EFFECT OF DIFFERENT ADDITIVES ON THE PRODUCTION OF BACTERIAL CELLULOSE FROM PINEAPPLE WASTE

MUHAMMAD FUAD FIKRI BIN AMIR

FACULTY OF CHEMICAL & NATURAL RESOURCES ENGINEERING

UNIVERSITI MALAYSIA PAHANG

EFFECT OF DIFFERENT ADDITIVES ON THE PRODUCTION OF BACTERIAL CELLULOSE FROM PINEAPPLE WASTE

MUHAMMAD FUAD FIKRI BIN AMIR

A thesis submitted in partial fulfillment
of the requirements for the award of the Degree of
Bachelor of Chemical Engineering (Biotechnology)

FACULTY OF CHEMICAL & NATURAL RESOURCES ENGINEERING

UNIVERSITI MALAYSIA PAHANG

FEBRUARY 2013

I declare that I have read this thesis and in my opinion this thesis is adequate in terms of scope and quality for the purpose awarding a Bachelor's Degree of Chemical Engineering (Biotechnology)."

Signature :…….....

Supervisor's Name : Junaidi bin Zakaria

Date :

I declare that this thesis entitiled – "EFFECT OF DIFFERENT ADDITIVES ON PRODUCTION OF BACTERIAL CELLULOSE FROM PINEAPPLE WASTE" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree

Signature :……..

Name : Muhammad Fuad Fikri bin Amir

Date :

Dedicated, in thankful appreciation for support, encouregemnt, and understanding to my beloved family, friends, and my supervisor. May Allah s.w.t bless our life.

ACKNOWLEDGEMENT

First and foremost, I would like to express my sincere appreciation to my supervisor, Mr Junaidi bin Zakaria, for constantly guiding and encouraging me throughout this study. Thanks a lot for giving me a professional training, advice and suggestion to bring this project to its final form. I am very thankful to the staff of Chemical & Natural Resources Engineering Faculty, Universiti Malaysia Pahang for their guiding and cheerfulness and professionalism in handling their work. In preparing this project, I was in contact with many people, researches, academicians and practitioners. They have contributed towards my understanding and thoughts.

In particular, my sincere thankful is also extends to all my colleagues and others who have provided assistance at various occasions. Their views and tips are useful indeed. Unfortunately, it is not possible to list all of them in this limited space. And last, but not least a special thanks to my parents and other family members for their continuous support while completing this project.

TABLE OF CONTENT

APPENDICES

EFFECT OF DIFFERENT ADDITIVES ON THE PRODUCTION OF BACTERIAL CELLULOSE FROM PINEAPPLE WASTE

ABSTRACT

The pineapple peel wastes contain high concentration of biodegradable organic material such as carbohydrate that can be utilized for the production of organic acid. With the goal of being economically competitive and overcome the problem of disposal of the waste, pineapple waste can be potentially used to enhance the production of bacterial cellulose. Bacterial cellulose is known as polysaccharide and usually it been used traditionally in food industry and the latest it is used as a material for medical application. In this research, bacterial cellulose was produced by *Acetobacter xylinum* from pineapple waste as a fermentation medium with three different types of additives that is sodium alginate, microcrystalline cellulose and carboxymethylcellulose. Hence, the purpose of this experiment is to investigate the effect of three different additives on the production of bacterial cellulose by *Acetobacter xylinum*. Generally, there are three major parts in completing the research on production of bacterial cellulose by *Acetobacter xylinum*. The first part is preparation of inoculum by *Acetobacter xylinum* strain. The inoculum of *Acetobacter xylinum* is mother culture where later on will be used for the next fermentation process for the synthesis of bacterial cellulose. In the second part, the experiment was proceed for bacterial cellulose synthesis. Firstly, the inoculum of *Acetobacter xylinum* was prepared in the HS-medium. After obtaining the optimum yield of bacterial celullose production with different parameter of pH medium, three different additives were used at various concentrations to enhanced bacterial cellulose production. All the medium fermentation was incubated 30°C for 3 days. The optimum pH value was determined by the weight of bacterial cellulose, where the medium fermentations were incubated for three days. The production bacterial cellulose was relatively high with similar properties to that produced in HS medium. Wet weight of bacterial cellulose production by *Acetobacter xylinum* without microcrystalline cellulose was 0.1097g, while bacterial cellulose production was the highest, 0.589 g, at 0.2% MCC. Besides that, the results show that these samples consist of ultrafine fibrils, which form the reticulated structure. The additions of additives into the medium does not effect the structure of the cellulose. These results

suggest that it is possible to produce bacterial cellulose from low-cost resources in order to increase its production to a larger scale.

KESAN ADITIF YANG BERLAINAN TERHADAP PENGHASILAN BAKTERIA SELULOSA DARIPADA SISA NANAS

ABSTRAK

Sisa nanas mengandungi kepekatan tinggi bahan organik terbiodegradasi seperti karbohidrat yang boleh digunakan untuk penghasilan asid organik. Berlandaskan matlamat untuk menjadi ekonomi yang lebih berdaya saing selain mengatasi masalah pelupusan sisa-sisa nanas yang berpotensi digunakan untuk meningkatkan pengeluaran selulosa bakteria. Selulosa bakteria dikenali sebagai polisakarida dan biasanya telah digunakan secara tradisional dalam industri makanan dan yang terbaru ianya digunakan sebagai bahan aplikasi perubatan. Dalam kajian ini, selulosa bakteria telah dihasilkan oleh *Acetobacter xylinum* dengan menggunakan sisa nanas sebagai medium penapaian dengan tiga jenis aditif iaitu alginat natrium, microcrystalline cellulose dan carboxymethycellulose. Justeru itu, tujuan eksperimen ini adalah untuk mengkaji kesan tiga bahan aditif yang berbeza kepada penghasilan selulosa bakteria oleh *Acetobacter xylinum*. Secara umumnya, terdapat tiga bahagian utama dalam menyempurnakan penyelidikan mengenai penghasilan selulosa bakteria oleh *Acetobacter xylinum*. Bahagian pertama adalah penyediaan inokulum *Acetobacter xylinum*. Inokulum *Acetobacter xylinum* adalah sumber inokulum yang mana kemudiannya akan digunakan untuk proses penapaian dan seterusnya untuk sintesis bakteria selulosa. Dalam bahagian kedua, eksperimen telah diteruskan untuk penghasilan bakteria selulosa. Pertama, inokulum *Acetobacter xylinum* telah disediakan dalam medium jus kelapa. Selepas mendapatkan hasil optimum pengeluaran bakteria selullose dengan beberpa nilai parameter media yang berbeza, kemudian tiga aditif yang berbeza telah digunakan pada kepekatan yang berbeza untuk mempertingkatkan pengeluaran bakteria selulosa. Semua media penapaian telah dieram 30 °C selama 3 hari. Nilai pH optimum ditentukan oleh berat daripada bakteria selulosa, dimana media penapaian telah dieram selama tiga hari. Penghasilan bakteria selulosa adalah agak tinggi dengan mempunyai ciri-ciri yang sama yang dihasilkan dalam media-HS. Berat basah penghasilan selulosa bakteria oleh *Acetobacter xylinum* tanpa selulosa adalah 0.1097g, manakala penghasilan selulosa bakteria adalah yang tertinggi, 0,589 g, pada kepekatan selulosa 0.2% w/w. Selain itu, keputusan menunjukkan bahawa sampel ini terdiri daripada gentian yang

amat halus, yang membentuk struktur retikulasi. Penambahan aditif ke dalam medium tidak mempengaruhi struktur selulosa. Keputusan ini menunjukkan bahawa ia adalah tidak mustahil untuk menghasilkan bakteria selulosa daripada sumber kos rendah dalam usaha untuk meningkatkan pengeluaran kepada skala yang lebih besar

LIST OF TABLES

LIST OF FIGURES

LIST OF NOMENCLATURES

ATP	adenosine triphosphate
BC	bacterial cellulose
CJ-M	coconut juice medium
CMC	caoboxymethylcellulose
FTIR	Fourier Transform Infrared Spectroscopy
HS-medium	Hestrin and Schramm medium
MCC	microcrystalline cellulose
PW-M	pineapple waste medium
SA	sodium alginate
SEM	scanning electron microscopy
TGA	thermogravimetric analysis

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Environmental pollution by waste generated from economic activities are common problems faced by the industries nowadays. World pineapple trade had shown increasing trend for the past three decades. Malaysia, once ranked as one of the top 3 pineapple producers in the world in the 60's and early 70's, has only a relatively modest industry today. The total area under pineapple in the last 5 years was only around 7,000 - 8,000 ha and 5,000 ha are managed by three prominent estates which grow pineapple for canning. Nowadays, Malaysia was listed number 15 of the world fresh pineapple exporter, while for canned pineapple Malaysia was listed as number 9 (MARDI, 2012). Pineapple canning industry is one of the many food industries producing large quantities of waste such as fresh peels. The disposal of the waste is becoming a major problem to many food processing industries. There is a potential for food processing waste such as pineapple waste to be used for conversion into useful and higher value added products. The pineapple peel wastes

contain high concentration of biodegradable organic material such as carbohydrate that can be utilized for the production of organic acid. With the goal of being economically competitive and overcome the problem of disposal of the waste, pineapple waste can be potentially used to enhance the production of bacterial cellulose.

Bacterial cellulose is an organic compound and is a form of cellulose that is produced by bacteria. The bacterial cellulose and plant cellulose have same chemical structure but different in physical and chemical properties. Bacterial cellulose is mainly a protective coating while plant cellulose only plays a structural role (Bielecki *et al*, 2000). Bacterial cellulose is synthesized by bacteria belonging to the genera *Acetobacter*, *Rhizobium*, *Agrobacterium*, and *Sarcina* (Jonas and Farah, 1998). However, the only species that can produce enough cellulose for commercial interest is *Acetobacter* species, *Acetobacter xylinum* is able to produces cellulose in high quantity. There are many features of the bacterial cellulose from the methods of the production and one of the most important features of bacterial cellulose its chemical purity. The bacterial cellulose produced by genera *Acetobacter* differs from plant cellulose is its crystallinity and purity (Pourramezan *et al*, 2009).

Bacterial cellulose is known as polysaccharide and usually it been used traditionally in food industry and the latest it is used as a material for medical application (Grande *et al.* 2005). Besides that, bacterial cellulose has application in paper, textile and food industries and also as a biomaterial in cosmetic and medicine (Ring *et al*., 1986). In medical field, several application of bacterial cellulose have been reported such as an artificial skin for human with extensive burns, artificial blood vessel for microsurgery and wound dressing (Czaja *et al.* 2005). While in food industries, bacterial cellulose produced by *Acetobacter xylinum* at the air-liquid

interface of coconut water is popularly known as nata-de-coco which use in desserts, fruit cocktails and friut jellies (Jagannath *et al.*, 2008).

In the fermentation process, bacterial cellulose production depends heavily on several factors such as the growth medium and the formation of byproducts. Bacteria are most efficient when supplied with an abundant carbon source and minimal nitrogen source (Ramana *et al*, 2000). Carbon sources played important role for cell growth and bacterial cellulose production as their growth medium, and at the same time cost for bacterial cellulose production must be considered as a main objective. This work aimed to optimize the bacterial cellulose production by using pineapple waste as a substrate with the addition of additives. In this research, three different additives are used to study their effect on bacterial cellulose production.

Recently, there are a few reports stating that addition of water-soluble polymers can increase relative viscosity, hinder coagulation to transfer nutrients and oxygen into bacterial cells, and promote bacterial productivity into the medium (Zhou *et al*., 2007). Addition of different chemicals to fermentation medium in bacterial cellulose production was found to enhance bacterial cellulose production both static and submerged cultivation (Cheng *et al*., 2009).

1.2 PROBLEM STATEMENT

In Malaysia, there are a lot of organic wastes from different stages of agroindustrial productions that, in many cases, cannot be marketed due to their poor quality. However, they are rich in sugars such as glucose, fructose and sucrose, as well as nitrogen and vitamins that are useful for cellulose biosynthesis (Castro *et al.*,

2010). Pineapple peel is the principal solid waste product of the juice processing industry. If these waste discharges to the environment are left untreated they could cause a serious environmental pollution. Then, the industry have to provide proper treatment regarding waste disposal and unfortunately illegal disposal may remain a positive expected present value decision if the penalties are small relative to proper treatment costs (Muoghalu *et al.*, 1990). Rather than allocate lot of money in waste disposal, why not the waste itself is used to generate side income to the company. The used of pineapple residue as raw material in bacterial cellulose production can prevent from the environmental issues.

Meanwhile at the same time, the increasing demand of industrialization for cellulose has imposed extreme negative pressure on the delicate ecoligical balance (Cheng *et al.*, 2009) and one approach to reduce the demand from plants is the production of cellulose using a microbial system (Lynd *et al.*, 2002). However if waste can be transformed into valuable products, this would optimize the profits and competitiveness of the industry. So, the pineapple waste produced from the pineapple canning industries can be used as a substrate for bacterial cellulose production.

In past few years, many researchs has been perform to develop culture media based on other sources of sugars like fruits and vegetables in order to decrease the costs of bacterial cellulose production (Castro *et al.*, 2010). Usage of pineapple residue as substrate in bacterial cellulose production not only optimizing the production of bacteria cellulose, but also lowering the cost of bacterial cellulose production. In addition, the used of pineapple waste as raw material in fermentation medium in bacterial cellulose production also can prevent environmental pollution caused by agroindustrial waste.

1.3 OBJECTIVES OF RESEARCH

i) To investigate the effect of three different additives on the production of bacterial cellulose by *Acetobacter xylinum*.

ii) To optimize the production of bacterial cellulose from pineapple waste by manipulating the pH value of the medium and the concentration of the additives.

1.4 SCOPES OF RESEARCH

i) To produce bacterial cellulose by *Acetobacter xylinum* from pineapple waste as a fermentation medium with three different types of additives which are sodium alginate, microcrystalline cellulose and carboxymethylcellulose.

ii) To examine the relationship between the bacterial cellulose yield and the pH of the fermentation media.

iii) To analyse the production of bacterial cellulose by using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM).

1.5 RATIONAL OF SIGNIFICANT STUDY

i) Reduce the environmental pollution by reuse pineapple waste produced from food and beverage industries in order to optimize the production of bacterial cellulose.

ii) Low cost of baecterial cellulose production.

iii) To obtain high bacterial cellulose yield from the pineapple waste.

CHAPTER 2

LITERATURE REVIEW

2.1 BACTERIAL CELLULOSE

Bacterial cellulose is the basic material of all plant substances and the most abundant polysaccharide on earth. Bacterial cellulose belongs to specific products of primary metabolism (Bielecki *et al.*, 2000). Bacterial cellulose can be extracellularly synthesized into nano-sized fibrils by the bacteria *Acetobactor xylinum*, in presence of glucose in the fermentation medium. One of the characteristics of bacterial cellulose is plant cellulose and bacterial cellulose have the same chemical structure. Recent investigation reveals that bacterial cellulose is chemically identical to plant cellulose (Bielecki *et al*., 2000). Cellulose can be synthesized by plants, some animal and a large member of microorganisms by the bacteria *Acetobacter xylinum* (Castro *et al.*, 2010).

However, bacterial cellulose possesses particular physicochemical properties different from plant cellulose. There are several aspects that differentiate bacterial cellulose with plant cellulose, which make bacterial cellulose has unique

characteristic compared to plant cellulose, including high purity, high crystallinity, high mechanical strength, high water holding capacity, good biocompatibility, and high porosity (Grande *et al*., 2009). Cellulose is the main part in the cell wall and act as protective and coating, whereas plant cellulose (PC) plays a structural role in plant (Bielecki *et al.,* 2000). There are no lignin, hemicellulose or other natural components in bacterial cellulose. The unique characteristics make bacterial cellulose an interesting raw material for applications and become potentially important industrial and biomedical material. Tsuchida & Yoshinaga, 1997, suggested that bacterial cellulose is expected to be used for many industrial applications as a high-strength construction material, food additive and a component of biodegradable products and paper. But, one of the bacterial cellulose application problems in industry is its low productivity (Pourramezan *et al*.,2009).

Since the cellulose obtained from the plant is not pure caused it have lignin and hemicellulose, bacterial cellulose used as an alternative instead of plant cellulose in order to produce high purity cellulose and in the same time to reduce the forest depletion as the most cellulose is produced by vascular plant, the increasing demand of industrialization has imposed negative pressure on the plant world (Cheng *et al*.,2009). Besides that, cellulose obtained from wood stock requires the removal of impurities and the process of removal impurities requires lot of the total energy and cause high cost of seperation process. Wider application of this polysaccharide is obviously dependent on the scale of production and its cost (Bielecki *et al*., 2000).

Nowadays, bacterial cellulose used as an alternative instead of plant cellulose in order to produce high purity cellulose and in the same time to reduce the forest depletion (Sherif, 2008). Cellulose is synthesized by bacteria belonging to the genera of *Acetobacter*, *Rhizobium*, *Agrobacterium*, and *Sarcina* (Retegi *et al*., 2009). The

ability to produce high levels of polymer in a large range of carbon and nitrogen sources that caused *Acetobacter xylinum* has applied as a model for the basic and was applied studies on cellulose. To obtain optimum production of bacterial cellulose, it is very critical in selecting the substrates, cultivation conditions, various additives, and finally the bacterial strain (Retegi *et al*., 2009).

2.2 *ACETOBACTER XYLINUM*

Acetobacter xylinum was used as a model bacterium in bacterial cellulose studies by Hestrin *et al*. (1954), who proved that resting and lyophilized *Acetobacter* cells synthesized cellulose in the presence of glucose and oxygen (Bielecki *et al*., 2000). Many strains of *Acetobacter xylinum* are capable of producing cellulose on varying amount and growing on a wide variety of substrates like glucose, sucrose, fructose, invert sugar, ethanol and glycerol (Jagannath *et al*., 2008) and it produced cellulose in the form of extracellular pellicle composed of ribbons. Besides that, oxygen is an important factor for bacterial cellulose production by *Acetobacter xylinum* since it is aerobic microbe (Suwannapinunt *et al*., 2007).

One approach to reduce the demand of bacterial cellulose produced from plants is the production of cellulose using a microbial system (Lynd *et al*., 2002). In this study, cellulose is synthesized by bacteria belonging to the genera *Acetobacter.* This is gram-negative bacterium, strictly aerobic capable of producing extracellulose using glucose, sucrose or others carbon source (Castro *et al*, 2010). *Acetobcater xylinum* is also acetic microbe that growth very well in acid condition from broth medium but *Acetobacter xylinum* still growth because it is a type of acetic microbe

and involve in fermentation process to convert glucose to cellulose. Gluconic, acetic or lactic acid is produced by *Acetobacter xylinum* in fermentation process caused the pH decrease from pH 6 to pH 4 in culture medium and at the same time the yield of cellulose decrease in fermentation (Chawla *et al*., 2008). In alkaline condition, *Acetobacter xylinum* will grow slowly and bacterial cellulose yield will decreasing (Pourramezan *et al*., 2009). The pH value of the fermentation is one of the most critical parameter since high or low pH value would cause decreasing in cellulose production.

The polymer structure depends on the organism, although the pathway of biosynthesis and mechanism of its regulation are probably common for the majority of bacterial cellulose producing bacteria (Ross *et al*., 1991). Bielecki *et al*., 2000, stated the bacterial cellulose is synthesized by several bacterial genera, of which *Acetobacter* strains are best known. Table 2.1 present an overview of bacterial cellulose producers.

Table 2.1 Bacterial cellulose producers

Genus	Cellulose Structure
Acetobacter	extracellular pellicle composed of ribbons
Achromobacter	fibrils
Aerobacter	fibrils
Agrobacterium	short fibrils
Alcaligenes	fibrils
Pseudomonas	no distinct fibrils
Rhizobium	short fibrils
Sarcina	amorphous cellulose
Zoogloea	not well defined

(Source : Jonas and Farah, 1998)

Acetobacter xylinum which is the most efficient producer of cellulose, has been recently reclassified and included within the novel genus *Gluconacetobacter xylunis* (Bielecki *et al*., 2000).

