OPTIMIZATION OF BACTERIAL CELLULOSE PRODUCTION BY USING RESPONSE SURFACE METHODOLOGY (RSM): EFFECT OF PH, TEMPERATURE AND CONCENTRATION OF FERMENTATION MEDIUM.

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SUPERVISOR'S DECLARATION

"I hereby declare that I have read this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology)"

Signature	:	…….
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Date	:	20 January 2012

STUDENT'S DECLARATION

I hereby declare that the work in this thesis entitled "Optimization of Bacterial Cellulose production using response surface methodology (RSM): Study the effect of pH, temperature and concentration" is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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Name : Muhammad Azlan Bin Nazeri

Date : 20 January 2011

To my beloved mother, father and sister

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We do hope that this report will give the readers some insight as to the maze of activities associated with the optimization of bacterial cellulose production using response surface methodology (RSM): Study the effect of pH, temperature and concentration from its planning stages until the final analysis and report.

Abstract

Bacterial cellulose is a type of biopolymer that produced by *Acetobacter xylinum* in high purity, high water holding capacity, good mechanical strength, elasticity and high crystallinity. In this research, pineapple residue was used as the carbon sources to replace the pure carbon sources as the substrate for the synthesis of bacterial cellulose. The objective of this study was to investigate the effect of temperature, pH and concentration in the production of bacterial cellulose by *Acetobacter xylinum*. The important part in this research, including of preparation HS-Medium and agar plate as a medium for breeding the stock culture that was taken from Malaysia Agricultural Research and Development Institute (MARDI), Serdang, Selangor. Ideal condition in this research that investigated was varied from 40% to 100% for the concentration while the temperature was between 28°C to 32°C and pH were 4.5 to 8.5. Besides, this is study also aims to optimize the production of bacterial cellulose from pineapple residue using response surface methodology (RSM) based on the central composite design (CCD). Before RSM is used, the known value of the parameters was estimated based on one factor at that time (OFAT). The results obtained from the OFAT showed the optimum condition at pH 5.50, temperature 30°C and concentration of pineapple residue was 80 % where the amount of dry weight bacterial cellulose produced was 3.3948 g. According to the RSM result, the optimal set cultural conditions for bacterial cellulose were pH 5.15, temperature 30.51°C and concentration of pineapple residue was 83.32%. Bacterial cellulose production of 3.4368 g was achieved by using these optimal conditions. The existence of bacterial cellulose was proven by Fourier Transform Infrared (FT-IR) Spectroscopy analysis based on the appearance of absorbance peak for the C-C bonding, C-O bonding, C-OH bonding and C-O-C bonding. In addition, Scanning Electron Microscopy (SEM) was used to observe surface and cross section of the bacterial cellulose film. In short, the data presented in this paper showed that pineapple residue has a great potential as the carbon source in production of bacterial cellulose.

Abstrak

Selulosa bakteria ialah sejenis biopolimer yang dihasilkan oleh *Acetobacter xylinum* dengan ketulinan yang tinggi, keupayaan pegangan air, kekuatan mekanikal yang baik, keanjalan dan kristaliniti yang tinggi. Dalam kajian ini, sisa-sisa nanas digunakan sebagai sumber karbon untuk menggantikan sumber karbon tulen sebagai substrat untuk sintesis selulosa bakteria. Objektif kajian ini adalah untuk menyiasat kesan suhu, pH dan kepekatan dalam penghasilan selulosa bakteria oleh *Acetobacter xylinum*. Peranan penting dalam kajian ini termasuklah penyediaan medium HS dan plat agar sebagai medium untuk pembiakan stok kultur yang telah diambil daripada Institut Penyelidikan dan Kemajuan Pertanian Malaysia (MARDI), Serdang, Selangor. Keadaan ideal dalam penyelidikan ini yang disiasat telah di variasi dari 40% kepada 100% untuk kepekatan manakala suhu 28°C hingga 32°C dan pH 4.5-8.5. Selain itu, kajian ini juga bertujuan untuk mengoptimumkan penghasilan selulosa bakteria dari sisa-sisa nanas menggunakan kaedah respons permukaan (RSM) berdasarkan reka bentuk pusat komposit (CCD). Sebelum menggunakan RSM dalam penyelidikan ini, nilai yang diketahui daripada parameter-parameter telah dianggarkan berdasarkan *one factor at that time (OFAT)*. Keputusan yang diperolehi daripada OFAT menunjukkan keadaan optimum pada pH 5.50, suhu 30°C dan kepekatan sisa-sisa nanas adalah 80% di mana jumlah berat kering selulosa ialah 3.3948 g. Berdasarkan hasil RSM , syarat yang ditetapkan kultur optimum untuk selulosa bakteria pada pH 5.15, suhu 30.51°C dan kepekatan sisa-sisa nanas adalah 83.32%. Penghasilan selulosa bakteria dengan 3.4368 g telah dicapai dengan menggunakan syarat-syarat yang optimum ini. Kewujudan selulosa bakteria telah dibuktikan oleh analisis *Fourier Transform Infrared (FT-IR) Spectroscopy* berdasarkan kemunculan puncak absorbansi bagi ikatan C-C, ikatan C-O, ikatan C-OH dan ikatan C-O-C. Tambahan pula, *Scanning Electron Microscopy (SEM)* telah digunakan untuk memerhati permukaan and keratan rentas daripada kepingan selulosa bakteria. Kesimpulannya data yang diperolehi ini menunjukkan bahawa sisa-sisa nanas mempunyai potensi yang besar sebagai sumber karbon dalam penghasilan selulosa bakteria.

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LIST OF SYMBOLS

BC	Bacterial Cellulose
°C	Degree C
C	Carbon
C_3	Carbon 3
C_6	Carbon 6
cm	Centimetre
g	Gram
ε	Error
IR	Infrared
ml	Millilitre
Y	Dry weight of Bacterial Cellulose
%	Percentage

LIST OF ABBREVIATIONS

Adj R^2	*Adjusted R^2*
ANOVA	Analysis of variance
CCD	Central composite design
DOE	Design of experiment
Et al	An others
FTIR	Fourier Transform Infrared
MARDI	Malaysia Agricultural Research and Development Institute
$MgSO_4$.7H2O	Magnesium Sulphate Heptahydrate
NaOH	Sodium hydroxide
Na_2HPO_4	Disodium hydrogen phosphate
OFAT	One factor at a time
pH	Potentiometric hydrogen ion concentration
RSM	Response surface methodology
SEM	Scanning electron microscopy

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Cellulose is the most abundance polymer that is present as the major component of plant biomass and a representative of microbial extracellular polymers. *Acetobacter xylinum* is the bacteria that able to grow on the waste material to produce cellulose. The several genera that has shown the ability to synthesize cellulose include *Sarcina, Agrobacterium, Rhizobium and Acetobacter* (Barbara *et al*., 2008). However, species that able to produces cellulose in high quantity is *Acetobacter xylinum* and over a century ago this organism and its produce were first identified based on the research (Iguchi and Yamanaka, 1997).

Stationary culture, agitated culture, cultivation in the horizontal fermenters or cultivation in the internal-loop airlift reactors are the few techniques that have been reported for economic and commercial bacterial cellulose production (Prashant *et al*., 2009). The monosaccharide or simple sugar such as glucose, xylose and glucose that act as a substrate (Ishihara *et al*., 2002) or other carbon sources such as ethanol and glycerol are the sources in a medium for bacterial cellulose production (Sherif and Kazuhiko, 2005). *Acetobacter xynlinum* synthesizes cellulose by fully utilizing the monosaccharide of carbohydrate or simple sugar such as glucose, fructose, sucrose or lactose.

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). Synthesis of bacterial cellulose is one of the methods in producing cellulose biopolymers. Another popular method is by extracting and isolating the cellulose from

plant but synthesis of bacterial cellulose is more popular because time consumption to produce is shorter than plant synthesis (Klemn *et al*., 2001) thus it also has the chemical purity as one of the most important features of bacterial cellulose that differentiate it from the plant cellulose (Surma *et al*.,2000).

The aim of this study is to optimize the bacterial cellulose production by using pineapple residue as a substrate that consists of pineapple core, the peeling skin and the pineapple crown. In this experiment, three parameters are used, which are pH, temperature and concentration of pineapple residue are evaluated with intention to find the optimum condition to generate the maximum mass of bacterial cellulose.

1.2 PROBLEM STATEMENT

Annually, there are approximately 15000 tonnes of pineapple residues produced from the pineapple cannery industries in Malaysia. The pineapple residue left over from the production processes are abundant and still contain the large amount of sugar content (Akhiro *et al*., 2008). Usage of pineapple residue as raw material in bacterial cellulose production not only reduces waste material created from the pineapple industry, but also lowers the cost of bacterial cellulose production. In addition, the used of pineapple residue as raw material in bacterial cellulose production also can prevent from the environmental issues. Besides that, most cellulose is obtained from the plant cell wall is not pure, and it is difficult to purify the cellulose from lignin and hemicelluloses (Klemn *et al*., 2001). Bacterial cellulose is used as an alternative instead of plant cellulose in order to produce high purity cellulose and to reduce the forest depletion (Barbara *et al*., 2008). Pineapple residue is used in this research as a raw material of carbon source in order to reduce the peel, crown and core from pineapple that discharged from food and beverage industries.

The carbon sources in pineapple residue consist of sucrose, glucose, fructose and other nutrients (Sasaki *et al*., 1991; Krueger *et al*., 1992). Therefore, it can be consumed to produce the value added product such as bacterial cellulose (Abdullah and Hanafi, 2008).

1.3 OBJECTIVES OF RESEARCH

i) To investigate the effect of temperature, pH and concentration of pineapple residue in the production of bacterial cellulose by *Acetobacter xylinum*.
ii) To optimize the production of bacterial cellulose from pineapple residue by using response surface methodology (RSM)

1.4 SCOPES OF RESEARCH

In order to achieve the objective, scope of study was divided into three as the following:

i) To produce bacterial cellulose from pineapple waste residue as a fermentation medium with different temperature, pH and concentration.
ii) To optimize the parameter by using response surface methodology (RSM)
iii) To analyse the bacterial cellulose produced by using FTIR.
iv) To characterize the morphology of the bacterial cellulose by using Scanning Electron microscope (SEM).

1.5 RATIONAL AND SIGNIFICANT OF STUDY

i) Reuse pineapple residue that produced from food and beverage industries to produce bacterial cellulose.
ii) Low cost bacterial cellulose production.
iii) High productivity of bacterial cellulose production

CHAPTER 2

LITERATURE REVIEW

2.1 BACTERIAL CELLULOSE

Bacterial cellulose is a polymer that produced by *Acetobacter xylinum* in presence of glucose. Bacterial cellulose has high purity cellulose where it was free from lignin and hemicelluloses not like plant cellulose that has low purity cellulose and containing lignin and hemicelluloses (Klemn *et al*., 2001). There are several aspects that differentiate bacterial cellulose with plant cellulose, which are bacterial cellulose has unique characteristic, including good mechanical strength, high water absorption capacity, high crystalline, ultra-fine and highly pure fibre network structure that caused bacterial cellulose more preferred than plant cellulose (Keshk and Sameshima, 2006). There are four different pathways in forming the cellulose biopolymer (Klemn *et al*., 2001). 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*. In the synthesis process of cellulose, bacteria that can produce the highest cellulose amount than other bacteria is *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).

Cellulose is the main part in the cell wall plant and act as protective and coating, whereas plant cellulose (PC) plays a structural role in plant (Bielecki *et al.*, 2000). However cellulose is obtained in the plant is not pure caused it have lignin and hemicellulose, so the separation process to remove lignin and hemicellulose is the most popular and industrial important isolation of cellulose from plants (Klemn *et al*., 2001), but it is difficult to purify the cellulose from lignin and hemicelluloses through the separation process. 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). Most of the paper production used cellulose pulp from plant and thus gives a problem on forest depletion and now many researches has been conducted on producing paper from bacterial cellulose and as a result, there is an improvement of the paper's strength properties and protect the surface of paper (Barbara *et al*., 2008). The form of its size, crystallinity and purity had differentiated between bacterial cellulose (BC) produced by bacteria that has unique physical and chemical properties with cellulose that produced from plant (Prashant *et al*., 2008). Bacterial cellulose also has unique characteristic, including good mechanical strength, high water absorption capacity, high crystallinity, ultra-fine and highly pure fibre network structure that caused it has been preferred than plant cellulose (Andelib and Nuran, 2009).

Bacterial cellulose also has disadvantages that need to encounter although bacterial cellulose has a unique characteristic than the plant cellulose, the problem is the price for the sugar as a substrate is very expensive but low in the quantity production of the process. Using the pineapple residue and fruit waste such as fruit peel is one of the alternatives that can overcome this problem. The mango peel, pineapple core, watermelon peel and other fruit wastes are the example of fruit waste that can be utilized as substrates to produce cellulose (Akihiro *et al*., 2008). Besides that lactose which has a lower price also potential to be used as a substrate for cellulose production using static culture.

2.2 WASTE AS A SUBSTRATE

One of the advantages bacterial cellulose is it can produce 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). Carbon and nitrogen sources played important role for cell growth and bacterial cellulose production, and in the same time cost for bacterial cellulose production must be considered as a main objective. Traditionally, bacterial cellulose production that using pure glucose as a carbon source and other nutrient sources was resulting in very high production cost. One of the solutions to overcome this problem by using waste as a substrate to replace pure glucose and that was proven by previous research about waste effective in bacterial cellulose production. Using cheap carbon and other nutrient sources such as from agro-forestry industrial residues is an interesting strategy to overcome the high production cost. Many of researchers were applied various wastes in bacterial cellulose production by using beet molasses (Kesk *et al*., 2006), sugar cane molasses and corn steep liquor (El-saied *et al*., 2008), several fruit juices, including orange, pineapple, apple, Japanese pear and grape (Kurosumi *et al*., 2009), pineapple waste (Ch'ng and Muhamad, 2000) and coconut water (Kongruang, 2008) was already successfully used as carbon sources for the bacterial cellulose production. An addition the value of industrial waste will have added value and give positive impact by reducing waste material in the environment for bacterial cellulose production (Pedro *et al*., 2011).

2.3 MEDIUM CONDITION

Medium condition was played as a main role for ensure bacterial cellulose production successfully. In production of bacterial cellulose using *Acetobacter xylinum*, temperature needs to be maintained at 30°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. 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. In this research, concentration was varied with 40%, 50%, 60%, 70%, 80%, 90% and 100% as a percentage for pineapple waste concentration and supported by the other nutrients for *Acetobacter xylinum* growth. Based on the previous research during the fermentation process, the pH of the medium change throughout the process. This caused by side product was produced during conversion of glucose to cellulose by synthesis of *Acetobacter xylinum*. This microbe also converted glucose in pineapple waste to gluconic, lactic and acetic acid as a side product. These side products will be accumulated and affecting the condition of the culture medium thus decrease the bacterial cellulose production (Chawla *et al*., 2008).

2.4 BACTERIAL CELLULOSE SYNTHESIZE

Cellulose is the most abundant earth biopolymer and also known as the major component of plant biomass and a representative of bacterial polymers in extracellular condition. An efficient producer of cellulose are acetic acid bacteria *Acetobacter xynilum*. Bacterial cellulose free of lignin and hemicelluloses (Barbara *et al*., 2008). In addition, with extracellular synthesized bacterial cellulose will be different with plant cellulose with respect to its high crystallinity, high water absorption capacity, and mechanical strength in the wet state and ultra fine network structure (Budhiono *et al*., 1999). 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. The precisely and specifically synthesis of bacterial cellulose was regulated multi step process, that mean involving a large number of both individual enzymes and complexes of catalytic and regulatory proteins, whose supramolecular structure has not yet been defined (Klemn *et al*., 2001). 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.

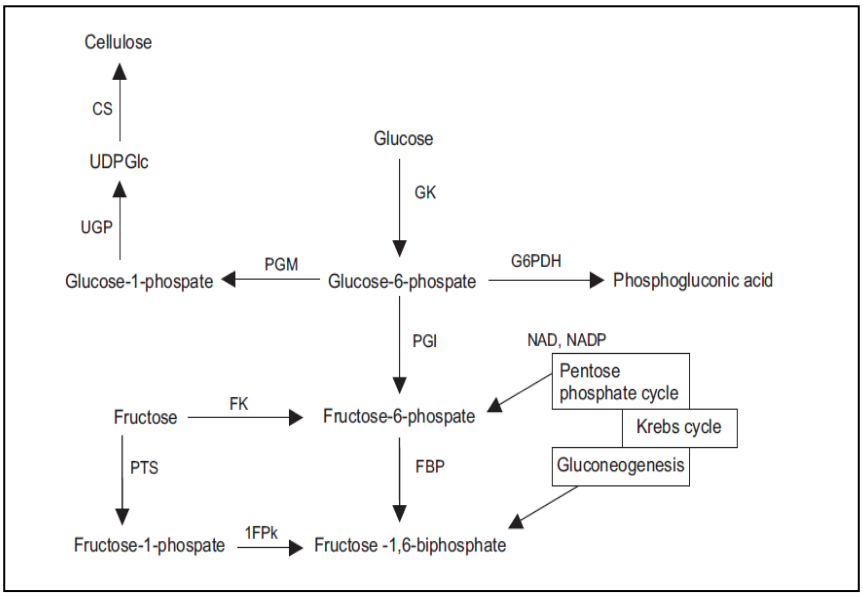


Figure 2.1: Simplified model for the biosynthetic pathway of Bacterial Cellulose.

