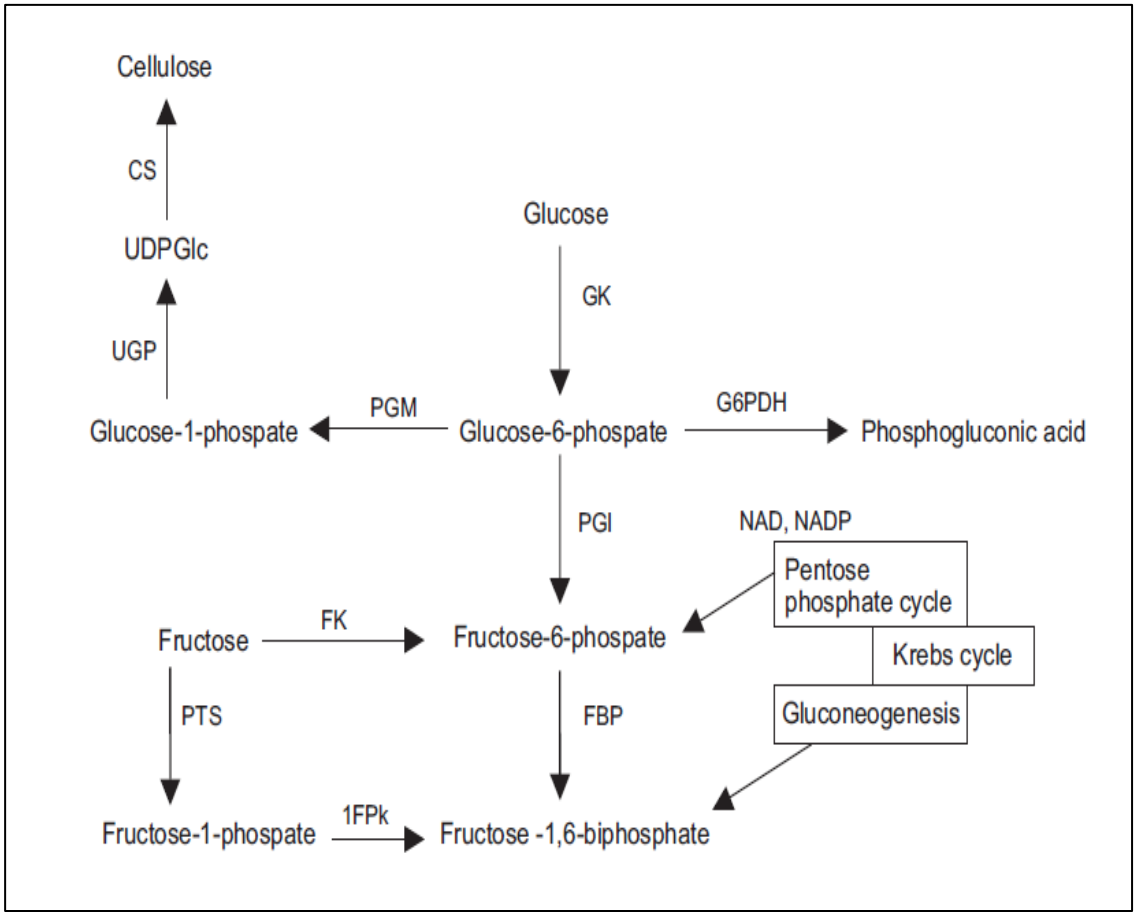

Figure 2.1: Biochemical pathway for cellulose synthesis by *Acetobacter Xylinum.* CS cellulose synthesis , GK glucokinase , FBP fructose -1,6-bi-phosphate phosphatase, FK frucktokinase, 1FPK fructose-1-phosphate kinase, PGI phosphoglucoisomerase, PMG phosphoglucumutase, PTS systems of phosphotransferases, UGP pyrophosphorylase uridine diphosphoglucose , UDPglc urdiene diphosphosglucose , G6PDH glucose-6-phospate dehydrogenase, NAD ricotinamide adenine dinucleotide , NADP nicotinamide adenine dinucleotide phosphate

Sources: Cooper *et al.* (1985), Saxena *et al.* (2000)

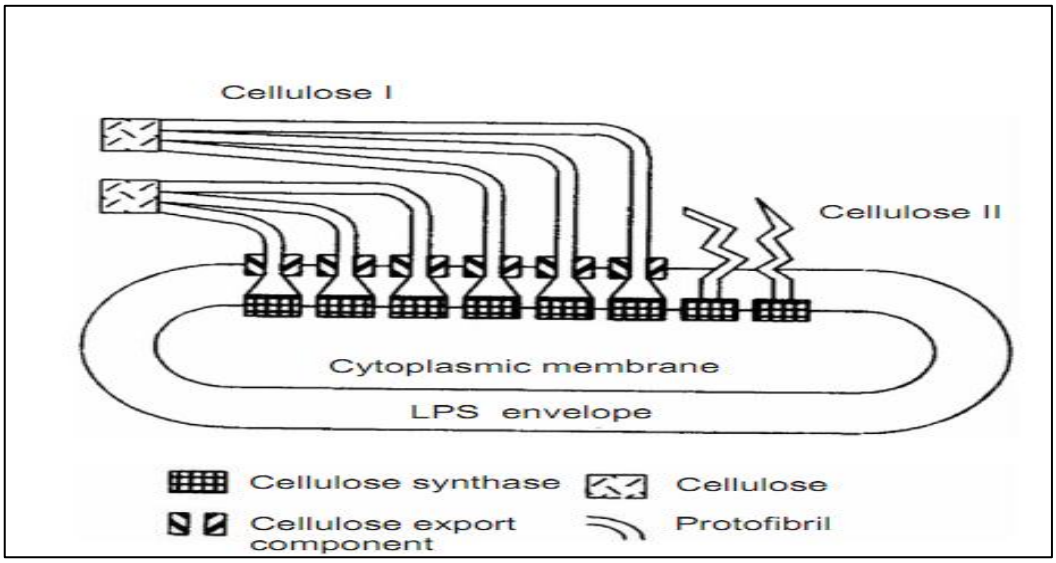

Figure 2.2: Assembly of cellulose microfibrils by *Acetobacter Xylinum*

Sources: Hagler *et al*. *(*1987), Delmer (1987), Ohe *et al*. (1993), Iguchi *et al*. (2000)

2.5 PROPERTIES OF BACTERIAL CELLULOSE

Bacterial cellulose possesses a very high crystallinity, high tensile strength, extremely insolubility in most of the solvents, moldability and high degree of polymerization (Ramana *et al*., 2005). The thickness of cellulose fibrils is commonly 0.1- 10µm, one hundred times thinner than cellulose fibrils obtained from the real plant (White *et al*., 1981). The water holding capacity is 100 times higher (Schrecker *et al*., 2005). The bacterial cellulose is far stronger than plant cellulose (George *et al*., 2005). The macroscopic morphology of cellulose is depending on the condition of the culture which can easily be tailored for the physicochemical properties (Wanichapichart *et al*., 2005). Cellulose is soluble in concentrated acids like sulphuric, hydrochloric or nitric acid. It also soluble in 8.5% NaOH solution. The solubility of cellulose in the alkali can be increases by adding 1% of urea to the solution (Sabapathy *et al*., 2005).

George *et al.,* (1996) studied the swelling property of cellulose under different condition. NaOH at lower concentration caused greater swelling in fibers as compared to the other alkalis at the same concentration (Takahashi *et al*., 1994). The percentage mass gain by the cellulose membranes after the immersion process in the different alkali solutions was to be found in order of NaOH>KOH>Na_2CO_3>K_2CO_3 (Yoshinaga *et al*., 1997). The evaporation characteristics of bacterial cellulose membrane were investigated over a wide range of water- ethanol feed composition and it was found to be promising for dehydration of azeotropes of ethanol (Ishida *et al*., 2002). It has a very high selectivity towards water at a reasonable flux.

The most attractive feature of bacterial cellulose production is the ability to control and modify not only the physical characteristics but also the chemical composition of the cellulose fiber (Sameshima *et al*., 2006). The structure of the cellulose assembly can be altered by using the dyes or derivatives. *Acetobacter Xylinum* was cultured in the HS medium and in the medium contains acetyl glucomannan. The presences of acetyl glucomannan in the medium prevent the assembly of cellulose microfibrils and change the crystal structure of cellulose (Dubey *et al*., 2002). Cultivation of the Acetobacter Xylinum in the HS medium contains glucuronoxylan showed loosed bundles of cellulose microfibrils in the medium (Nakagaito *et al*., 2005). In contrast, the cellulose ribbons were formed in the pectin medium. Glucuronoxylan in the medium prevented the assembly of cellulose microfibrils and changed the crystal structure of cellulose, whereas pectin in the medium scarcely had an effect (Haigler *et al*., 1993).

2.6 CHEMICAL STRUCTURE OF BACTERIAL CELLULOSE

Cellulose is a homopolymer consisting of glucose glycosidically linked in the β-1-4 conformation. The Figure 2.3 shows the repeating unit of cellulose.

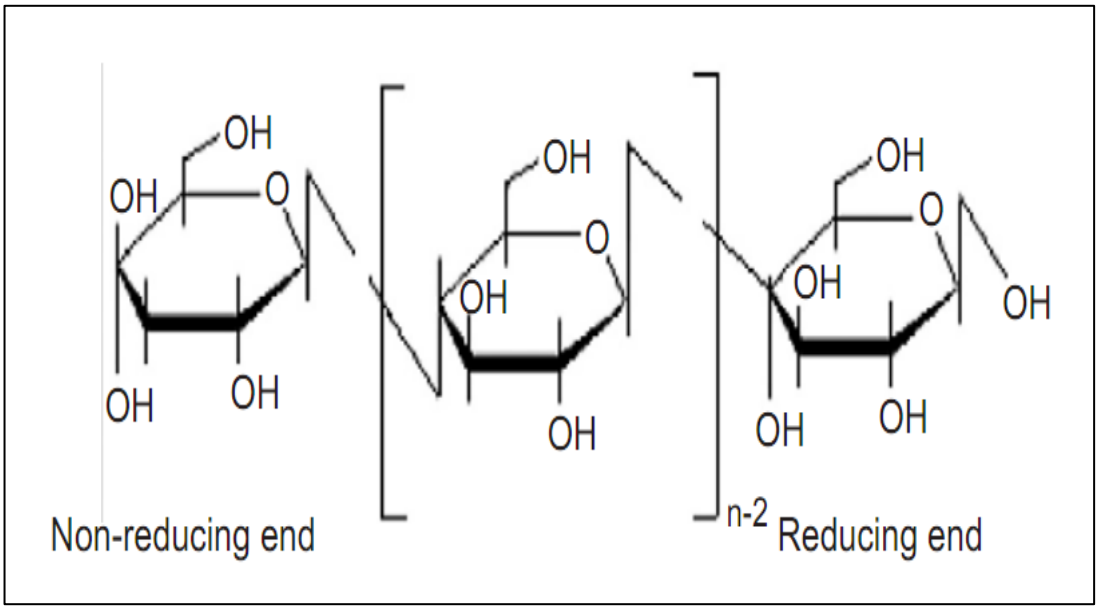

Figure 2.3: The repeating units of cellulose

Source: Yoshino *et al*. (2000)

The repeating unit of the polymer synthesis consists of two glucose molecules bonded together in formation where that one molecule is rotated 180 degrees with respects to the other (Valla *et al*., 1989). The chemical structure of bacterial cellulose is similar to the plant cellulose but the different is at degree of polymerization. In plant is about 13000 to 14000 and for bacterial cellulose is about 2000-6000 (Kobayahsi *et al*., 2006). The glucose units in cellulose are bound together to produce the long straight chain of unbranched polymer chain and the capacity to form intermolecular hydrogen bonds between adjacent glucan chains is extremely high (Ishihara *et al*., 2002). The shape of bacterial cellulose sheet seems to be maintained by hydrophobic bonds. It is reported that, the molecular hydrogen bonds initially occur in each cellulose sheet and then the cellulose crystalline structure is formed with the development of hydrogen bonds between cellulose sheets (Nguyen *et al*., 2008). The microfibrils structure of bacterial and plant cellulose under the scanning electron microscope (SEM) is shown in Figure 2.4

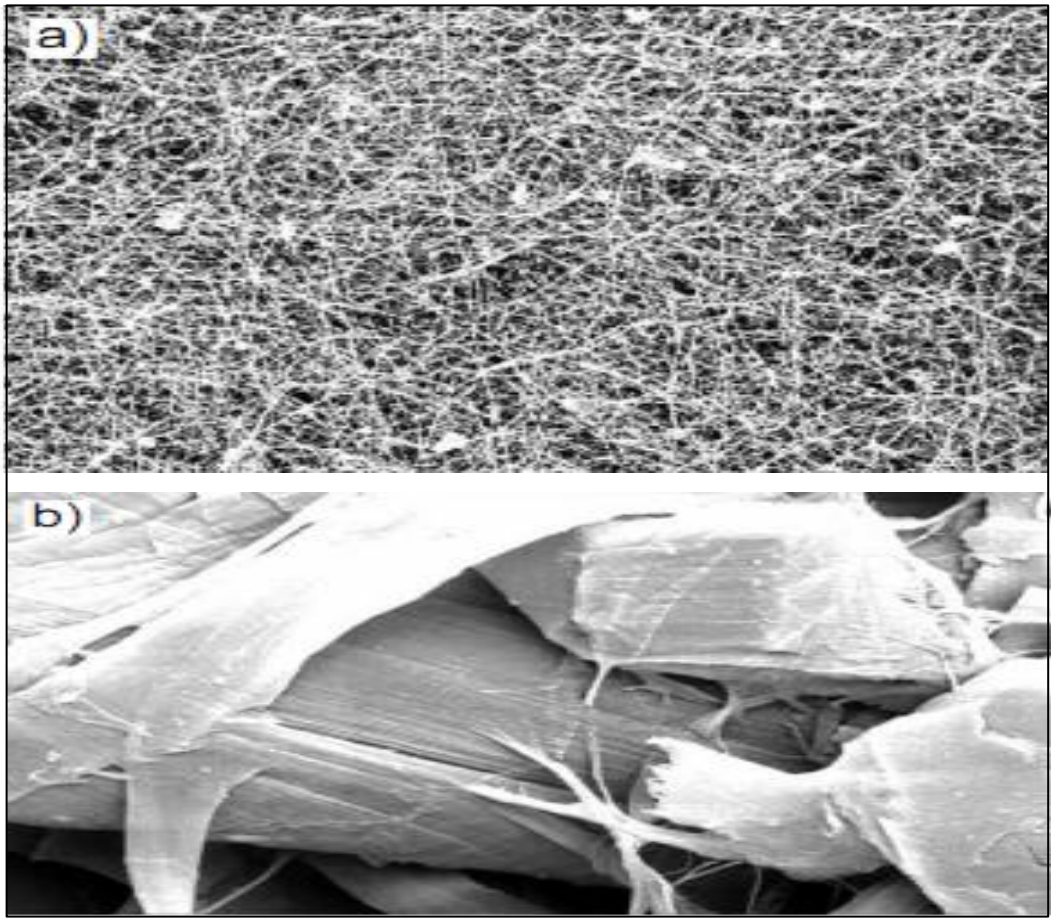

Figure 2.4: Microfibrillar organization between (a) *Acetobacter Xylinum* cellulose, and (b) wood pulp 5000x

Source: Gidley *et al*. (2003)

The bacterial cellulose observed under the SEM showed a significant difference in appearance of the external and internal surfaces of the pellicles (Takabe *et al*., 1995). The external surfaces had irregular clusters of fibrils meanwhile internal surfaces were organized in the fractured sections. At higher magnification, layers of tunnels in the bacterial cellulose of about 7µm in diameter were found (Brown *et al*., 1994).