2.3 PINEAPPLE WASTE AS A SUBSTRATE

In recent years, in order to produce bacterial cellulose at lower cost, there has been many reports to develop method or culture media. One of the advantages of bacterial cellulose is it can be produced from various carbon and nitrogen sources. Various carbon sources including D-glucose, sucrose, fructose, D-galactose, lactose, mannitol and ethanol while for various nitrogen sources are ammonium sulphate, ammonium nitrate, riboflavin, glycine, peptone, sodium nitrate and methionine (Panesar *et al*., 2009).

An alternative method for bacterial cellulose production is using fernentation medium from organic waste. In Malaysia, there are a lot of organic waste from different stages and also type of agroindustrial productions that cannot be marketed due to their poor quality. However, they are rich in sugars such as glucose, fructose, and sucrose as well as nitrogen and vitamins that are essential for fermentation medium in cellulose biosynthesis (Castro *et al*., 2010). Using the pineapple residue and fruit waste such as fruit peel is one of the alternatives that can overcome this problem. The cost of collecting the pineapple rind waste is much lower than buying the pure glucose medium for the cellulose production and these wastes can caused environmental pollution problems if it not is utilized.

Table 2.2 The characteristics of solid pineapple waste reported by different authors.

Composition (%)	Bardiya et al. (1996)	Viswanath (1992)	Chandapillai and Selvarajah (1978)
Moisture	92.80	87.69	89.70
Total solid	7.80	12.31	10.30
Ash	10.60	6.20	3.90
Organic carbon	51.85	38.9	-
Nitrogen free extract	-	-	75.10
Total carbohydrates	35.00	-	-
Ether extract	-	-	0.20
Cellulose	19.80	-	-
Crude fibre	-	-	14.70
Hemicelluloses	11.70	-	-
Phosphorus		0.08	0.10
Total soluble	30.00	-	-
Total nitrogen	0.95	0.90	-

Krueger et al. (1992) have been reported that major constituents of fresh pineapple waste juice are glucose, fructose, sucrose, citric acid, malic acid and mineral potassium.

In Malaysia, the pineapple industry is the oldest agro-based export-oriented industry dating back to 1888. Though relatively small compared to palm oil and rubber, the industry also plays important role in the country's socio-economic development of Malaysia, particularly in Johore. The three registered canneries situated in Johore currently produce all the Malaysian canned pineapple (KPUM, 1990). Malaysian Cannery of Malaysia Sdn. Bhd is a location to obtain the pineapple residue. The canning factory is the first place for the fresh pineapple fruits to be submitted. Then they will be peeled, core removed, sliced, sorted and canned. All the peeled skin, unwanted fruits and the core will be sent to the crush machine for crushing. After crushing, the solid waste will be sent to cattle feeding and in the same

time the liquid waste is send to storage for fermentation process (Abdullah and Hanafi, 2008).

Although pineapple can be grown all over the country, the planting of pineapple for canning purpose is presently confined to the peat soil area in the state of Johore, which is the only major producer of Malaysian canned pineapple. In other states such as Selangor, Perak, Kelantan, Terengganu, Negeri Sembilan and Sarawak, pineapples are planted specifically for domestic fresh consumption (KPUM, 1990).

2.4 FERMENTATION MEDIUM CONDITION

Recently, there are many report that tried to increase the productivity of bacterial cellulose using various biochemical (Keshk and Sameshima, 2005). Hence, further study shows that culture conditions are essential to achieve industrial levels of cellulose production (Pourramezan *et al.*, 2009). Bacterial cellulose yield, structure and properties are different depending on the cultivation method or the type of medium used for the fermentation medium. In general, a medium containing different rich in nutrition could promote cell growth and bacterial production differently. Based on reported Son *et al*., 2003, was mentioned that increasing amount of glucose into the medium will enhance bacterial cellulose production but the yield will decrease when medium containing more glucose excess.

Fermentation is the enzymatic decomposition and utililization of foodstuffs, particularly carbohydrates, by microbes. During fermentation,pyruvate is metabolized to various compounds. Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of

abundant oxygen, yeast cells greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption. Sugars are the most common substrate of fermentation. Oxidative phosphorylation is a metabolic pathway that uses energy released by the oxidation of nutrients to produce ATP. During oxidative phosphorylation, electrons are transferred from electron donors to electron acceptors such as oxygen, in redox reactions. These redox reactions release energy, which is used to form ATP. Fermentation acid-resistant bacteria are in most cases Gram-positive bacteria with a high intracellular potassium concentration, and even acid-sensitive bacteria like E. coli have increased potassium levels when fermentation acids are present. Intracellular potassium provides a counteraction for fermentation acid anions, and allows bacteria to tolerate even greater amounts of fermentation anions. The delta pH-mediated anion accumulation provides a mechanistic explanation for the effect of fermentation acids on microbial ecology and metabolism (Russell and Diez-Gonzalez, 1998).

In recent years, in order to reduce the costs of bacterial cellulose production, there has been a growing concern to develop culture media based on other sources of sugars like fruits and vegetables (Castro *et al*.,2010). Unfortunately, bacterial cellulose production also has disadvantages that need to encounter such as the high price for the sugar as a substrate and low production of sugar. Cellulose production by *Acetobacter xylinum* has been noted both in static as well as agitated culture and is known to be affected by the type and concentration of sugar, nitrogen source and pH (Jagannath *et al*., 2008). In production of bacterial cellulose using *Acetobacter xylinum*, temperature needs to be maintained at 30^{o}C and pH will be measured at 5.5 by pH meter to ensure optimized growth of this microbe, that gives the highest dry weight of bacterial cellulose (Pourramezan *et al*., 2009).

Normally, the previous research, most of the researchers study the effect concentration of glucose into the fermentation medium, but in this research study the effect concentration pineapple residue to replace pure glucose as a parameter into the medium.

2.5 MECHANISM OF SYNTHESIS OF BACTERIAL CELLULOSE FROM *ACETOBACTER XYLINUM*

Bacterial cellulose can be extracellularly synthesized into nano-sized fibrils by the bacteria *Acetobactor xylinum*, using glucose as a common substrate. Cellulose synthesis by *Acetobacter* is a complex process and involves the polymerization of single glucose residues into linear β-1,4-glucan chains, the extracellular secretion of these linear chians and the assembly the crystallization of the glucan chains into hierarchically composed ribbons (Wojciech *et al*., 2006).

Brown, 1987 stated that the synthesis of bacterial cellulose is a multistep process that involves two main mechanisms: the synthesis of uridine diphosphoglucose (UDPGIc), followed by the polymerization of glucose 4 glucan chain).◊into long and unbranched chains, the β-1,4-glucan chains. The cellulose formation includes five fundamental enzymes mediated steps: the transformation of glucose to UDP-glucose via glucose-6-phosphate and glucose-1-phosphate and finally, the addition of UDP-glucose to the end of a growing polymer chain by the cellulose synthase (Prashant *et al*., 2008). The overall mechanism for cellulose biosynthetic pathway is illustrated in Figure 2.1.

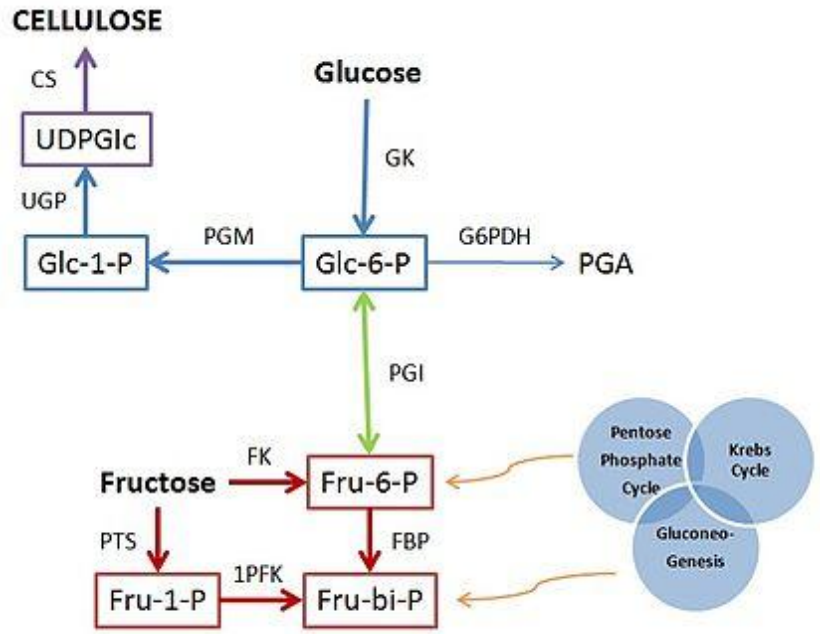


Figure 2.1 Biochemical pathway for cellulose synthesis
(Source : Bielecki *et al*., 2000)

The first pathway is by the isolation of cellulose from plants. This pathway involved another separation process step to remove lignin and hemicelluloses. The second pathway is the synthesis of cellulose by *Acetobacter xylinum*. *Acetobacter xylinum* produced cellulose in the form of the 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 a well defined form (Barbara *et al*., 2008). The third and fourth methods 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 (Klemn *et al*., 2001).

Tsuchida & Yoshinaga, 1997, stated that there have been some studies on breeding bacterial cellulose producing bacteria which increasing the bacterial

cellulose synthase activity by genetic engineering, with branches of their metabolic pathway blocked to decrease the amounts of by-products. They bred mutant cells whose growth was increased to increase the enhancement of bacterial cellulose productivity.

2.6 ADDITIVES IN BC PRODUCTION

There are a few reports stating that addition of additives such as water-soluble polimer such as xanthan, agar, polyacrylamide-co-acylic acid (PCA) and acetan are known to interfere with the aggregation of microfibrils into a normal ribbon assembly, affect the crystallization of cellulose, and introduce the characteristic properties of theses additives to attain bacterial cellulose composite materials (Zhou *et al*., 2007).

Addition of Calcoflour White ST, a stilbene derivative used as optical brightner for cellulose increase the rate of glucose polymerization into cellulose, but disrupt the assembly crystalline cellulose when the concentration is above 01 mM (Benziman *et al*., 1980). Peng *et al*., 2006, suggested that considering that sodium alginate is also a water-soluble polysaccharide and there are many -COOH and -OH groups, sodium alginate has some effect on cellulose production and the modification of the cellulose occurs during microbiological synthesis. The negatively charged water-soluble cellulose derivative, carboxymethylcellulose, was widely used to enhance bacterial cellulose production in agitated culture (Cheng *et al*., 2009).

Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to over ten

thousand β(1→4) linked D-glucose units (Updegraff, 1969). Cellulose is the structural component of the primary cell wall of green plants. Some species of bacteria secrete it to form biofilms. Microcrystalline cellulose is used to make water-soluble adhesives and binders such as methyl cellulose and carboxymethyl cellulose. Microcrystalline cellulose and powdered cellulose are used as inactive fillers in tablets and as thickeners and stabilizers in processed foods. Cellulose consists of crystalline and amorphous regions. CMC is used in food science as a viscosity modifier or thickener, and to stabilize emulsions in various products. Last but not least, sodium alginate (NaAlg) also was used as an additives in this study. The chemical compound sodium alginate is the sodium salt of alginic acid. Its empirical formula is $NaC_6H_7O_6$.Sodium alginate is used by the foods industry to increase viscosity and as an emulsifier. Nowadays, it is also used in the biological experiments for the immobilization of cells to obtain important products like alcohols and organic acids.

2.7 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

Fourier Transform Infrared (FTIR) absorption spectroscopy is an analytical technique based on the frequency at which chemical bonds vibrate when subjected to electromagnetic radiation passed through. FTIR is a one of the equipment that used to analyze bacterial cellulose by detecting the chemical bonding that present in the polyme or reflected off a subject of interest. The whole and expanded FTIR spectra revealing characteristic's absorption band of bacterial cellulose. As functional groups and polar bonds of elements absorb radiation at specific wavelengths, FTIR

spectroscopy can be used to both qualitatively and quantitatively measure these elements. An important advance in FTIR spectroscopy in the past decade has been the advent of spectroscopic imaging a technique that allows a map of an element or molecule of interest to be constructed over a large (sub mm) area from multiple spectra collected by transmission or reflection mode. Table 2.3 show that the characteristics of infrared absorption according to the functional group.

Table 2.3 Table of characteristic IR Absorptions

Functional Group	Molecular Motion	Wave number (cm^{-1})
Alkanes	C-H stretch	2950-2800
	CH2 bend	~1465
	CH3 bend	~1375
	CH2 bend (4 or more)	~720
	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
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

(Souce : Sherif, 2008)

An absorbance spectrum that can detect much higher absorbance than the UV-visible spectrometer is the Fourier-transform infrared spectrometer and it has long been a valuable tool for identifying functional groups based on of their characteristic vibrational frequencies. Based on the report Oh *et al*., (2005) was mentioned that the changing of the structure in bacterial cellulose will give effect to the some of the absorbance peak either change to the decrease or shifted to greater or lower wave number. The result shown that the absorbance peak of wave number is either decreased or shifted to higher value or to lower value because of the change or rearrangement of the cellulose structure. Infrared spectroscopy is routinely used for the analysis of samples in the gas, liquid, and solid states. Examples of solids that have benn analyzed by FTIR include polymers, fibers, fabrics, powders, and biological tissue samples. Transparent solid samples can be analyzed directly by placing them in the IR beam. Bacterial cellulose no need to dispersed in a more transparent medium before recording a transmission spectrum since it is not opaque. Identification of the unknown organic or inorganic, mixture of microscopic compound, detection or characterization of organic or inorganic and some inorganic additive in polymers at the level as low as few percent, characterization of changes in chemical structure of organic material as a result of polymer cure, sterilization, heat treatment, plasma treatment and else are some of the common application of FT-IR (EAGLABS Fourier Transform Infra Red Spectroscopy (FT-IR) Services, 2009).

2.8 SCANNING ELECTRON MICROSCOPY (SEM)

A Scanning Electron Microscope (SEM) is a type of electron microscope that images a sample by scanning it with a beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition, and other properties.

For SEM, a specimen is normally required to be completely dry, since the specimen chamber is at high vacuum. Living cells and tissues and whole, soft-bodied organisms usually require chemical fixation to preserve and stabilize their structure. In this study, the bacterial cellulose was dried first before being analyzed using SEM. There are divided into three signal which are the secondary electrons, backscattered electrons, and X-rays for provide the greatest amount of information in SEM as the high energy electron is strike the sample and variety of signal are generated. For imaging sample, the signal of secondary electron is commonly used while the backscattered electron signal will determine the crystal structure and orientation of mineral and also for imaging sample. Detector are collected these x-rays, backscattered electorons, and secondary electron and convert them into a signal that is sent to a screen for the final images. In a typical SEM, an electron beam is thermionically emitted from an electron gun fitted with a tungsten filament cathode. The electron beam, which typically has an energy ranging from 0.2 keV to 40 keV, is focused by one or two condenser lenses to a spot about 0.4 nm to 5 nm in diameter. The beam passes through pairs of scanning coils or pairs of deflector plates in the electron column, typically in the final lens, which deflect the beam in

the *x* and *y* axes so that it scans in a raster fashion over a rectangular area of the sample surface.

Bacterial cellulose is a nanomaterial produced by various strains of *Acetobacter* species and also strains of *Pseudomonas*, *Achrobacter, Alcaligine, Aerobacter*, and *Azotobacter* (Kongruang *et al*., 2008). The observation the microfibrils of wet bacterial cellulose pellicle using light microscope is difficult due to the ultrafine microstructure of the bacterial cellulose. Scanning electron microscope was used as it has the combination of higher magnification, larger depth of field and greater resolution that make it as the most heavily used instrument in the observation chemical composition, crystalline structure and crystalline orientation. The morphology of bacterial cellulose composite is a very important parameter because it is closely related with their mechanical performances and the usage of the bacterial cellulose can be specifically known. Many biological process and structures occur at surfaces and if antibodies are available, their components can be located within the surface structure.

The morphology of bacterial cellulose composite is a very important parameter because it is closely related with their mechanical performances. The morphology of bacterial cellulose depends on the growing culture environment. That mean for a static culture and an agitated medium, the bacteria morphology were different between each culture medium. For a static culture, a leather-like pellicle of overlapping and intertwined ribbons forms (Jonas and Farah, 1998) and formed irregular bacterial cellulose granules and fibrous strands in agitated medium (Vandamme *et al*., 1998).

CHAPTER 3

METHODOLOGY

3.1 OVERVIEW OF RESEARCH METHODOLOGY

Generally, there are three major parts in completing research on this research. The first part is preparation of inoculum by *Acetobacter xylinum* strain. The inoculum of *Acetobacter xylinum* is mother culture where later on will be used for the next fermentation process for the synthesis of bacterial cellulose. In the second part, the experiment was proceed for bacterial cellulose synthesis. Firstly, the inoculum of *Acetobacter xylinum* was prepared in the coconut juice medium. After obtaining the optimum yield of bacterial celullose production with different parameter of culture medium, three different additives were used at various concentrations to enhance bacterial cellulose production. Finally, the films were characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM). Figure 3.1 shows an overview of overall process on research methodolgy, while Figure 3.2 shows research design for the production of

bacterial cellulose by *Acetobacter xylinum*. All the chemicals used were of analytical grade.

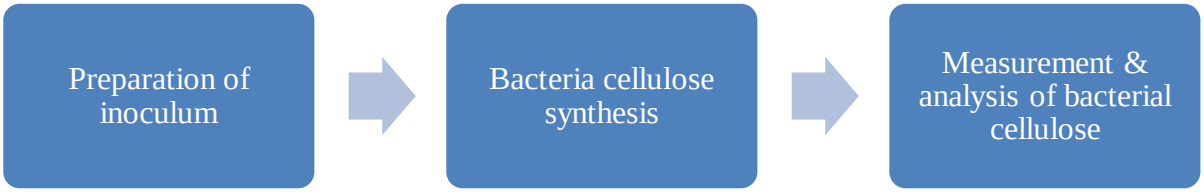


Figure 3.1 Overall process of production of bacterial cellulose with additives

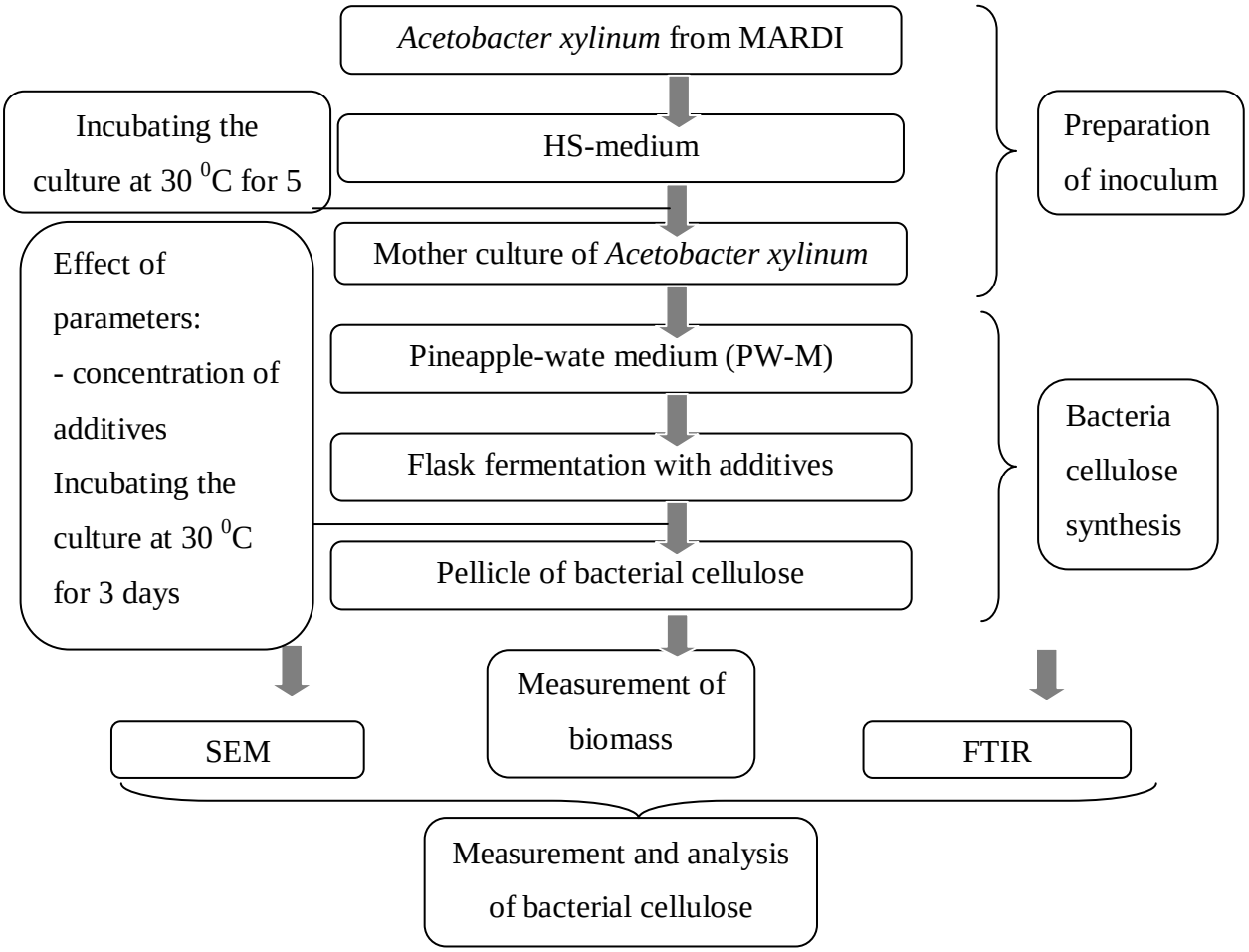


Figure 3.2 Research methods for the production of bacterial cellulose by *Acetobacter xylinum*

3.2 PREPARATION OF INOCULUM

In this research, mother culture must be prepared first by using coconut juice medium as fermentation medum before proceed with using pineapple waste medium. The bacterial strain used in this study was *Acetobacter xylinum* obtained from the Malaysian Agricultural Research and Development Institute (MARDI). Culture media used for preparation of inoculum was modified HS-medium, which contain 90 ml of modified HS-medium, 2%, w/v, glucose, 0.5%, w/v, yeast extract, 0.5%, w/v pepton from milk, 0.115%, w/v, citric acid, 0.27%, w/v, disodium hydrogen phosphate, and 10 ml of *Acetobacter xylinum* bacteria. The mother culture was incubated for 3 days at 30 ^{0}C.