Sources: Prashant *et al*., 2008

2.5 *ACETOBACTER XYLINUM*

Acetobacter xylinum is a gram negative microbe that produced bacterial cellulose in aerobic condition. *Acetobcater xylinum* also acetic microbe that growth very well in acid condition from broth culture 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) but *Acetobacter xylinum* still growth because it is a type of acetic microbe. In alkaline condition, *Acetobacter xylinum* will grow slowly and bacterial cellulose yield will decreasing (Pourramezan *et al*., 2009). *Acetobacter xylinum* can produces cellulose from a variety of carbon sources including glucose, ethanol, sucrose, and glycerol and it produced cellulose in the form of extracellular pellicle composed of ribbons. This microbe can be obtained from the nectar of flowers, decaying fruit, fresh apple cider and unpasteurized beer which have not been filtering sterilized (Barbara *et al*., 2008).

2.6 PINEAPPLE RESIDUE

2.6.1 Pineapple Canning Industry

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. After that the fruits will be graded into several sizes according to the fruit diameter. 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). Figure 2.2 shows the pineapple canning process.

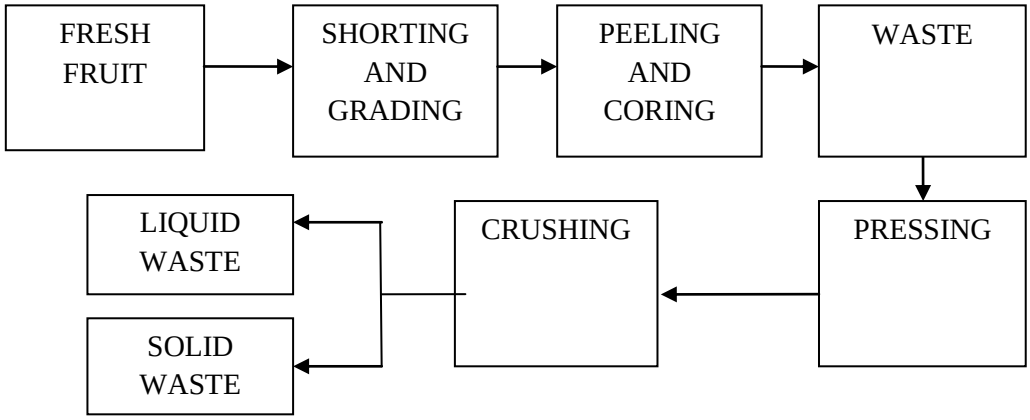


Figure 2.2: The pineapple canning process

Sources: Abdullah and Hanafi Mat 2000

2.6.2 Pineapple Residue Characteristics

Primarily solids in the form of peels, stem, pits, culls and organic matter suspension are the wastes that generated by fruit processing. Identify and characterise the wastes either solid or liquid that were produced is the first stage in the optimization of waste reduction. Each particular food industry generates specific type and amount of waste. For example, more solid waste were generated by the fruit and vegetables industry than the dairy industry. The problem of suspended organic matter in the waste

water is the characteristics of the waste load of various fruit processing industries (Moon and Woodroof, 1986). The comparison between pineapple waste to other fruit processing wastes is given in table 2.1. The solid waste from pineapple processing was about 45% from fresh fruit, followed by citrus, apple, pear, peach and cherry were 43,32, 30, 24, 17, and 14% respectively. The suspended and organic matter in the waste water is higher than other fruits processing for pineapple processing. It can be indicated by the BOD and suspended solid contained in the rinse water which are 4.8 kg/m^3 and 2.4kg/m^3 respectively.

Table 2.1 : The comparison between pineapple waste to other fruit processing waste.

Fruit	Raw (tones)	Waste water (m^3)	BOD (kg/m^3)	Suspended solid (kg/m3)	Solid residual (tonnes)
Apple	1,000,000	18,920,000	0.95	0.11	320,000
Apricot	120,000	2,270,000	1.39	0.20	21,000
Cherry	190,000	1,130,000	1.60	0.40	27,000
Citrus	7,800,000	87,050,000	0.16	0.28	3,390,000
Peach	1,100,000	16,650,000	1.79	0.29	270,000
Pear	400,000	6,050,000	2.09	0.74	120,000
Pineapple	1,000,000	1,890,000	4.80	2.40	450,000

Sources: Moon and Woodroof (1986)

The characteristics of solid waste from pineapple processing are shown in table 2.2 reported by different authors. The moisture content of solid waste was found to be at the range of 87.50-92.80%. The difference of moisture content might be due to the sample obtained from various geographical origins and of varying degree of ripeness. The total nitrogen and ash content in the wastes were between 0.90-0.95% and 3.9

10.6% respectively. Pineapple that consist of rind, crown and core that prepared in juice form is one of the unconventional media indentified that can promote a low cost substrate for the production of bacterial cellulose. Although the amount of sugars in the pineapple rind, crown and core is much lower than the total sugar in the pineapple flesh, it still can act as a carbon source for *Acetobacter xylinum* to produce bacterial cellulose. 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	-

Sources: Abdullah and Hanafi Mat (2000)

2.7 PURIFICATION OF BACTERIAL CELLULOSE

The purification is the important step in the production of any cellulose product, which is traditionally in the paper making industry known as chemical pulping process. This process is aimed to remove essentially all of the undesired residual insoluble lignin and other chemical bound to cellulose fibre as well as impurities occurring during processing and converting them into compounds which are soluble in alkaline water. The photo-oxidation that caused discolouration probably will occur if lignin is not removed and the final paper product is fragile (Saharman *et al*., 2011). Therefore various method have been developed and different chemical have been used to produce a good quality paper as well as to overcome low pulp yields (Bajpai, 2005). Plant cellulose as a naturally is bound with lignin and hemicelluloses and also other chemicals but bacterial cellulose is not bound to other chemicals and it has high purity (Klemn *et al*., 2006). Therefore the purification process that was applied on the bacterial cellulose differs from plant cellulose purification.

There are two stages in the purification process, firstly bacterial cellulose was washed in 2.5wt% NaOH solution for one day and then washed with 2.5wt% NaOCI also for 1 day (Saharman *et al*., 2011) and it had known as the two step purification process. An addition using 2.5wt% NaOH solution will prevent lower mechanical properties in bacterial cellulose but using 6% NaOH can potentially change the crystal structure of bacterial cellulose (Gomes *et al*., 2007) by breaking of many inter and intra molecular hydrogen bonds, which are naturally present in cellulose. Protein and nucleic acid that were derived from bacterial cellulose, and the culture broth were known as the non cellulose material will be removed from the pellicle by using two step purification process and in the same time the pellicle will form inter and intra fibrillar hydrogen bonds that strong. Normally, within a few weeks for untreated bacterial cellulose sheet can present mould on the surface and the result it changes the colour and becoming darker but bacterial cellulose that was experience two step purification it can stored for a long time and without change in colour and quality. The importance for two step purification process is to allow observation easily the internal structure bacterial cellulose using FT-IR and it show that NaOCI as a bleaching agent is effectiveness in removing impurities, which are not removed with NaOH alone (Saharman *et al*., 2011).

2.8 APPLICATION OF BACTERIAL CELLULOSE

Nowadays, in new commercial application of secondary and third degree burn, bacterial cellulose will be shown to be very successful in the medical treatment (Prashant *et al*., 2008). To prove this triumph a clinical study has been performed on 34 patients and in this analysis the bacterial cellulose wound dressing materials were directly applied on the fresh burn covering up to 9-18% of the body surface. Then macroscopic observation of the wound and wound extract, epidermis growth, microbiological test, and histopathological are diagnoses that were considered in this clinical study. The result is bacteria cellulose is to be one of the best material to promote wound healing from burns caused a moist environment for tissue regeneration, specific cellulose nano-morphology which promotes cell interaction and tissue re-growth and significant reduction of scar tissue formation (Prashant *et al*., 2008). Bacterial cellulose also is used in wound care such as surgical wounds, ulcers, tissue and organ engineering and it can be used in various areas including pharmaceutical and waste treatment (Prashant *et al*., 2008).

The chemically pure cellulose in bacterial cellulose is also applied in food application to food process as thickening and stabilizing agent (Prashant *et al*., 2008). In 1992, bacterial cellulose is used into diet drinks in Japan and the first use of bacterial cellulose in the food industries was in nata de coco in the Philippines (Budhiono et *al*., 1999).

The application of papermaking using bacterial cellulose is also popular in the paper industry (Surma *et al*., 2008). Cellulose pulp from plant were used in most of paper production and thus gives a problem on forest depletion. As a result to improve of the paper's strength properties and protect the surface of paper (Surma *et al*., 2008) and in the same time to reduce forest depletion, many researches has applied bacterial cellulose in producing paper.

2.9 OPTIMIZATION PARAMETERS ON PRODUCTION OF BACTERIAL CELLULOSE BY USING RESPONSE SURFACE METHODOLOGY (RSM)

One-at-a-time factorial design experiment as a single factor is varied while others are kept constant. It often expensive and time consuming and do not take into account the possible interaction of various independent factors that would skew the result. This disadvantages was realised during the preliminary investigations into this research. For these reasons, Response Surface Methodology as highly successful method have been developed to reduce the cost and duration of experiment that also allow for the observation of any interacting factors in the final process response. Response Surface Methodology is defined as a statiscal method that uses quantitative data from appropriate experimental designs to determine and simultaneously solve multivariate equations that specify the optimum product for a specified set of factors through mathematical models. It involves four important steps, identification of critical factors factors for the product or process, determination of the range of factor levels, selection of specific test samples by the experimental design, analysis of the data by RSM and data interpretation (Mutanda *et al*., 2008).

Response Surface Methodology (RSM) is a systematic approach that can be obtained by using an inverse process of first as basically. Specifying the criteria and then computing the best design according to a formulation. Response Surface Methodology encompasses a point selection method (also referred to as Design of Experiment, Approximations methods and Design Optimization) to determine optimal settings of the design dimensions. It has important applications in the design, development and formulation of new products as well as in the improvement of existing product designs. RSM which includes factorial design and regression analysis can build models to evaluate the effective factors and study their interactions and select the optimum conditions in the limited number of experiments. RSM also is a collection of statiscal and mathematical techniques useful for developing, improving and optimizing the design process (Chauhar and Gupta, 2004).

There have been few report on optimization of bacterial cellulose production with *Acetobacter Xylinum.* Based on reported Jagannath *et al*., 2008, Response Surface Methodology was used to study the effect of three variables for bacterial cellulose production by using coconut water medium. Maximum thickness of bacterial cellulose was obtained at pH 4.0 with 10% sucrose and 0.5% ammonium sulphate concentrations. These conditions also produced good quality bacterial cellulose with a smooth surface and soft chewy texture (Jagannath *et al*., 2008).

Optimization of bacterial cellulose production from the batch and non-agitated condition was conducted by Castro *et al*., 2010. The effects of several factors such as fructose and sucrose concentration, pH and temperature of incubation was evaluated that affected cellulose yield significantly. A-five level, four-factor central composite design was used in Response Surface Methodology (RSM) to determine the relationship of four factor (fructose and sucrose concentration, pH and temperature of incubation) to the response of cellulose yield (in g of crude cellulose/l of medium). Maximum product yield could be obtained by the following predicted optimum fermentation conditions: fructose concentration:24.8g/l, sucrose concentration:76.5g/l, pH:4.49 and temperature of incubation:29.3°C. The predicted cellulose yield was 13.24 g/l was found to be very close to the average experimental yield 12.67 g/l. This indicating that the mathematical model obtained was an adequate predictor of cellulose yield (Castro *et al*., 2010).

Optimization of bacterial cellulose production by *Acetobacter xylinum* BPR 2001 using maple syrup as a carbon sources was conducted by Xiaobo *et al*., 2011. Optimal condition for fermentation in a rotary shaker were optimized by the Response Surface Methodology (RSM) using a three-level, four-factor Box-Behnken design. In this research, optimization was carried out for twelve culture parameter were screened by the Plackett-Burman design.Optimal condition were maple syrup 30g carbohydrate/l, (NH4)2SO4 3.3 g/l, KH2PO4 1 g/l, yeast extract 20g/l, citric acid 1.6 g/l, trisodium citrate dehydrate 2.4g/l, ethanol 0.5% (v/v), acetic acid 0.5g/l, MgSO4.7H20 0.8g/l, inoculums age 3 days, inoculums volume 6% (v/v), shaking speed 135 rpm, and incubation temperature 25°C. Comparison of bacterial cellulose production with maple syrup or pure sugars showed maple syrup was a suitable carbon sources (Xiaobo *et al*., 2011).

Evaluation and optimization of microbial cellulose production using pineapple waste as substrate was conducted by Ch'ng and Muhammad, 2000. This research was evaluated the production of bacterial cellulose in static and shaken culture conditions by using Response Surface Methodology (RSM). Optimum condition were fixed at pH 5.0 and temperature of 28°C for all the experiments in this research project and 80 rpm, 120 rpm and 160 rpm were carried out for shaken culture technique. Response Surface Methodology was used to study the effect of five variables for bacterial cellulose production by using pineapple waste medium. Maximum thickness of bacterial cellulose was obtained at yeast extract 6.0g, sucrose 20.0 g, bactopeptone 1.49g, potassium dihydrogen phosphate (KH2PO4) 1.08g and Magnesium sulfate (MgSO4) 0.06 g with wet cellulose mass of 176.47g. From statically analysis on design experiments it is found that Magnesium sulfate is not affecting toward cellulose formation. It can be just ignore to use in prepared medium culture for future (Ch'ng and Muhammad, 2000)

2.10 BACTERIAL CELLULOSE ANALYSIS

In this research, bacterial cellulose has two analysis, which are by using Fourier transform infrared spectroscopy (FT-IR) to check type of bonding that presents in bacterial cellulose and Scanning Electron Microscope (SEM) to check morphology of bacterial cellulose.

2.10.1 Bacterial Cellulose Wavelength Region

FT-IR is a one of the equipment that used to analyze bacterial cellulose by using the chemical bonding that present in the polymer. The whole and expanded FT-IR spectra revealing characteristic's absorption band of bacterial cellulose. The characteristics bands that appeared were list in table 2.3.

Table 2.3: 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 to 1290 cm^{-1}	Hwang, 2007
C-0 strecthing at C3	1060 cm^{-1}	Sun *et al*., 2008
C-O stretching at C6	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

All the absorbance stated above are associated with the chemical bonding that present in cellulose polymer. 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 previous studies were investigates about of the crystalline structure of cellulose and showed that some of the chemical bond on the surface of cellulose in is broken down in the reaction and exposing the hidden internal chemical bond after treated with enzyme, diluted acid, sodium hydroxide and carbon dioxide. 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. For example the effect of acid first was on the surface and amorphous zone, the hydrogen bonds was broken and more bond types C-OH, C-O-C, and C-C were exposed, thereby the stretching absorbency increase (Sun, *et al*., 2008). Some of the absorbance peak will change either decrease or shifted to greater or lower wave number when the cellulose structure is changing. Table 2.4 show that the characteristics of infrared absorption according to the functional group.