2.7 FERMENTATIVE PRODUCTION OF BACTERIAL CELLULOSE

2.7.1 Effects of medium components

In general, there are several factor affecting cellulose production mainly include the growth medium, environmental condition and formations of byproducts. Commonly, medium containing high carbon to limiting nutrient ratio is favorable for polysaccharide production (Farah *et al*., 1990). The fermentation medium contains carbon, nitrogen and other macro and micronutrients required for the growth of microorganism and thus enhance the formations of product. The change in the medium components effects growth and the yield production of bacterial cellulose indirectly. Sometime a complex medium supplying amino acids and vitamins is also used to enhance the cell growths and yield productions (Johnson *et al*., 1989). According to the Brown *et al*., (2003), the carbon sources in each medium play a very important role to enhance the production of bacterial cellulose. Each medium have different nutrient component that affect the yield that bacterial cellulose produce (Ishihara *et al*., 2001). The quality of bacterial cellulose produce also depends on the component of the medium. For synthesis the bacterial cellulose from the *Acetobacter Xylinum*, the glucose and nitrogen contain are the most vital component needs to be provide enough in order to get a full concentrations of cellulose yield (Yamashita *et al*., 2003). The table 2.2 and 2.3 summarize the chemical composition in Hestrin Schramm medium and banana peel medium. From this comparison, the banana peel consists of more glucose content than Hestrin Schramm medium. According to Brown *et al*., (2002), the concentration of glucose in a medium will affect the production of bacterial cellulose. The more glucose in the medium will produce more concentrate bacterial cellulose but report from Vershuleen *et al*., (2001), the exceeds of glucose content also can give the effect to the yield of bacterial cellulose. It state that, the inhibit substrate growth occur when the glucose is exceeds in the medium and this problem will reduce the yield of bacterial cellulose produce.

Table 2.2: Chemical composition in the Hestrin Schramm fermentation medium

Parameter	Concentration (% w/v)
Glucose	2.0
Peptone	0.5
Yeast extract	0.5
Disodium phosphate	0.27
Citric acid	0.115

Source: Hestrin and Schramm (1954)

Table 2.3: Proximate chemical composition content in the banana peel

Parameter	Concentration
Carbohydrate (%)	59.00
Iron (%)	0.610
Crude lipid (%)	1.700
Crude fiber (%)	31.70
Oxalate (mg/g)	0.510
Manganese (mg/g)	76.20
Potassium (mg/g)	78.10
Calcium	23.65
Protein	23.95
Insoluble fiber	36.23
Soluble fiber	NA

Source: Anhwange *et al*. (2009)

Table 2.4: Chemical composition of *hibiscus roselles sinesis*

Parameter	Concentration (g/100g)
Protein	31.02
Ash	6.89
Carbohydrate	36.37
Crude fiber	4.12
Insoluble fiber	NA
Moisture	9.25

Source: Ismail *et al*. (2008)

Table 2.5: Chemical composition in coconut water

Parameter	Concentration (g/100g)
Water	94.99
Protein	0.72
Sucrose	10.70
Glucose	2.02
Fructose	2.48
Total sugar	15.02

Source: Santoso *et al*. (1996)

2.7.2 Effect of carbon sources

Usually, glucose and sucrose are used as a carbon sources for cellulose production, other carbon sources hydrates such as fructose, maltose, xylose, starch and glycerol have also been tried (Stephens *et al*., 2005). *G.hansenii* PJK (KCTC 10505 BP) produced 1.72 g/L of cellulose when glucose was provided as carbon glucose (Westland *et al*., 1990). *Acetobacter Xylinum sp*. V6 strain produced 4.16 g/L cellulose in a complex medium containing glucose as a carbon sources (Son *et al*., 1994). The effect of initial glucose concentration on cellulose production is also important, since the formation of gluconic acid as a byproduct in the medium decrease the pH of the culture and slowly decreases the bacterial cellulose production. Ishihara *et al*., (2004) used xylose as a carbon source for the production of cellulose by *Acetobacter Xylinum* IFO 15606 and obtained a yield of 3.0g/L. Sucrose, mannitol and glucose were found to be the optimal carbon sources for cellulose production by *Acetobacter Xylinum* NCIM 2526. *Gluconacetobacter Xylinus* strain gave a maximum cellulose production with mannitol as a carbon sources. The problem associated with the use of glucose as a carbon sources for cellulose production is the formation of gluconic acid as a byproduct in the medium which decrease the pH of the medium. Keshk and Sameshima, (2003) investigated the formation of gluconic acid and bacterial cellulose in the presence of

lignosulphonate. Gluconic acid production was decreased and the bacterial cellulose production was increase when the medium was added with the lignosulphonate. This was attributed to the inhibition of gluconic acid formation in the presence of antioxidant in the lignosulphonate.

2.7.3 Effects addition of ethanol

Ethanol is used as additional carbon sources and also to degenerate the cellulose non-producing cells of *G.hansenii* which can be appear under submerged culture conditions. Addition of ethanol increased on cellulose production from 1.30 to 2.31g/L in *G.hansenii* (Guttman *et al*., 2005). Son *et al.,* (2001) studied the effect of addition of ethanol on cellulose production by using isolated Acetobacter sp. A9 strain. It was observed that with the addition of 1.4% ethanol to the medium increase the production of cellulose to 15.2g/L which is four times higher from the medium without ethanol. Addition of ethanol was also found to eliminate the mutation of cellulose non-producing cells. The mutation in bacterial cellulose occurs due to the strain that having a contamination with other substance and this will lead to the non-producing membrane of cellulose. This will also can affect the environment pollution (Schmidt *et al.,* 1997)

2.7.4 Effects of precursors

The addition of precursor molecules is very importance in the polysaccharide synthesis. Amino acids have been used by some researcher as nitrogen sources to improve the biopolymer yield (Lee *et al*., 2003). Methionine has an important effect on the cellulose production by *Acetobacter Xylinum ssp. sucrofermentans,* which increase 90% of cell growth and cellulose production (Matsuoka *et al*., 1996). Nicotinamide has also been used in the bacterial cellulose production. Using the Nicotinamide show the maximum bacterial cellulose production was at 0.000005% (Son *et al*., *2003*). Vitamins like pyridoxine, nicotinic acid and biotin were also found to be important for the cells growth and cellulose

production, but vitamins like pantothenate and riboflavin were found to have contradictory effects (Tsuchida *et al*., 1996). The sugar nucleotides had an important effect on the cell growth and bacterial cellulose production. UDPGlc has enhanced effect on the production of bacterial cellulose (Kim *et al*., 2003, Sugano *et al*., 2004)

2.7.5 Effect of the temperature

Temperature is one of the environmental factors. Temperature is crucial parameters that effect both growth and also the bacterial cellulose production. In the most of the experiment, the maximum cellulose production was obtained between 28 to 30 ^{0}C (Hestrin and Elanann, 1954, Hestrin and Schramm, 1963)**.**

2.7.6 Effect of pH

The optimum pH of the culture medium for bacterial cellulose production is in the range 4.0 to 6.0. The yield of cellulose decreasing below the pH 4.0 (Sakota *et al*., 1993). The pH is decreasing during the production of bacterial cellulose is due to the formation of gluconic, acetic and lactic acids in the culture broth (Kongruan, 2008). Therefore, it is very important to control the pH within the optimal range. Noro *et al*., (2002) reported used the buffering capacity of corn steep liquor to maintain the pH (Smitchd *et al*., 2001).

2.7.7 Effect of operation mode

Operation mode is one of the major factors that will affect the fermentation of bacterial cellulose. The report from the Brown *et al*., (1989), the fed batch mode consistently produces a high number of bacterial cellulose in 7 days of fermentation. In that report, Brown *et al.,* (1989) state that, fed batch mode of operation control the nutrient supply to the *Acetobacter Xylinum*. This method had prevent or reduces the substrate- associated growth inhibition. This method will stimulate the

Acetobacter Xylinum to get enough nutrients in a optimum condition. Thus, it will increase the bacterial cellulose production time by time. One of the report was in 2004 by Sangok *et al*., (2004), in this report include the formation of 7.82 g/L of bacterial cellulose was obtained when 0.2 L of molasses medium was added five times.

2.8 MICROCRYSTALLINE CELLULOSE (MCC)

Microcrystalline cellulose is obtained from the hydrolysis of wood and cotton cellulose using dilute mineral acids. Preparation of microcrystalline cellulose from materials other than wood and cotton such as hyacinth (Gaonkar and Kulkarni, 1987), coconut water shells (Gaonkar and Kulkarni, 1989) , sugar cane bagasse (Padmadisastra and Gonda, 1989) are among the sources that produce good quality of the microcrystalline cellulose. Microcrystalline cellulose has a relatively low chemical reactivity combine with excellent compactibility at low pressure. Microcrystalline cellulose was rated the most useful filler for direct compression tableting (Shangraw and Demarest, 1993). However, a number of limitations to the use of microcrystalline cellulose have been reported (Bolhuis and Chowhan, 1996), the most important of which were considered to be its low bulk density, high lubricant sensitivity, poor flow characteristics and the influence of moisture on the compression characteristics. The microcrystalline cellulose is very pure and it's not a chemical derivative and there has been no chemical modification of the cellulose molecule (Thomas and Pourcelot, 1993). The comparison of the bacterial cellulose and microcrystalline cellulose has been made by Goh *et al*. (2012). They reported the comparison in the Fourier Transform Infrared Spectroscopy analysis and Scanning Electron Microscope analysis. This two comparison is very important because, the microcrystalline possess a very good mechanical strength (Mathew *et al*., 2002), thus from the FTIR and SEM analysis will characterize the bacterial cellulose features.

2.8.1 Fourier Transformed Infrared Spectroscopy of MCC

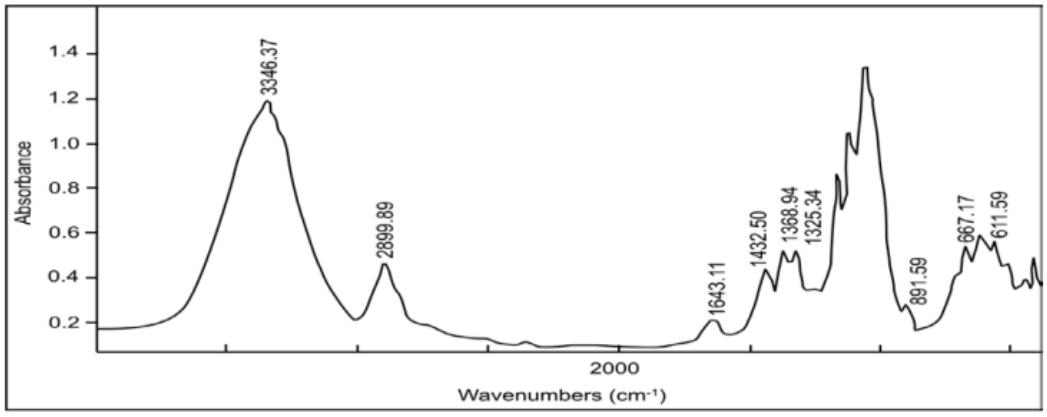

Figure 2.5: Commercial Microcrystalline cellulose

Source: Goh *et al*. (2011)

Figure 2.5 show the FTIR analysis from the commercial microcrystalline cellulose. Absorbance spectra for MCC in the region 4000-450 cm^{-1}. The characteristic of anomeric carbons were at the region of 960-730 cm^{-1}. The band of 891.59 cm-1 shows the β, 1-4 linkages. The strong bond for MCC is at 1432.50 cm^{-1}. This relationship with the cellulose was very important because the FTIR spectra bands will characterize the bacterial cellulose (Brown *et al.*, 1989). Table 2.6 summarizes the wavelength of spectra band.