3.3 BACTERIA CELLULOSE SYNTHESIS

Experiments were prepared by adding 10 vol.% inoculums to the different media and statically incubating at 30 ^{0}C for 3 days. Culture medium used for bacterial cellulose production was pineapple peel juice (2.14%, w/v, glucose, 2.4%, w/v, fructose, 2.10%, w/v, sucrose, 0.31, w/v, total nitrogen). The quantification of total nitrogen in pineapple peel juice medium showed that it was not necessary to add any source of nitrogen (Castro *et al.*, 2010). The pH value of the fermentation medium was adjusted from 4 to 7 and autoclaved at 121^{0}C. The temperature of incubator was set to 30^{0}C. Ten mililiter of the prepared inoculum was added to 90 ml of fresh pineapple juice medium with various additives at different concentrations. A control experiment without addition of additives was also performed simultaneously.

3.4 MEASUREMENT OF BACTERIAL CELLULOSE

3.4.1 Treatment of BC

At the end of 3-days incubation, pellicle of bacterial cellulose was treated to remove the bacteria and eliminate the remaining culture. The pellicles that formed were treated with 2.5% natrium hydroxide, NaOH for 1 days and 2.5% NaOCl also for 1 day.Finally, films of cellulose were prepared by dried in oven-dried at 60 ^{0}C for 6 hours.

3.4.2 Measurement of Biomass and Bacterial Cellulose

After the pellicle of bacterial cellulose was treated to remove the bacteria and eliminate the remaining culture medium, the bacterial cellulose was weight by using analytical balance. The reading consists of wet weight and also dry weight of pellicle bacterial cellulose.

3.5 ANALYSIS SAMPLE OF BACTERIAL CELLULOSE

3.5.1 Scanning Electron Microscopy (SEM)

SEM was used to generate high resolution images of surface and cross section of the sample and for this study, it was operating at various acceleration voltage from 7kV - 10 kV. The surface of the sample were coated with gold under vacuum for SEM analysis. Then, the image was taken at various magnification ranging from 1,000x to 10,000x.

3.5.2 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the films measured in the wavelength range from 1000 to 4000 cm^{-1} (Jatupaiboon *et al*., 2008).

3.6 MATERIALS FOR BACTERIAL CELLULOSE PRODUCTION

The bacterial strain used in this study was *Acetobacter xylinum* obtained from Malaysian Agricultural Research And Development Institute (MARDI). All the chemicals used were of analytical grade and commercially available. In this research, *A. xylinum* and pineapple waste was used as raw material.

For inoculum of *A. xylinum* strain, HS-medium was used which contains D-glucose, yeast-extract, peptone from milk, citric acid, disodium hydrogen phosphate. The culture medium for the growth of bacterial cellulose consisted of distiled water, pineapple waste juice, D-glucose, fructose, sucrose (food-grade white sugar), yeast extract, and becto-pepton. For the treatment of bacterial cellulose's pellicle, natrium hydroxide, NaOH, and sodium hypochloride, NaOCl was used.

The main equipment used in this experiment were incubator, blender, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). The main apparatus used were conical flask, autoclave, beaker, petri dish, water bath, freezer, filter funnel and filter paper in bacterial cellulose production.

3.7 EXPERIMENTAL PROCEDURE

3.7.1 Preparation of Inoculum Using HS-Medium (HS-M)

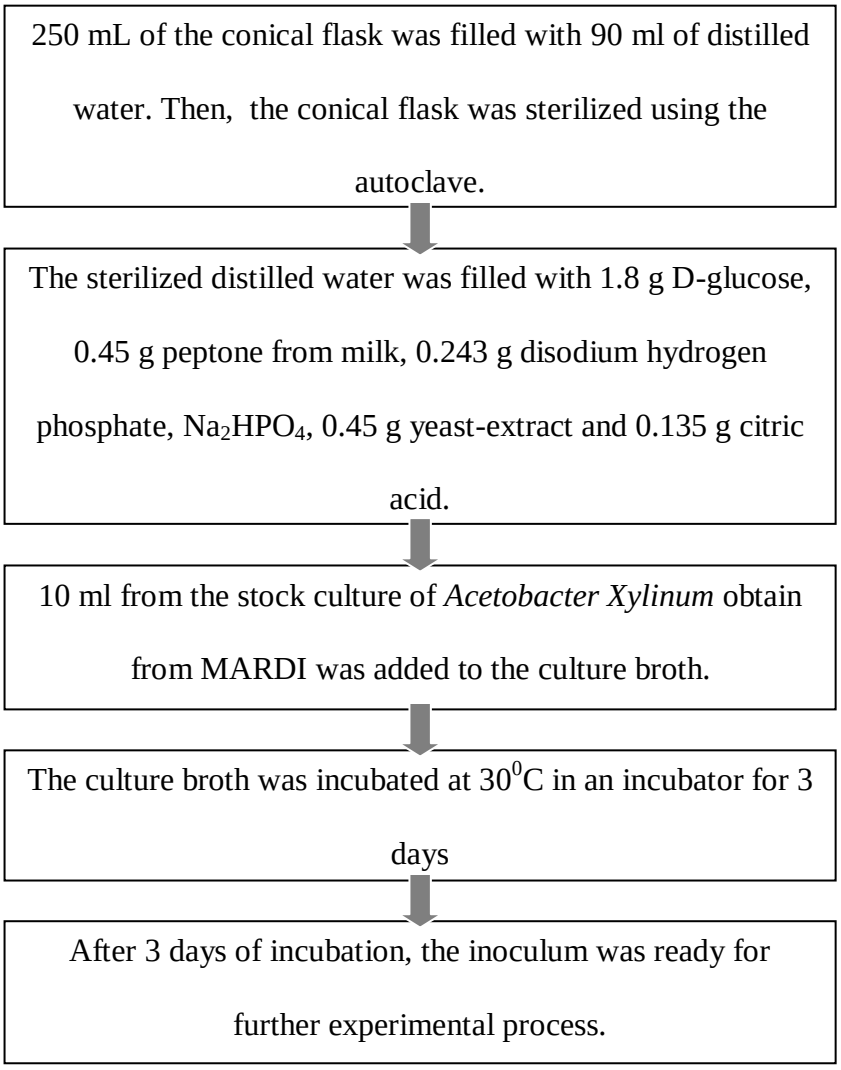


Figure 3.3 Preparation of inoculum using HS-M

3.7.2 Preparation of Pineapple Peel Juice

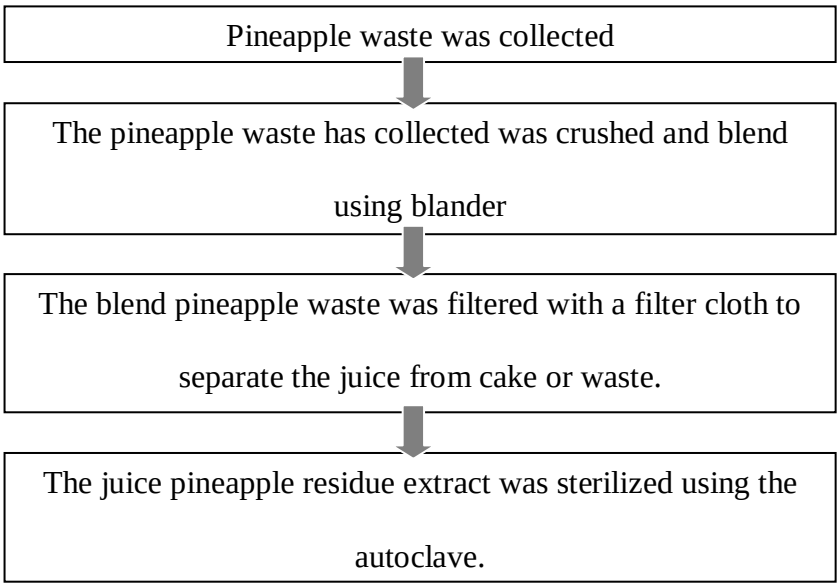


Figure 3.4 Preparation of pineapple waste juice

3.7.3 Production of Bacterial Cellulose

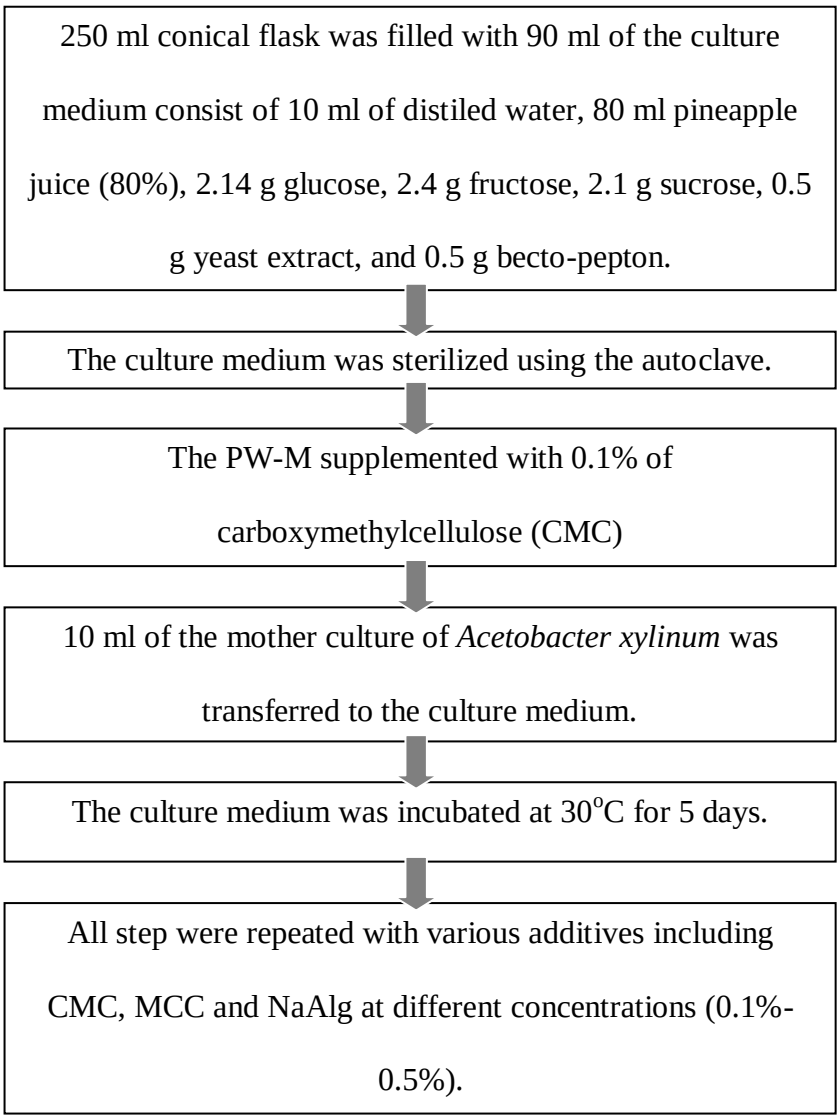


Figure 3.5 Production of bacteria cellulose using PW-M

3.7.4 Measurement & Analysis Sample of Bacterial Cellulose

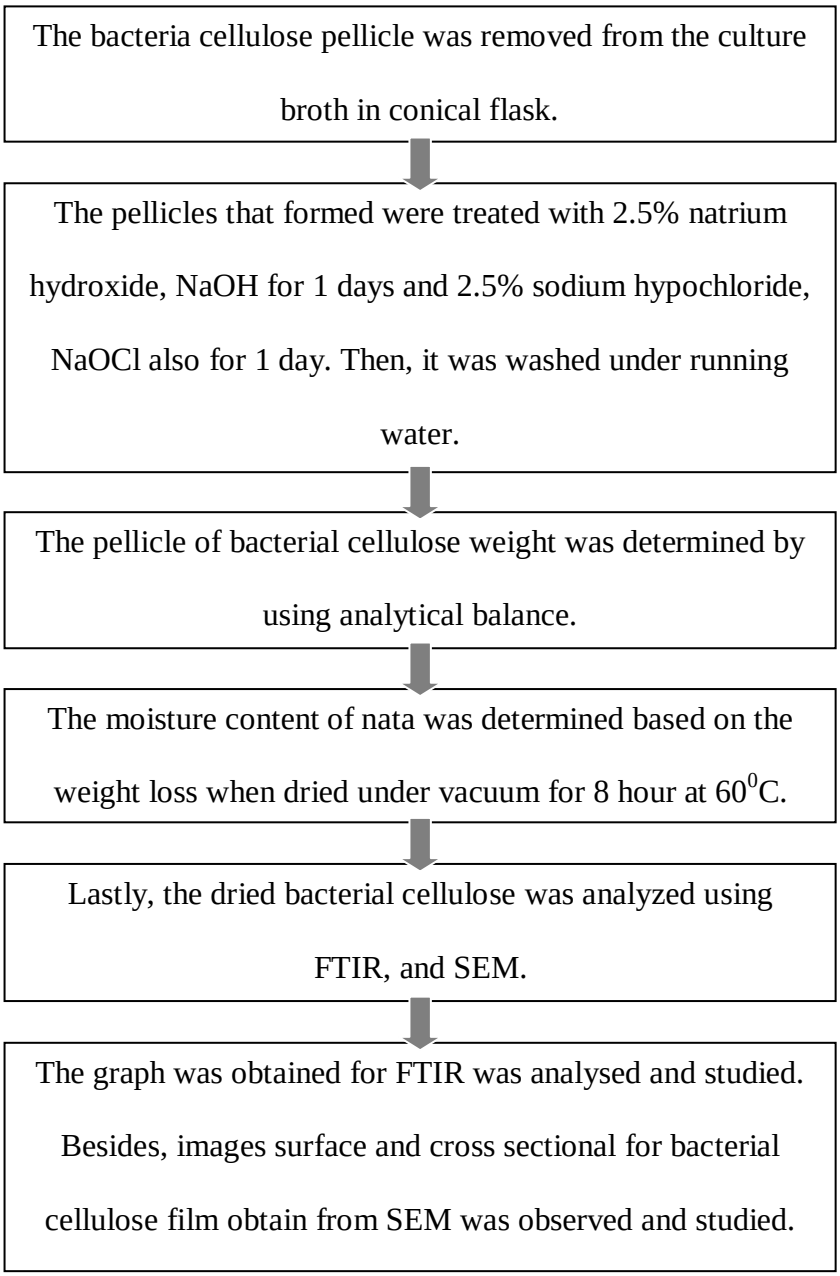


Figure 3.6 Bacterial cellulose measurement & analysis

CHAPTER 4

RESULT & DISCUSSION

4.1 THE EFFECT OF pH VALUE OF FERMENTATION MEDIUM

All the medium fermentation was incubated for 30°C by using a various pH medium in range 4.0-7.0. The optimum pH value was determined by the weight of bacterial cellulose, where the medium fermentations were incubated for five days. In the previous study, it was found that pH bears an important role in the production of bacterial cellulose when sucrose was used as the carbon source (Jagannath *et al*., 2008). The result obtained from the experiment was tabulated in the Table 4.1 as below.

Table 4.1 Dry weight of bacterial cellulose produced by different pH value.

pH Value	Dry Weight of Bacterial Cellulose Pellicle (g)
4.0	1.183
5.0	3.3948
6.0	2.1055
7.0	0.4886

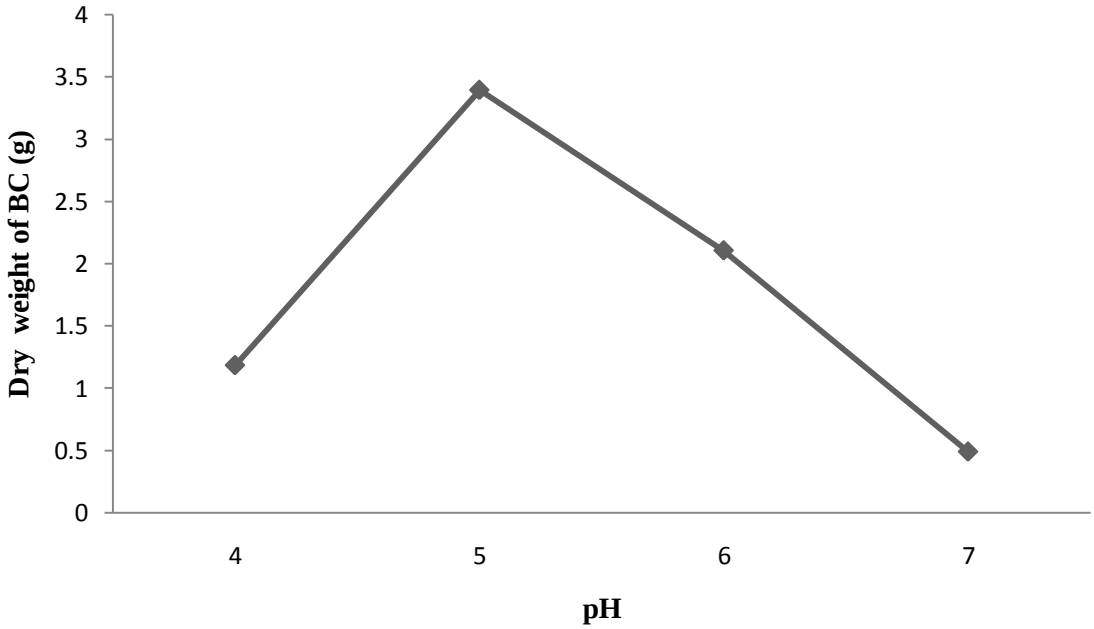


Figure 4.1 Graph of dry weight of BC vs pH

Optimization on the pH of fermentation medium in bacterial cellulose production is performed using Least-Square Regression method. The parameters involved are concentration of each additives. The data of the parameters are determined from the previous result.