Table 2.4: Table of characteristic IR Absorptions

Functional Group	Molecular Motion	Wave number (cm^{-1})
alkanes	C-H stretch	2950-2800
	CH_2 bend	~1465
	CH_3 bend	~1375
	CH_2 bend (4 or more)	~720
alkenes	=CH stretch	3100-3010
	C=C stretch (isolated)	1690-1630
	C=C stretch (conjugated)	1640-1610
	C-H in-plane bend	1430-1290
	C-H bend (monosubstituted)	~990 & ~910
	C-H bend (disubstituted - E)	~970
	C-H bend (disubstituted - 1,1)	~890
	C-H bend (disubstituted - Z)	~700
	C-H bend (trisubstituted)	~815
Alkynes	acetylenic C-H stretch	~3300
	C,C triple bond stretch	~2150
	acetylenic C-H bend	650-600
aromatics	C-H stretch	3020-3000
	C=C stretch	~1600 & ~1475
	C-H bend (mono)	770-730 & 715-685
	C-H bend (ortho)	770-735
	C-H bend (meta)	~880 & ~780 & ~690
	C-H bend (para)	850-800
alcohols	O-H stretch	~3650 or 3400-3300
	C-O stretch	1260-1000
ethers	C-O-C stretch (dialkyl)	1300-1000
	C-O-C stretch (diaryl)	~1250 & ~1120
aldehydes	C-H aldehyde stretch	~2850 & ~2750
ketones	C=O stretch	~1715
	C-C stretch	1300-1100
carboxylic acids	O-H stretch	3400-2400
	C=O stretch	1730-1700
	C-O stretch	1320-1210
	O-H bend	1440-1400
esters	C=O stretch	1750-1735
	C-C(O)-C stretch (acetates)	1260-1230
	C-C(O)-C stretch (all others)	1210-1160
acid chlorides	C=O stretch	1810-1775
	C-Cl stretch	730-550
anhydrides	C=O stretch	1830-1800&1775-1740
	C-O stretch	1300-900

amines	N-H stretch (1 per N-H bond)	3500-3300
	N-H bend	1640-1500
	C-N Stretch (alkyl)	1200-1025
	C-N Stretch (aryl)	1360-1250
	N-H bend (oop)	~800
amides	N-H stretch	3500-3180
	C=O stretch	1680-1630
	N-H bend	1640-1550
	N-H bend (1o)	1570-1515
alkyl halides	C-F stretch	1400-1000
	C-Cl stretch	785-540
	C-Br stretch	650-510
	C-I stretch	600-485
nitriles	C,N triple bond stretch	~2250
isocyanates	-N=C=O stretch	~2270
isothiocyanates	-N=C=S stretch	~2125
imines	R2C=N-R stretch	1690-1640
nitro groups	-NO2 (aliphatic)	1600-1530&1390-1300
mercaptans	S-H stretch	~2550
sulfoxides	S=O stretch	~1050
sulfones	S=O stretch	~1300 & ~1150
sulfonates	S=O stretch	~1350 & ~11750
	S-O stretch	1000-750
phosphines	P-H stretch	2320-2270
	PH bend	1090-810
phosphine oxides	P=O	1210-1140

Sources: Sherif (2008)

2.10.2 Fourier Transform Infrared Spectroscopy

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. To identify a chemical or a mixture of chemical compound either organic or inorganic in the form solid, liquid or gases, Fourier transform infrared spectroscopy (FT-IR) is used as one of the equipment. Another that it also can identify unknown materials, determine the quality or consistency of a sample and determine the amount of components in a mixture.

FTIR also differentiated bacteria cellulose from one another. Before the invention of nuclear magnetic resonance (NMR) spectroscopy in structural identification, IR was the technique that was used. Infrared spectroscopy is still widely used when there is only a limited amount of sample but for the certain case when there is a sufficient amount of sample, NMR spectrometers will supplant infrared spectrometers for routine structural determination of liquids and solids. 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).

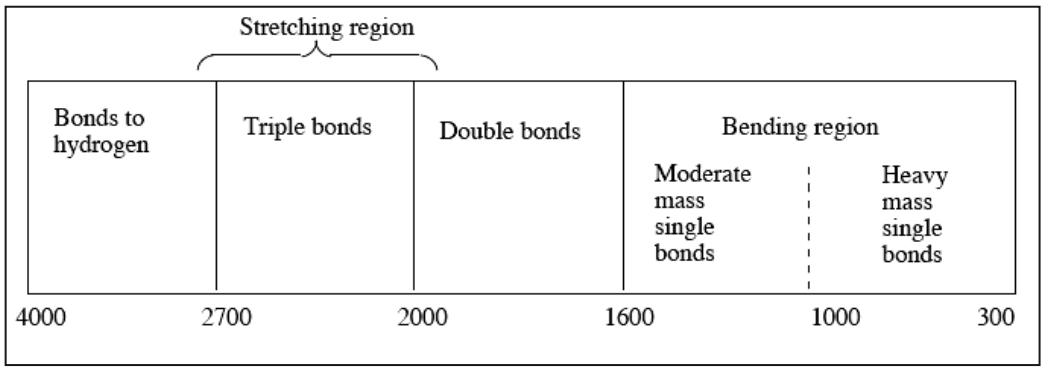


Figure 2.3 : Characteristic wavelength regions (in wave numbers, cm^{-1}) for different vibrations

Sources: Modern Techniques in Chemistry: Infrared Spectroscopy 2005

2.10.3 Scanning Electron Microscopy (SEM)

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. For solving this problem, the scanning electron microscope

was used due 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. Another that it also represent of the combination between a few essential components such as electron sources, electron lenses and detectors for all signals of interest.

There are divide three signal which are the secondary electrons, backscattered electrons, and X-rays for provide the greatest amount of information in SEM and 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.

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 RESEACRH METHODOLOGY

Basically, there are three steps to complete all this research on production of bacterial cellulose. The first step is preparation of medium fermentation by using pineapple residue as a medium for synthesis bacterial cellulose by *Acetobacter xylinum.* Secondly, the experiment is done by using three different parameter, which are temperature, pH of the medium and concentration of pineapple residue. Then the optimization is done using Response Surface Methodology (RSM). Finally the films were characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM). Figure 3.1 shows research design for the production of bacterial cellulose.

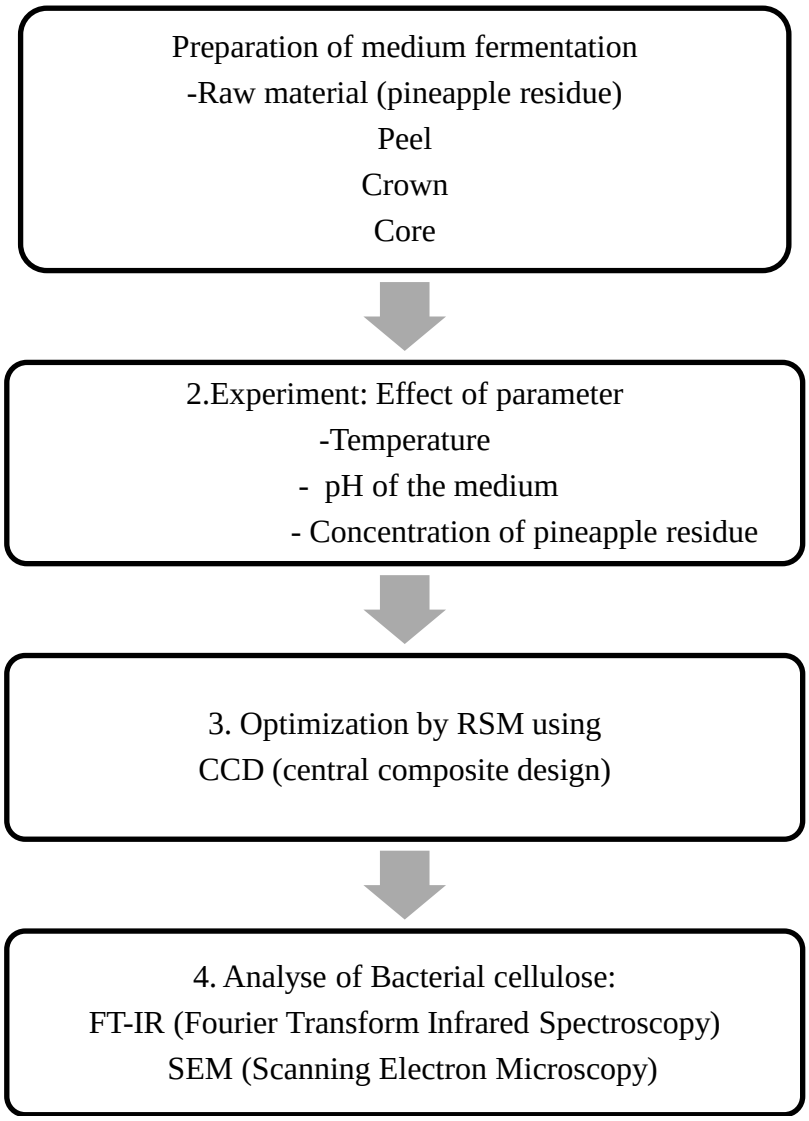


Figure 3.1: Research design for the production of Bacterial Cellulose

3.2 SYNTHESIZE OF BACTERIAL CELLULOSE

Medium fermentation was prepared by using pineapple residue as a carbon source to replace glucose. In this research, mother culture must be prepared first by using HS-medium before proceed with preparation pineapple residue medium. Mother culture was incubated for 5 days at 30°C. After that, 10 ml from mother culture is added into the 100 ml medium fermentation for the synthesis of bacterial cellulose.

3.3 EFFECT OF PARAMETERS

In the production of bacterial cellulose, the yield of the product depends on the parameters used in the experiments. There are various parameters that affect the yield of bacterial cellulose such as temperature incubation, pH medium and concentration pineapple residue, agitation rate and also time incubation

However, in this research, only the effect of temperature, pH medium and concentration pineapple residue are studied. It is believe that these three parameters give a big impact on yield of bacterial cellulose.

3.3.1 Effect of Temperature

Experiment was carried out with different temperature and the other parameter is kept constant. Medium fermentation was placed at incubator at temperature of 28°C, 29°C, 30°C, 31°C and 32°C for 5 days. The pH is fixed at 5.5 and concentration of pineapple residue was 80%. After incubated for five days, the bacterial cellulose formed was treated using natrium hydroxide for one days to remove excess of biomass and then the yield is weighed.

3.3.2 Effect of PH Medium

Experiment was carried out with different pH medium and the other parameter is kept constant. Medium fermentation was calibrated using pH meter at pH 4, 5, 6, 7 and 8 . The temperature is fixed at 30°C and concentration of pineapple residue was 80%.

After incubate for five days, the bacterial cellulose formed was treated using natrium hydroxide for one days to remove excess of biomass and then the yield is weighed.

3.3.3 Effect of Concentration Pineapple Residue

Experiment was carried out with different concentration pineapple residue and the other parameter is kept constant. Medium fermentation was measured at concentration pineapple residue were 60%, 70%, 80%, 90% and 100%. The pH is fixed at 5.5 and temperature is 30°C. After incubate for five days the bacterial cellulose formed was treated using natrium hydroxide for one days to remove excess of biomass and then the yield is weighed.

3.4 RESPONSE SURFACE METHODOLOGY (RSM)

Design Expert Software (State-Ease Inc, Statistic Made Easy, Minneapolis, MN, USA, (Version 6.0.4) is applied to execute the response surface methodology. The central composite design (CCD) is chosen in the optimization process due to its ability in providing factorial analysis in 3 levels of the factors involves (temperature incubation, pH medium & concentration pineapple residue), which will be from the centre point to the star point. The relation between the coded values and actual values is described in Equation 3.1.

$$Xi = \frac{Ai - Ao}{\Delta A} \tag{3.1}$$

Where;

X_i = Coded value of the variable
A_i=Actual value
A_o= Actual value of A_i at the centre point
The quadratic model to predict the optimal point is coded in Equation 3.2:

$$Y = \beta o + \sum \beta iXi + \sum \beta iiXi2 + \sum \beta ijXiXj \tag{3.2}$$

Where;

Y= Predict response variable
β_o= Offset term
β_i= Linear effect
β_{ii}= Interaction effect
X= Coded levels of the independent variables

The regression equation above was optimized for the optimal values using Design Expert Software. An experiment will be conducted in randomized order to stay away from systematic bias. Next, the ANOVA test is used to analyzed the experimental data obtained. P-value will be determined to identify the significance of the quadratic model. A P-value ≤ 0.05 are considered to be significant.

3.5 ANALYSED SAMPLE OF BACTERIAL CELLULOSE

3.5.1 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectroscopy was used to identify the chemical bonds are specific functional group of the membrane. The FTIR spectra of the films measured in the wavelength range from 1000 to 4000 cm^{-1} (Jatupaiboon *et al*., 2008).

3.5.2 Scanning Electron Microscopy (SEM)

SEM was used to generate high resolution images of surface and cross section of the film. The films sample was frozen in liquid nitrogen. Immediately, the sample was snapped, vacuum dried and then sputter with gold. Images were take on scanning electron microscope (Phisalapong *et al*., 2010).

3.6 MATERIALS OF BACTERIAL CELLULOSE PRODUCTION

Acetobacter xylinum and pineapple residue juice were the raw materials used in this study. HS-medium, agar plate, inoculums and pineapple residue juice also was prepared for bacterial cellulose production. The yeast extract, peptone, magnesium sulfate hepatahydrate, disodium hydrogen phosphate and citric acid were chemical used

for the preparation of the medium. 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, laminar flow, petri dish, water bath, freezer, filter funnel and filter paper in bacterial cellulose production. After that analysed the bacterial cellulose that formed using FT-IR and scanning electron microscope. *Acetobacter xylinum* was the bacterium that used in this experiment and was purchased from Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor. Residue from the peel, crown and core was collected and separated from its flesh and the pineapple residue was washed using tap water. The pineapple was purchased from Tunas Manja Supermarket.

3.7 EXPERIMENTAL PROCEDURE

3.7.1 Preparation Inoculum Using HS-Medium

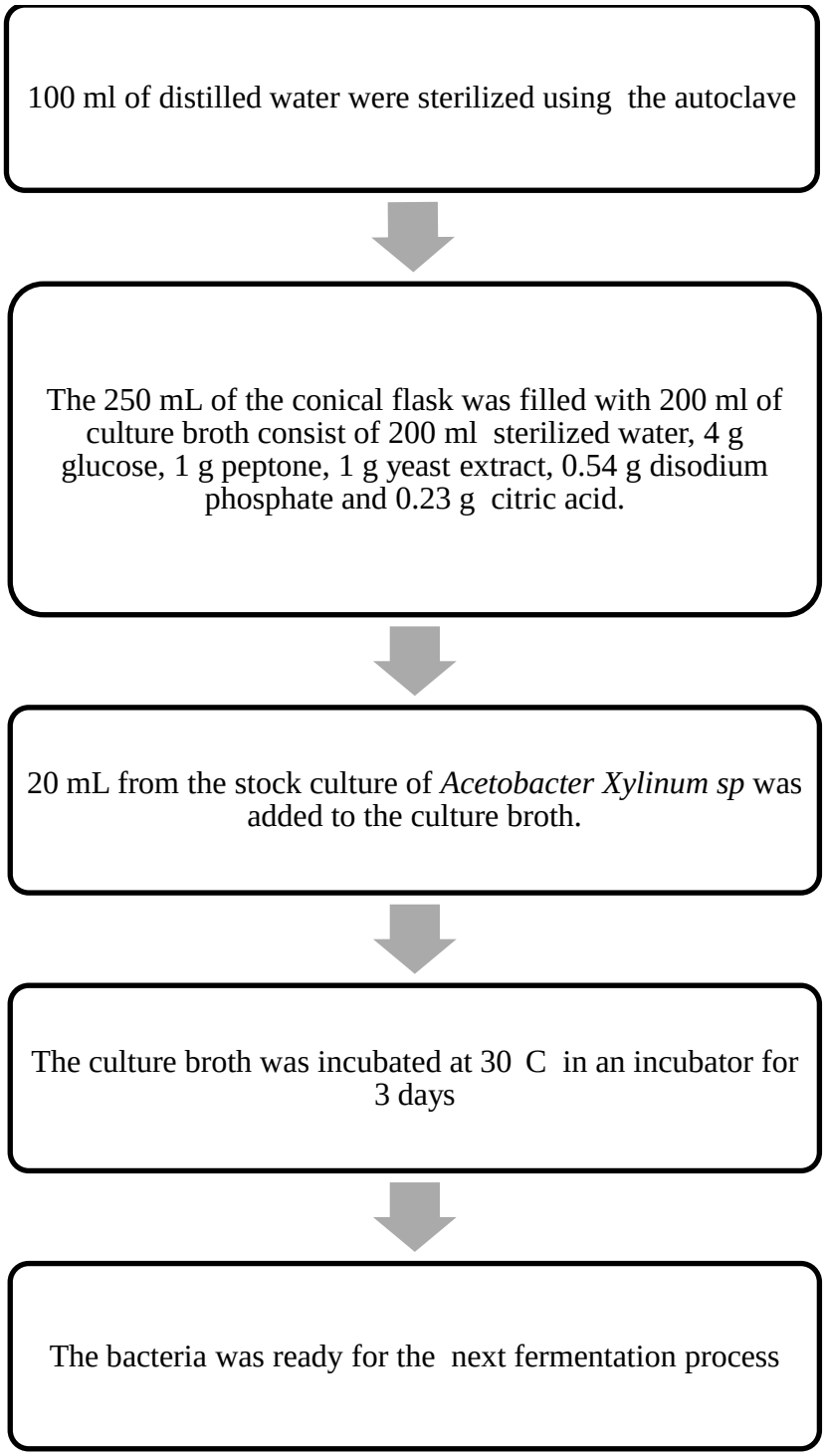


Figure 3.2: Preparation inoculum using HS-Medium.