Table 2.6: The FTIR wavelength bands

Frequency (cm^{-1})	Bands	Bond Orientation
1740	(C = O)	-
1426	CH_2	-
1115	(C-O), (C-C)	C-2-O-2
895	(C-l-H)	B-anomeric link
1371	CH_2w	-
1362,1317	CH_2w	-
1160	(C-O-C)	Glycosidic link,ring

1146	(C-O-C)	Glycosidic link,ring
1130	(C-O-C)	Glycosidic link,ring
1100	(C-O),(C-C)	ring
1075	(C-O),(C-C)	ring
1060	(C-O),(C-C)	C-3-O-3
1042	(C-O),(C-C)	ring
1030	(C-O),(C-C)	C-6-H_2-O-6
1015,1000	(C-O),(C-C)	C-6-H_2-O-6
1019	(C-O),(C-C)	C-2-C-3, C-2-O-2,C-1-O-1
960	(CO)	-

Source: Wilson *et al*. (2000)

2.8.2 Scanning Electron Microscope of MCC

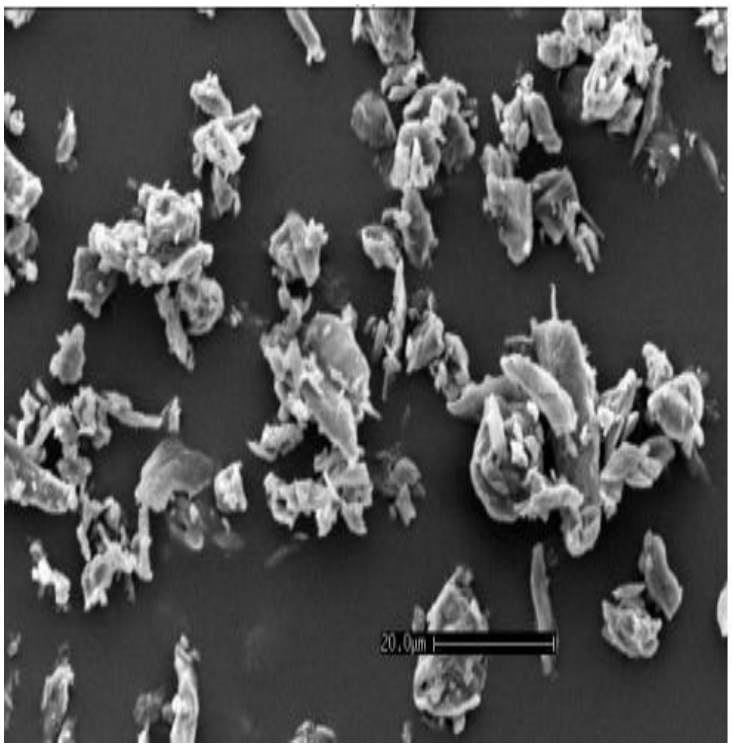

Figure 2.6: Scanning electron micrograph of surfaces commercial MCC, magnification 1000x

Source: Rajeev *et al*. (2008)

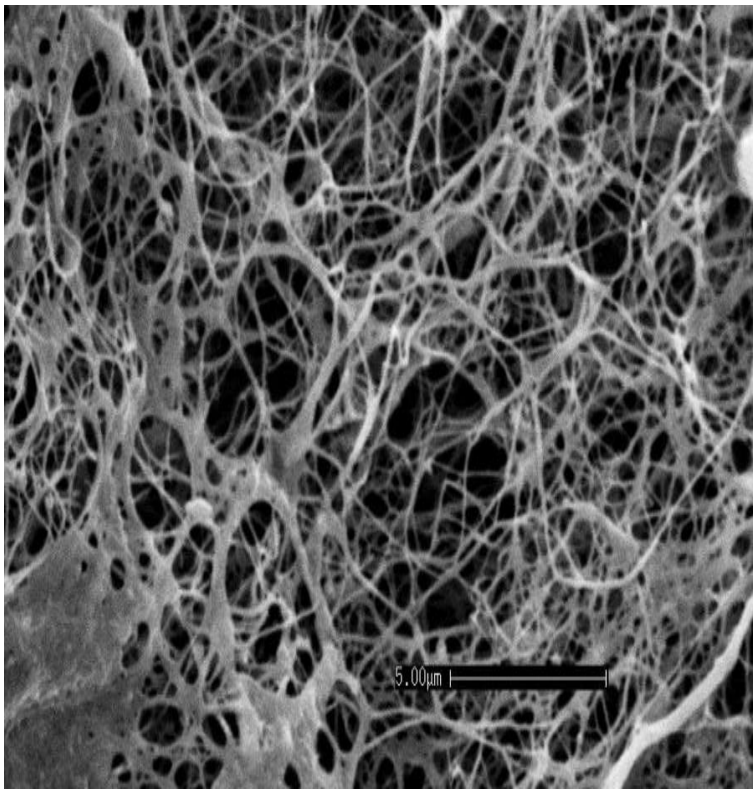

Figure 2.7: Bacterial cellulose from scanning electron micrograph, magnification 5000x

Source: Rajeev *et al*. (2008)

Figure 2.6 and 2.7 describe about the bacterial cellulose and microcrystalline cellulose based on the scanning electron microscope analysis. Surface of MCC is different from the bacterial cellulose. MCC surface are less dense and contain less ribbon structure network. The primary particles that form the MCC are bigger than bacterial cellulose (Kothari *et al*., 2002). In addition the surface of MCC aggregates showed less smooth and also less dense.

The structure of bacterial cellulose formed is more like to ribbon structure. It is dense packly in the surface of BC. From the Figure 2.7, the link of every ribbon

structure will form a surface of bacterial cellulose with strong mechanical characteristics (Iguchi *et al*., 2007)

2.9 ADVANTAGE AND DISADVANTAGE

Cellulose is the major part of plant wall and act as protector and coating (Bielecki *et al*., 2000) .However cellulose that produce by the plant wall is not pure because the lignin and hemicellulose have to be removed first in order to get the cellulose (D. Klemn *et al.,* 2001) but bacterial cellulose is very pure because it doesn't consist of lignin and hemicellulose, so it is easy to get the cellulose in bacteria if compare to plant cellulose (Brown, 1986). Bacterial cellulose have a properties include high crystanility, high mechanical strength and high water absorption (D. Klemn *et al*., 2001). Bacterial cellulose also have the disadvantage include the high price of production and very low of production.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

The production of bacterial cellulose was investigated in the four different medium in the three categories of operation mode. The medium use in this study was Hestrin Schramm medium (control), banana peel medium, old coconut water medium and hibiscus waste mean while the operation mode use in this study were batch, fed-batch and repeated batch. The *Acetobacter Xylinum sp.* was fermented in the medium for 10 days. After 10 days, the cellulose produce was harvested and the analysis was done using the Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM)

3.2 MATERIAL AND APPARATUS

Acetobacter xylinum sp. and the HS medium were the raw materials that will be used in this study. Yeast extract, peptone, disodium hydrogen phosphate and citric acid are the chemical that will be used for the preparation of the culture medium and using the Hestrin Schramm Medium for the preparation of the culture medium in this experiment. The main equipment for this experiment are incubator and Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM).