From the Figure 4.1, a curve would be better suited to fit the data. The least-squares procedure can be readily extended to fit the data to a higher-order polynomial. For this data, a second-order polynomial or quadratic will be fit into Equation 4.1,

$$y = a_0 + a_1 x + a_2 x^2 + e \tag{4.1}$$

For this case, the sum of the squares of the residuals using Equation 4.2,

$$S_r = \sum_{i=1}^{n} (y_i - a_0 - a_1 x_i - a_2 x_i^2)^2 \tag{4.2}$$

The set of normal equations as Equation 4.3,

$$(n)a_0 + \left(\sum x_i\right)a_1 + \left(\sum x_i^2\right)a_2 = \sum y_i$$

$$\left(\sum x_i\right) + \left(\sum x_i^2\right)a_1 + \left(\sum x_i^3\right)a_2 = \sum x_i y_i$$

$$\left(\sum x_i^2\right) + \left(\sum x_i^3\right)a_1 + \left(\sum x_i^4\right)a_2 = \sum x_i^2 y_i \quad (4.3)$$

where all summations are from i = 1 through n. The above three equations are linear and have three unknowns a_0, a_1, and a_2. The coefficients of the unknowns can be calculated directly from the data.

The standard error is formulated by Equation 4.4,

$$s_{y/x} = \sqrt{\frac{S_r}{n - (m+1)}} \quad (4.4)$$

From the given data,

m = 2	$\sum x_i$ = 22	$\sum x_i^4$ = 4578
n = 4	$\sum y_i$ = 7.1719	$\sum x_i y_i$ = 37.7592
y_{mean} = 1.792975	$\sum x_i^2$ = 126	$\sum x_i^2 y_i$ = 203.5374
x_{mean} = 5.5	$\sum x_i^3$ = 748	

Therefore, the simultaneous linear equations as Equation 4.5

$$\begin{bmatrix} 4 & 22 & 126 \\ 22 & 126 & 748 \\ 126 & 748 & 4578 \end{bmatrix} \begin{Bmatrix} a_0 \\ a_1 \\ a_2 \end{Bmatrix} = \begin{Bmatrix} 7.1719 \\ 37.7592 \\ 203.5374 \end{Bmatrix} \quad (4.5)$$

Solving these equations gives Equation 4.6 until Equation 4.8,

$$a_0 = -24.1102 \quad (4.6)$$

$$a_1 = 10.1917 \quad (4.7)$$

$$a_2 = -0.9572 \quad (4.8)$$

The least-square quadratic equation for the graph by substituting Equation 4.6 until Equation 4.8

$$y = -24.1102 + 10.1917x - 0.9572x^2 \quad (4.9)$$

The standard error of the estimate based on the regression polynomial is

$$s_{y/x} = 0.7096 \quad (4.10)$$

The Newton-Raphson method is an open method that finds the root x of a function such that $f(x) = 0$. The method is summarized as

$$x_{i+1} = x_i - \frac{f(x_i)}{f'(x_i)} \quad (4.11)$$

A similar open approach can be used to find an optimum of $f(x)$ by defining a new function, $g(x) = f'(x)$. Thus, because the same optimal value x satisfies both

$$g(x) = f'(x) = 0 \quad (4.12)$$

it can use the following,

$$x_{i+1} = x_i - \frac{f'(x_i)}{f''(x_i)} \quad (4.13)$$

as a technique to find the minimum or maximum of $f(x)$.

From the Equation 4.9 with an initial guess of $x_0 = 4.5$,

The first and second derivatives of the function can be evaluated as

$$y' = 10.1917 - 1.9144x \quad (4.14)$$

$$y'' = -1.9144 \quad (4.15)$$

After the process was repeated, the optimum value is

$x_{opt} = 5.3237$

From the equation, the optimum value of pH for the fermentation medium is 5.32. Hence, the pH of the fermentation medium for this study was set to 5.32 to obtain optimum yield of bacterial cellulose.

Prashnant *et al*., (2009) reported that *Acetobacter xylinum* is an acetic acid microbe and activity for this microbe to synthesize cellulose will be optimum in acetic condition. The catalyst activity was proportional to H^+ concentration. The more hydrogen ions formed in the medium fermentation and more rapid the cellulose synthesizes process occurred. *Acetobacter xylinum* was active in synthesize bacterial cellulose during acidic condition because *Acetobacter xylinum* is a type of acetic acid microbe that needs an acidic condition for growth (Chawla *et al.,* 2008). The acetic acid was used to bring down the pH of PW-M as acetic acid was suitable for growing of *A. xylinum* and bacterial cellulose formation which had a better effect as compared to other acids (Jagannath *et al*., 2008). Vandamme *et al.*, (1998) opined that the role of acetic acid is that of an in situ control pH. Jagannath *et al.*, (2008) mentioned that the acetic acid breaks down to CO_2 and water generating extra ATP and thereby leading to more efficient utilization of sugars for cellulose synthesis.

However, at the the higher value of pH value of medium (alkaline), the weight of bacterial cellulose started to be decreased. Based on the report Bielecki *et al*., (2000) in alkaline condition the production of bacterial cellulose will still be occurred but in a lower yield because the bacterial growth rate will decrease and eventually the bacteria will die.

4.2 THE EFFECT OF DIFFERENT ADDITIVES ON BACTERIAL CELLULOSE PRODUCTION

Acetobacter xylinum was grown in PW-M with different types and concentration of additives for BC production in static cultures. Besides that, a control

run without additives was also performed. In this research, different additives including sodium alginate, carboxymethylcellulose and microcrystalline cellulose were compared for bacterial cellulose production by *Acetobacter xylinum*. Addition of carboxymethylcellulose and microcrystalline cellulose demonstrated an improvement of bacterial cellulose production (Cheng *et al.*, 2009). The percentage of water content inside the bacterial cellulose was calculated by using the following equation,

$$moisture\ content\ (\%) = \left|\frac{wet\ weight - dry\ weight}{wet\ weight}\right| \times 100\% \qquad (4.16)$$

4.2.1 Effects of Addition of Microcrystalline Cellulose on BC Production

The optimum concentration of additives was determined by the weight of bacterial cellulose, where the medium fermentations were incubated for three days, whereas concentration of cellulose are varied the range of 0.1% - 0.5%. The effects of diffrent concentration of additives on weight of bacterial cellulose are shown in Figure 4.2.

Table 4.2 Dry weight of bacterial cellulose produced by different concentration of MCC

Concentration of MCC (%)	Wet Weight of BC (g)	Dry Weight of BC (g)	Water Content (%)
0.0	7.0680	0.1097	98.45
0.1	10.3472	0.1587	98.47
0.2	8.1608	0.589	92.78
0.3	4.2588	0.445	90.02
0.4	5.0817	0.425	91.24
0.5	2.3108	0.1792	92.25

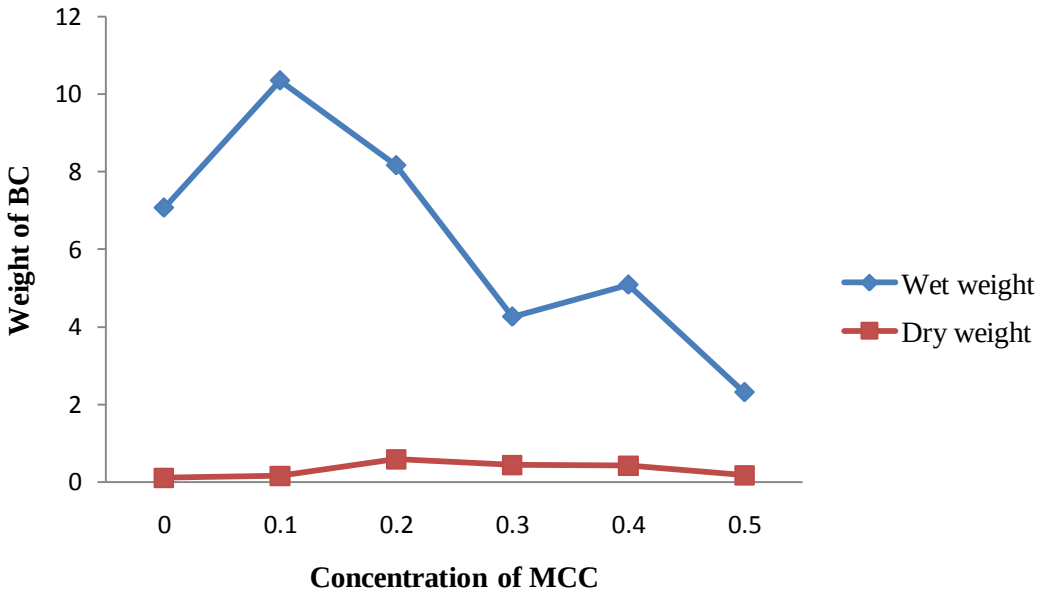


Figure 4.2 Graph of weight of BC vs concentration of MCC

Based on the Figure 4.2, the weight of bacterial cellulose produced increased at concentration of MCC of 0.1%-0.2%. However, the weight of bacterial cellulose decrease as a result of addition of MCC higher than 0.2%. Although the weight of bacterial cellulose decrease as addition of MCC higher than 0.2%, there is slight increase of bacterial cellulose yield as the concentration of MCC increase from 0.3%-0.5%.

Wet weight of bacterial cellulose production by *Acetobacter xylinum* without cellulose was 0.1097g, while bacterial cellulose production was the highest, 0.589 g, at 0.2% MCC. However, there is no enhancing trend toward higher bacterial cellulose yield with increasing MCC concentration. It is suggests that MCC with higher concentration may do harm to bacterial cellulose production due to the increase of medium viscosity. The maximum bacterial cellulose produced by *Acetobacter xylinum* was in the 0.2% MCC addition medium in the conical flask, which is 5.46-fold greater than g in the control medium without MCC.

From the tabulated data, it can be seen that fermentation with addition of microcrystalline cellulose as additives give advantages as it could enhancing bacterial yield but addition of microsrystalline cellulose to the fermentation media cause the water content inside bacterial cellulose lesser than fermentation without cellulose. The percentage of water content inside bacterial cellulose with addition of cellulose ranging 90-92% which is 6-8% lesser than fermentation without MCC.

4.2.2 Effects of Addition of Carboxymethylcellulose (CMC) on BC Production

The optimum concentration of additives was determined by the weight of bacterial cellulose, where the medium fermentations were incubated for three days, whereas concentration of carboxymethylcellulose are varied the range of 0.1% - 0.5%. The effects of diffrent concentration of additives on weight of bacterial cellulose are shown in Figure 4.3.

Table 4.3 Dry weight of bacterial cellulose produced by different concentration of CMC

Concentration of CMC (%)	Wet Weight of BC (g)	Dry Weight of BC (g)	Water Content (%)
0.0	7.0680	0.1097	98.44
0.1	3.8919	0.1075	97.24
0.2	4.1395	0.1139	97.25
0.3	5.1803	0.1295	97.50
0.4	5.8045	0.1320	97.73
0.5	7.0488	0.1778	97.48

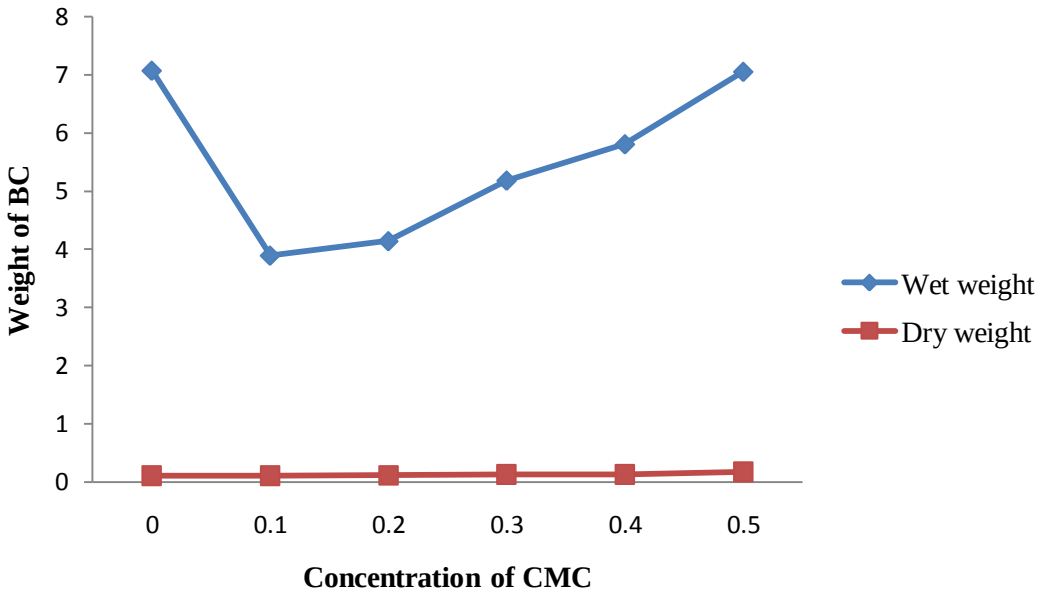


Figure 4.3 Graph of weight of BC vs concentration of CMC

Based on the Figure 4., the weight of bacterial cellulose produced steadily increased at concentration of carboxymethylcellulose of 0.1%-0.5%. The results indicated bacterial cellulose production reached g at an optimal CMC concentrations of 0.5% which is 1.62-fold greater than the control. The production of bacterial cellulose where CMC acted as an additives does not gives significant increasing in bacterial cellulose yield compared to cellulose.

From the tabulated data, it can be seen that fermentation with addition of cellulose as additives give advantages as it could contain more water compared to a medium without cellulose as addition. Although CMC does not enhancing the production of bacterial cellulose as high as cellulose but production of bacterial cellulose have the same percentage of water content inside the bacterial cellulose compared to fermentation without additives. The ability of bacterial cellulose to contain more water would extend new applications of bacterial cellulose.

From this study, the negatively charged water-soluble cellulose derivative, carboxymethylcellulose, was also can be used to enhance bacterial cellulose production in static culture.

4.2.3 Effects of Addition of Sodium Alginate on BC Production

The optimum concentration of additives was determined by the weight of bacterial cellulose, where the medium fermentations were incubated for three days, whereas concentration of sodium alginate are varied the range of 0.1% - 0.5%. The effects of diffrent concentration of additives on weight of bacterial cellulose are shown in Figure 4.4.

Table 4.4 Dry weight of bacterial cellulose produced by different concentration of sodium alginate

Concentration of NaAlg (%)	Wet Weight of BC (g)	Dry Weight of BC (g)	Water content (%)
0.0	7.0680	0.1097	98.45
0.1	6.9703	0.1680	97.59
0.2	4.4205	0.1522	96.56
0.3	3.8237	0.1352	96.46
0.4	3.7363	0.1188	96.82
0.5	3.6209	0.1118	96.91

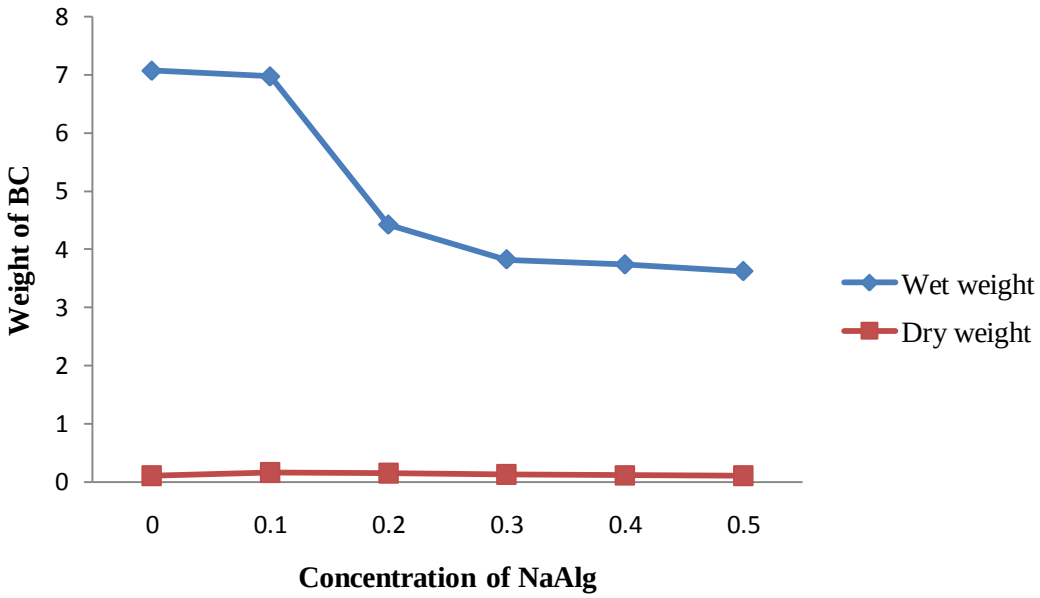


Figure 4.4 Graph of weight of BC vs concentration of NaAlg

From the graph, the BC yield increasing when 0.1% w/w of sodium alginate added into the medium. Unfortunately, the trend of graph decreasing when 0.2% w/w or more of sodium alginate added into the medium.

Zhou *et al.*, (2007) stated that the addition of 0.04% sodium alginate, NaAlg promoted cell growth and enhanced bacteria cellulose production. However, there is no enhancing trent toward higher bacterial cellulose production with increasing sodium alginate, concentration, which suggests that sodium alginate with higher concentration may do harm to bacterial cellulose production due to an increase of broth viscosity (Zhou *et al.*, 2007). Addition of sodium alginate higher than 0.2% seemed to have a negative effect on *Acetobacter xylinum* strain and resulted in a decrease of bacterial cellulose production (Cheng *et al*., 2009).

4.2.4 Determination of The Optimum Of Parameter On Bacterial Cellulose Production

Optimization on the parameters in bacterial cellulose production is performed using Least-Square Regression method. The parameters involved are concentration of each additives. The data of the parameters are determined from the previous result.

From the Figure 4.5, a curve would be better suited to fit the data. The least-squares procedure can be readily extended to fit the data to a higher-order polynomial. For this data, a second-order polynomial or quadratic will be fit.

$$y = a_0 + a_1 x + a_2 x^2 + e \tag{4.1}$$

For this case, the sum of the squares of the residuals is

$$S_r = \sum_{i=1}^{n} (y_i - a_0 - a_1 x_i - a_2 x_i^2)^2 \tag{4.2}$$

The set of normal equations,

$$\begin{aligned} (n)a_0 + \left(\sum x_i\right) a_1 + \left(\sum x_i^2\right) a_2 &= \sum y_i \\ \left(\sum x_i\right) + \left(\sum x_i^2\right) a_1 + \left(\sum x_i^3\right) a_2 &= \sum x_i y_i \\ \left(\sum x_i^2\right) + \left(\sum x_i^3\right) a_1 + \left(\sum x_i^4\right) a_2 &= \sum x_i^2 y_i \end{aligned} \tag{4.3}$$

where all summations are from i = 1 through n. The above three equations are linear and have three unknowns a_0, a_1, and a_2. The coefficients of the unknowns can be calculated directly from the data.

The standard error is formulated

$$s_{y/x} = \sqrt{\frac{S_r}{n - (m+1)}} \tag{4.4}$$

The Newton-Raphson method is an open method that finds the root x of a function such that $f(x) = 0$. The method is summarized as

$$x_{i+1} = x_i - \frac{f(x_i)}{f'(x_i)} \tag{4.11}$$

A similar open approach can be used to find an optimum of $f(x)$ by defining a new function, $g(x) = f'(x)$. Thus, because the same optimal value x satisfies both

$$g(x) = f'(x) = 0 \tag{4.12}$$

it can use the following,

$$x_{i+1} = x_i - \frac{f'(x_i)}{f''(x_i)} \tag{4.13}$$

as a technique to find the minimum or maximum of $f(x)$.