3.7.2 Preparation Agar Plate (Stock culture)

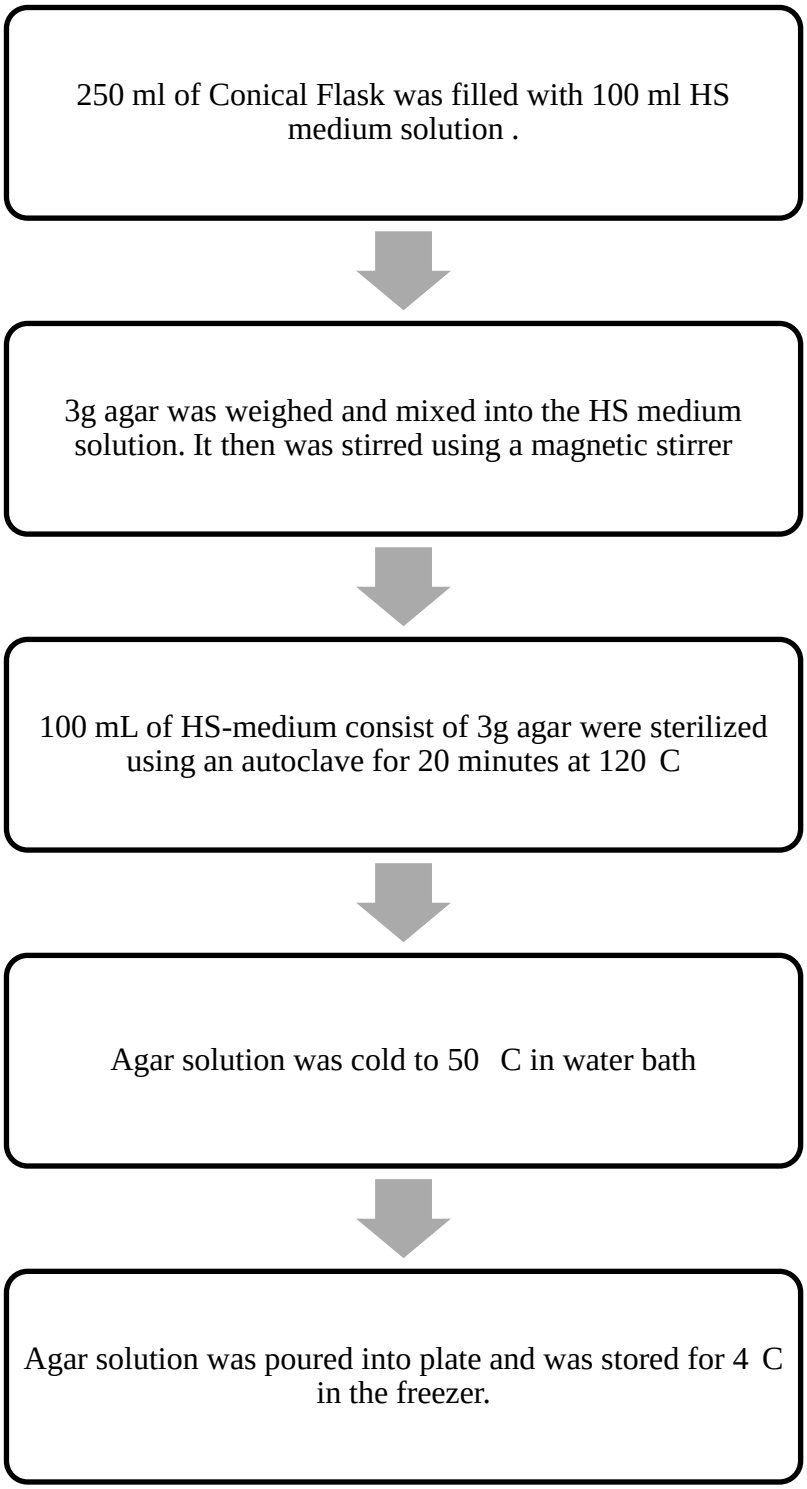


Figure 3.3: Preparation agar plate (stock culture)

3.7.3 Preparation Pineapple Residue Extract

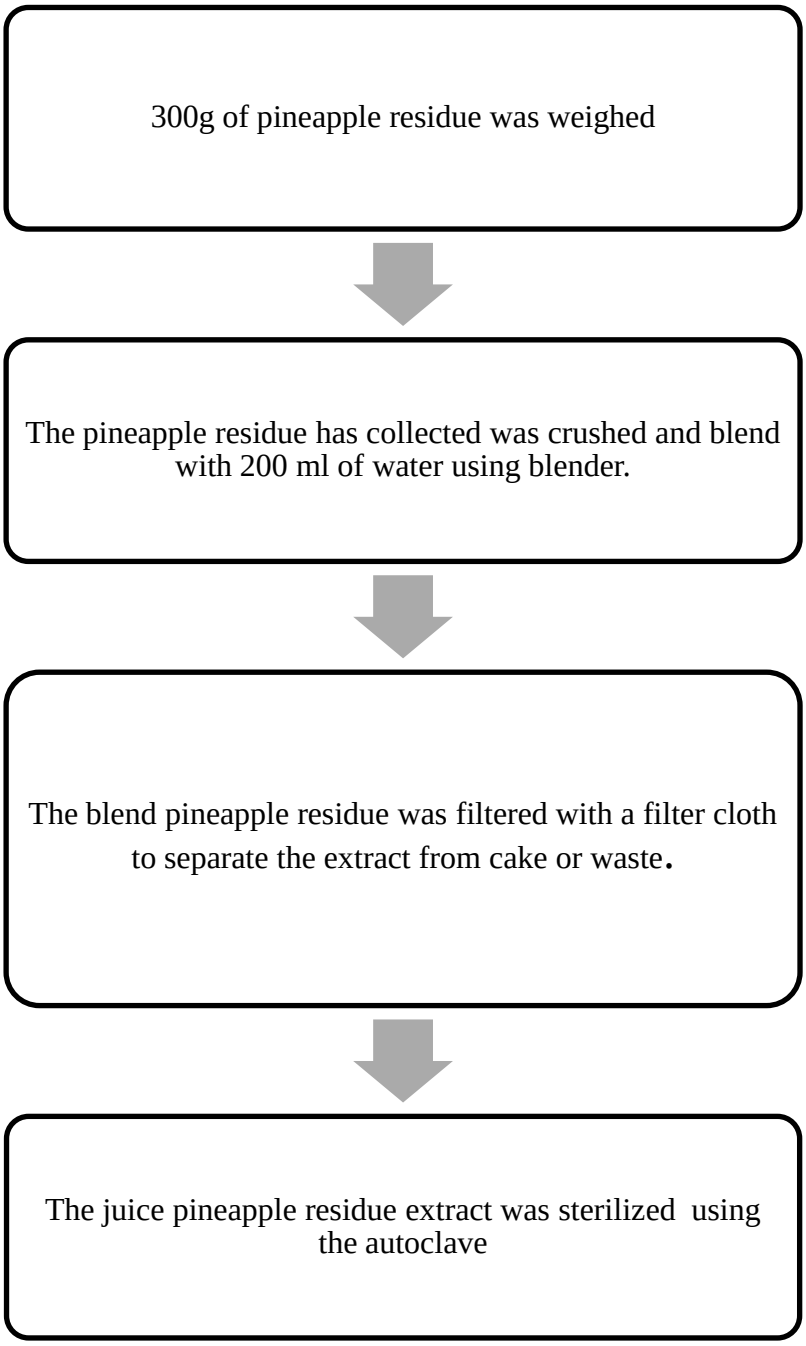


Figure 3.4: Preparation of pineapple residue juice

3.7.4 Synthesis of Bacterial Cellulose

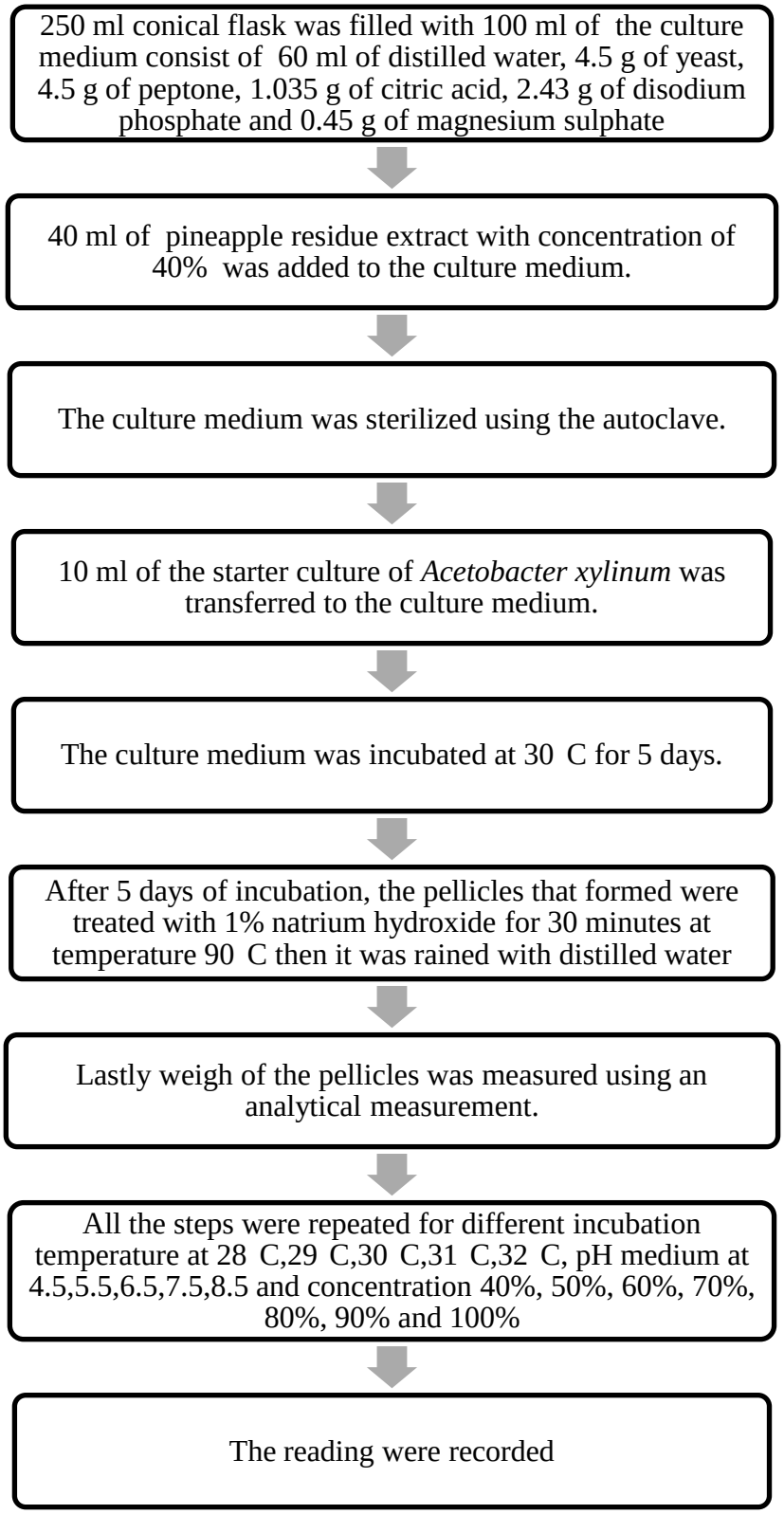


Figure 3.5: Synthesis of Bacterial Cellulose

3.7.5 Bacterial Cellulose Analysis

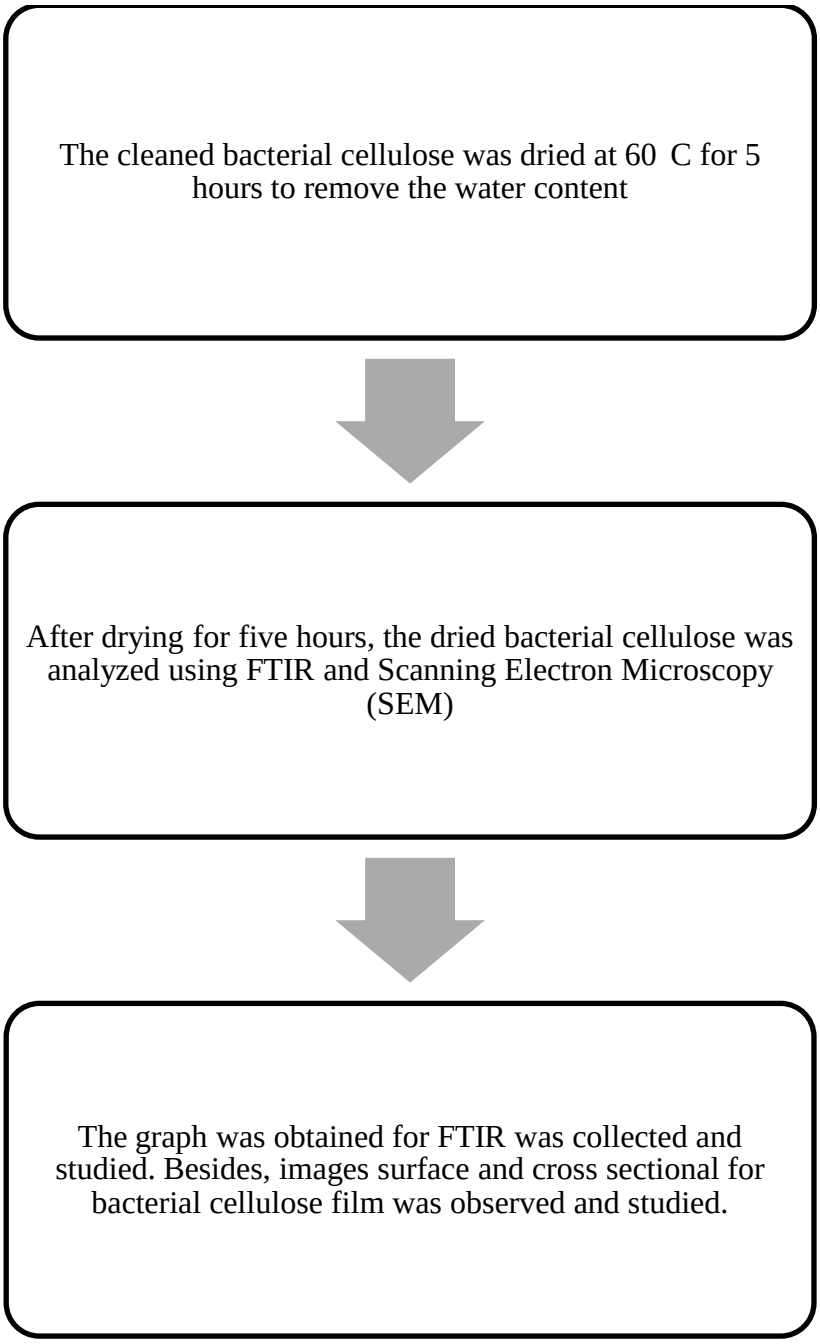


Figure 3.6: Bacterial Cellulose analysis

CHAPTER 4

RESULT AND DISCUSSION

4.1 THE EFFECT OF TEMPERATURE

The optimum temperature was determined by the weight of bacterial cellulose, where the medium fermentations were incubated for five days, whereas incubation temperature is varied the range of 28-32°C. The effects of incubation temperature on weight of bacterial cellulose are shown in Figure 4.1.

Based on the result in Figure 4.1, initially the weight of bacterial cellulose produced increased with the temperature. Based on Pourramezan *et al*., 2009, the weight of bacterial cellulose increased with the increase in incubation temperature because more energy is supplied to the bacteria to convert the glucose molecule into cellulose. The conversion of glucose to cellulose was regulated through a multi step carbon metabolism pathway involving a large number of both individual enzymes and complexes of catalytic and regulatory proteins (Chawla *et al*., 2008). Moreover based on Prashnant *et al*., 2008, enzyme that involves for conversion of glucose to cellulose will work at optimum performance between 28°C to 30°C.

However, as the incubation temperature is over than the temperature range, the reaction for enzyme will be slowed. Based on Figure 4.1, at the temperature 31°C to 32°C the weight of bacterial cellulose started to decrease. According to the report Pourramezan *et al*., 2009, *Acetobacter xylinum* that involved in bacterial cellulose production was not resistance to heat and will die at the high temperature.

The best temperature to obtain the maximum yield of weight bacterial cellulose is 30°C. This condition will then be used to study the effect of pH medium and concentration of pineapple residue.