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

3.3 PREPARATION OF INOCULUM

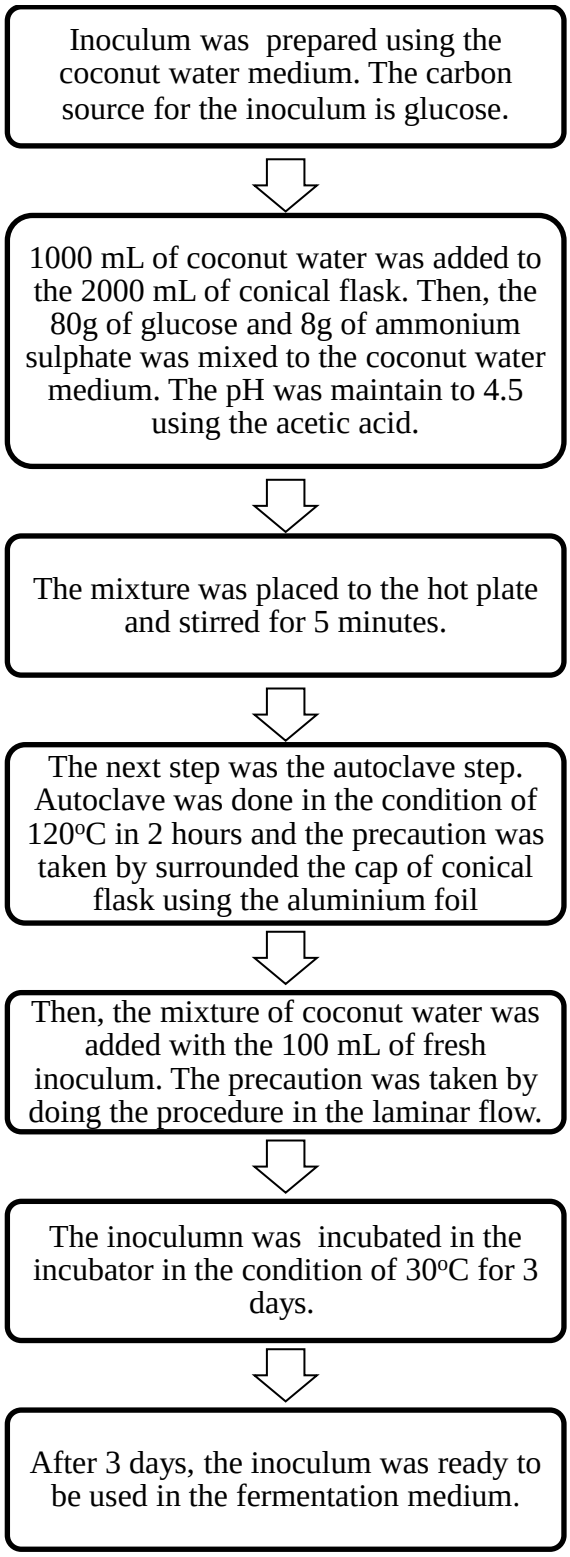

Figure 3.3: Preparation of inoculum using the coconut water

3.4 PREPARATION OF FERMENTATION MEDIUM

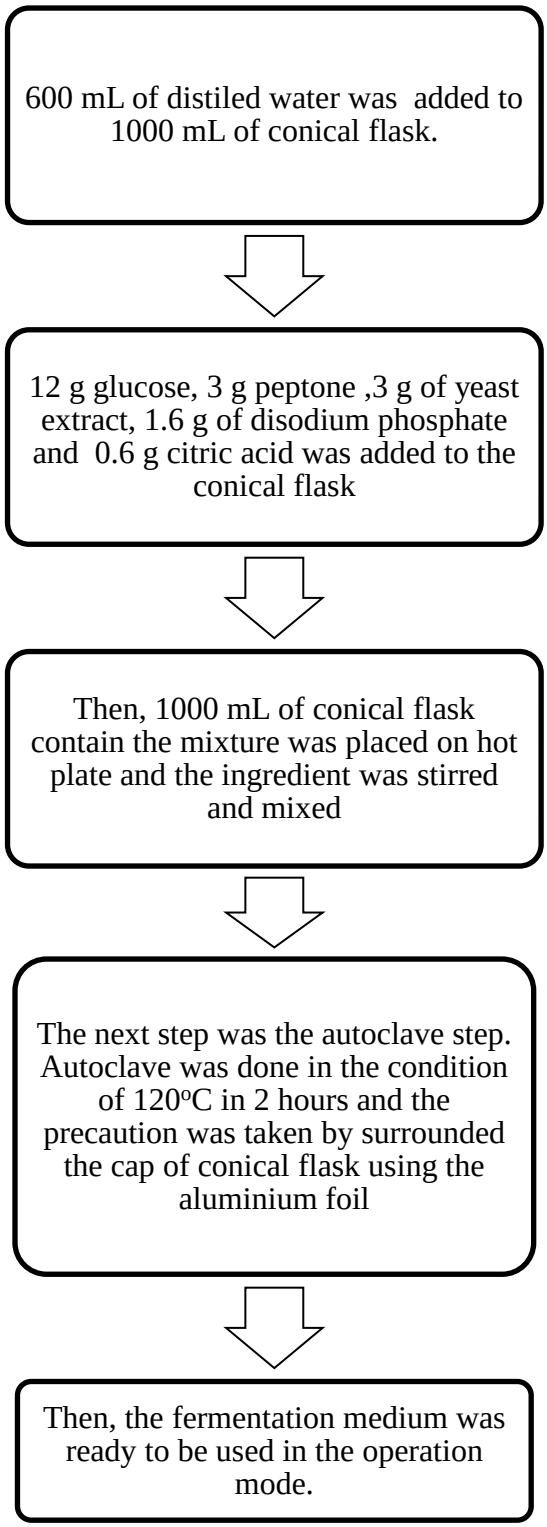

Figure 3.4.1: Preparation of Hestrin Schramm medium

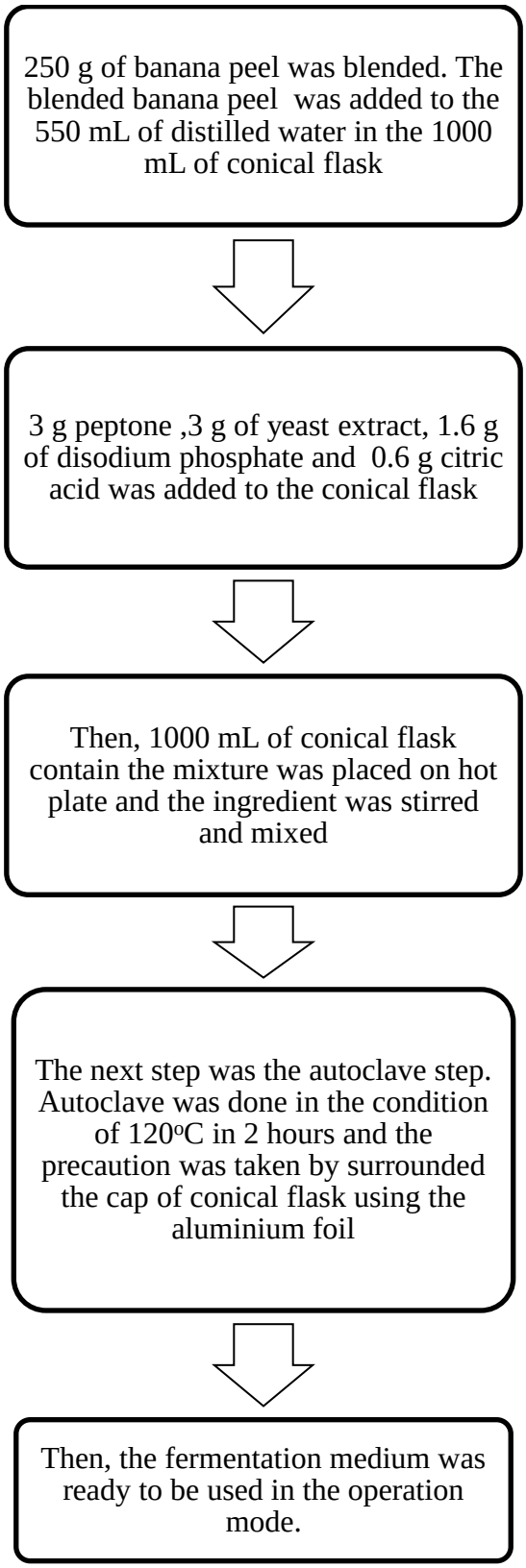

Figure 3.4.2: Preparation of banana peel fermentation medium

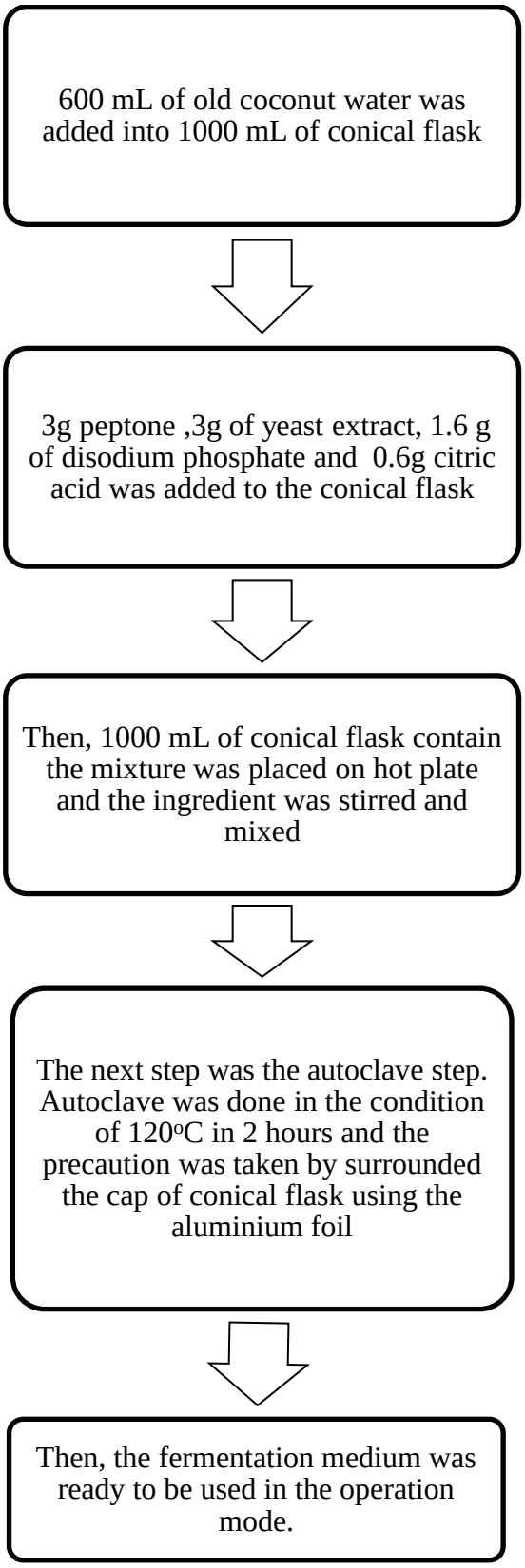

Figure 3.4.3: Preparation of old coconut water fermentation medium

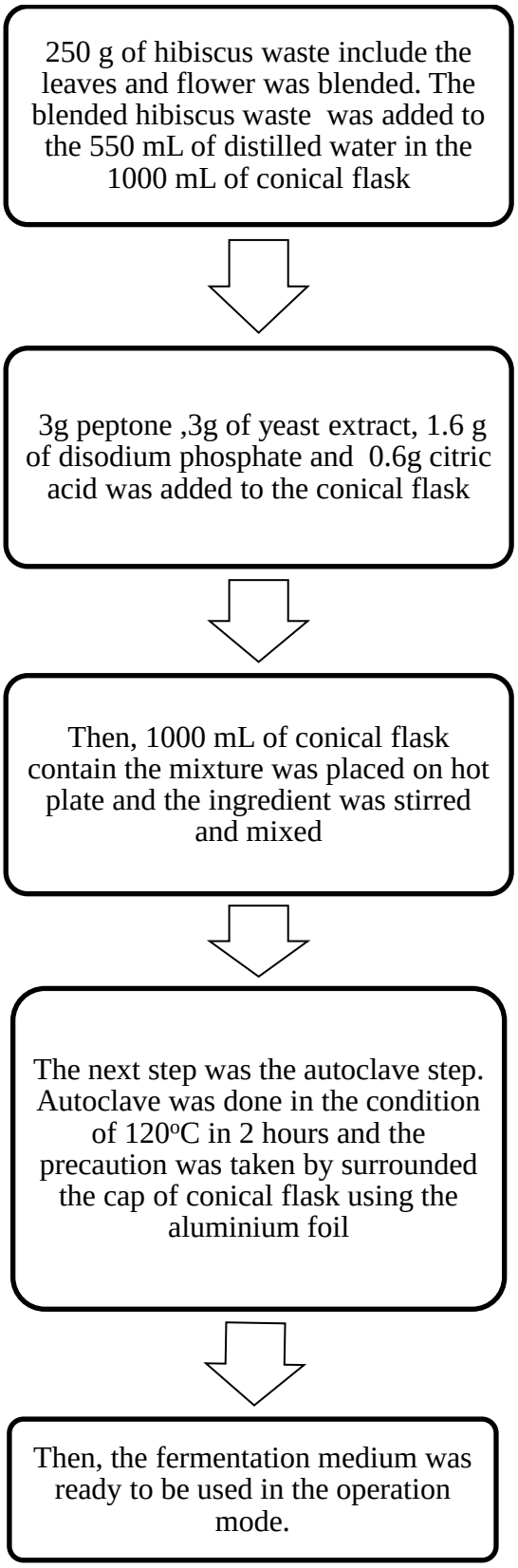

Figure 3.4.4: Preparation of hibiscus waste fermentation medium

3.5 EXPERIMENTAL PROCEDURE

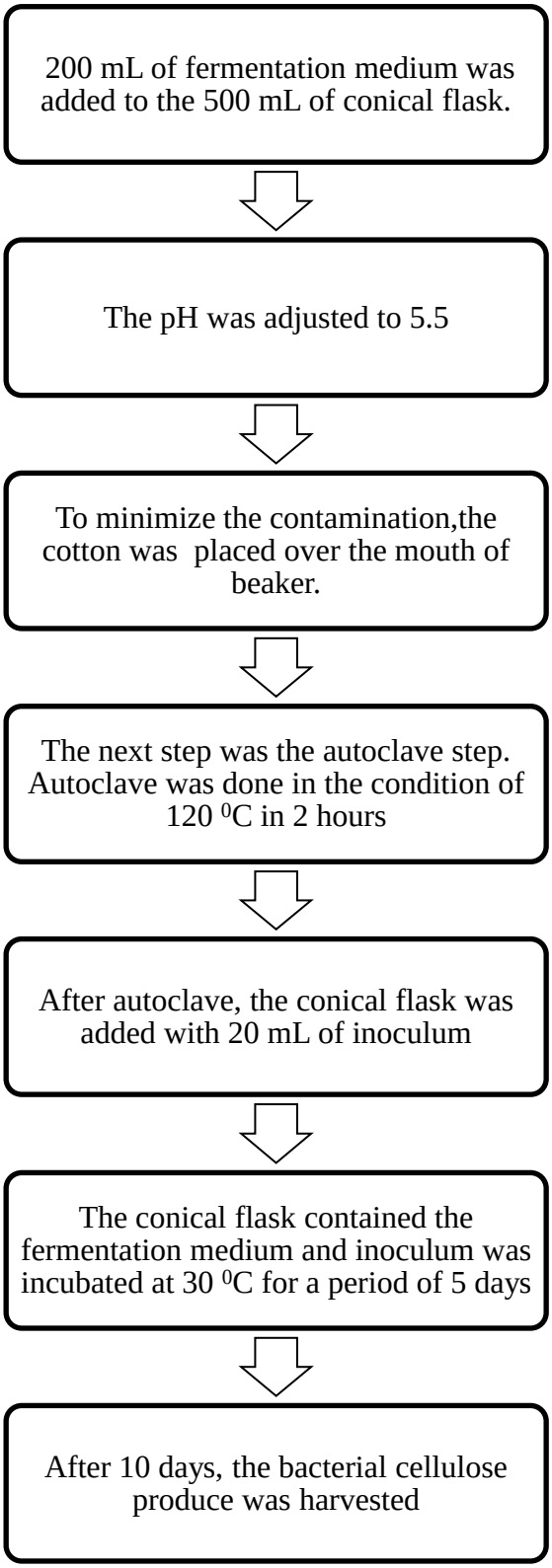

Figure 3.5.1: Production of bacterial cellulose in batch mode

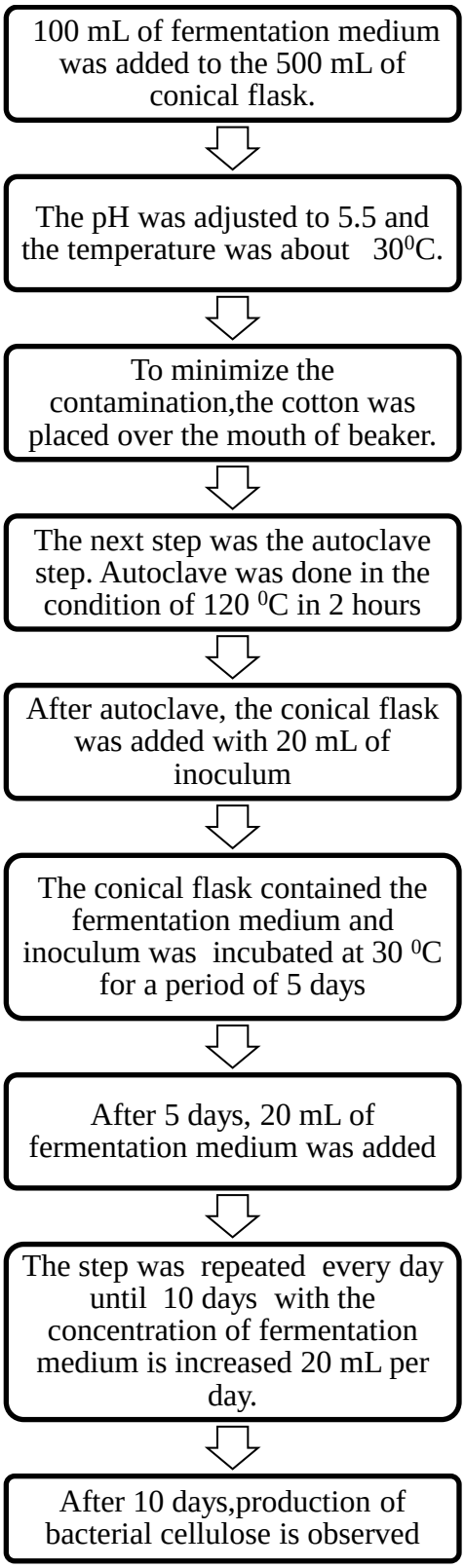

Figure 3.5.2: Production of bacterial cellulose in the fed-batch mode

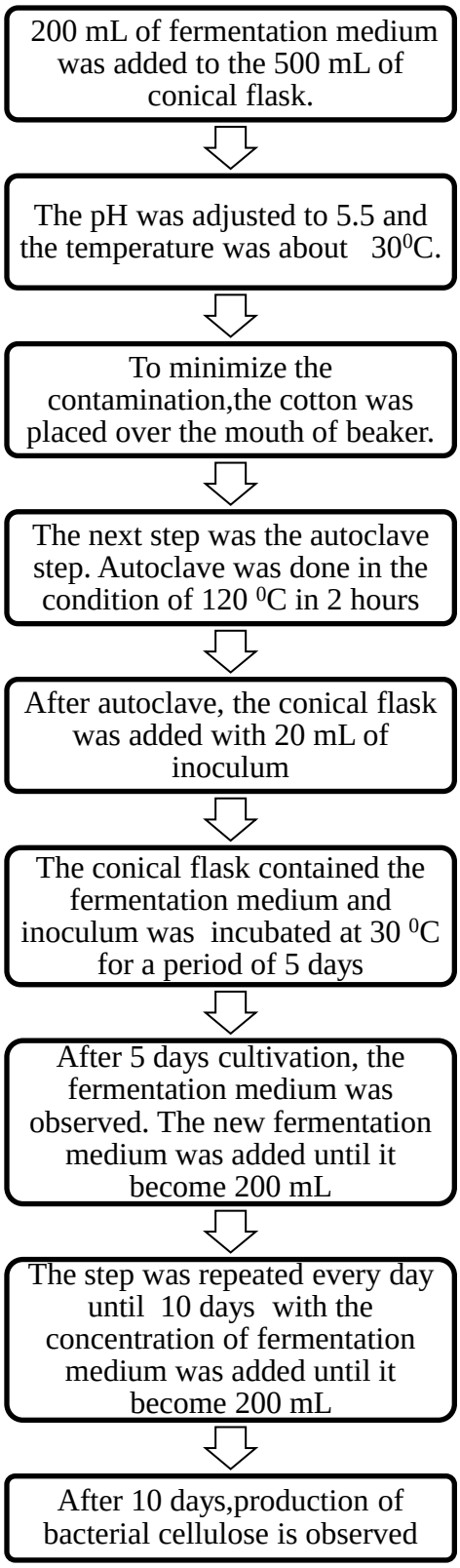

Figure 3.5.3: Production of bacterial cellulose in the repeated batch mode

3.6 MEASUREMENT AND ANALYSIS

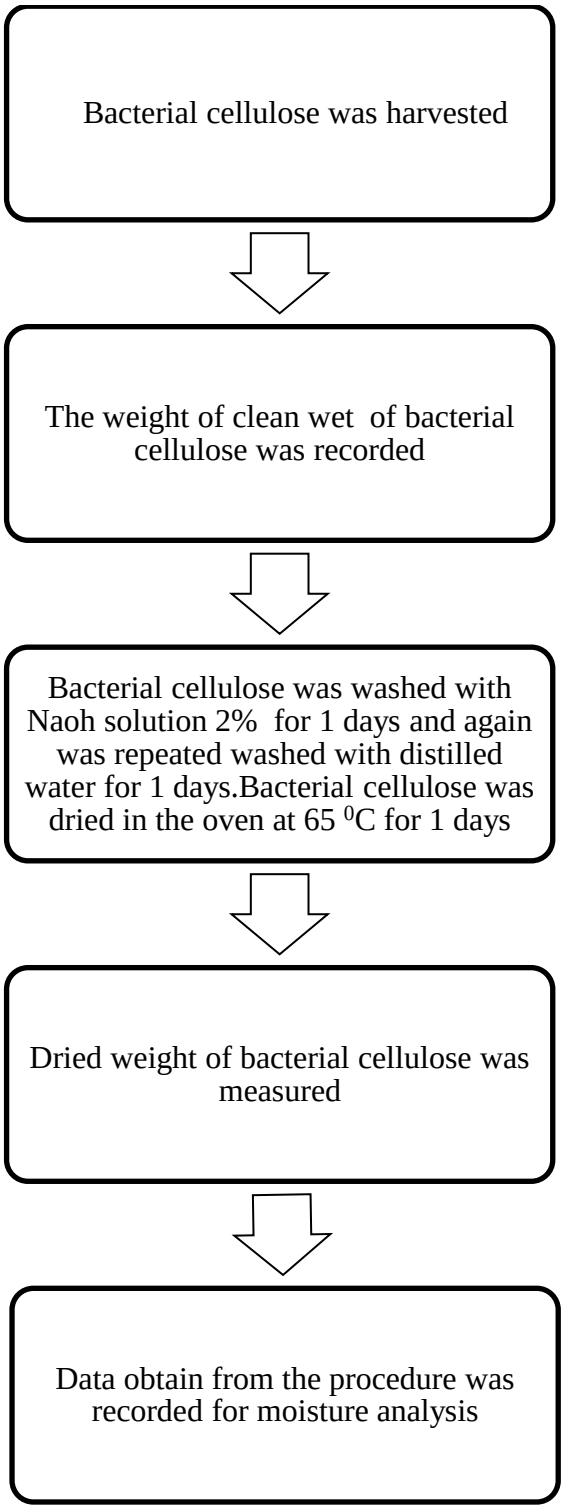

Figure 3.6.1: Analysis of moisture content in bacterial cellulose

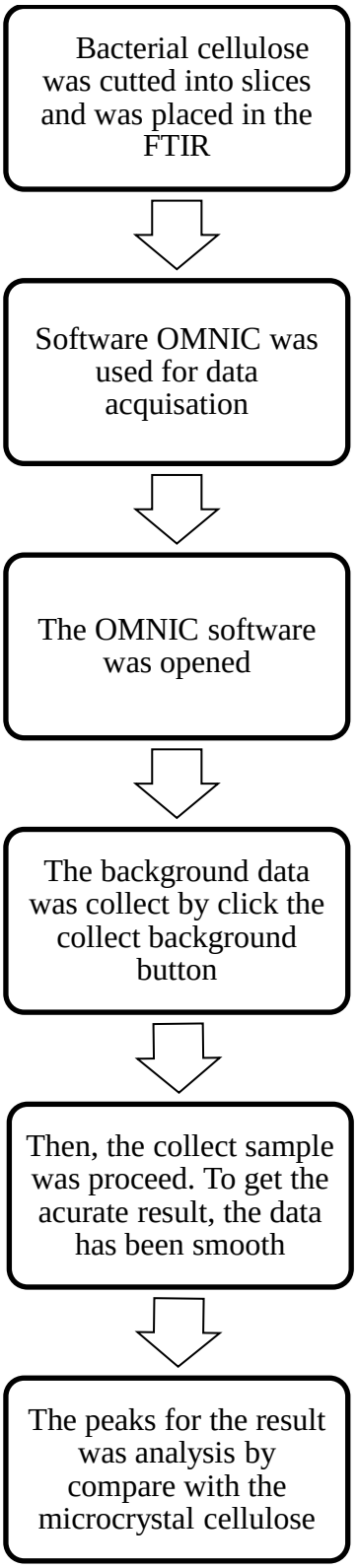

Figure 3.6.2: Analysis the characteristic of bacterial cellulose using the FTIR

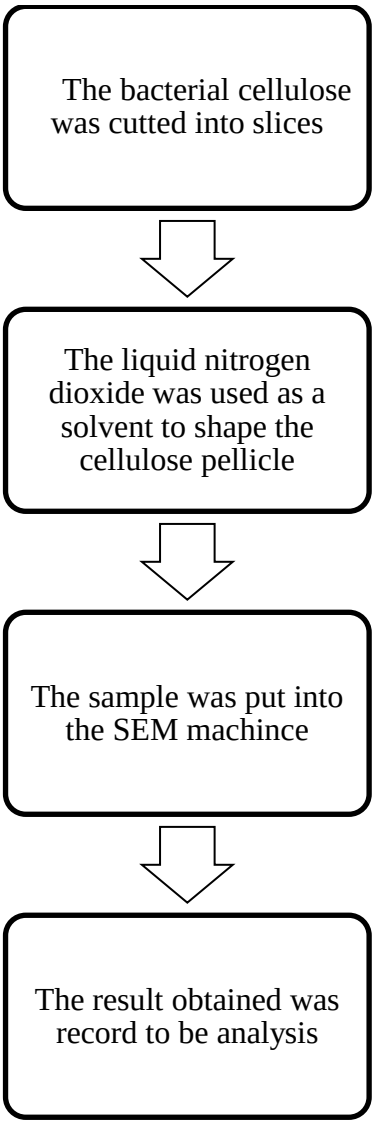

Figure 3.6.3: Analysis of morphology of bacterial cellulose using the SEM

CHAPTER 4

RESULT AND DISCUSSION

4.1 INTRODUCTION

After completing a few months of research period, the scheduled experiments were accomplished. Important data to be analyses was produced, such as mass of bacterial cellulose. Figure 4.1 below shows the obtained of bacterial cellulose membrane. In this study, the bacterial cellulose was produce in the static culture under different mode and different fermentation medium. The experiments were performed in 10 days at 30^0C under pH 5.5. The analysis of bacterial cellulose was done using the Scanning Electron Microscope and Fourier Transform Infrared Spectroscopy.

Figure 4.1: The membrane of bacterial cellulose obtained from the experiment

4.2 ANALYSIS OF MOISTURE CONTENT

Table 4.1: Wet and dry weight of bacterial cellulose in the Hestrin Schramm fermentation medium

Weight	Batch	Fed Batch	Repeated Batch
Wet weight of bacterial cellulose (g)	7.16	18.63	9.60