Microcrystalline Cellulose (MCC)

m = 2	$\sum x_i$ = 1.5	$\sum x_i^4$ = 0.0978
n = 6	$\sum y_i$ = 2.886	$\sum x_i y_i$ = 0.6623
y_{mean} = 0.481	$\sum x_i^2$ = 0.54	$\sum x_i^2 y_i$ = 0.2525
x_{mean} = 0.25	$\sum x_i^3$ = 0.224	

Therefore, the simultaneous linear equations are

$$\begin{bmatrix} 6 & 1.5 & 0.54 \\ 1.5 & 0.54 & 0.224 \\ 0.54 & 0.224 & 0.0978 \end{bmatrix} \begin{Bmatrix} a_0 \\ a_1 \\ a_2 \end{Bmatrix} = \begin{Bmatrix} 2.886 \\ 0.6623 \\ 0.2525 \end{Bmatrix} \tag{4.17}$$

Solving these equations gives

$$a_0 = -0.2385 \tag{4.18}$$

$$a_1 = 6.2496 \tag{4.19}$$

$$a_2 = -11.7898 \tag{4.20}$$

The least-square quadratic equation for the graph is

$$y = -0.2385 + 6.2496x - 11.7898x^2 \quad (4.21)$$

The standard error of the estimate based on the regression polynomial using Equation 4.4,

$$s_{y/x} = 0.2714 \quad (4.22)$$

From the Equation 4.22, with an initial guess of $x_0 = 0.15$,

The first and second derivatives of the function can be evaluated and substituting into Equation 4.13,

$$y' = 6.2496 - 23.5796x \quad (4.23)$$

$$y'' = -23.5796 \quad (4.24)$$

After the process was repeated, the optimum value is

$$x_{opt} = 0.2713$$

From the calculation above, the optimum % w/w of cellulose is 0.2713.

Carboxymethycellulose (CMC)

From the graph, the optimum value is $x_{opt} = 0.5$

Sodium Alginate (NaAlg)

Substituting the data into Equation 4.3,

m = 2	$\sum x_i = 1.5$	$\sum x_i^4 = 0.0978$
n = 6	$\sum y_i = 0.7957$	$\sum x_i y_i = 0.1744$
$y_{mean} = 0.1326$	$\sum x_i^2 = 0.54$	$\sum x_i^2 y_i = 0.0652$
$x_{mean} = 0.25$	$\sum x_i^3 = 0.224$	

Therefore, the simultaneous linear equations are

$$\begin{bmatrix} 6 & 1.5 & 0.54 \\ 1.5 & 0.54 & 0.224 \\ 0.54 & 0.224 & 0.0978 \end{bmatrix} \begin{Bmatrix} a_0 \\ a_1 \\ a_2 \end{Bmatrix} = \begin{Bmatrix} 0.7957 \\ 0.1744 \\ 0.0652 \end{Bmatrix} \quad (4.25)$$

Solving these equations gives

$$a_0 = 0.4258 \quad (4.26)$$

$$a_1 = -3.2278 \quad (4.27)$$

$$a_2 = 5.7085 \quad (4.28)$$

The least-square quadratic equation for the graph is

$$y = 0.4258 - 3.2278x + 5.7085x^2 \quad (4.29)$$

The standard error of the estimate based on the regression polynomial is

$$s_{y/x} = 0.2621 \quad (4.30)$$

From the Equation 4.29, with an initial guess of $x_0 = 0.10$

The first and second derivatives of the function can be evaluated and substituting into Equation 4.13,

$$y' = 41.3624\,x - 11.5141 \quad (4.31)$$

$$y'' = 41.3624 \quad (4.32)$$

After the process was repeated, the optimum value is

$$x_{opt} = 0.2827$$

From the calculation above, the optimum % w/w of cellulose is 0.2827.

Comparison of the bacterial cellulose production are shown in the Figure 4.5 while in Figure 4.6 shows the maximum yield of bacterial cellulose yield for each different type of fermentation medium and additives.

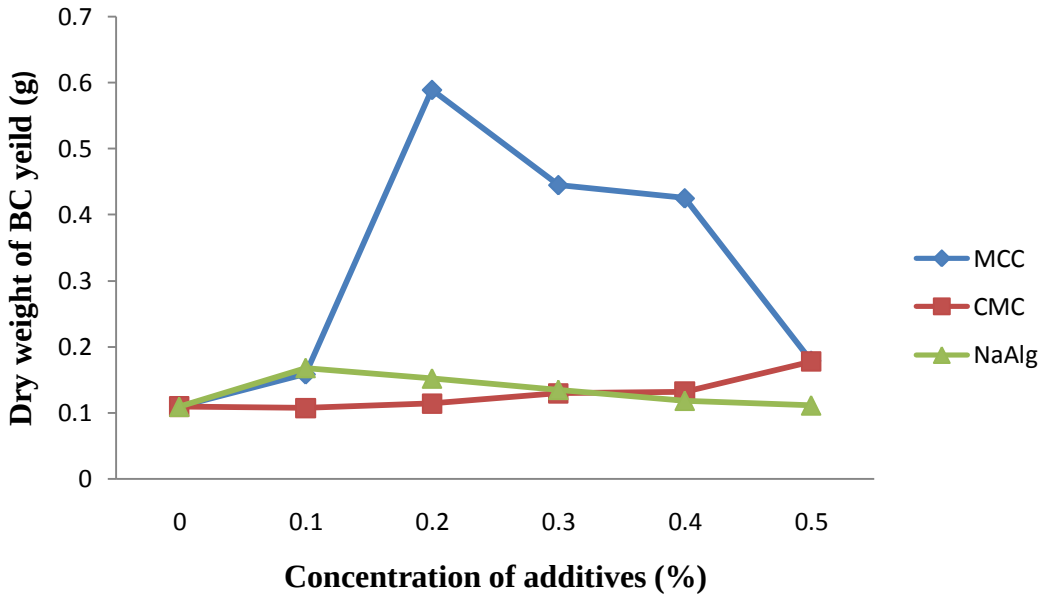


Figure 4.5 Comparison of BC yield for each concentration of additives.

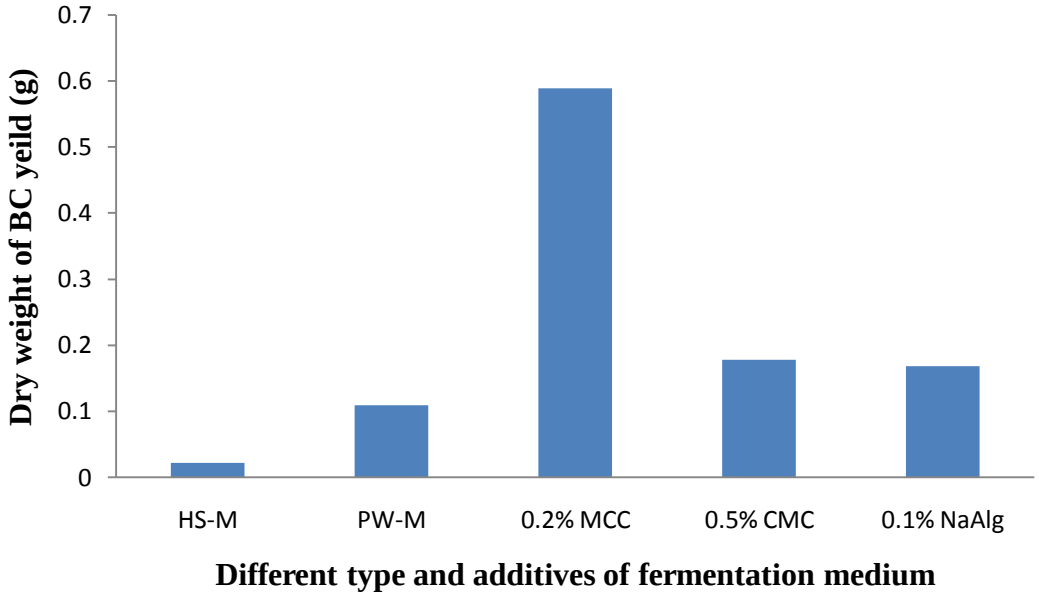


Figure 4.6 Comparison of the maximum yield of bacterial cellulose yield for each different type of fermentation medium and additives.

Every cellulose pellicle fermented in different type of fermentation medium and additives having different percentage of water content. Figure 4.7 shows the comparison of water content in each medium.

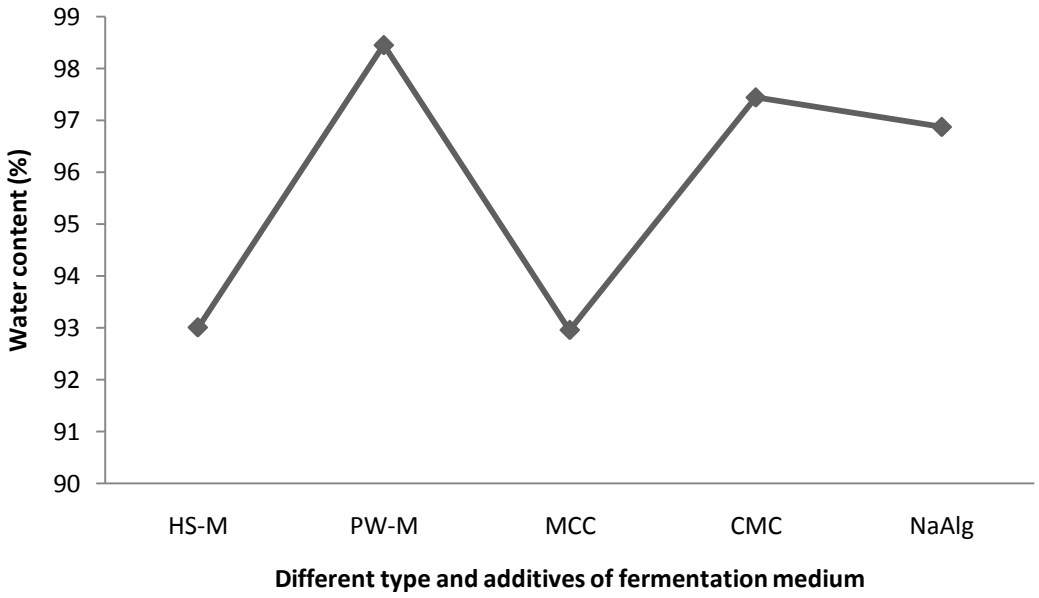
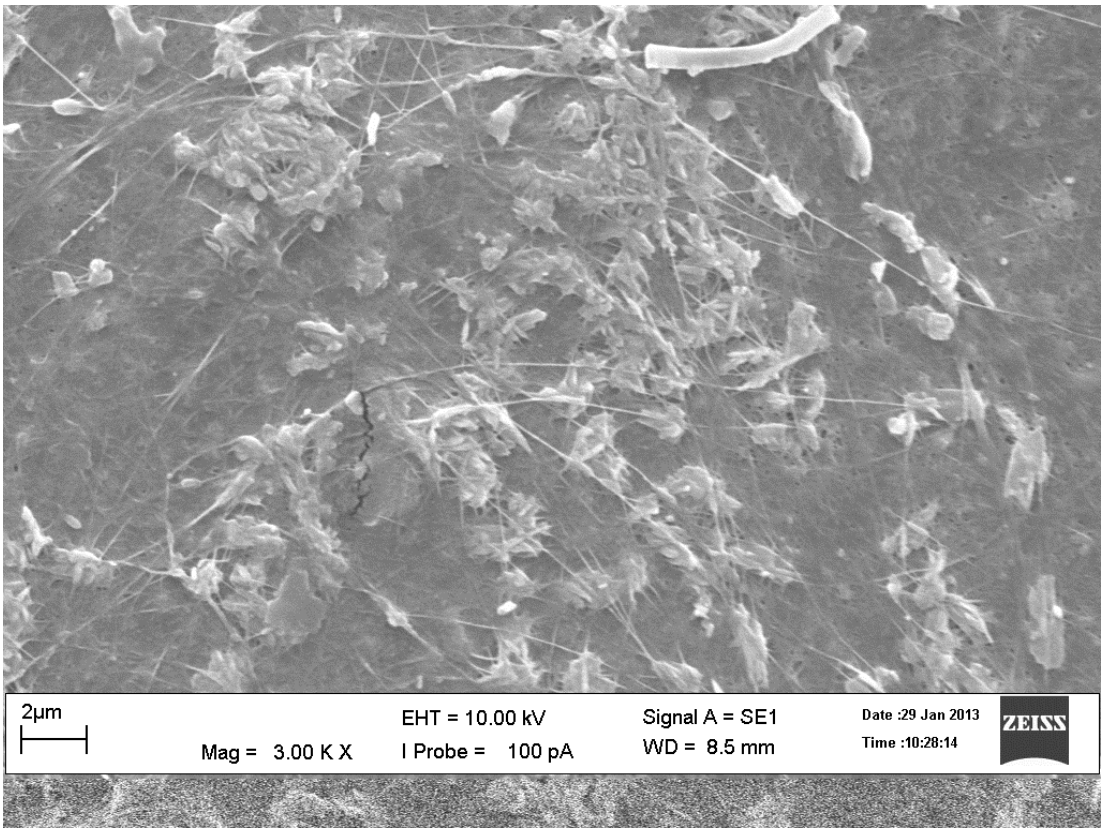


Figure 4.7 Comparison of water content in different type and additives of fermentation medium

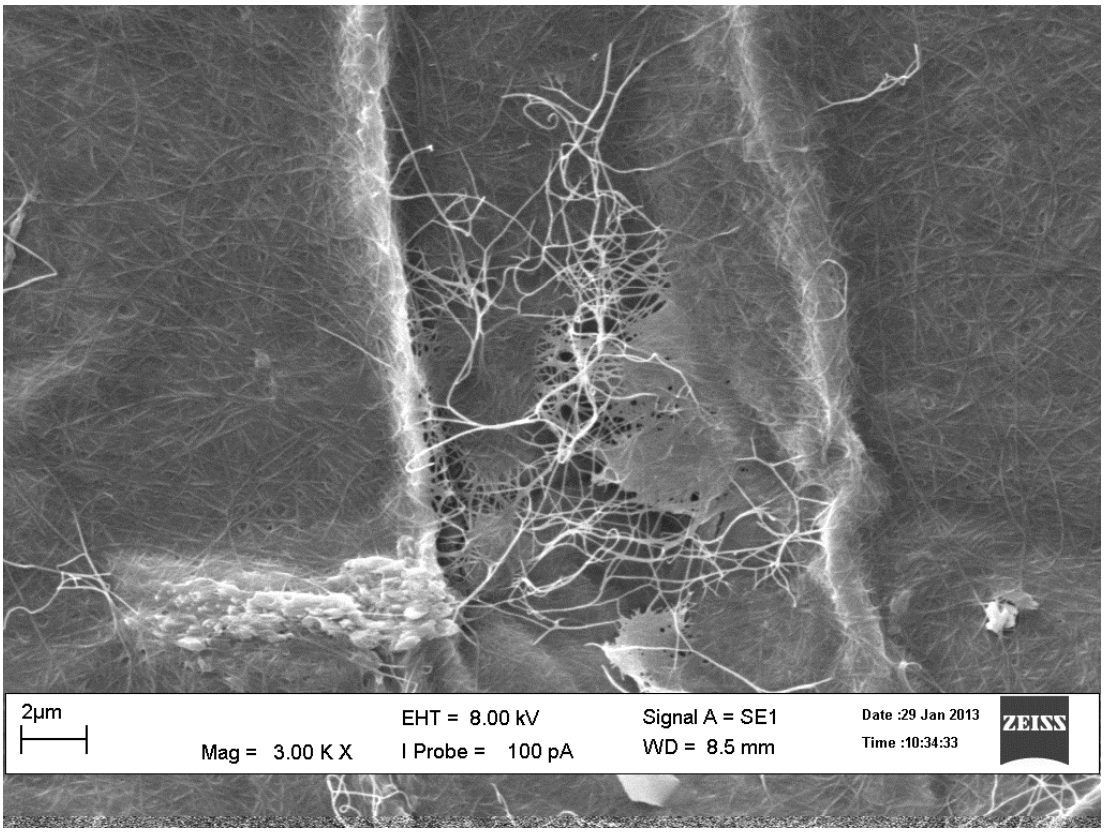
From the Figure 4.5 and Figure 4.6, the best additives and its concentration in enhancing bacterial cellulose production is 0.2% w/w of MCC. From the Figure 4.6, the addition of additives into the fermentation medium proved that it could enhance the production of bacterial cellulose. Besides that, bacterial cellulose produced by adding different additives having different level of water content, which would extend new applications of bacterial cellulose, because some of the bacterial cellulose applications depend on its water content. From the Figure 4.7, bacterial cellulose produced with addition of MCC had low percentage of water content compared to addition of CMC or NaAlg. Although addition of MCC enhance BC production greatly, but the percentage of water content was the lowest. Hence, it can be conclude that the production of bacterial cellulose can be enhance with addition of CMC, MCC or NaAlg, but the best additives to be used depends on the application of the bacterial cellulose.

4.3 BACTERIAL CELLULOSE STRUCTURE

The morphological structures of bacterial cellulose obtained with and without addition of additives were analyzed by Scanning Electron Microscopy (SEM). The morphology of bacterial cellulose strictly depends on culture condition (Watanabe *et al*., 1998; Yamanaka *et al*., 2000). In static conditions, bacteria was accumulated cellulose mats on the surface of medium fermentation at the liquid interface with oxygen-rich air. The results show that these samples consist of ultrafine fibrils, which form the reticulated structure (Zhou *et al*., 2007).



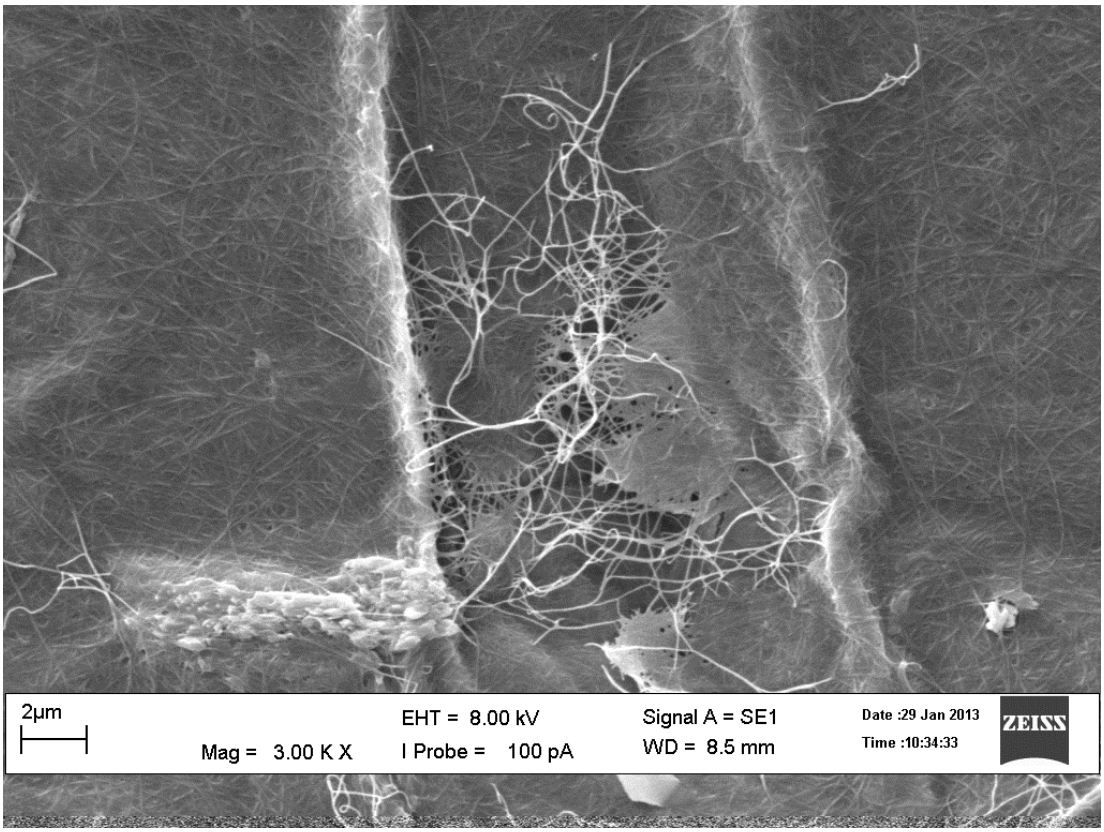
(a)