Table 4.1: Result of dry weight bacterial cellulose at constant pH and concentration medium

Temperature °C	Dry weight of Bacterial Cellulose(g)
28	0.615
29	1.156
30	3.3948
31	0.369
32	0.0957

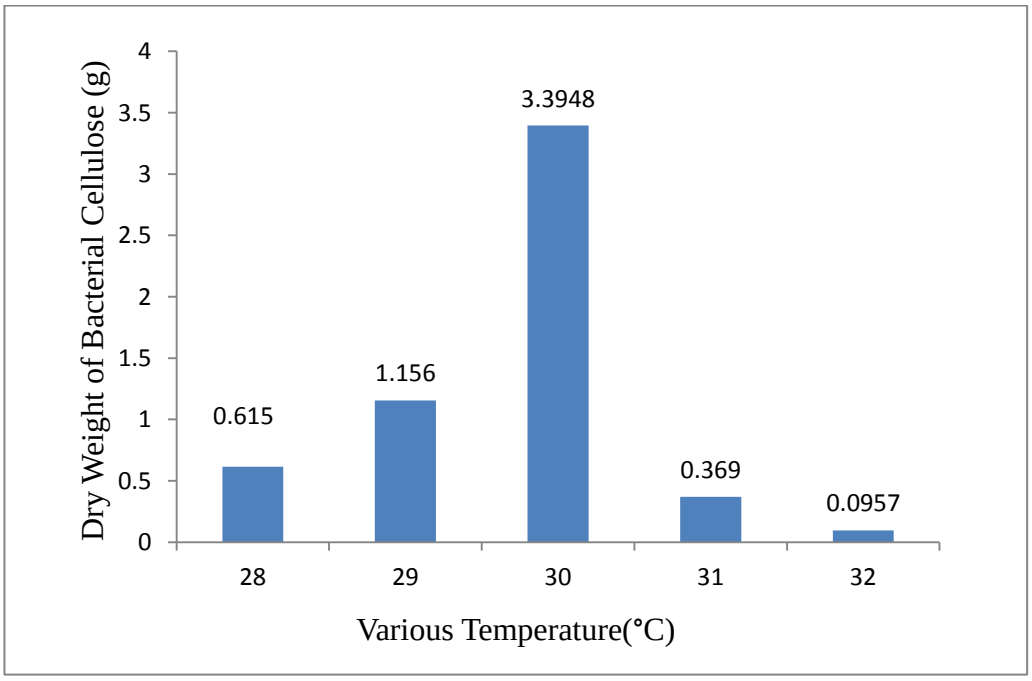


Figure 4.1: The effect of various temperature toward Bacterial Cellulose yield

4.2 THE EFFECT OF PH MEDIUM

All the medium fermentation was incubated for 30°C by using a various pH medium in range 3.5-7.5. The effect of pH medium on the weight of bacterial cellulose are shown in Figure 4.2.

Based on the Figure 4.2, the weight of bacterial cellulose produced increased at pH medium 4.5-5.5. 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. Therefore, the weight of bacterial cellulose increased with increased of pH medium.

However, at the pH medium of 5.5-7.5 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.

As a result, the best pH medium was at the pH 5.5. This condition was used to study the effect of concentration pineapple residue.

Table 4.2: Result of dry weight bacterial cellulose at constant temperature and concentration medium

pH	Dry weight of Bacterial Cellulose (g)
3.5	1.0837
4.5	1.183
5.5	3.3948
6.5	2.1055
7.5	0.4886

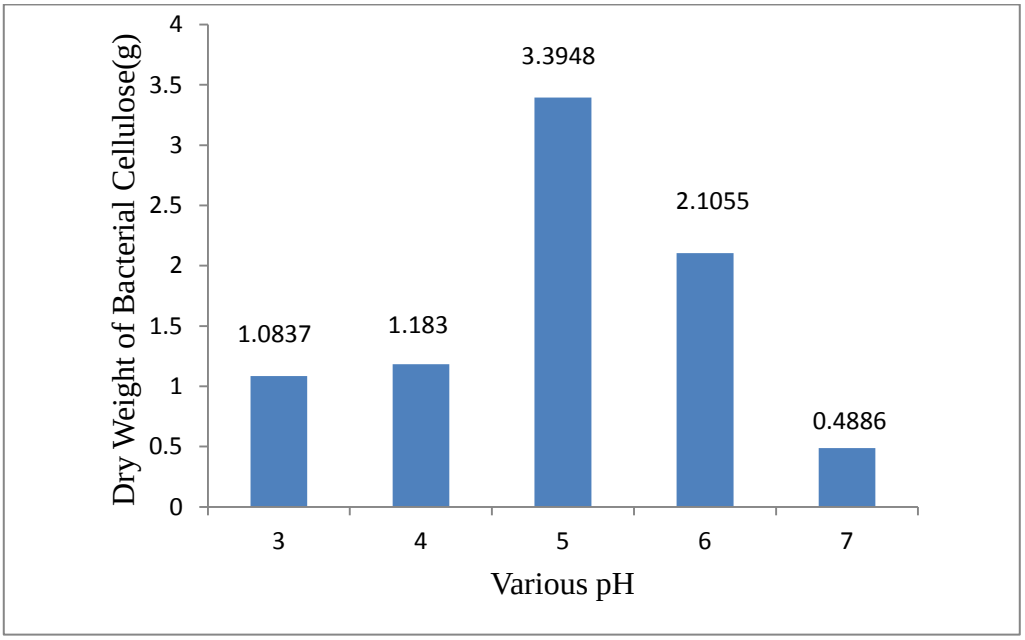


Figure 4.2: The Effect of various pH medium toward Bacterial Cellulose Yield

4.3 THE EFFECT OF PINEAPPLE RESIDUE CONCENTRATION.

All the medium fermentation was incubated for 30°C and pH of 5.5 by using a various pineapple residue concentration in range 40%-100%. The effect of concentration pineapple residue on the weight of bacterial cellulose is shown in figure 4.3.

Based on the Figure 4.3, initially the weight of bacterial cellulose produced increased at concentration pineapple residue 40%-80%. Based on the report Son *et al*., 2010, the weight of the bacterial cellulose increase with an increase of glucose content in the fermentation medium because more carbon sources is supplied to the bacteria to convert the glucose molecule into cellulose.

However, at the concentration pineapple residue of 80%-100% the weight of bacterial cellulose started to decrease. Hwang *et al*., 1999, reported that during the fermentation, the pH of the medium change throughout the process. Besides the conversion of glucose to cellulose, *Acetobacter xylinum* also convert glucose to gluconic acid. The accumulation and consumption of gluconic acid contribute to the changes of the pH medium and excess sugar in fermentation media also lowered down the cellulose yield because of conversion of excess of glucose into gluconic acid, therefore the yeild of bacterial cellulose decrease because more gluconic acid into the medium fermentation (Vandamme *et al*., 1998).

As a conclusion, the best concentration pineapple residue was at the 80%. Clearly, these three parameters; incubation temperature, pH medium and concentration pineapple residue is very important parameters in the production of bacterial cellulose. Thus, these factors are used for further study in the optimization to obtain the higher yield of weight bacterial cellulose.

Table 4.3: Result of dry weight Bacterial Cellulose at constant pH and temperature

Concentration medium (%)	Dry weight of Bacterial Cellulose (g)
40	1.1772
50	2.5057
60	2.9955
70	3.0257
80	3.3948
90	2.7792
100	1.6572

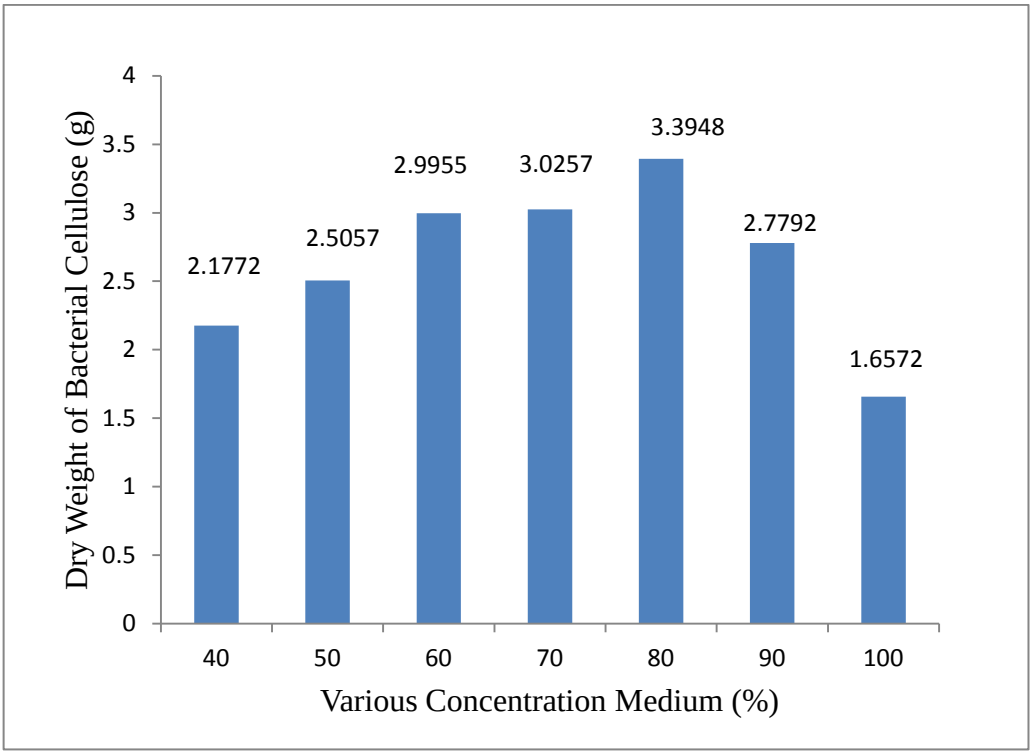


Figure 4.3: The effect of concentration medium toward Bacterial Cellulose yield

4.4 DETERMINATION OF THE OPTIMUM OF PARAMETER ON BACTERIAL CELLULOSE PRODUCTION USING RESPONSE SURFACE METHODOLOGY (RSM)

Optimization on the parameters in bacterial cellulose production is performed using response surface methodology (RSM). The parameters involved are pH, temperature and concentration media. The low level and high level of the parameters are determined from the previous result. Table 4.4 shows the low level and high level of each parameter.

Table 4.4: Low level and high level of parameters

Parameters	Low level	High level
pH	5	6
Temperature (°C)	29	31
Concentration medium (%)	70	90

By using central composite design (CCD), the experiments with different combination of pH, temperature and concentration medium was performed. Experiments arranged by Design Expert Software and the yield of bacterial cellulose produced are listed table 4.5.

Run 8 gave the highest production of bacterial cellulose which is 3.563 g. The parameters of run 8 are: pH medium (5.5), temperature (30°C) and concentration medium (80%). Conversely run 5 gave the lowest production which produced only 0.102 g of dry weight bacterial cellulose. The parameters of run 5 are: pH medium (6.5), temperature (30°C) and concentration medium (80%). According to Ishihara *et al*., 2002, the value that gave the highest weight of bacterial cellulose in static culture is 5 or 6 of pH medium and 30°C of temperature. An addition to obtain the better cellulose yield, most of the researchers used pH 5 or pH 6 in their research and the optimum temperature is 30°C because more energy is supplied to the microbe to convert of a glucose molecule into cellulose (Pourramezan *et al*., 2009). In the other case, the increasing temperature will decrease the yield of bacterial cellulose production because most of the bacteria were not resistance to heat and will die at the high temperatures. As

stated above, this can be observed the changes of dry weight of bacterial cellulose at run 4 and run 8 which produced 1.5782g and 3.563 g respectively. Although both of the runs have the same optimum parameter with 5.5 of pH medium and 80% of concentration medium but there is some difference in dry weight resulted from the different temperature with 32°C of run 2 and 30°C of run 8.

In the synthesis of bacterial cellulose, the amount of carbon sources in the fermentation medium played the main role to determine the yield of bacterial cellulose. The pineapple residue was used in this research containing glucose that functions as the carbon sources. The highest weight of bacterial cellulose at 80% of the concentration mediums is caused by more glucose content into the medium, therefore *Acetobacter xylinum* was synthesized glucose in the large amount into cellulose. In the same time, the yield of bacterial cellulose was decreased when a concentration medium is very high. This was observed from the dry weight of bacterial cellulose at run 11 and 8, which produced 0.4392g and 3.563 g respectively. For the both run have the same optimum of pH 5.50 and temperature 30°C but the different parameters in concentration, which are 100% and 80% at run 11 and run 8 respectively ,therefore also resulted from the different weight of bacterial cellulose. According to the report by Masaoka *et al*., 1993, decreasing the weight of bacterial cellulose is caused by increasing in the glucose concentration into the medium. Based on the Sherif and Keshk, 2005, during the fermentation process, *Acetobacter xylinum* was synthesized glucose into cellulose but in the same time acetic, lactic and gluconic acids were produced as the side product from *Acetobacter xylinum*. The accumulation of these acids into the medium fermentation is inefficiency of the bacterial cellulose production and was prevented from development of the large scale fermentation system. In addition, based on a report Vandamme *et al*., 1998, excess sugar in fermentation media decreased the cellulose yield because of conversion of excess glucose into gluconic acid which is an intermediate product of cellulose production by *Acetobacter* species and this conversion is not beneficial for overall cellulose productivity (Pourramezan *et al*., 2009).

The optimum pH of the culture medium for bacterial cellulose production is in range of 4.0 to 6.0 (Chawla *et al*., 2009; Prashant *et al*., 2008; Fontana *et al*., 1990).

This was observed from the dry weight of bacterial cellulose at run 5 and 8 which produced 0.102 g and 3.563 g respectively. Although concentration and temperature are same with concentration 80% and temperature 30°C for both run but also have the different weight of bacterial cellulose because have the different parameter in pH, which are 6.50 of run 5 and 5.5 of run 8. According to the report Bielecki *et al*., 2000, synthesizes cellulose will still be occurred in alkaline condition but in a lower yield and bacterial growth rate will decrease, eventually the bacterial will die at the pH medium is above pH 7. As stated above, the suitable condition for bacterial cellulose production was at pH 4 to pH 6, so in this research, it was observed from response surface methodology at pH 6.50 the weight of bacterial cellulose is lower and this is caused by pH 6.50 was approached to alkaline condition whereby not enable to enhance increasing of weight bacterial cellulose. As the state earlier, during fermentation process, gluconic, acetic or lactic acid was accumulated into the medium and decreases the pH medium thus decrease the yield of bacterial cellulose (Chawla *et al*.,2008), so bacterial cellulose production was decreased as the pH decrease from pH 6 to pH 4. This was observed from the dry weight of bacterial cellulose at run 19 and 8, which produced 2.506g and 3.563 g respectively. Although concentration and temperature are same for the both run with concentration 80% and 30°C but also have the different weight of bacterial cellulose because have the different parameter in pH, which are 4.50 of run 19 and 5.50 of run 8.

Table 4.5: Experiments designed by Design Expert Software

Run	Factor 1A: pH	Factor 2B: Temperature (C)	Factor 3C: Concentration medium (%)	Response : Dry weight of bacterial cellulose (g)
1	5.00	31.00	90.00	3.114
2	5.50	30.00	80.00	3.038
3	6.00	29.00	90.00	0.113
4	5.50	32.00	80.00	1.5782
5	6.50	30.00	80.00	0.102
6	5.50	30.00	80.00	3.2419
7	5.50	30.00	80.00	3.1834
8	5.50	30.00	80.00	3.563
9	5.50	30.00	60.00	0.9106
10	5.00	29.00	90.00	1.123
11	5.50	30.00	100.00	0.4392
12	5.50	28.00	80.00	0.2541
13	5.50	30.00	80.00	3.4252
14	6.00	31.00	90.00	1.4703
15	5.00	31.00	70.00	1.44
16	6.00	29.00	70.00	2.191
17	5.00	29.00	70.00	2.091
18	6.00	31.00	70.00	0.6335
19	4.50	30.00	80.00	2.506
20	5.50	30.00	80.00	3.154

Table 4.6 shows the ANOVA and regression analysis for the production of bacterial cellulose. The precision of a model can be checked by determination of coefficient (R2) and correlation confession (R). As a rule, a regression model having as R2 value higher than 0.9 is considered to have a very high correlation (Haaland, 1989). The value of R indicates better correlation between the experimental and predicted values.

Using multiple regression analysis on the experimental data, the following second order polynomial equations (4.1) was found to shown the dry weight of bacterial cellulose (Y)

$$Y = 3.26 - 0.51A + 0.24B - 0.092C - 0.49A^2 - 0.59B^2 - 0.65C^2 - 0.19AB - 0.24AC + 0.69BC \quad (4.1)$$

Where Y(g) was the response factors for dry weight of bacterial cellulose. A, B, and C were values of independent factors for temperature, pH and concentration. In order to verify the validity of the models, it is necessary to conduct an analysis of variance (ANOVA) as shown in table 4.6.