Dry weight of bacterial cellulose (g)	0.06	0.3925	0.186

Table 4.2: Wet and dry weight of bacterial cellulose in the old coconut water fermentation medium

Weight	Batch	Fed Batch	Repeated Batch
Wet weight of bacterial cellulose (g)	17.70	42.48	21.84
Dry weight of bacterial cellulose (g)	0.378	1.682	0.624

Table 4.3: Wet and dry weight of bacterial cellulose in the banana peel fermentation medium

Weight	Batch	Fed Batch	Repeated Batch
Wet weight of bacterial cellulose (g)	39.64	98.42	47.21
Dry weight of bacterial cellulose (g)	1.044	3.191	1.073

Table 4.4: Wet and dry weight of bacterial cellulose in the hibiscus waste fermentation medium

Weight	Batch	Fed Batch	Repeated Batch
Wet weight of bacterial cellulose (g)	26.66	40.48	37.20
Dry weight of bacterial cellulose (g)	0.485	1.205	0.523

Table 4.1 until 4.4 describes the wet weight and dry weight of bacterial cellulose that was produce after 10 days of fermentation. From the result, the wet weight for every bacterial cellulose was significantly high if compare to the dry weight. This is due to the water content in the bacterial cellulose. The water content will give the result to the moisture content. The moisture content of bacterial cellulose was determined by

$$\text{Moisture content} = \frac{Ww - Wd}{Ww} x\ 100 \qquad \text{(eq 4.1)}$$

*W_w: Wet weight of bacterial cellulose

*$W_{d:}$ Dry weight of bacterial cellulose

Table 4.5: Moisture content in a bacterial cellulose

Fermentation Medium	Batch	Fed Batch	Repeated Batch
Hestrin-Scharmm	91.48%	97.63%	98.06%
Old Coconut Water	97.86%	96.04%	97.14%
Banana Peel	97.36%	96.75%	97.22%
Hibiscus Waste	98.17%	97.02%	98.03%

Table 4.5 is a summary of moisture content that was calculate using the above equation. This result show the moisture content in bacterial cellulose is very high. For each parameter, the average moisture content is approximately 97%. This was due to the water holding capacity of bacterial cellulose that can hold until 100 times of their mass.

4.3 EFFECT OF FERMENTATION MEDIUM

The fermentation medium use in this experiment were the HS medium that act as a basis, old coconut water medium, banana peel medium and hibiscus waste medium. Each medium contain different chemical composition that can affect the production of bacterial cellulose.