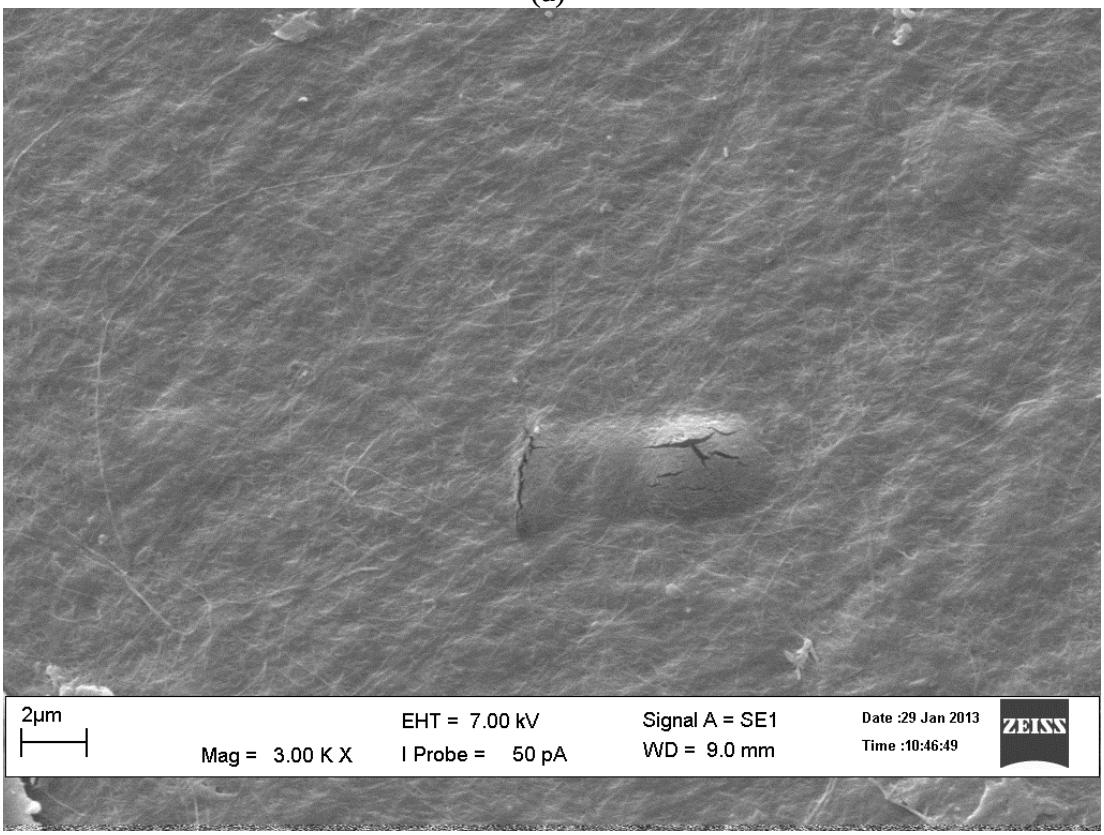
(b)

Figure 4.8 Scanning Electron microscopy (a) surface of bacterial cellulose from HS-medium and (b) surface of bacterial cellulose from pineapple waste

SEM images for the surface morphology of the film sample were shown in Figure 4.8. Figure 4.8 (a) present the surface image of the film from the sample cultured in the HS-medium while Figure 4.8 (b) present the surface image of the film from the sample that optimized in PW-M. In static conditions, bacteria was accumulated cellulose mats on the surface of medium fermentation at the liquid interface with oxygen-rich air. The results show that these samples consist of ultrafine fibrils, which form the reticulated structure. But, both figure reveal some obvious differences in surface structures and ribbons width. BC produced in the PW-M was characterized by more compact and highly extended structure than that in the HS-medium. In contrast, BC produced in HS-medium contained nets and many particles, which probably composed of impurities.



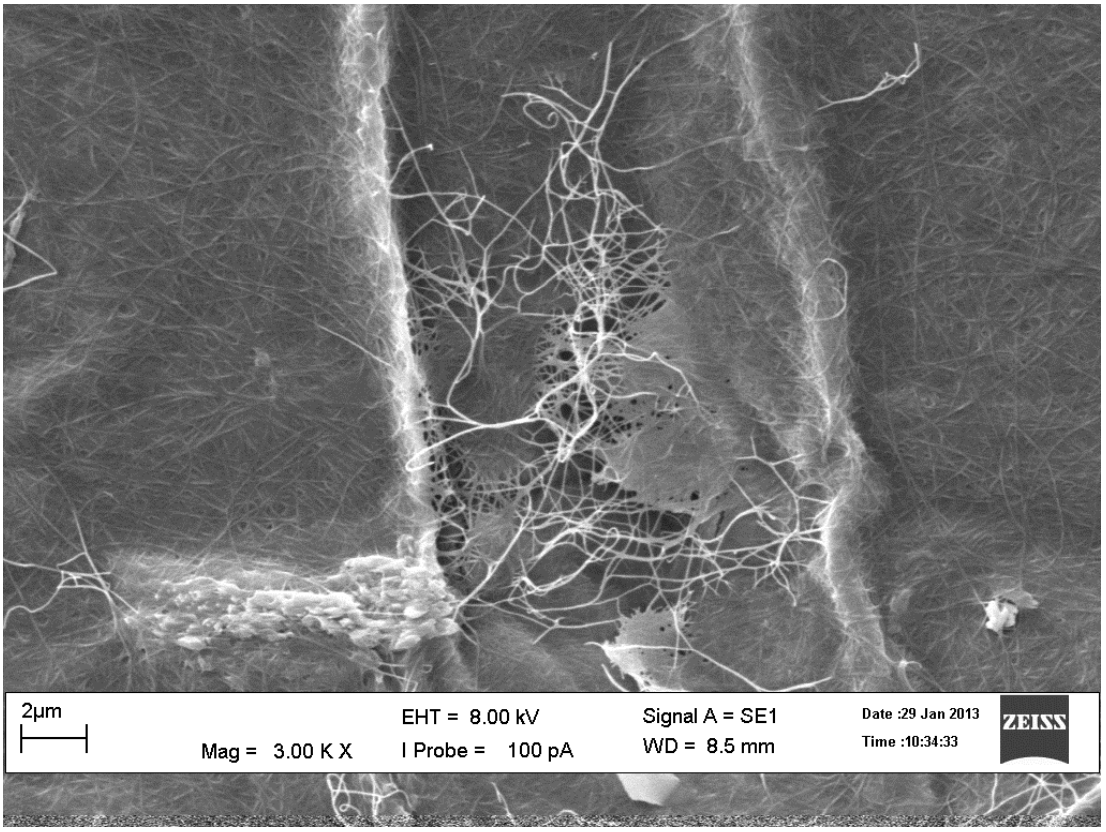
(a)



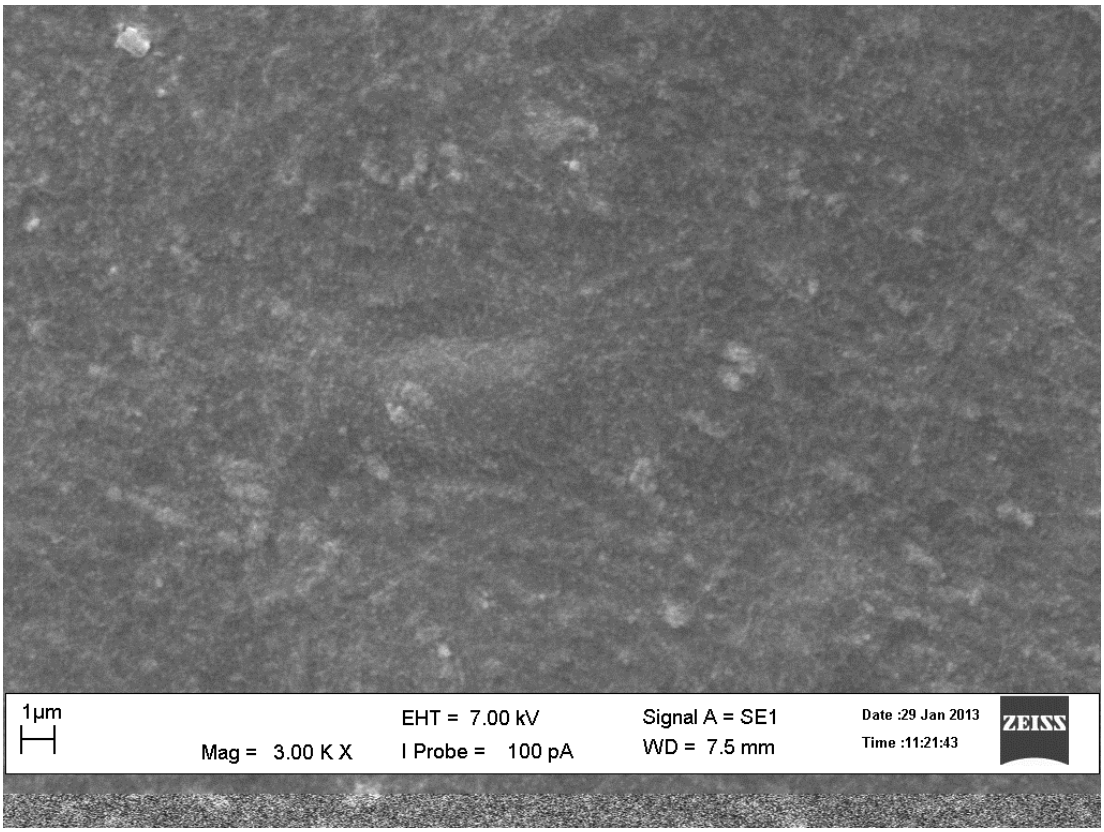
(b)

Figure 4.9 Scanning Electron microscopy (a) surface of bacterial cellulose from pineapple waste and (b) surface of bacterial cellulose in the 0.2% MCC-added medium

Figure 4.9 (a) present the surface image of the film from the sample cultured in the PW-M while Figure 4.9 (b) present the surface image of the film from the sample that having optimum yield of 0.2% MCC-added medium. The results show that these samples having quite similar structure but careful observations of the photograph show some difference in surface structure. BC produced in the conical flask in the PW-M with 0.2% MCC-added was characterized by more compact.



(a)

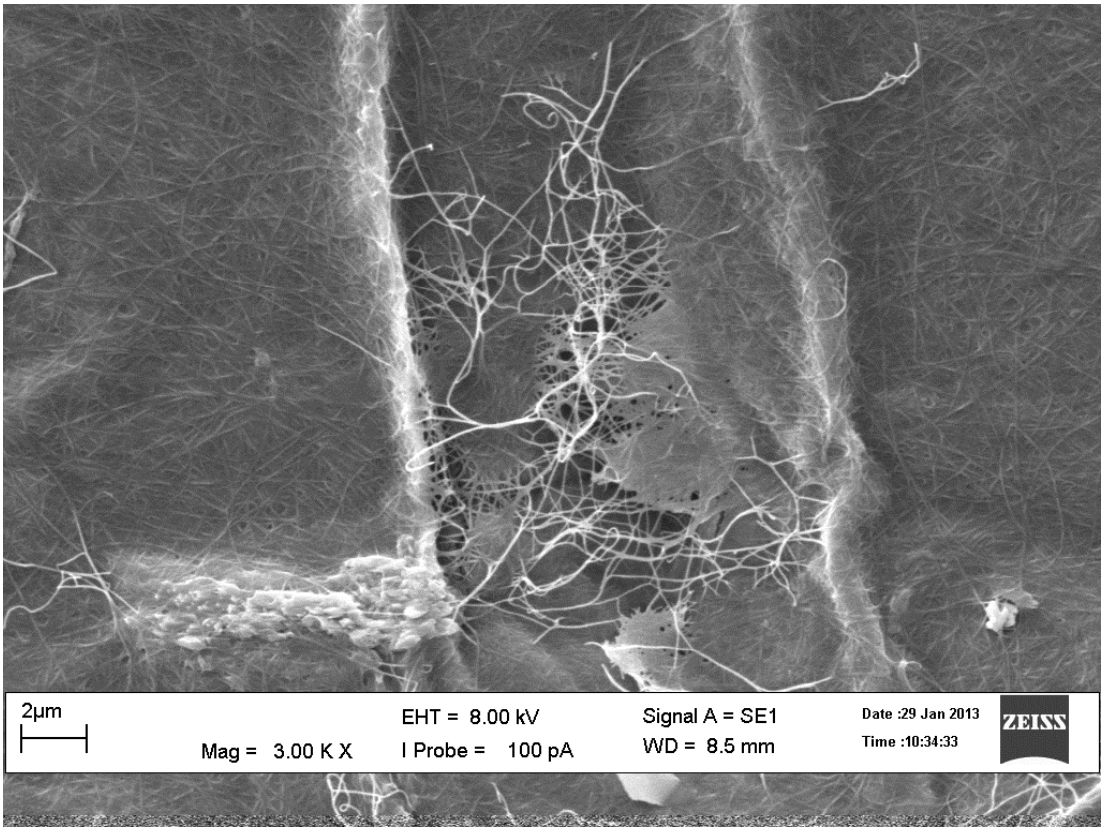


(b)

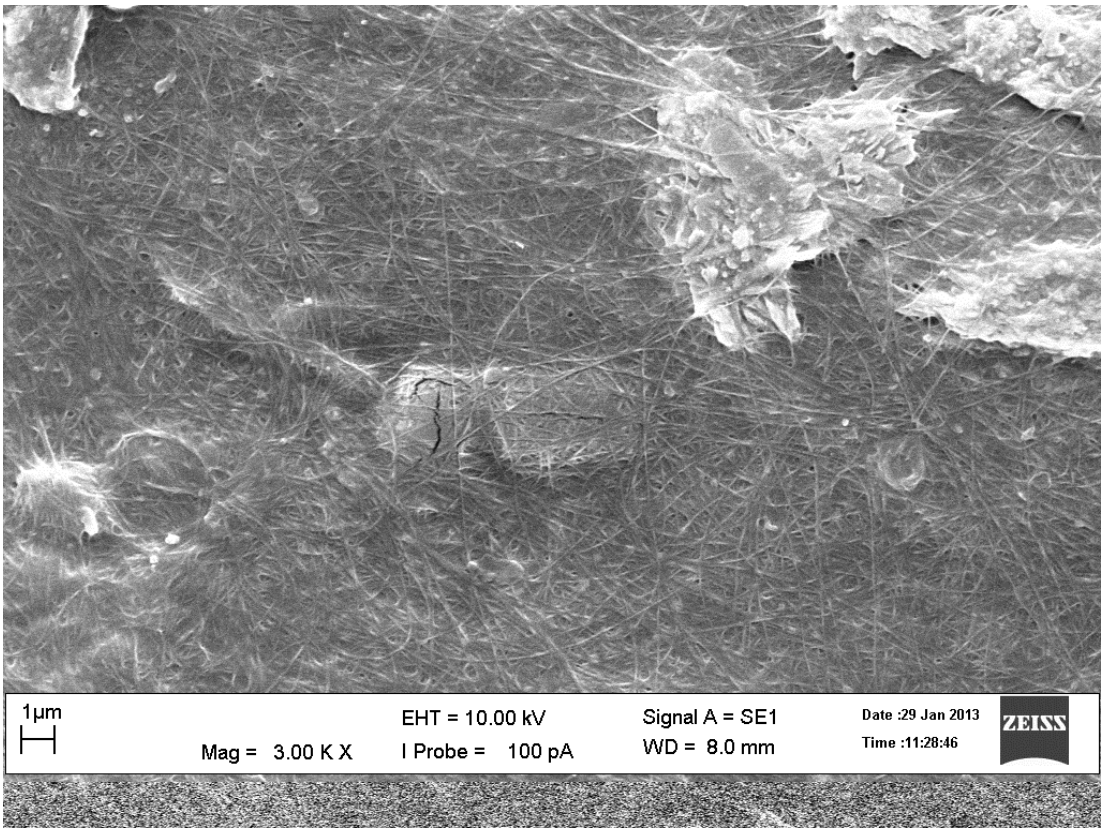
Figure 4.10 Scanning Electron microscopy (a) surface of bacterial cellulose from pineapple waste and (b) surface of bacterial cellulose in the 0.5% CMC-added medium

Figure 4.10 (a) present the surface image of the film from the sample cultured in the PW-M while Figure 4.10 (b) present the surface image of the film from the sample that having optimum yield of 0.5% MCC-added medium. As other polymeric additives, CMC exhibited its ability to enhanced BC production. This can possibly be attributed to an increase in the solubility of BC in the presence of the highly soluble CMC, which may bind to the surface of the BC fibrils (Cheng *et al*., 2009). The results show that these samples having quite similar structure but careful observations of the photograph show some difference in surface structure. BC produced in the conical flask in the PW-M with 0.5% MCC-added was characterized by more compact. These samples showed interweaving BC fibers. As CMC concentration increased, the width of cellulose fiber decreased slightly and the

photographs revealed some differences in surface structure. The CMC-added BC kept its interweaving property and had some debris on its surface.



(a)



(b)

Figure 4.11 Scanning Electron microscopy (a) surface of bacterial cellulose from pineapple waste and (b) surface of bacterial cellulose in the 0.1% NaAlg-added medium

SEM images for the comparison of surface morphology of the film sample were shown in Figure 4.11. Figure 4.8 (a) present the surface image of the film from the sample cultured in the PW-M without containing additives while Figure 4.8 (b) present the surface image of the film from the sample containing 0.1% NaAlg. BC produced in the conical flask in the control medium without NaAlg was characterized by more compact and highly extended structure than that in the NaAlg-added medium. While, BC produced in the NaAlg-added medium contained nets with many holes. In the surface of net there were many particles, which were probably composed of NaAlg. The inside structure of BC was made up of many ultrafine ribbons, which were curve and entangled with each other. The ribbons of BC with NaAlg had a broader range of width than those without NaAlg.

The bacterial cellulose extracellular excretion can form aggregated fibrils which crystallize into ribbons and assemble into thick cellulosic mat known as pellicle (Suwannapinunt *et al*., 2007). For a static culture, a leather-like pellicle of overlapping and intertwined ribbons forms (Jonas and Farah, 1998). As carboxymethylcellulose concentration increased, the width of cellulose fiber decreased slightly (Cheng *et al*., 2009). Haigler *et* al., (1982) suggested that the addition of carboxymethycellulose can change morphology of bacterial cellulose into separate, intertwining bundles of microfibrils but do not prevent microfibril crystallization. Bacterial cellulose produced in the NaAlg-added culture contained nets with many hole which shows that the ribbons of bacterial cellulose with NaAlg had a broader range of width (Zhou *et al*., 2007). The morphology structures of BC obtained will influenced BC properties such as water-holding capacity, viscosity of wet BC suspension and mechanical properties.

4.4 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY ANALYSIS

Fourier Transform Infrared (FTIR) spectroscopy of bacterial cellulose sample was carried out in order to detect any peak shift that could be attributed to weak interactions between the two polymers (Zhou *et al*., 2007). The absorption maxima of stretching vibration of hydroxyl bonding shifted toward lower wavenuumbers and the hrdroxyl stretching bands became much broader in the presence of sodium alginate (Zhou *et al*., 2007). The characteristics bands that appeared were list in Table 4.5.