Table 4.6 recorded that the regressions for dry weight of bacterial cellulose were significant at <0.0001 and those lacked of fit was not significant at p is 1.59 and the values are greater than 0.1000 that indicate the model terms are not significant. The fits of the model were checked by the correlation coefficient, R^2. The R^2 value provided a measure of how much variability in the observed response values can be explained by the experimental factors and their interactions. The R^2 value always lied between 0 and 1. The closer R^2 value to 1.00, the stronger the model was and the better it predicted the response. In this case, the R^2 value for dry weight of bacterial cellulose is 0.9831. These values showed that 31.22% of the total variable were not explained by the model. The 'Pred R^2' of 0.9040 for dry weight of bacterial cellulose was reasonable agreement with 'Adj R^2' of 0.9678. This indicated a good agreement between the experimental and predicted values for dry weight of bacterial cellulose. The adjusted R2 corrected the R2 value for the sample size and for the number of terms in the model. If there are many terms in the model and the sample size is not very large, the adjusted R2 may be noticeable smaller than R2. This should be the caution signal as too many terms were present in the model (Haaland, 1989). The plot of predicted versus experimental dry weight of bacterial cellulose are shown in figure 4.4 with R2=0.9831, thus indicating an excellent adequacy of the proposed model.

In table 4.6, the P-value obtained for regression model <0.0001 compared to a desired significant level of 0.05. This signified that the regression model is precise in predicting the pattern of significance to the production of bacterial cellulose.

Table 4.6 : Annova for response surface quadratic model for the production of bacterial cellulose

Sources	Sum of square	Degree of freedom	Mean square	F-value	P-Value (Prob> F)	R^2	
Model	27.84	9	3.09	64.45	<0.0001	0.9831	Significant
A-pH	4.17	1	4.17	86.89	<0.0001		
B-Temperature	0.90	1	0.90	18.69	0.0015		
C-Concentration medium	0.14	1	0.14	2.85	0.1226		
A2	6.10	1	6.10	127.13	<0.0001		
B2	8.74	1	8.74	182.11	<0.0001		
C2	10.62	1	10.62	221.27	<0.0001		
Residual	0.48	10	0.048				
Lack of Fit	0.29	5	0.059	1.59	1.59		Not significant
Pure Error	0.19	5	0.037				
Correlation Total	28.32	19					

Dry weight of bacterial cellulose: R2 0.9831; adjusted R2 0.9678; predicted R2 0.9040

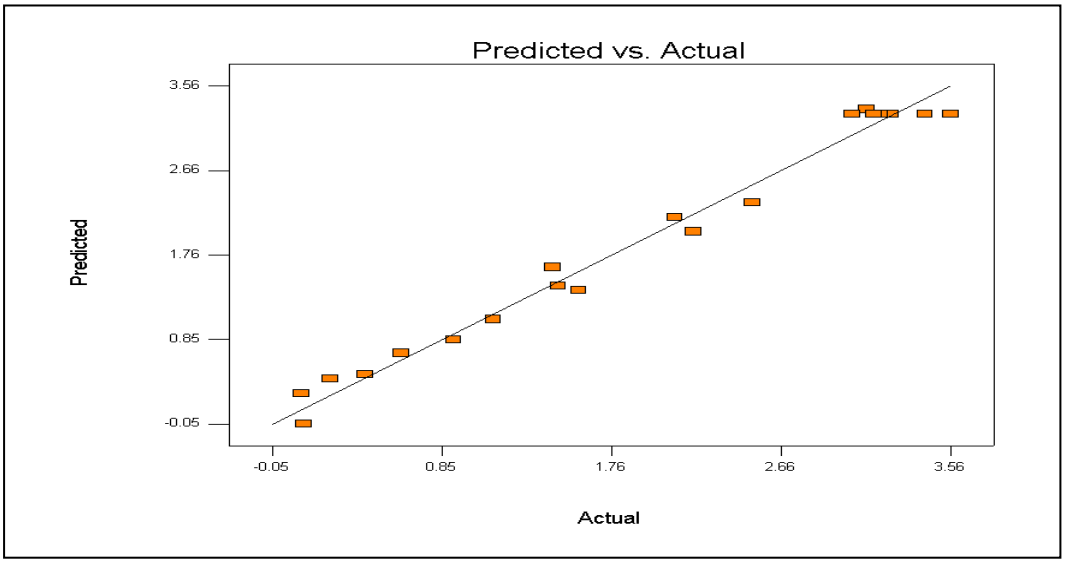


Figure 4.4: Plot of predicted versus experimental data for dry weight of Bacterial Cellulose.

4.4.1 Interaction Of Parameters

To investigate the effects of the parameter on the dry weight of bacterial cellulose, the response surface methodology was used and the tree-dimensional plot was drawn. Figure 4.5,4.6 and 4.7 shows the response surface plot for the parameters studied; pH medium, temperature and concentration medium.

PH of medium, temperature and concentration medium gave the significant effect to the production of bacterial cellulose. From the figure 4.5, 4.6 and 4.7, the data indicated that the increase in pH medium, temperature and concentration resulted in the increase in dry weight of bacterial cellulose within the range 5.25-5.75 of pH medium 29.50°C -30.50°C of temperature and 70%-80% of the concentration mediums. After this range, dry weight of bacterial cellulose slightly decreased. The maximal production 3.48818 g of dry weight bacterial cellulose was obtained when using 5.15 of pH medium, 30.51°C of temperature incubation and 83.32% of medium concentration.

Based on reported Chawla *et al*., 2008, *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 and the highest value of bacterial cellulose yield was a pH 5.25-5.75 as showed at Figure 4.5 and 4.6. The data indicated that as the pH either decrease or increase outside from the level optimum pH range 5.25-5.75 resulted decrease in cellulose production (figure 4.5 and 4.6). This was shown that more gluconic acid that was interfered for bacterial cellulose production was formed in range 5.00-5.25 for pH medium during synthesized glucose to bacterial cellulose. According to the Klemn *et al*., 2001, during cultivation, gluconic acid and 5-keto-gluconic acid are responsible for the decrease of the pH value from the culture medium and gluconic acid is not beneficial for overall cellulose productivity. For the other side in range 5.75-6.00 for pH medium also resulted in decrease in cellulose production. Although in alkaline condition has a minimum conversion of glucose into gluconic acid that enables increase's cellulose production (Pourramezan *et al*., 2009) but the result still lower for bacterial cellulose yield. This indicated that in alkaline condition, the production of cellulose will still be occurred but in a lower yield

and the bacterial growth rate will decrease, eventually the bacteria will die (Bielecki *et al*., 2000).

Based on report by Jonas and Farah., 1998 the optimal growth temperature for cellulose production is 25°C -30°C, although most researchers used 28°C -30°C and 30°C was observed as the better temperature for cellulose yield. This was showed by the figure 4.5 and 4.7 where it was revealed that the highest value of bacterial cellulose yield was a temperature 29.00°C -30.00°C and the ideal production for bacterial cellulose is 30°C. According to the report by Chawla *et al* 2008., the conversion of glucose to cellulose was regulated a multi step carbon metabolism pathway involving a large number of both individual enzymes and complexes of catalytic and regulatory proteins. For the temperature in range 30.00°C -31°C from the figure 4.5 and 4.7, the bacterial cellulose yield was decreased because the enzyme cannot perform well when the temperature is over than optimum temperature range. As stated above, the various enzyme that was involved for conversion of glucose to cellulose that occurs in a metabolic pathway and the suitable temperature was between 28°C to 30°C for the enzyme to work optimum performance. Most of the bacteria was not resistance to heat and will die at the high temperatures as well as *Acetobacter xylinum sp*.

In the synthesis of bacterial cellulose, *Acetobacter xylinum* used natural glucose that presents in the pineapple residue medium and converts it into value products, which are bacterial cellulose in pellicle form. In the normal condition, *Acetobacter xylinum* produced thicker of layer bacterial cellulose by synthesize more glucose and was resulted in higher weight of bacterial cellulose as shown in figure 4.6 and 4.7. For the other case, the data indicated that as the concentration medium either decrease or increase outside from the optimum concentration range 70%-80%, resulted in the decrease in cellulose production due to more gluconic acetic or lactic acid formed when more glucose was synthesized during the fermentation process. This side product will accumulate into the medium and decreases the pH level of the culture medium. In the same time affecting the condition of the culture medium thus decrease the bacterial cellulose production (Prashnant *et al*., 2009).

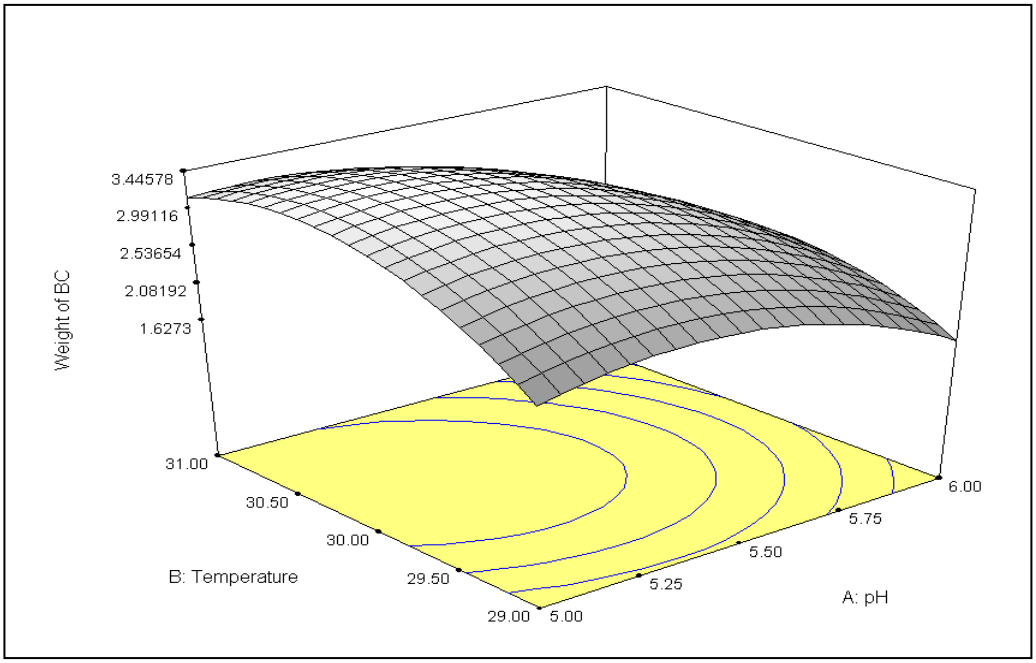


Figure 4.5: Response surface plot of bacterial cellulose production: Interaction of temperature and pH towards Bacterial Cellulose yield

Figure 4.5 showed the response of dry weight bacterial cellulose with respects to temperature and pH. This figure was mentioned that when increasing the pH from 5.00 to 5.50 and the temperature from 29.00°C to 30.00°C, thus resulted increase in the weight of bacterial cellulose due to this is the optimum condition for *Acetobacter xylinum* to growth very well and synthesize cellulose in high condition. The highest dry weight of bacterial cellulose obtain from the figure 4.5 was in pH (5.50) and temperature (30°C) with 2.53654g. In the highest yield, *Acetobacter xylinum* will need more energy from the outside to synthesize sugar content into cellulose and medium in acid condition also needed because *Acetobacter xylinum* is a type of acetic acid microbe (Prashnant *et al*., 2008).

The increasing temperature and pH over the optimum ranges which are 30.50°C to 31.00°C and pH 5.50 to pH 6.00, resulted decreased in the dry weight of the bacterial cellulose. The lowest dry weight of bacterial cellulose obtained from the figure 4.5 was in temperature (30°C) and pH (6.00). In the pathway synthesize glucose into cellulose was regulated through the multi step carbon metabolism involving a large number of the individual enzyme and enzyme cannot perform very well when the temperature increase

over the temperature range (Chawla *et al*., 2008). *Acetobacter xylinum* is a type of the acetic acid microbe, so when medium condition was the approach to the alkaline condition, synthesize cellulose will be slow thus decrease the yield of the bacterial cellulose due to this microbe not resistance to the alkaline condition. As a conclusion interaction between temperature and pH is significant based on the yield of bacterial cellulose.

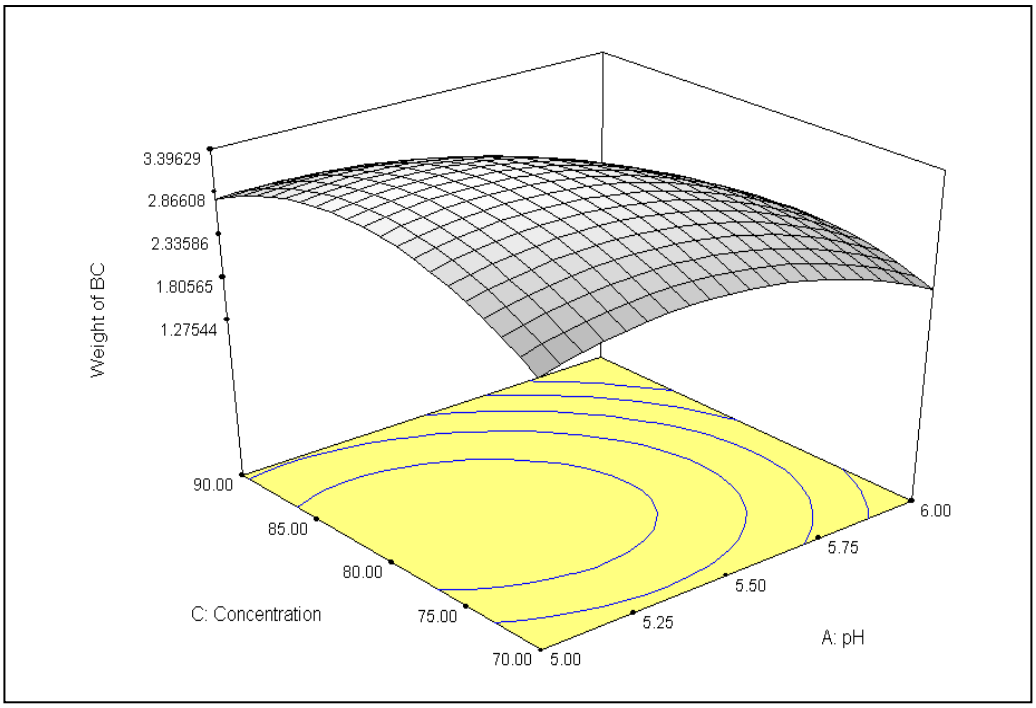


Figure 4.6: Response surface plot of bacterial cellulose production: Interaction concentration and pH towards Bacterial Cellulose yield

Figure 4.6 showed the response of dry weight bacterial cellulose with respects to concentration and pH. This figure was mentioned that when increasing the concentration from 70% to 80% and the pH from 5 to 5.50, thus resulted increase in the weight of bacterial cellulose due to this is the optimum condition for *Acetobacter xylinum* to growth very well and synthesize cellulose in high condition. The highest dry weight of bacterial cellulose obtained from the figure 4.6 was in concentration (80%) and pH (5.50) with 2.33586 g. In the highest yield, more sugar content was supply to *Acetobacter xylinum* to synthesize glucose into cellulose and medium in acid condition also needed because *Acetobacter xylinum* is a type of acetic acid microbe.

The increasing concentration and pH over the optimum ranges which are 70% to 80% and pH 5.50 to pH 6.00, resulted decreased in the dry weight of the bacterial cellulose. The lowest dry weight of bacterial cellulose obtained from the figure 4.6 was in concentration (90%) and pH (6.00). In the pathway to synthesize sugar content into cellulose, *Acetobacter xylinum* not just convert sugar content into cellulose but in the same time also converted it into gluconic acid, lactic acid and acetic acid as the side product. That mean more side products was formed when more amounts of sugar content present into the media. The accumulation and consumption of side product contribute to the decrease of the pH media thus decrease the dry weight of bacterial cellulose (Hwang *et al*., 1999). In alkaline condition *Acetobacter xylinum* also will grow slowly and eventually will die, so yield of bacterial cellulose will be low (Bielecki *et al*., 2000). As a conclusion interaction between concentration and pH is significant based on the yield of bacterial cellulose.