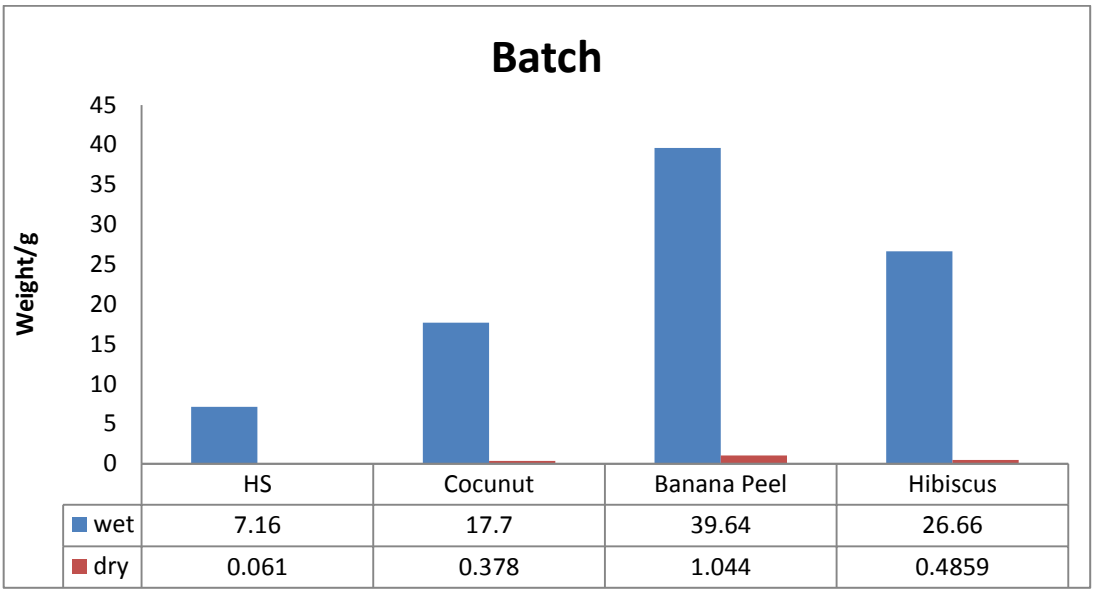

Figure 4.2: Graph effect of fermentation medium under batch mode

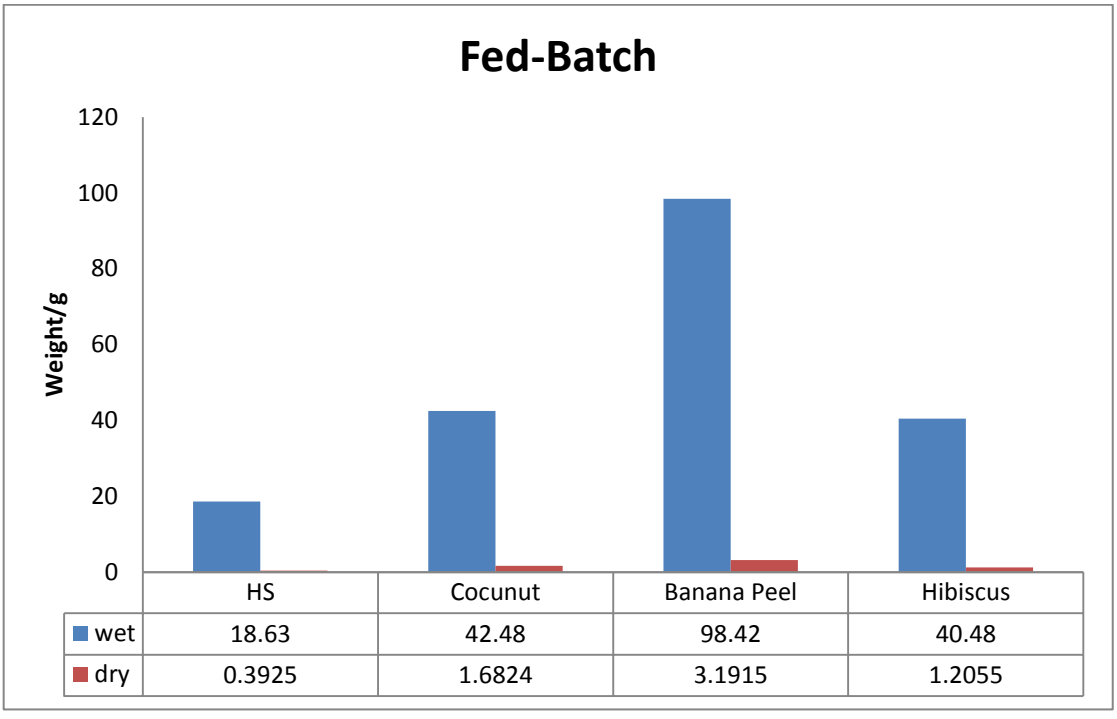

Figure 4.3: Graph effect of fermentation medium under fed batch mode

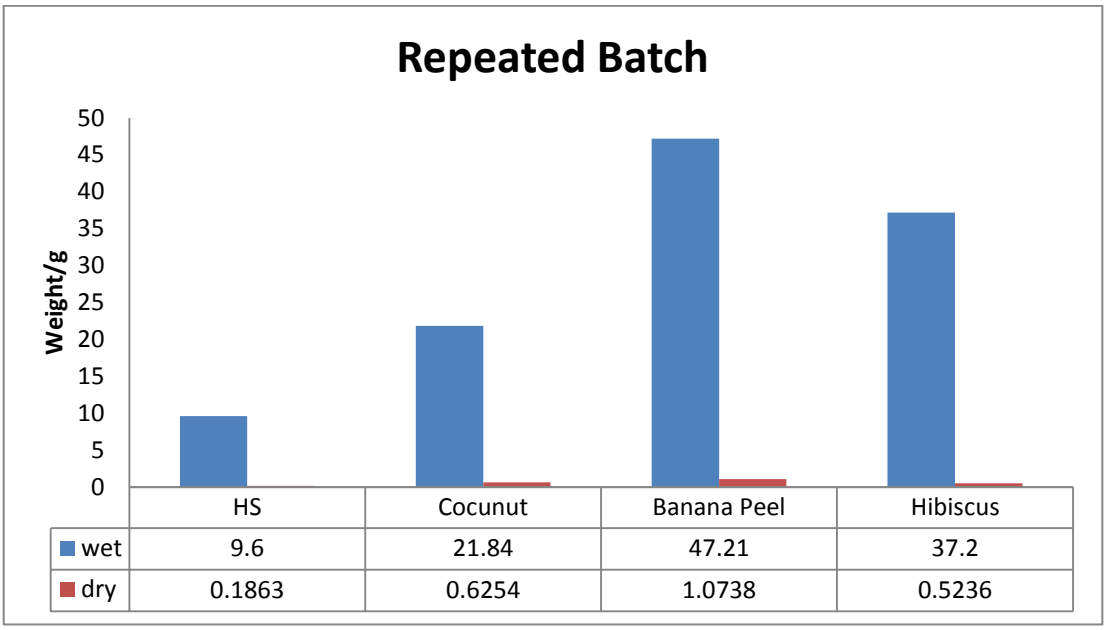

Figure 4.4: Graph effect of fermentation medium under repeated batch mode

The fermentation contains different chemical composition will affect the production of bacterial cellulose. From the result obtain, the production of bacterial cellulose was different from medium to medium. The highest yield of bacterial cellulose was produce in banana peel fermentation medium which was 98.42g for wet weight and 3.1915g for dry weight under the fed batch operation mode. Meanwhile the lowest yield of bacterial cellulose was produce under the Hestrin and Schramm fermentation medium which was 7.16g for wet weight and 0.061g for dry weight under the batch operation mode. In the old coconut water fermentation medium, the highest yield of bacterial cellulose produce was about 42.48g for wet weight and 1.6824g for dry weight under the fed batch operation mode. In addition, in the hibiscus waste fermentation medium, the highest yield of bacterial cellulose was obtained to be 40.48g for wet weight and 1.205g for dry weight under fed batch operation mode. The different production of bacterial cellulose in the fermentation medium is due to the chemical composition in each medium. Anhwange *et al.* (2009) report that the concentrations of glucose in the banana peel is about 59%, if compare to the report from Hestrin and Schramm, the glucose contain in the Hestrin

and Schramm medium is 2% meanwhile in the hibiscus waste medium, it consist of 38% of glucose and the coconut water consist of 15.2% of glucose content. This relation proved that, the chemical composition effects the production of bacterial cellulose. In this study, it also proved that the synthesis of bacterial cellulose needs more carbons sources to enhance the bacteria metabolism.

The highest number of carbon sources in the banana peel is contributing to high production of bacterial cellulose. According to the Frank (1995) the 'mother' culture of bacterial cellulose depends on the supply of a carbon sources as it cannot produce the cellulose in adequate quantities on its own. If compare to the lowest production of bacterial cellulose, the adequate of carbon sources is the cause for the low yield obtain from bacterial cellulose. The Hestrin and Schramm (1954) report the glucose contain in the Hestrin and Schramm medium is 2%. This amount is far different from the glucose in banana peel. This lead to the less yield production of bacterial cellulose as was obtained. It also noticed that the membrane layer of bacterial cellulose produces in this fermentation medium also very thin and white color in Hestrin Schramm fermentation medium.

4.4 EFFECT OF OPERATION MODE

The operation mode in this study was divided into three categories which are batch, fed batch and repeated batch. These three categories still can be considered as a static culture because there are no involve the agitation and shaking condition. Each of operation modes will produce different amount of bacterial cellulose. From the result obtain, the highest production was in fed-batch mode. About 98.42g yield of bacterial cellulose was produce under banana peel fermentation medium. The study also showed the lowest production of bacterial cellulose was obtained under batch operation mode using the Hestrin Schramm fermentation medium. The Figure 4.5 to 4.7 is a summary of the bacterial cellulose that was produce in the operation mode condition. This result significantly shows the highest production was in the fed- batch operation mode.

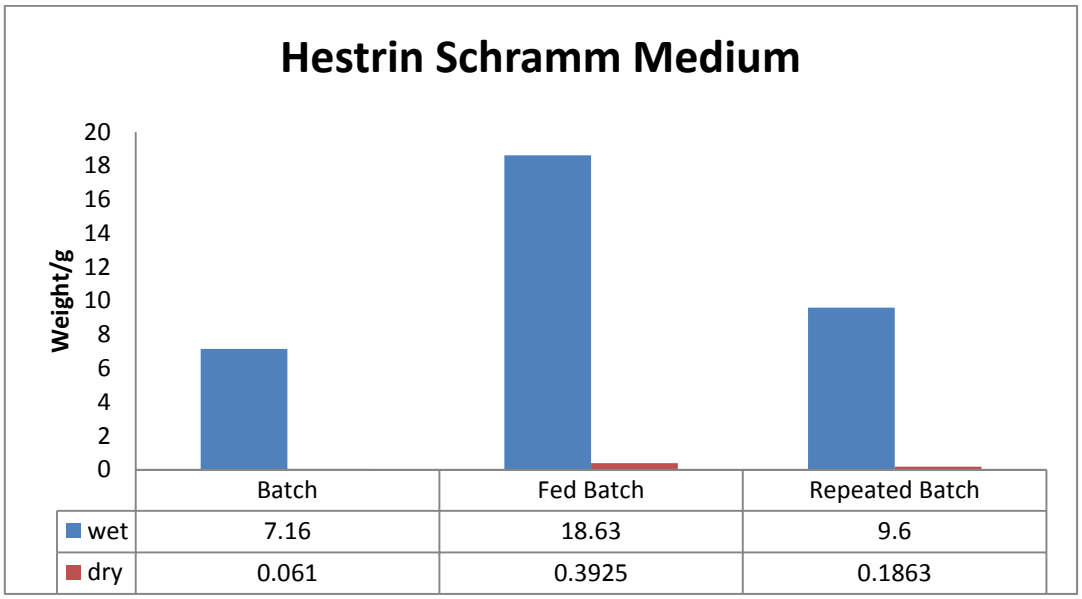

Figure 4.5: Graph effect of operation mode under Hestrin Schramm medium

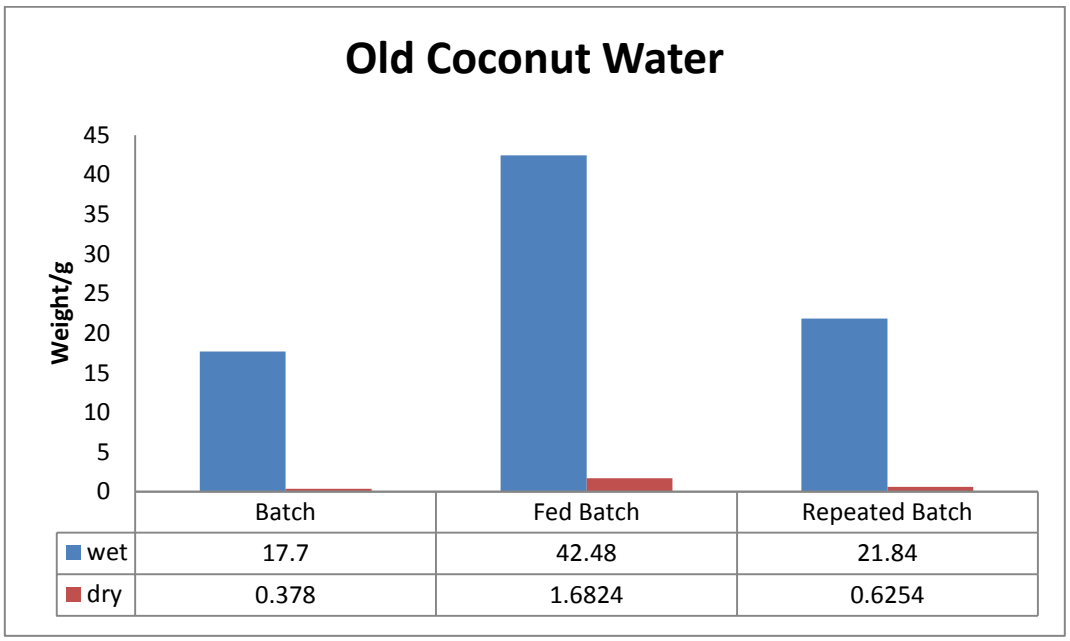

Figure 4.6: Graph effect of operation mode under old coconut water fermentation medium

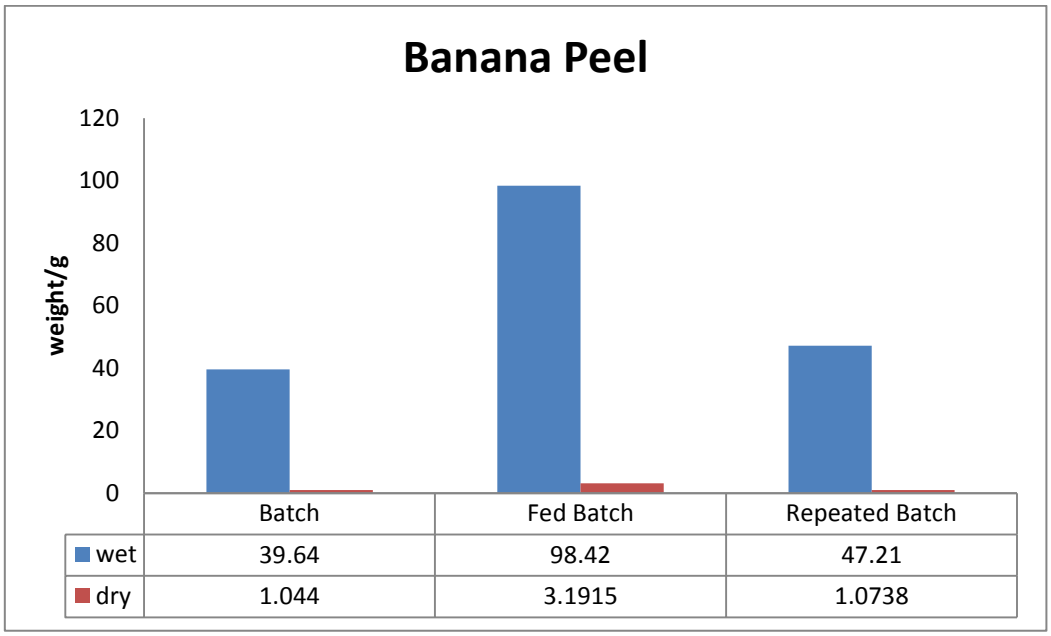

Figure 4.7: Graph effect of operation mode under banana peel fermentation medium

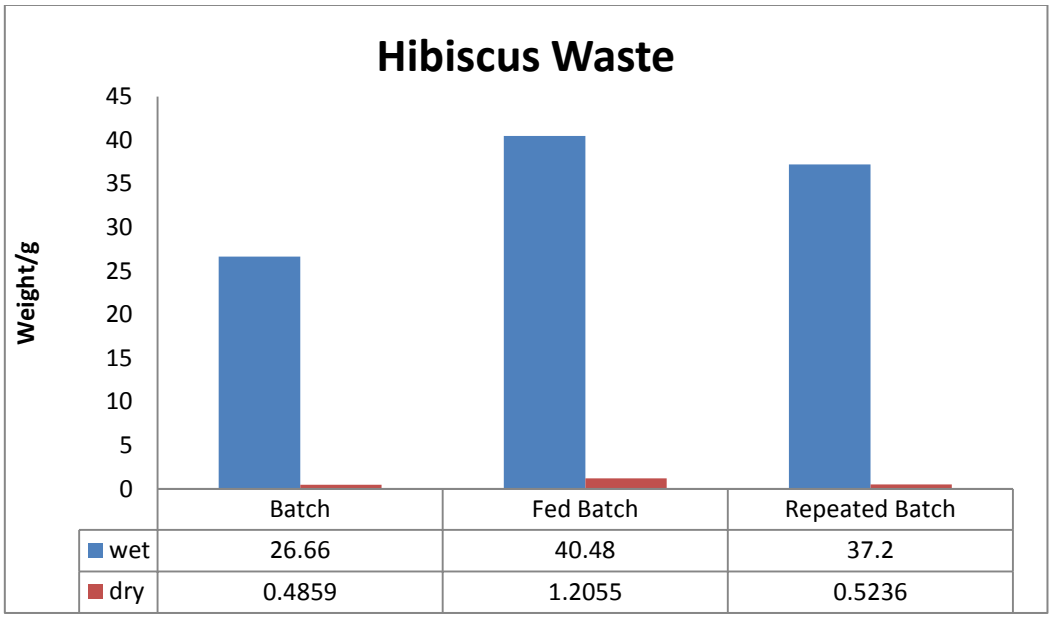

Figure 4.8: Graph effect of operation mode under banana peel fermentation medium

From the data observation, the batch mode production was very low if compare to the fed-batch and repeated batch mode. In the fed-batch mode fermentation, the controlling of nutrient supply reduces the substrate – associated growth inhibition. Thus, the bacterial cellulose was produced in the high amount. The cultivations method using the fed-batch fermentation was recommended by several researchers. The advantage of using the fed batch mode is reducing the cost and this method allows the production of desired product to high concentration with high productivity and yield. During the fed-batch cultivation, one or more nutrients are supplied to the fermenter while cells and products remain in the fermenter until the end of operation. In the batch mode, the nutrients are not supply until the end of fermentation. This will contribute to the substrate – associated growth inhibition. The formation of bacterial cellulose layer above the culture medium also one of the factor that decrease the production of bacterial cellulose. In addition, the repeated batch produce average amount of bacterial cellulose. There is the different between the fed-batch and repeated batch operation mode. The quantity of culture medium supply is different between the fed batch mode and the repeated batch. In the repeated batch mode, after 5 days of cultivation the volume culture medium was not inconsistently added but in the fed batch, the volume of culture medium was added consistently to the fix amount. Thus, this factor induced the decrease of bacterial cellulose production in repeated batch if compare to the fed batch. As a conclusion, fed batch is better operation mode in the static culture.