Table 4.5 List of bonding that present in Bacterial Cellulose

Chemical bonding	BC peak(cm)	References
Carbonyl group(C=O)	1650 cm^{-1}	Guo et al., 2008
C-O-H	672 cm^{-1} and 711 cm^{-1}	Sun *et al*., 2008
C-H Bonding	1430 cm^{-1} to 1290 cm^{-1}	Hwang, 2007
C-0 strecthing at C_3	1060 cm^{-1}	Sun *et al*., 2008
C-O stretching at C_6	1030 cm^{-1}	Sun *et al*., 2008
C-O-C stretching at b-glycosidic linkage	116 cm^{-1} and 900cm^{-1}	Sun *et al*., 2008

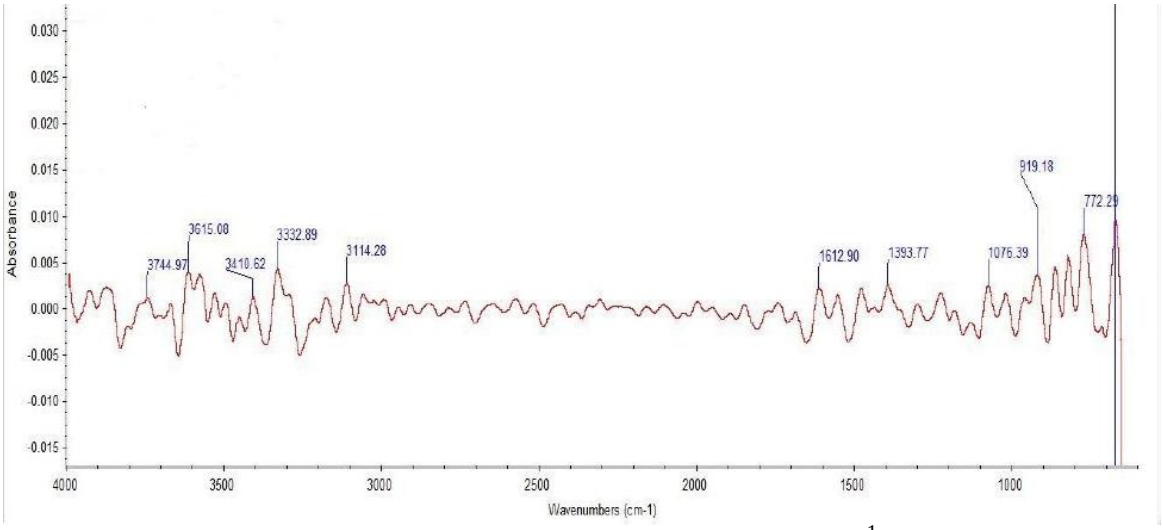


Figure 4.12 FTIR spectra for bacterial cellulose (cm^{-1})

Based on the result obtained from the FTIR analysis, the Figure 4.12 show the absorbance peak at between 3744.97 cm^{-1} - 3114.28 cm^{-1} that was originated from the OH strectching. This result had been proven from the previous study by Parmjit *et al.*, (2008) where the peaks that appear near in range of 3853 - 3256 cm^{-1} was hydroxyl functional group. There also showed also showed the absorbance peak at 1393.77 cm^{-1} where according to the Sun *et al.*, (2008) reported, several bands typical for bacterial cellulose were shown in the region of 1500-1235 cm^{-1} due to in plane bending vibration of CH_2, CH, or OH groups. From the analysis, absorbance also appear at 1076.39 cm^{-1} which the strong band in bacterial cellulose that appear near 1081 cm^{-1} wavenumber was the representative of the C-O-C asymmetric

stretching (Sun *et al*., 2008). Besides that there was also absorbance that appears at 1612.90 cm^{-1}. Based on the FT-IR analysis, it was proven that the gelatinous membrane that produced from the fermentation process using pineapple residue was bacterial cellulose.

C-OH bonding (Sun *et al*., 2008), anti-symmetric bridge stretching of C–O–C (Sun *et al*., 2008), H bond in OH group, aliphatic OH group (Guo *et al*., 2008), C-O stretching at C3, C-O stretching and C-C stretching at C6 (Sun *et al*., 2008) are the chemical bonding that present in cellulose molecular structure. Figure 4.12 show the molecular structure of the BC.

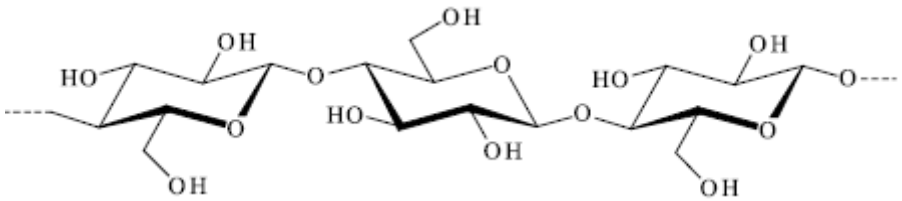


Figure 4.13 Molecular structure of cellulose
(Sources : Klemm *et al*., 2001)

CHAPTER 5

CONCLUSION & RECOMMENDATIONS

5.1 Conclusion

The morphological, chemical and structural characteristics of cellulose produced in non-conventional culture media like pineapple waste were examined. The production bacterial cellulose was relatively high with similar properties to that produced in HS-medium. These results suggest that it is possible to produce bacterial cellulose from low-cost resources in order to increase its production to a larger scale.

Acetobacter xylinum was cultured in pineapple waste medium with additives to produce high yield bacterial cellulose. Three different additives, microcrystalline cellulose, carboxymethylcellulose and sodium alginate, were applied. The results showed that additives affected BC yields. BC produced by *Acetobacter xylinum* can be enhanced and modified by introducing additives in fermentation medium. The optimal additive was chosen based on the amount of BC produced which is 0.27 % w/w of microcrystalline cellulose. Besides, in an economic point of view, the pineapple waste medium requires a low cost operation and easy to perform.

5.2 Recommendations

In this research, lab scale is used for bacterial cellulose production. For future research, it is highly recommended to scale up the bacterial cellulose production. In this research, pineapple residue was used as a substrate to replace sugar in bacterial cellulose production with different types of additions; so that it is recommended to extend the research by using a different kind of additions that have a high amount of sugar content.

Besides that before start the preparation of the media for the future research, pH and sugar content that presents in pineapple residue needs to be checked using the pH meter for pH and UV-Vis spectrophotometer for sugar content. From this results, the progress of additives effect on the bacterial cellulose production can be studied and determined which one have fastest effect on the BC yield.

REFERENCES

Abdullah and Hanafi, Mat. 2008. *Characterisation of solid and liquid pineapple waste.* Ph.D. Thesis. Department of Chemical Engineering, Faculty of Engineering Diponegoro University, Indonesia.

Bielecki, P. D., Krystynowicz, D. E., Mariannaturkiewicz, P. D. and Kalinowska, D. E. 2000. *Bacterial Cellulose*.39: 40-46.

Budhiono, A., Rosidi, B., Taher, H., and Iguchi, M. 1999. Kinetic aspect of bacterial cellulose formation in *nata-de-coco* culture system. *Carbohydrate Polymers*. 40: 137-143.

Castro, C., Robin, Z., Jean L. P., Gloria, C., Inaki, M. and Piedad, G. 2010. Structural characterization of bacteria cellulose produced by *Gluconacetobacter* wastes. *Carbohydrate Polymers*. 84: 96-102.

Chawla, P. R., Bajaj, I. B., Survase, S. A. and Singhal, R. S. 2008. Microbial cellulose: fermentative production and application. *Food Technology And Biotechnology* .47 (2): 107-124.

Cheng, K. C., Catchmark J. M., Demerci, A. 2009. Effect of ddifferent additives on bacterial cellulose production by Acetobacter xylinum and analysis of material property. *Cellulose* 16: 1033-1045.

Fontana, J, D., De Souzza, A. M., Fontana, C.K., Torriani, I, C., Moresch, J.C., and Gallotti, B..J *et al*., 1990. Acetobacter cellulose pellicle as a temporary skin substitute. *Aplied Biochemistry and Biotechnology*: 24(25):253-264.

Hestrin, H. and Schramm, M.1954. Synthesis of cellulose by Acetobacter xylinum. *Biochem. J*. 58:345-352.

Jagannath, A., Kalaiseluan, A., Manjunatha, S.S., Raju, P.S., and Bawa, A.S. 2008. The effect of pH, sucrose and ammonium sulphate concentrations on the production of bacterial cellulose (*Nata-de-coco*). *World J Microbial Biotechnology*. 24: 2593-2599.

Jonas, R. and Farah, L.F. 1998. Production and application of microbial cellulose. *Polym. Degrad. Stab*. 59: 101-106.

Krueger, D.A., Krueger, R.G. and Maciel. J. 1992. Composition of pineapple juice. *Journal International AOAC*. 75(2): 280-282

Lynd L. R., Weimer, P. J., van Zyl W. H. 2002. Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol Mol Biol* 66(3): 506-577.

Oh ,S.Y., Yoo D.l., Shin, Y. and Kim H.C. 2005. Crystalline structure analysis of cellulose treated with sodium hydroxide and carbon dioxide by means of X-ray diffraction and FTIR spectroscopy. *Carbohydr. Res*.340:2376-2391.

Pourramezan, G.Z., Roayaei, A.M., Qezelbash, Q.R. 2009. Optimization of culture conditions for bacterial cellulose production by *Acetobacter xylinum sp. 4B-2.Biotechnol*. 8: 150- 154.

Ring, D., Nashed, W. and Dow, T. 1986. Liquid loaded pad for medical applications.Us Patent. 4: 588-400

Vandamme, E.J., De Baets, S., Vanbalen, A., Joris, K., and De Wulf, P. 1998. Improved production of bacterial cellulose and its application potential. *Polymer degradation and stability*. 59:93-99.

Watanabe, K., Tabuchi, M., Moringa, Y., and Yoshinaga, F. 1998. Structural features and properties of bacterial cellulose produced in agitated culture. *Cellulose*. 5(3): 187-200.

Yamanaka, S. and Sugiyama, J. 2000. Structural modification of bacterial cellulose. *Cellulose*. 7(3): 213-225.

Zhou L. L. , Sun D. P., Hu L. Y. 2007. Effect of addition of sodium alginate on bacterial cellulose production by *Acetobacter xylinum*. *J Ind Microbiol Biotechnol* 34(7): 483-489.

APPENDICES

Appendix A Research methodology

Figure A.1 Pineapple waste was blended using blender

Figure A.2 Separation of pineapple residue from pineapple juice

Figure A.3 PW-M was autoclaved and ready for fermentation process

Figure A.4 After 3 days of incubation

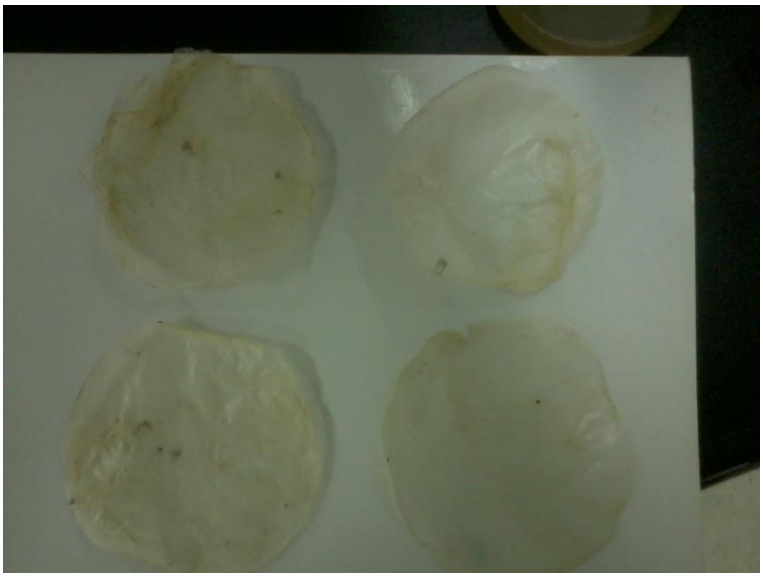


Figure A.5 Cellulose film after being treated and dried

Appendix B Calculation for optimization

Set of normal equations

$$(n)a_0 + \left(\sum x_i\right)a_1 + \left(\sum x_i^2\right)a_2 = \sum y_i$$

$$\left(\sum x_i\right) + \left(\sum x_i^2\right)a_1 + \left(\sum x_i^3\right)a_2 = \sum x_i y_i$$

$$\left(\sum x_i^2\right) + \left(\sum x_i^3\right)a_1 + \left(\sum x_i^4\right)a_2 = \sum x_i^2 y_i \quad (4.3)$$

Second-order polynomial equation

$$y = a_0 + a_1 x + a_2 x^2 + e \quad (4.1)$$

Sum of the squares of the residuals

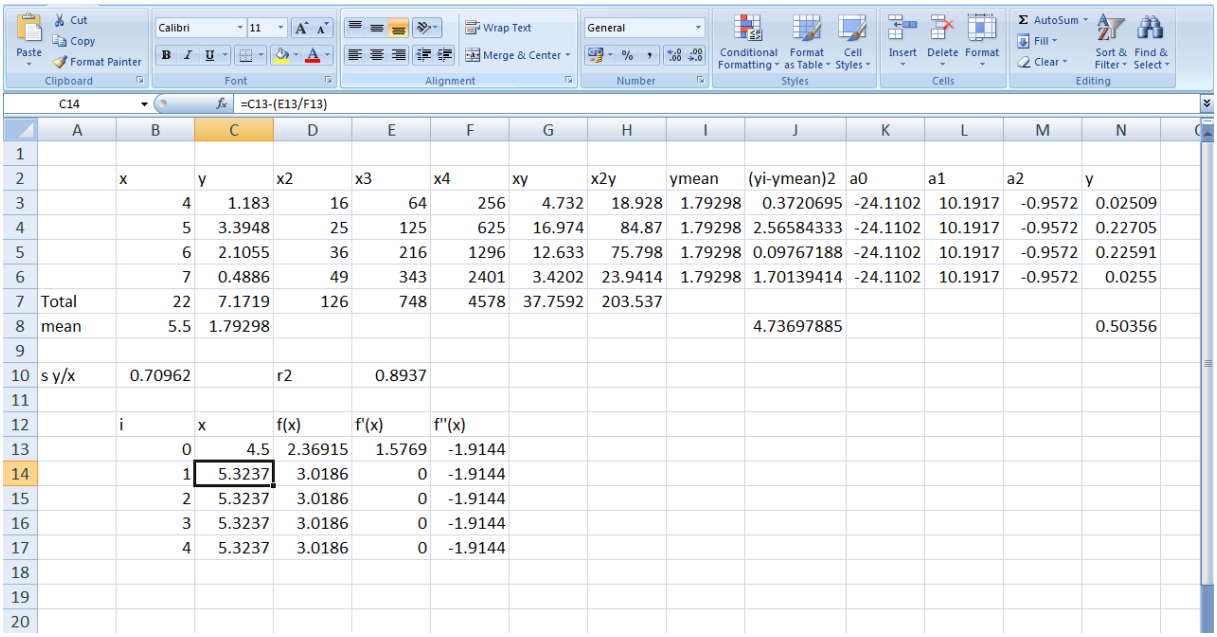
$$S_r = \sum_{i=1}^{n} (y_i - a_0 - a_1 x_i - a_2 x_i^2)^2 \quad (4.2)$$

Standard error

$$s_{y/x} = \sqrt{\frac{S_r}{n - (m+1)}} \quad (4.4)$$

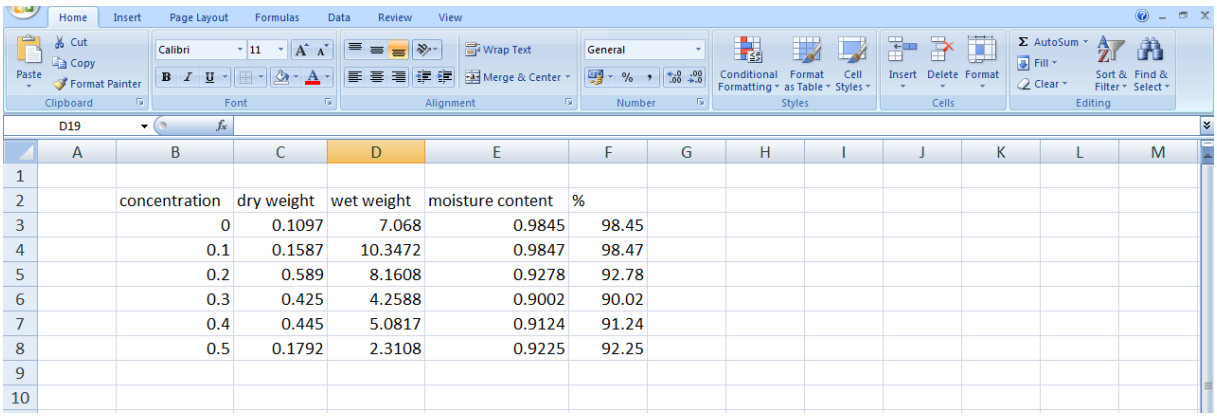
Newton-Raphson method

$$x_{i+1} = x_i - \frac{f'(x_i)}{f''(x_i)} \quad (4.13)$$



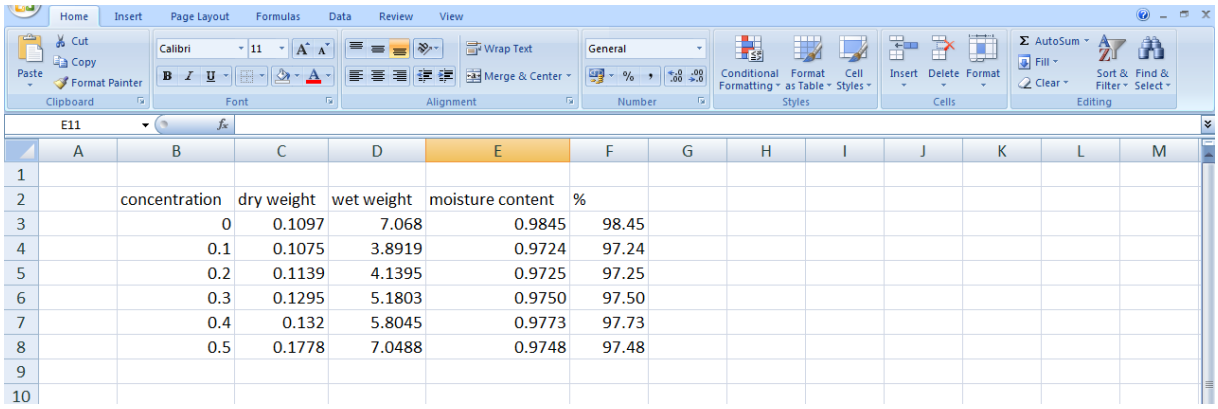
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1														
2		x	y	x2	x3	x4	xy	x2y	ymean	(yi-ymean)2	a0	a1	a2	y
3		4	1.183	16	64	256	4.732	18.928	1.79298	0.3720695	-24.1102	10.1917	-0.9572	0.02509
4		5	3.3948	25	125	625	16.974	84.87	1.79298	2.56584333	-24.1102	10.1917	-0.9572	0.22705
5		6	2.1055	36	216	1296	12.633	75.798	1.79298	0.09767188	-24.1102	10.1917	-0.9572	0.22591
6		7	0.4886	49	343	2401	3.4202	23.9414	1.79298	1.70139414	-24.1102	10.1917	-0.9572	0.0255
7	Total	22	7.1719	126	748	4578	37.7592	203.537						
8	mean	5.5	1.79298							4.73697885				0.50356
9														
10	s y/x	0.70962		r2	0.8937									
11														
12		i	x	f(x)	f'(x)	f''(x)								
13		0	4.5	2.36915	1.5769	-1.9144								
14		1	5.3237	3.0186	0	-1.9144								
15		2	5.3237	3.0186	0	-1.9144								
16		3	5.3237	3.0186	0	-1.9144								
17		4	5.3237	3.0186	0	-1.9144								
18														
19														
20														