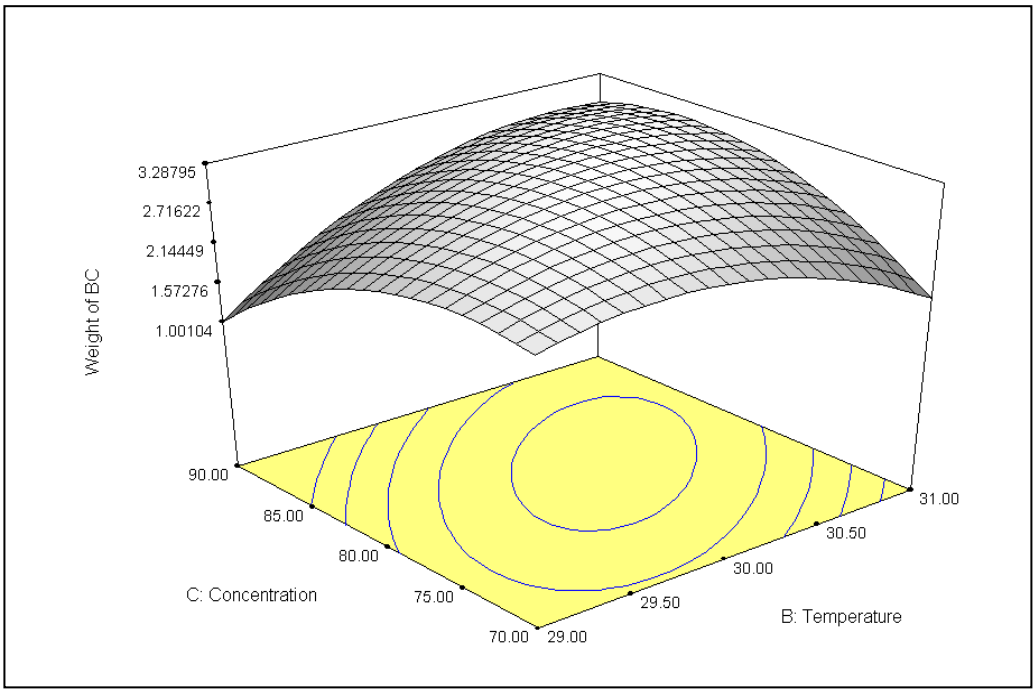


Figure 4.7: Response surface plot of bacterial cellulose production: Interaction concentration and temperature towards Bacterial Cellulose yield

Figure 4.7 showed the response of dry weight bacterial cellulose with respects to concentration and temperature. This figure was mentioned that when increasing the concentration from 70% to 80% and the temperature from 29°C to 30°C, thus resulted

an increase in the weight of bacterial cellulose due to this is the optimum condition for *Acetobacter xylinum* to growth very well and synthesize cellulose in high condition. The highest dry weight of bacterial cellulose obtained from the figure 4.7 was in concentration (80%) and temperature (30°C) with 2.1449 g.

The increasing concentration and temperature over the optimum range which are 80% to 90% and temperature 30°C to 31°C, resulted decreased in the dry weight of the bacterial cellulose. The lowest dry weight of bacterial cellulose obtained from the figure 4.7 was in concentration (90%) and temperature (31°C). In the medium fermentation, when the temperature too high, *Acetobacter xylinum* will die because it not resistance to heat thus yield of bacterial cellulose will be low (Pourramezan *et al*., 2009). As a conclusion interaction between concentration and temperature is significant based on the yield of bacterial cellulose.

4.5 VALIDATION OF THE MODEL

Based on Table 4.7, the production of bacterial cellulose was successfully optimized after the optimization process was carried out. The pH, temperature and concentration medium used was reduced after optimization. Table 4.7 shows summary of the optimized conditions for production. In order to validate the adequacy of the model equation 4.1, a total of three verifications experiments for dry weight bacterial cellulose responses were carried out under various fermentation conditions as shown in table 4.7. The verification of the results was accomplished by carrying out the experiments under optimal condition of pH 5.15, temperature 30.51 and concentration 83.32%. The agreement reached between the predicted and experimental result verifies the validity of the model and existence of an optimal point with error 0.0147 (table 4.7).

Table 4.7: Validation of the data and models constructed for Bacterial Cellulose yield.

Condition	**After Optimization**			**Before Optimization**	
	Value	**Dry weight Bacterial Cellulose (g)**		**Value**	**Dry weight of Bacterial Cellulose (g)**
pH	5.15	**Predict**	**Experimental**	6.00	
Temperature (°C)	30.51	3.48818	3.4368	31	1.4703
Concentration (%)	83.32			90.00	

Error (ε)= 0.0147

4.6 BACTERIAL CELLULOSE ANALYSIS

One of the most useful method to identify a chemical compounds based on the absorption of radioactive by the compounds chemical bonds is the Fourier Transform Infrared Spectroscopy. 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 C_3, C-O stretching and C-C stretching at C_6 (Sun, et al., 2008) are the chemical bonding that present in cellulose molecular structure. Figure 4.8 shows the molecular structure of cellulose.

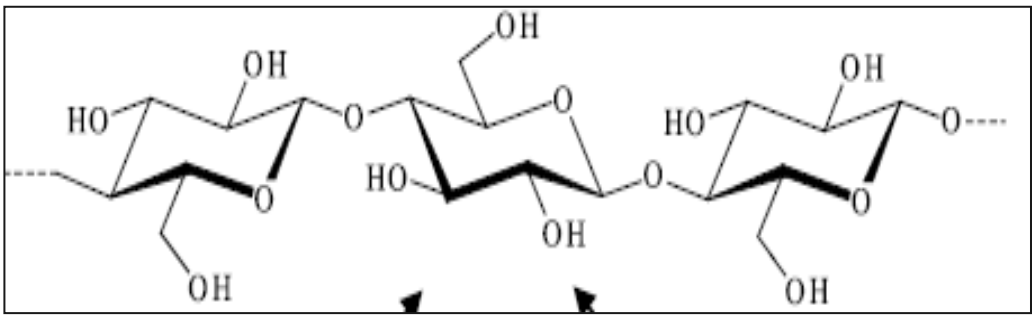


Figure 4.8: Molecular structure of cellulose

Sources: Klemm *et al*., 2001

Based on the result obtained from the FTIR analysis, the Figure 4.9 show the absorbance peak at 3744.97 cm^{-1}, 3615.08 cm^{-1}, 3410.62 cm^{-1} , 3332.89 cm^{-1} and 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} and 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, OH groups. An addition absorbance peak at 1393.77 cm^{-1} is appeared near in range 1235 cm that represent the strong bond in bacterial cellulose due to C-O symmetric stretching (Esin *et al*., 2011). From the analysis, absorbance also appear at 1076.39 cm^{-1}. The strong band in bacterial cellulose that appear near 1081 cm 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}. This absorbance peak was attributing to the bending mode of the absorbed water in the cellulose. After fermentation process, bacterial cellulose was treated using 1% of sodium hydroxide for purification step (Sherif *et al*., 2005) and some of the chemical bond was breaking during the treatment process. This was causing absorbance peak for cellulose that appeared on the FT-IR spectra was a not exact peak

as stated in literature review due to the breaking of some of the chemical bond thus decreasing the absorbance peak and shifting the absorbance peak to lower or higher wavenumber (Oh *et al*., 2005). 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.

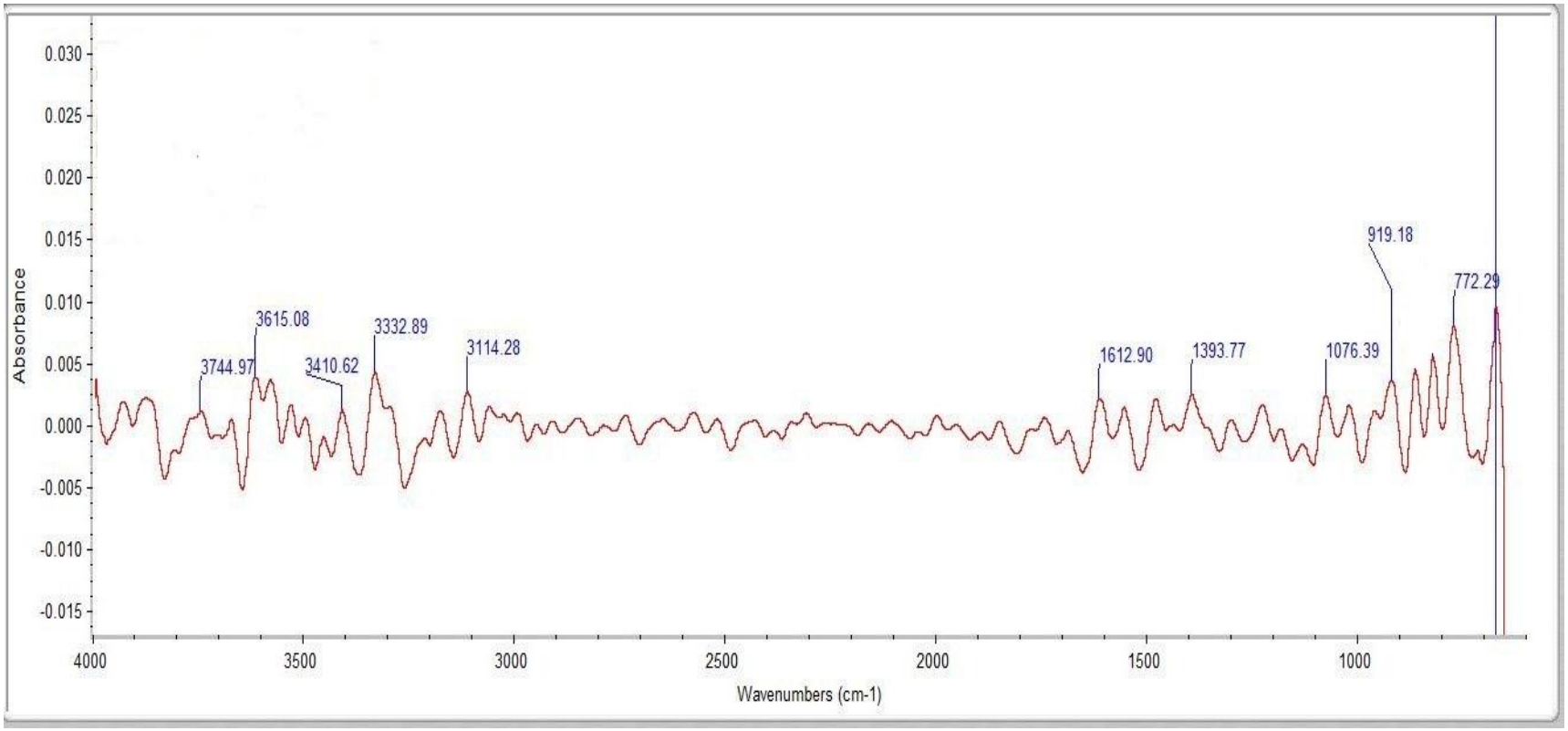
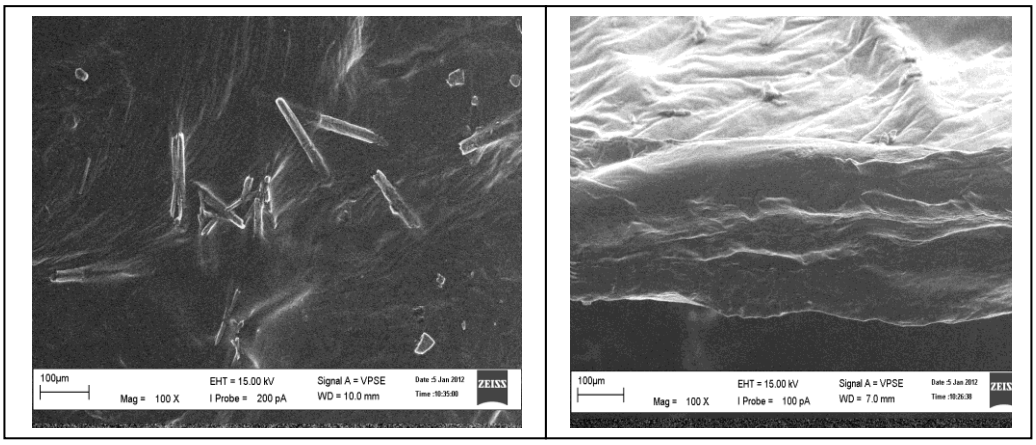


Figure 4.9: FT-IR spectra for bacterial cellulose (cm^{-1})

4.7 SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS



(A) (B)

Figure 5.0: Scanning Electron micrograph (A) surface of bacterial cellulose and (B) cross section of bacterial cellulose

SEM images for the surface and cross section morphology of the film sample were shown in figure 5.0. Figure 5.0 (A) present the surface image of the film from the sample that optimized using response surface methodology at pH 5.15, temperature 30.51 ^{O}C and concentration of 83.32%. The morphology of bacterial cellulose strictly depends on culture condition (Watanabe *et al*., 1998; Yamanaka *et al*., 2000). In static conditions (Figure 5.0 A), bacteria was accumulated cellulose mats on the surface of medium fermentation at the liquid interface with oxygen-rich air. From the Figure A, surfaces are not smooth, roughness and forming parallel but disorganized planes (Jonas and Farah, 1998). The surface image the sample showed the random orientation and good dispersion of the cellulose in the film surface.

Figure 5.0 (B) shows an image for cross section film samples of bacterial cellulose using the same sample with Figure 5.0 (A). The static conditions for bacterial cellulose fibrils are more extended and piled above one another in a criss-crossing manner. It has the larger cross-sectional width usually 0.05-0.10 um (Barbara *et al*., 2008).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

As a conclusion the results showed the optimum bacterial cellulose production is at temperature 30.51^{o}C, pH 5.15 and concentration pineapple residue is 83.32% with the amount of bacterial cellulose is 3.4368 g. Synthesis of bacterial cellulose and the bacterial growth involve with various parameter either major effect or minor effect. pH, temperature and concentration used in this research are the major parameters that give major effect in synthesis of bacterial cellulose. The other major parameters are carbon source concentration, agitation speed and dissolve oxygen concentration while the other gives minor effect. All of this parameter contributes in bacterial cellulose synthesis either increasing or decreasing the bacterial cellulose yield. Besides, this research used pineapple residue as the high potential carbon sources replacing the pure carbon sources as the substrate for the synthesis of bacterial cellulose and Response Surface Methodology (RSM) to optimize the production of bacterial cellulose in static conditions.

In addition, this result data also proved that pineapple residue is one of the high potentials of carbon sources for the synthesis of bacterial cellulose and it is more convenient than using the extraction from plants because it requires short time to produce the higher amount of bacterial cellulose.

5.2 RECOMMENDATION

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. Therefore, we can apply bioreactor for large scale production because it has high demand in industry. In this research, pineapple residue was used as a substrate to replace sugar in bacterial cellulose production; so that it is recommended to extend the research by using a different kind of waste that have a high amount of sugar content.

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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 check using the pH meter for pH and Uv-Vis spectrophotometer for sugar content. Another that, standard glucose calibration curve also needs to be prepare in the method.

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APPENDIX A

OFAT

A1) Effect of temperature

Temperature °C	Dry weight of Bacterial Cellulose(g)
28	0.615
29	1.156
30	3.3948
31	0.369
32	0.0957

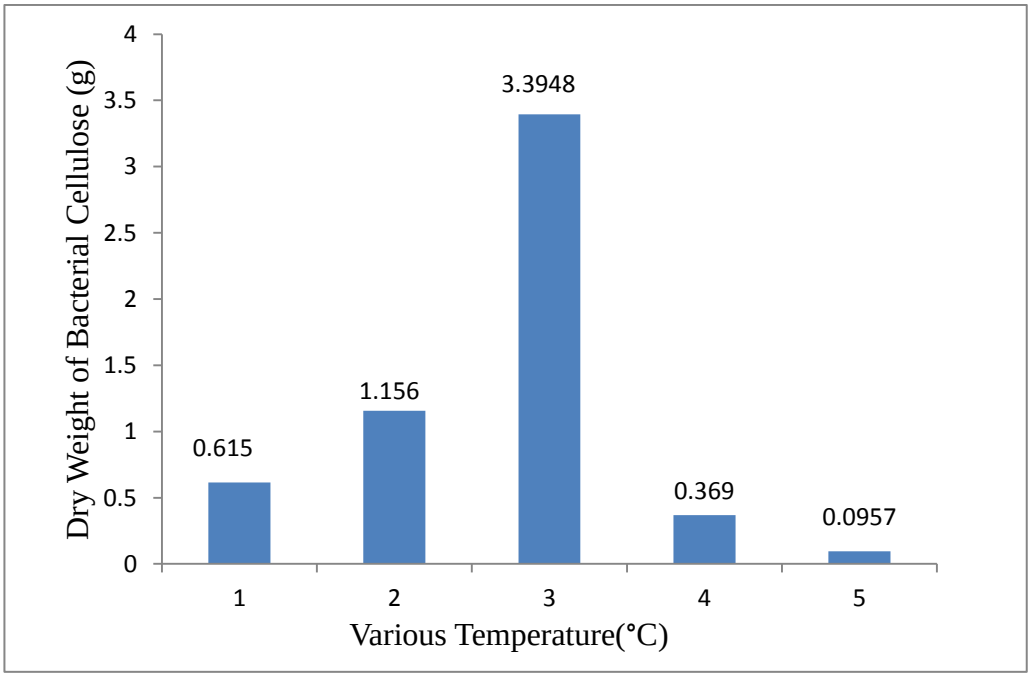


Figure A1:The effect of temperature toward Bacterial Cellulose yield

A2) Effect of pH

pH	Dry weight of Bacterial Cellulose (g)
3.5	1.0837
4.5	1.183
5.5	3.3948
6.5	2.1055
7.5	0.4886