4.5 ANALYSIS OF BACTERIAL CELLULOSE CHARACTERISTICS

FTIR analysis was performed to investigate the characteristic of bacterial cellulose produce. This is very important to ensure the bacterial cellulose produce exhibit the characteristics similarly to the microcrystalline cellulose.

Microcrystalline cellulose is basically cellulose that derived from high quality wood pulp (Rosma *et al*., 2003). Microcrystalline cellulose can only be extract from a special grade of alpha cellulose. It consists of a very high grade of

cellulose characteristics. In addition, the FTIR analysis result was compare with the MCC as a reference.

In this study, the chemical nature of bacterial cellulose produce was confirmed by the infrared spectra of the MCC in Figure 2.5. Figure 4.9 to 4.12 shows FTIR spectra of bacterial cellulose produce in this research. In general, both the spectra of MCC and bacterial cellulose possess a similar trend. Absorbance spectra for microbial cellulose and MCC in the region 4000-450 cm^{-1}. The characteristics region of anomeric carbons (960 – 730 cm^{-1}) was identified for each of bacterial cellulose produce .A band at 891.59 cm^{-1} confirms the presence of β, 1-4 linkages. There is a good correspond between the bacterial cellulose and the MCC. The only difference between bacterial cellulose and the MCC was restricted to the intensity of some bands.

According to the Nelson and Connor, (1964), a weak and broad band centered at 891.59 cm^{-1} and a strong band centered at 1424.18 cm^{-1}. From the comparison of the bacterial cellulose produce in different fermentation, the result obtain show the very similar spectra band was obtain under the fermentation in the old coconut water fermentation medium. In addition, it possesses a degree of crystallinity under the band at 1489 cm^{-1}.

Measurements of crystallinity of cellulose materials was performed by means of infrared crystallinity ratio (CR), given by the ratio of absorptivity at 1372 cm^{-1} (C-H bending) to that at 2900cm^{-1} (CH_2 and CH stretching). From the result obtained in the FTIR analysis most of the bacterial cellulose exhibit all spectra band for the crystallinity characteristics. Bacterial cellulose produce in the old coconut possess a spectra band 1398.52 cm^{-1} and 2940.46 cm^{-1} meanwhile in the fermentation under the Hestrin Schramm , there is no spectra band that possess the means of infrared crystallinity. In the hibiscus waste fermentation medium, the result obtained show the similar number of wavelength was determined at 1329.10 cm^{-1} and 2902.74 cm^{-1}. The fermentation of bacterial cellulose under the banana

peel has a similar to the crystallinity band which is 1324 cm^{-1} and 2841cm^{-1}. From these data, it appears that the bacterial cellulose exhibits a very high degree of crystallinity, comparable to that MCC.

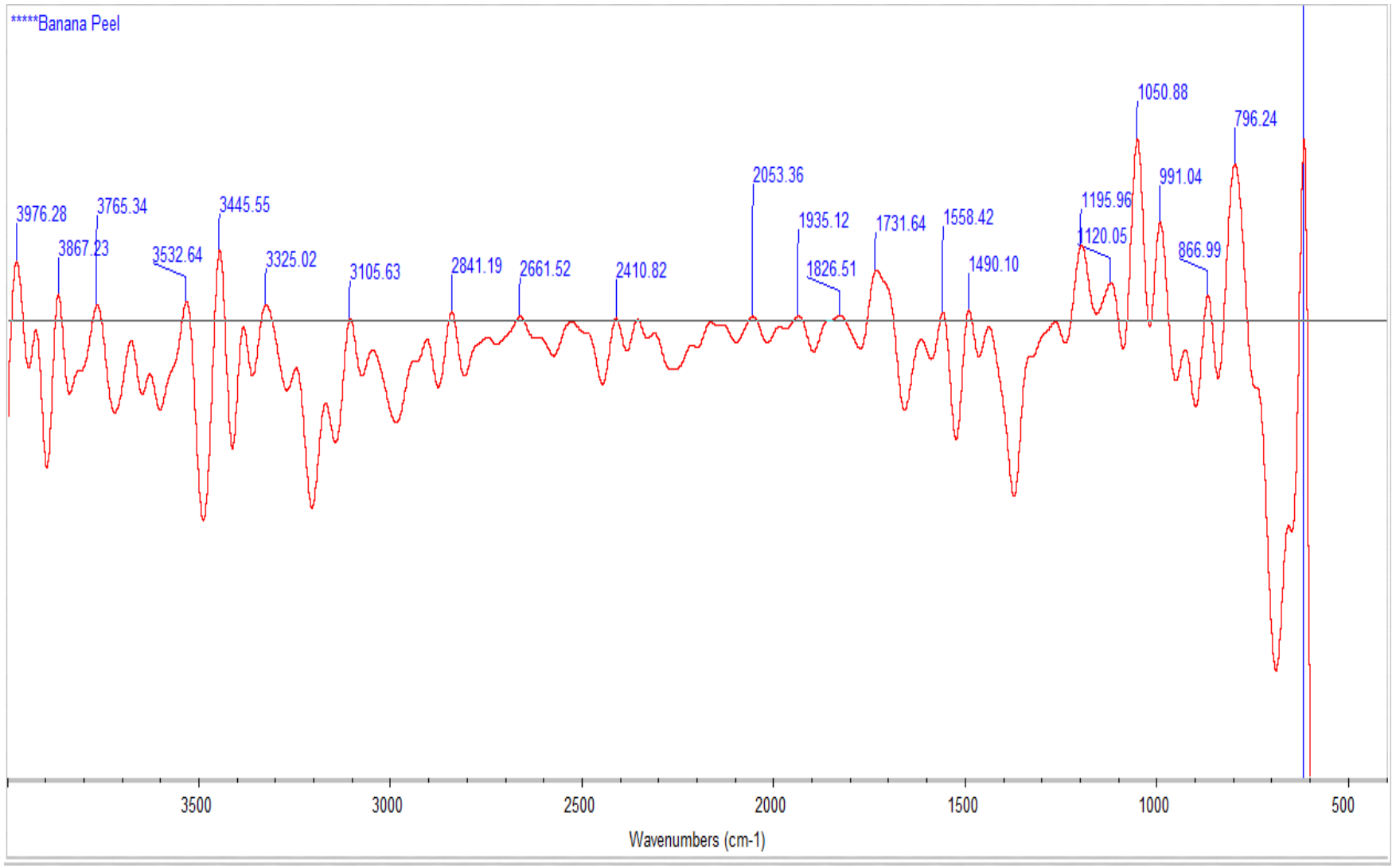

Figure 4.9: FTIR analysis in banana peel fermentation medium

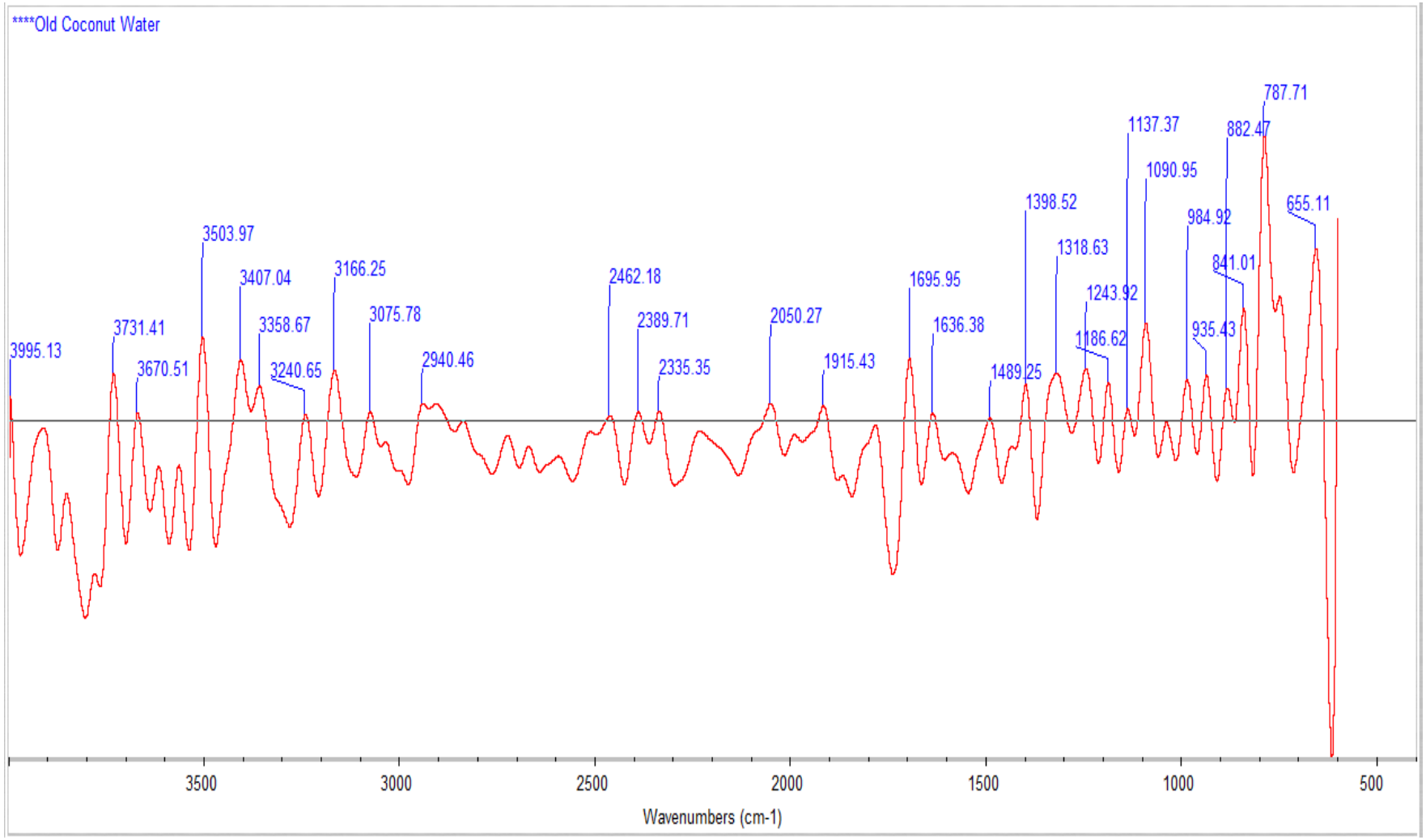

Figure 4.10: FTIR analysis in the old coconut water fermentation medium

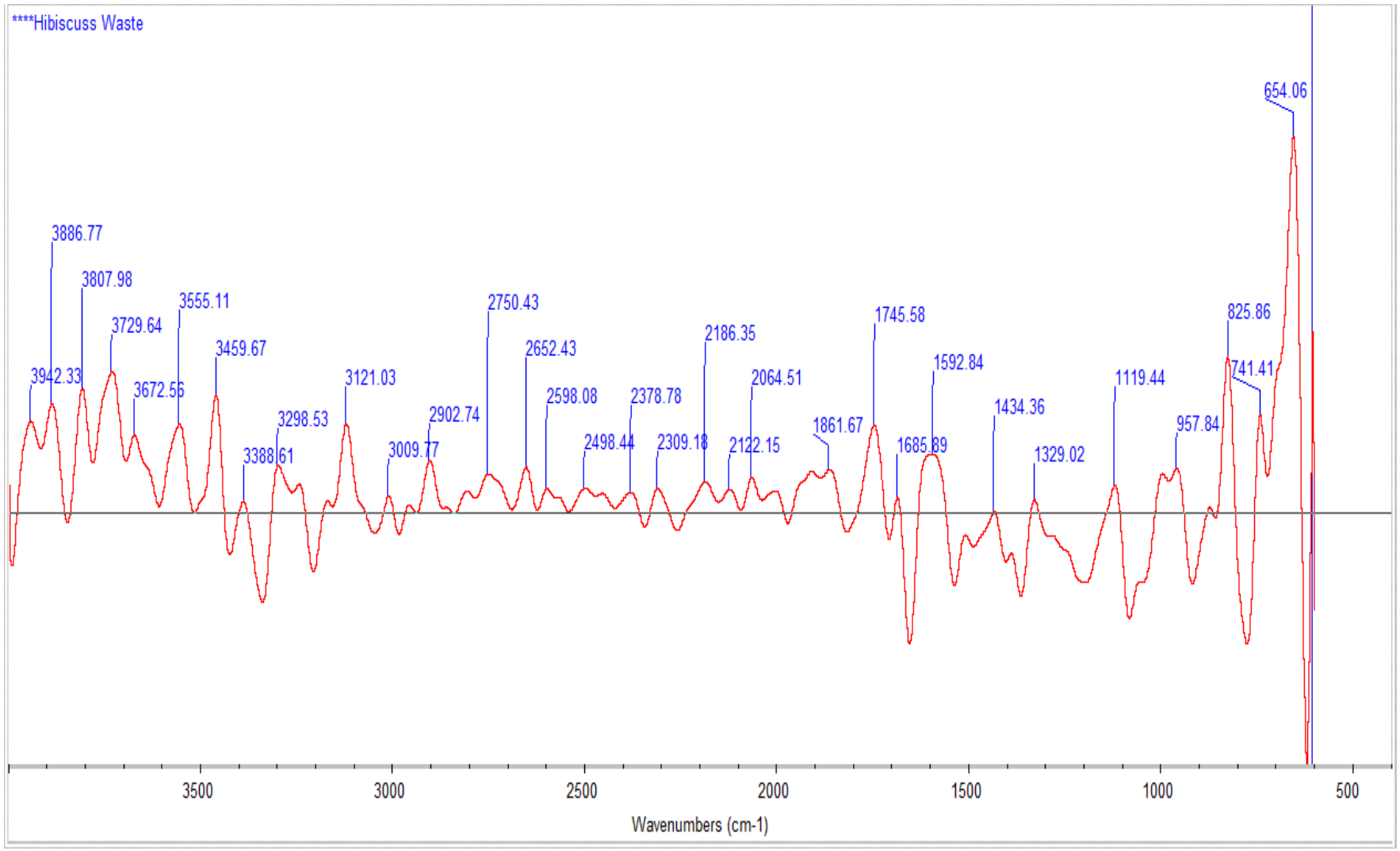

Figure 4.11: FTIR analysis in the hibiscus waste fermentation medium

Figure 4.12: FTIR analysis in the Hestrin Schramm fermentation medium

4.6 ANALYSIS THE MORPHOLOGY OF BACTERIAL CELLULOSE

Figure 2.6 shows the scanning electron micrograph of MCC which is characterized by an ultrafine network structure and the bacterial cellulose layer, the connection by a compact cellulose network structure which is on par with the observation of (Klemm *et al*., 2001). According to the Iguchi *et al.* (2000), the cellulose pellicle exhibits random assembly microfibrils of less than 100 A^0 in diameter.