Figure B.1 Excel worksheet for calculating the optimum value of pH



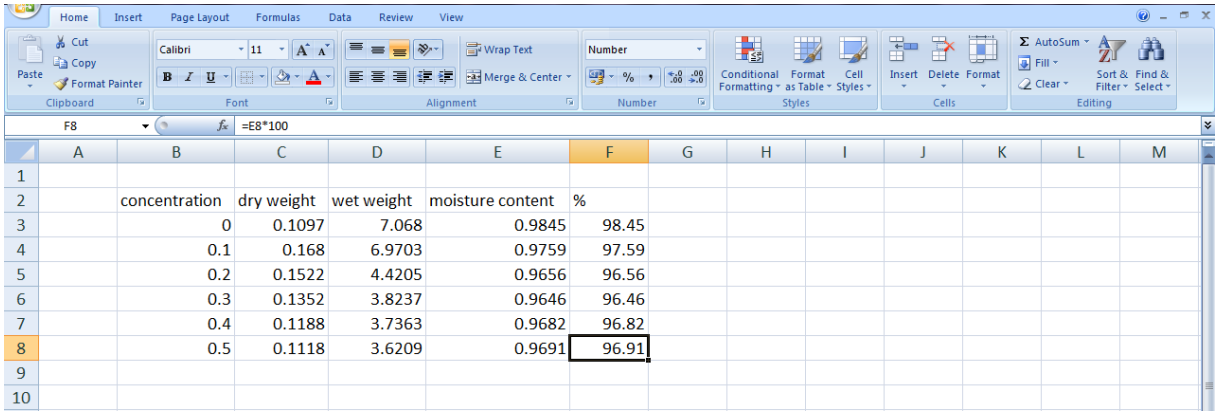
	A	B	C	D	E	F	G	H	I	J	K	L	M
1													
2		concentration	dry weight	wet weight	moisture content	%							
3		0	0.1097	7.068	0.9845	98.45							
4		0.1	0.1587	10.3472	0.9847	98.47							
5		0.2	0.589	8.1608	0.9278	92.78							
6		0.3	0.425	4.2588	0.9002	90.02							
7		0.4	0.445	5.0817	0.9124	91.24							
8		0.5	0.1792	2.3108	0.9225	92.25							
9													
10													

Figure B.2 Excel worksheet for calculating the water content of bacterial cellulose which cellulose act as an additives



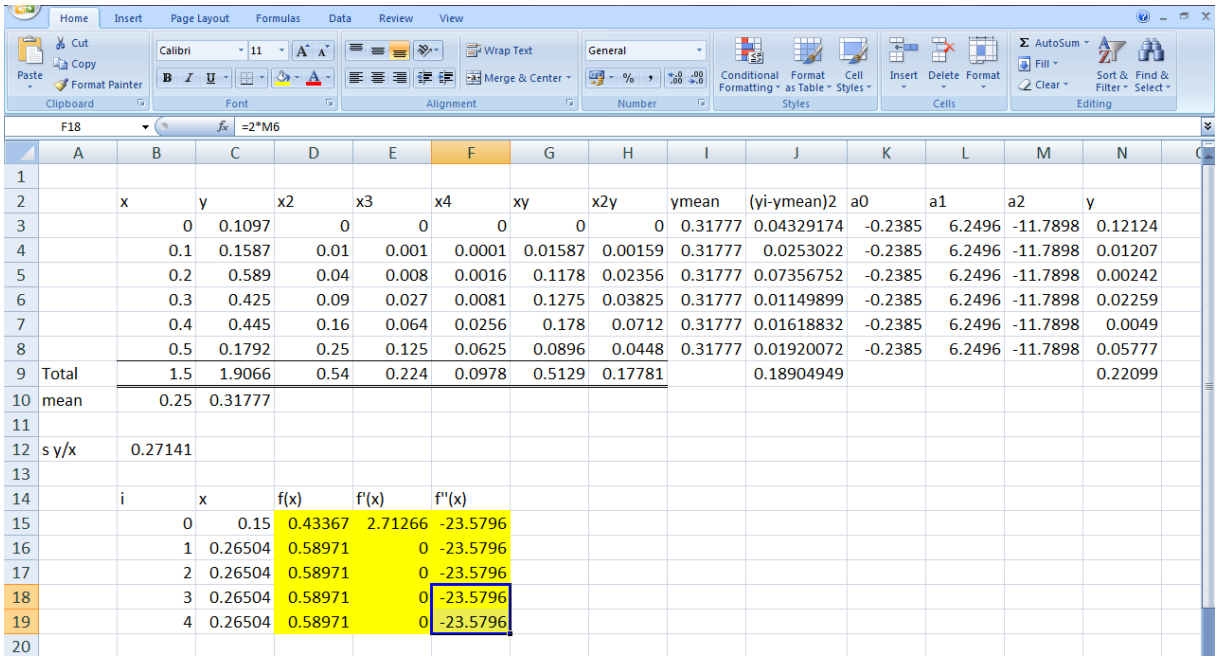
	A	B	C	D	E	F
1						
2		concentration	dry weight	wet weight	moisture content	%
3		0	0.1097	7.068	0.9845	98.45
4		0.1	0.1075	3.8919	0.9724	97.24
5		0.2	0.1139	4.1395	0.9725	97.25
6		0.3	0.1295	5.1803	0.9750	97.50
7		0.4	0.132	5.8045	0.9773	97.73
8		0.5	0.1778	7.0488	0.9748	97.48
9						
10						

Figure B.3 Excel worksheet for calculating the water content of bacterial cellulose which CMC act as an additives



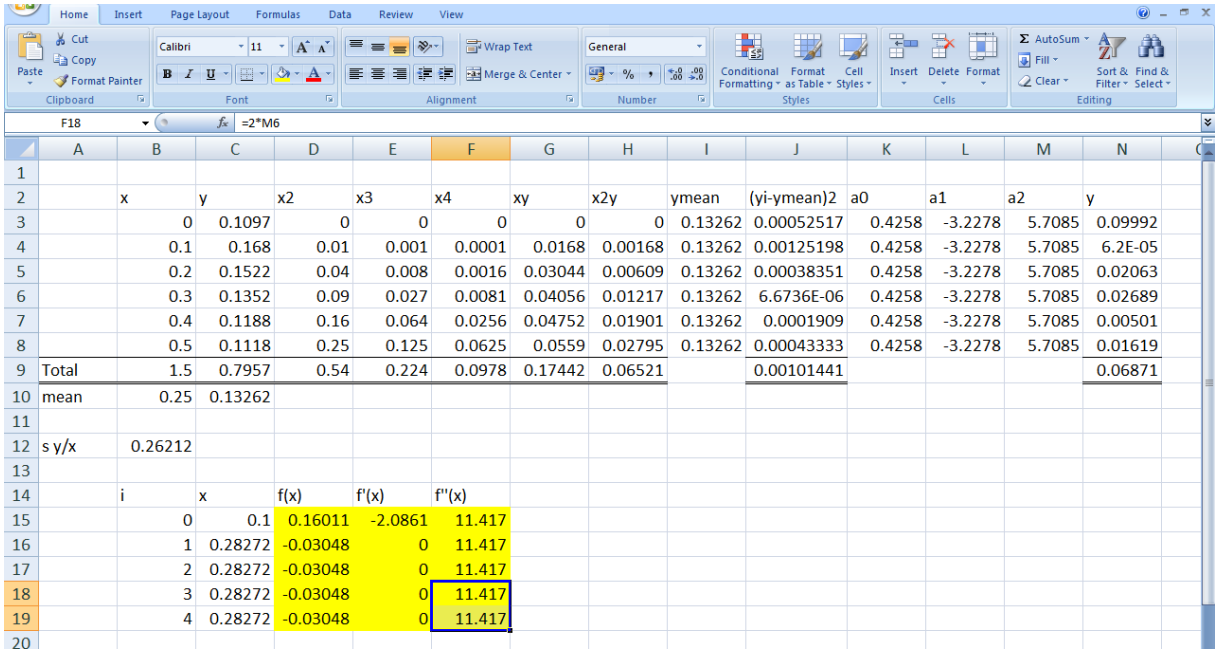
	A	B	C	D	E	F
1						
2		concentration	dry weight	wet weight	moisture content	%
3		0	0.1097	7.068	0.9845	98.45
4		0.1	0.168	6.9703	0.9759	97.59
5		0.2	0.1522	4.4205	0.9656	96.56
6		0.3	0.1352	3.8237	0.9646	96.46
7		0.4	0.1188	3.7363	0.9682	96.82
8		0.5	0.1118	3.6209	0.9691	96.91
9						
10						

Figure B.4 Excel worksheet for calculating the water content of bacterial cellulose which sodium alginate act as an additives



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1														
2		x	y	x2	x3	x4	xy	x2y	ymean	(yi-ymean)2	a0	a1	a2	y
3		0	0.1097	0	0	0	0	0	0.31777	0.04329174	-0.2385	6.2496	-11.7898	0.12124
4		0.1	0.1587	0.01	0.001	0.0001	0.01587	0.00159	0.31777	0.0253022	-0.2385	6.2496	-11.7898	0.01207
5		0.2	0.589	0.04	0.008	0.0016	0.1178	0.02356	0.31777	0.07356752	-0.2385	6.2496	-11.7898	0.00242
6		0.3	0.425	0.09	0.027	0.0081	0.1275	0.03825	0.31777	0.01149899	-0.2385	6.2496	-11.7898	0.02259
7		0.4	0.445	0.16	0.064	0.0256	0.178	0.0712	0.31777	0.01618832	-0.2385	6.2496	-11.7898	0.0049
8		0.5	0.1792	0.25	0.125	0.0625	0.0896	0.0448	0.31777	0.01920072	-0.2385	6.2496	-11.7898	0.05777
9	Total	1.5	1.9066	0.54	0.224	0.0978	0.5129	0.17781		0.18904949				0.22099
10	mean	0.25	0.31777											
11														
12	s y/x	0.27141												
13														
14		i	x	f(x)	f'(x)	f''(x)								
15		0	0.15	0.43367	2.71266	-23.5796								
16		1	0.26504	0.58971	0	-23.5796								
17		2	0.26504	0.58971	0	-23.5796								
18		3	0.26504	0.58971	0	-23.5796								
19		4	0.26504	0.58971	0	-23.5796								
20														

Figure B.5 Excel worksheet for calculating the optimum value of cellulose concenration



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1														
2		x	y	x2	x3	x4	xy	x2y	ymean	(yi-ymean)2	a0	a1	a2	y
3		0	0.1097	0	0	0	0	0	0.13262	0.00052517	0.4258	-3.2278	5.7085	0.09992
4		0.1	0.168	0.01	0.001	0.0001	0.0168	0.00168	0.13262	0.00125198	0.4258	-3.2278	5.7085	6.2E-05
5		0.2	0.1522	0.04	0.008	0.0016	0.03044	0.00609	0.13262	0.00038351	0.4258	-3.2278	5.7085	0.02063
6		0.3	0.1352	0.09	0.027	0.0081	0.04056	0.01217	0.13262	6.6736E-06	0.4258	-3.2278	5.7085	0.02689
7		0.4	0.1188	0.16	0.064	0.0256	0.04752	0.01901	0.13262	0.0001909	0.4258	-3.2278	5.7085	0.00501
8		0.5	0.1118	0.25	0.125	0.0625	0.0559	0.02795	0.13262	0.00043333	0.4258	-3.2278	5.7085	0.01619
9	Total	1.5	0.7957	0.54	0.224	0.0978	0.17442	0.06521		0.00101441				0.06871
10	mean	0.25	0.13262											
11														
12	s y/x	0.26212												
13														
14		i	x	f(x)	f'(x)	f''(x)								
15		0	0.1	0.16011	-2.0861	11.417								
16		1	0.28272	-0.03048	0	11.417								
17		2	0.28272	-0.03048	0	11.417								
18		3	0.28272	-0.03048	0	11.417								
19		4	0.28272	-0.03048	0	11.417								
20														

Figure B.6 Excel worksheet for calculating the optimum value of NaAlg concenration

Appendix C Scanning electron micrograph of bacterial cellulose from SEM

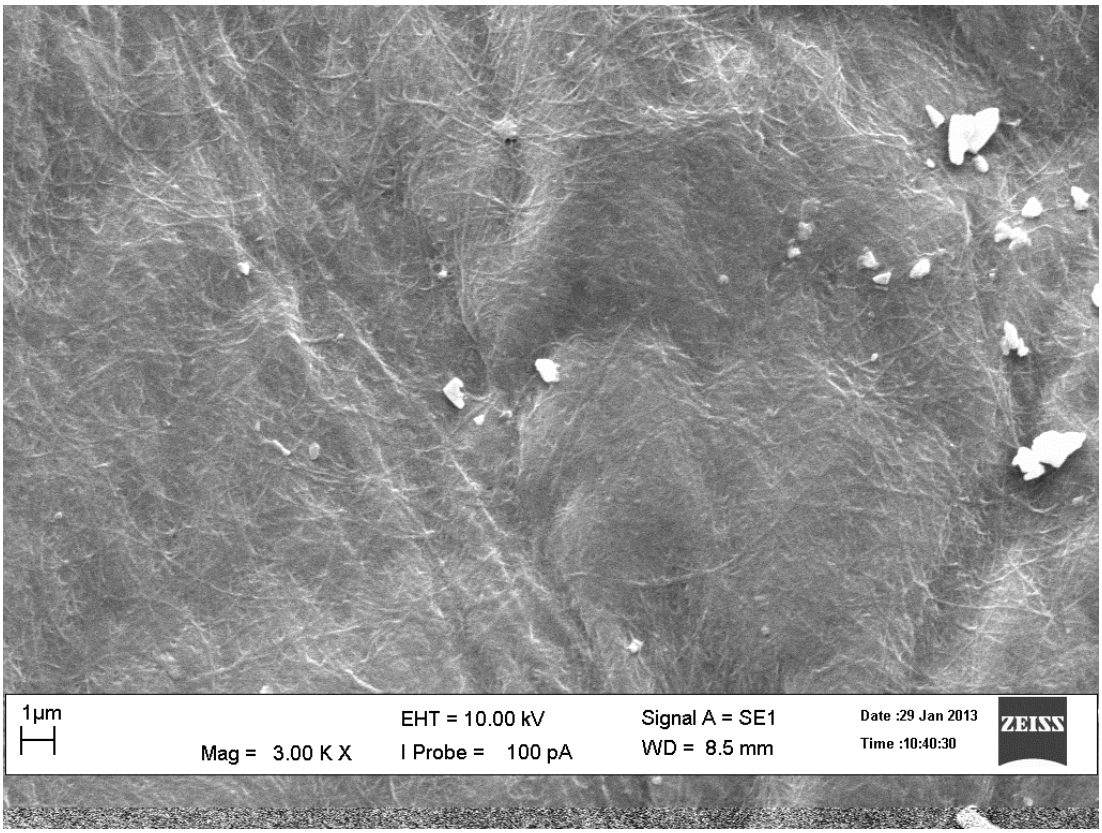


Figure C.1 Scanning electron micrographs of bacterial cellulose produced by *Acetobacter xylinum* in PW-M containing 0.1% MCC

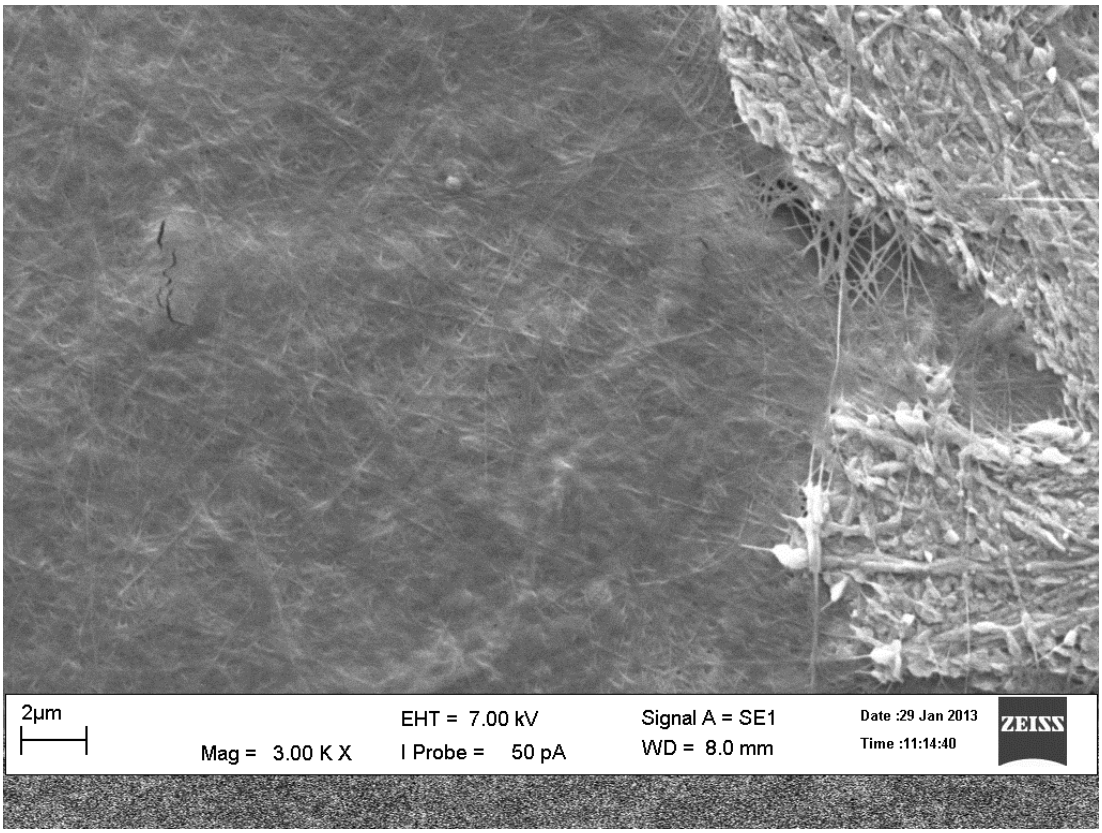


Figure C.2 Scanning electron micrographs of bacterial cellulose produced by *Acetobacter xylinum* in PW-M containing 0.1% CMC

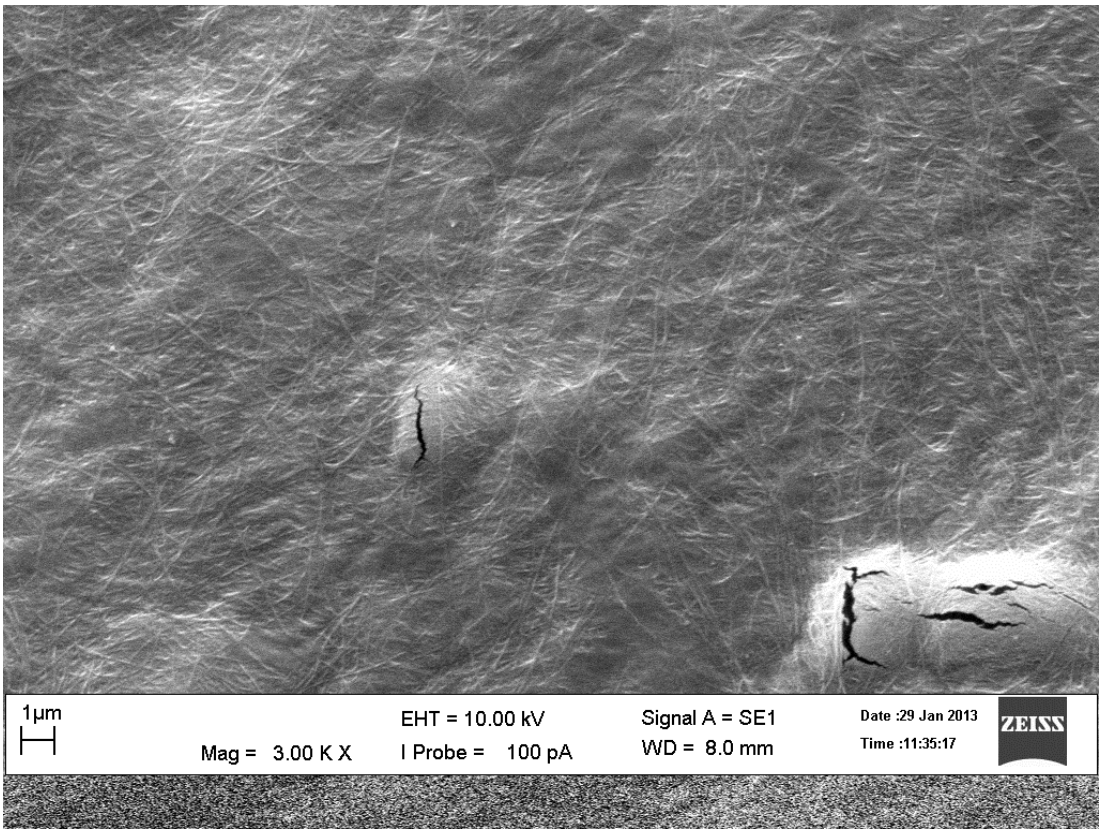


Figure C.3 Scanning electron micrographs of bacterial cellulose produced by *Acetobacter xylinum* in PW-M containing 0.5% NaAlg