MCC pose a very different structure. The primary particles forming the MCC exist in a bigger size (Kothari *et al*., 2002) .If compare with the bacterial cellulose the surface of bacterial cellulose is more dense and the surface are not smooth. The Figure 4.14 describes the surface of bacterial cellulose under fed-batch mode in banana peel fermentation medium. Under this observation, the particle of the cellulose shows the less number of ribbons under magnification of 1000x and 300x. It was reported by Yamanaka *et al*. (1989) at 10000x magnification, the ribbons structure will appear as crossed, superimposed layers of cellulose ribbons that are randomly oriented (Ben-Hayyim and Ohad, 1965)

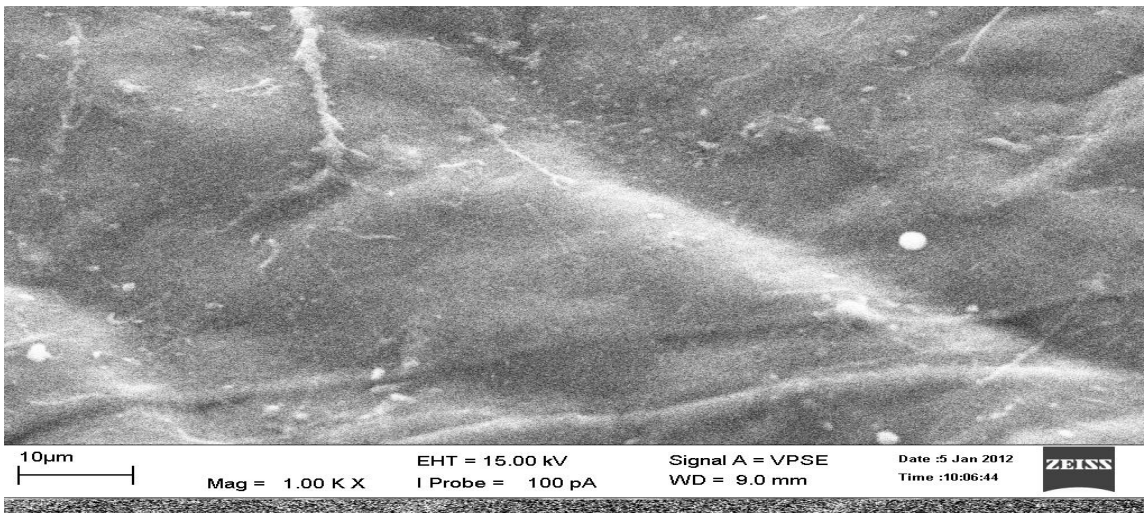

Figure 4.13: Surface of bacterial cellulose under 1000x magnification

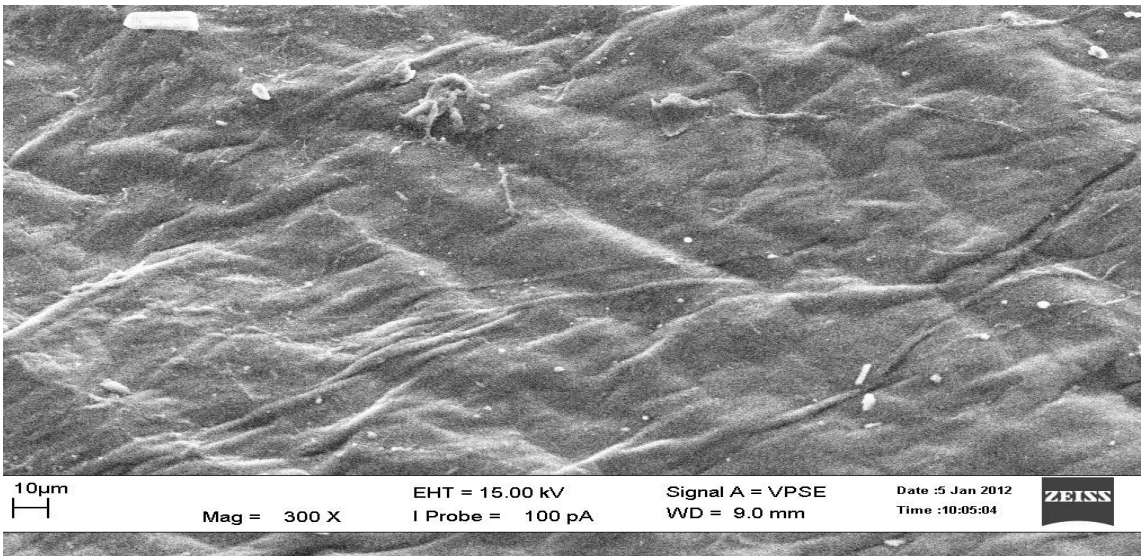

Figure 4.14: Surface of bacterial cellulose under 300 x magnifications

CHAPTER 5

CONCLUSION AND RECOMENDATION

5.1 CONCLUSION

The different medium use for fermentation of the bacterial cellulose can give the effect to the production of the yield. It was proven from the experiment that the different medium will produce different amount of the bacterial cellulose. The significant change is between the fermentation medium in banana peel and Hestrin Schramm fermentation medium. Banana peel produces the highest yield of bacterial cellulose which is 3.1915g for dry cellulose meanwhile the production of bacterial cellulose in the Hestrin Schramm was the lowest which is 0.0610g. In addition, the study of operation mode in bacterial cellulose fermentation also had shown an impact to the production of cellulose yield. Through the experiment, the fed-batch was determined as a better operation mode if compare to the other mode. For each of fermentation using the fed- batch mode, the production of bacterial cellulose will produce in high concentration. This has been proved that fed-batch is the better operation mode. Analysis of bacterial cellulose produces using the FTIR shown that, the bacterial cellulose appear to have similar characteristics with the MCC. They are very good in crystallinity and some of the bacterial cellulose possesses a spectra band that is very similar to the MCC.

5.2 RECOMMENDATIONS

In this research, there are many types of recommendations can be suggested. In order to obtain such a good results in the near future, first is regarding the strength of the cellulose. In future, the strength for every cellulose produce in each fermentation medium should be investigate. The strength of cellulose is different for each medium of fermentation. For the fermentation medium, it should be done properly by study the carbon sources in each medium such as sucrose, glucose, fructose and the ratio for each carbon sources that contribute to the high production of bacterial cellulose. Besides that, the fermentation medium that use in the project should be fairly compares. For example use the fresh banana peel, rotten banana peel or related to that to study the effect of different fermentation medium. In addition, it is necessary to maintain the pH of the medium to the optimum pH by checking the pH in a schedule time. A sudden reduction of pH in the medium will contribute to the less production of cellulose.

On industrial point of view, there is a need to find a suitable method using fed batch operation mode to produce a high yield of bacterial cellulose. This will help to reduce the cost of operation and lead to maximize the production. It also suggests adding some precursor to enhance and stimulate the bacteria to produce more cellulose.

REFERENCES

Anhwange, B. A, T.J. Ugye, T.D Nyiaatagher, 2009, Chemical composition of *Musa Sapientum* (Banana peel), EJEAFChe, volume 8, 2009, pp 437-442

A.Ismail, E.K.I Hainida, H.M.N Saadiah, (2008), Roselle (*Hibiscuss roselle sinesis L*.) Nutrional composition, Protein Quality and Health Benefits, 2008

A.R. White, R.M. Brown Jr., (1981), Enzymatic hydrolysis of cellulose: Visual characterization of the process, Proc. Natl. Acad. Sci. USA, 78, 1981, pp 1047-1051

A.B. Shirai, M. Takahashi, H. Kaneko, S. Nishimura, M. Ogawa, N Nishi, S. Tokura, (1994), Biosynthesis of novel polysaccharide by *Acetobacter Xylinum*, Int. J. Biol. Macromol. Volume 16, 1994, pp 297-300

C.C. Ng, Y.T Shyu, (2004), Development and production of cholesterol-lowering, Monascus-nata complex, World J. Microbiol. Volume 20 (2004) pp 875-879

D.C. Johnson, A.N. Neogi, (1989), Sheeted products formed from reticulated microbial cellulose. US patent 4863565, 1989

D.P. Delmer, Y. Amor, (1995), Cellulose biosynthesis, Plant Cell, Volume 7, 1995, pp 987-1000

Goh, W.N., Rosma, A. Kaur, B., Fazilah, A., Karim A.A. and Bhat, R. , (2011), Fermentation of black tea broth: Effect of sucrose concentration and fermentation time on the yield of microbial cellulose, International Food Research Journal, 2011

H. Backdahl, G. Helenius, A. Bodin, U. Nannmark, B.R. Johansson, B. Risberg, P. Gatenholm, (2006), Mechanical Properties of bacterial cellulose and interactions with smooth muscle cells, Biomaterials, Volume 27, 2006, pp 2141-2149

H.J Son, M.S Heo, Y.G Kim, S.J Lee,(2001), Optimization of fermentation conditions for the production of bacterial cellulose by newly isolated *Acetobacter sp.* A9 in shaking cultures, Bioethanol. Appl. Biochem. Volume 33, 2001, pp 1-5

J.A Westland, G.S. Penny, R.S Stephens, A.R Winslow, (1994), Method of supporting fractures in geologic formations and hydraulic fluid composition for same. US patent 5350528, 1994

K. Toda, T. Asakura, M. Fukaya, E. Entani, Y. Kawamura,(1997), Cellulose production by acetic acid resistant *Acetobacter Xylinum*, J. Ferment. Bioeng. Volume 84, 1997, pp 228-231

K.V. Ramana, A. Tomar, L. Singh, (2000), Effect of various carbon and nitrogen sources on cellulose synthesis by *Acetobacter Xylinum*, World J. Microbial. Biotechnol. Volume 16, 2000, pp 245-248

L.F.X Farah, (1990), Process of the preparation of cellulose film, cellulose film produced thereby, artificial skin graft and its use, US patent, 1990

M. Iguchi, S. Yamanaka, A. Budhiono, (2000), Bacterial cellulose a masterpiece of nature's arts, J. Mater. Sci. Volume 35, 2000, pp 261-270

M. Ishihara, M. Matsunaga, N. Hayashi, V. Tisler, (2002), Utilization of xylose as carbon sources for production of bacterial cellulose, Enzyme Microb. Technol. Volume 31, 2002, pp 986-991

M. Matsuoko, T. Tsuchida, K. Matsushita, O. Adachi, F. Yoshinaga,(1996), A synthetic medium for bacterial cellulose production by *Acetobacter Xylinum* subsp. sucrofermentants, Biosci. Bioethanol. Biochem. Volume 60, 1996, pp 575- 579

N. Noro, Y. Sugano, M. Shoda, (2004), Utilization of the buffering capacity of corn steep liquor in bacterial cellulose production by *Acetobacter Xylinum*, Appl. Microbiol. Biotechnol. Volume 64, 2004, pp 199-205

P. Wanichapichart, S. Kaewnopparat, K. Buaking, W. Puthai, (2002), Characterization of cellulose membranes produced by *Acetobacter Xylinum*, J. Sci. Technol. Volume 24, 2002, pp 855-862

R.M Brown, C.H. Haigler, J. Suttie, A.R.White, E. Roberts, C. Smith, T. Itoh, K. Cooper, (1983), The biosynthesis and degradation of cellulose, J. Appl. Polym. Sci. Volume 37, 1983, pp 33-78

R.M Brown Jr., (1987), The biosynthesis of cellulose, Food Hydrocolloids , Volume 1, 1987, pp 345-351

S. Hestrin, M. Schramm, (1954), Synthesis of cellulose by *Acetobacter Xylinum*: II. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose, Biochem. J. Volume 58, 1954, pp 345-352

S. Kongruang, (2008), Bacterial cellulose production by *Acetobacter Xylinum* strains from agricultural waste product, Appl. Biochem. Biotechnol. Volume 148, 2008, pp 245-256

Sangok Bae, Makoto Shoda, (2004), Bacterial cellulose production in a fed batch mode, Appl. Biochem. Biotechnol. Volume 200, 2004, pp 332-340

Santoso, U. Kubo, K. Ota, T. Tadokoro, T.Maekawa, (1996), A nutrient composition of kopyor coconuts (*Cocus nucifera L.*). Food Chem, 1996, volume 57, pp 299-304

T. Yoshino, T. Asakura, K. Toda, (1996), Cellulose production by *Acetobacter Pasteurianus* on silicone membrane, J. Ferment. Bioeng. Volume 81, 1996, pp 32-36

U. Geyer, T. Heinze, A. Stein, D. Klemm, S. Marsch, D. Schumann, H.P. Schmauder, (1994), Formation derivatization and applications of bacterial cellulose, Int. J. Biol. Macromol. Volume 16, 1994, pp 343-347

Z. Gromet-Elhanan, S. Hestrin,(1963), Synthesis of cellulose by *Acetobacter Xylinum*. VI. Growth on citric acid-cycle intermediates, J.Bacteriol. Volume 85, 1963, pp 284-292

APPENDIX A

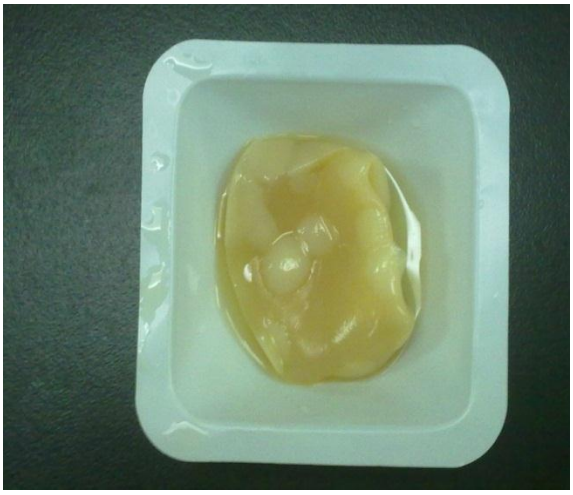

Figure A.1: Membrane of bacterial cellulose

Figure A.2: Fermentation medium and inoculum preparation