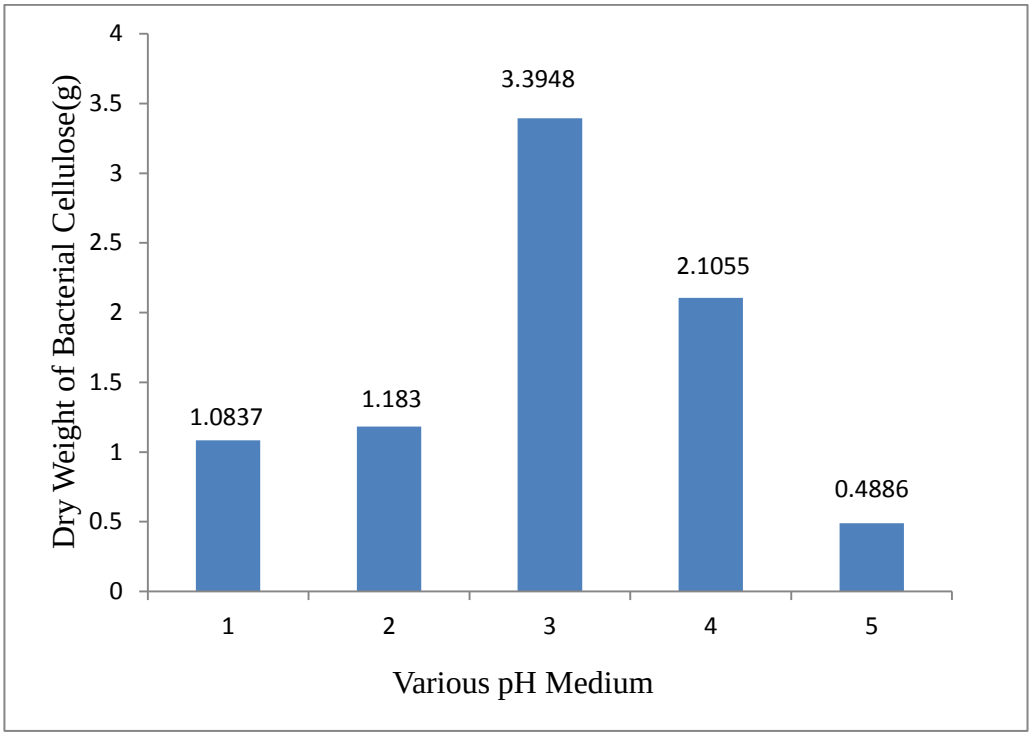


Figure A2:The effect of pH medium toward Bacterial Cellulose yield

A 3) Effect of concentration

Concentration medium (%)	Dry weight of Bacterial Cellulose (g)
40	1.1772
50	2.5057
60	2.9955
70	3.0257
80	3.3948
90	2.7792
100	1.6572

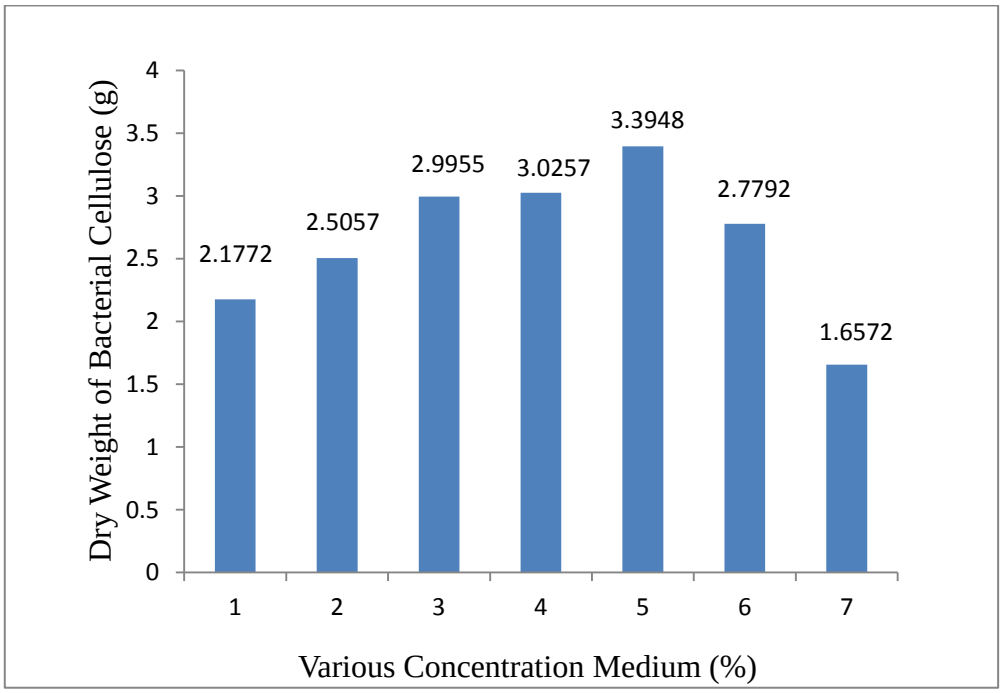


Figure A3: The effect of concentration medium toward Bacterial Cellulose yield

APPENDIX B

RESPONSE SURFACE METHODOLOGY (RSM)

ANNOVA Result for optimization of Parameters

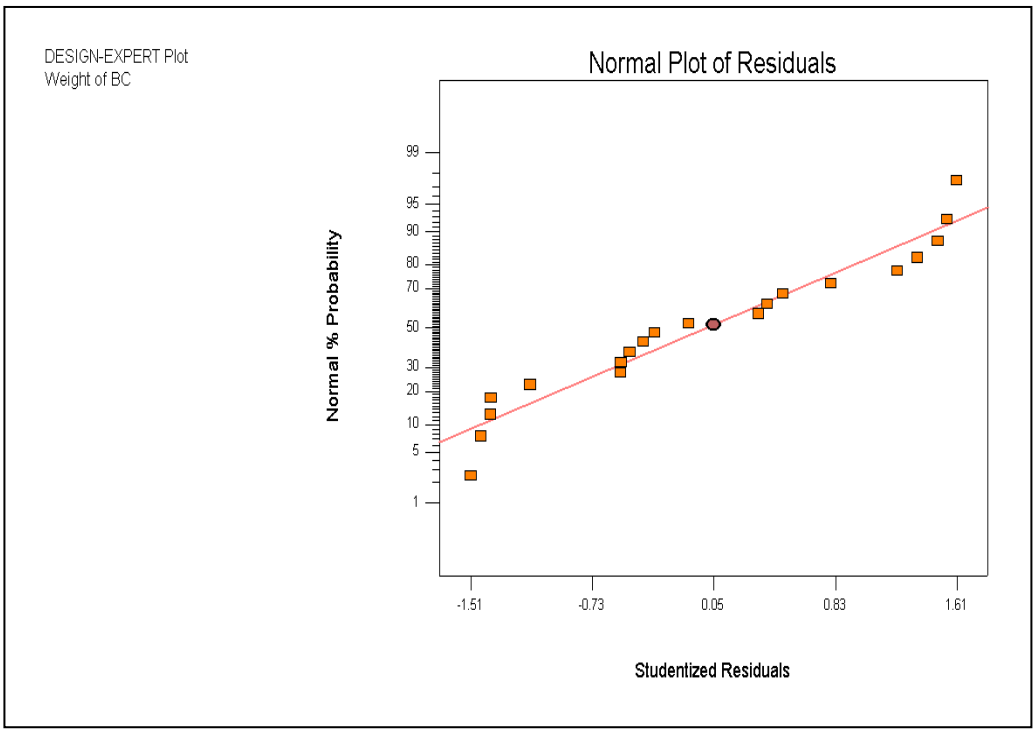


Figure B1: Graph of the normal plot of residual of Bacterial Cellulose production

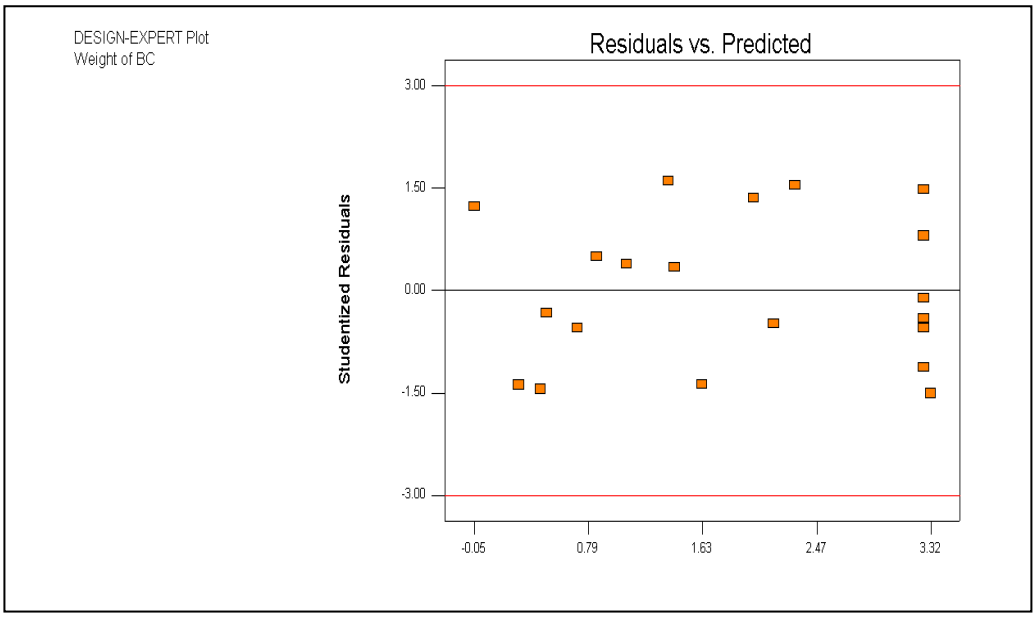


Figure B2: Residual Versus Predicted values of Bacterial Cellulose production

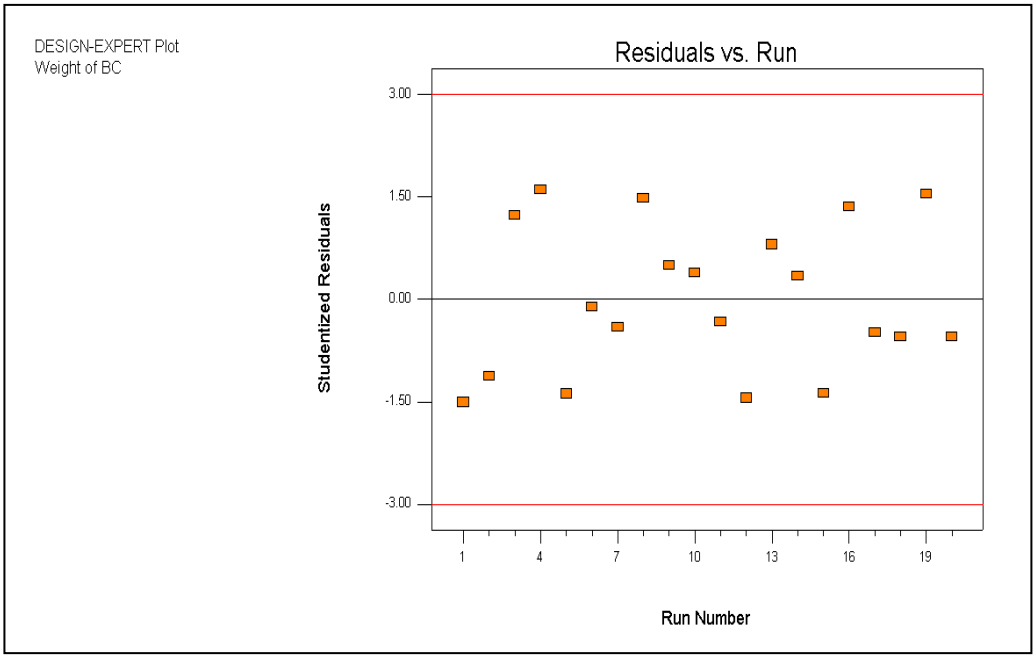


Figure B3: Residual values of Bacterial Cellulose production for each run experiment

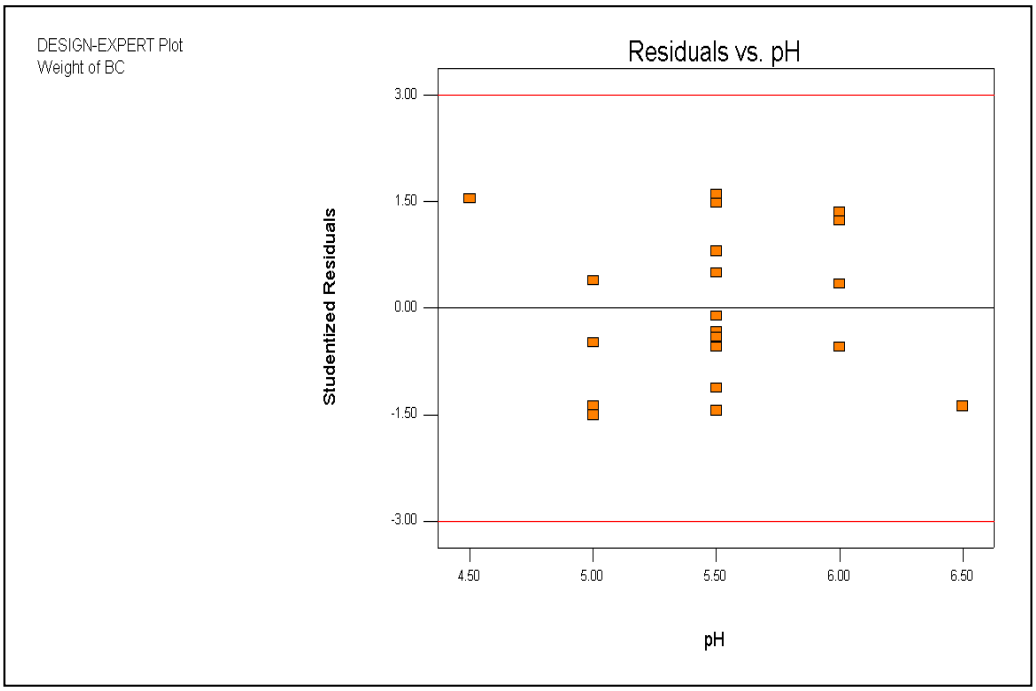


Figure B4: Residual versus pH values of Bacterial Cellulose production

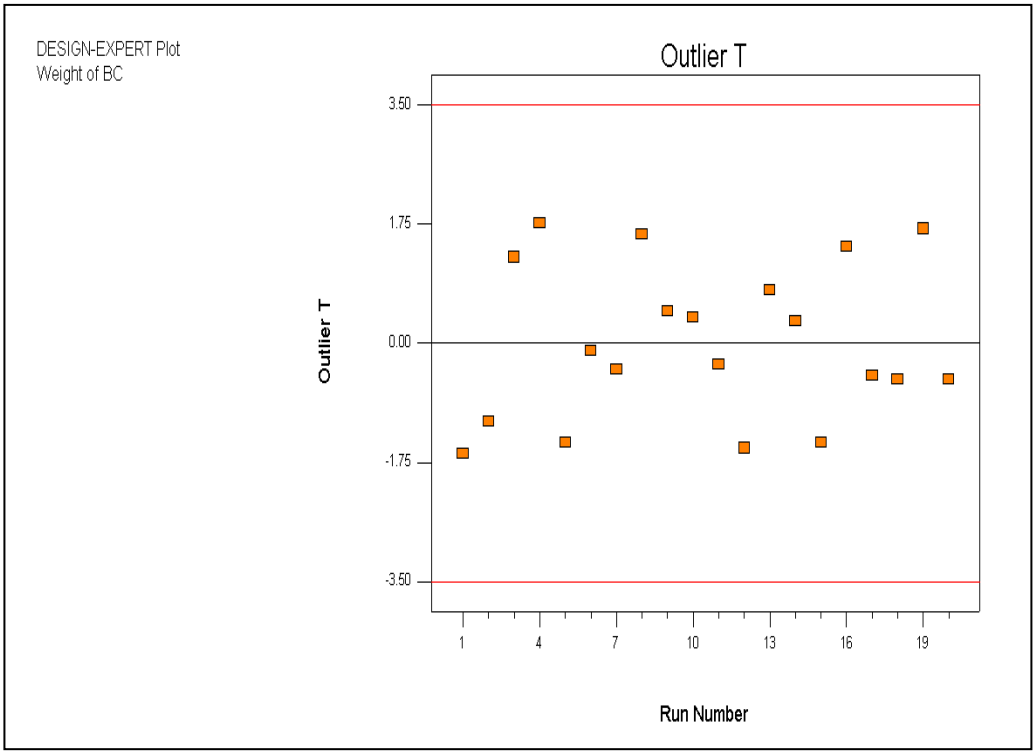


Figure B5: Graph of the outlier T of Bacterial Cellulose production

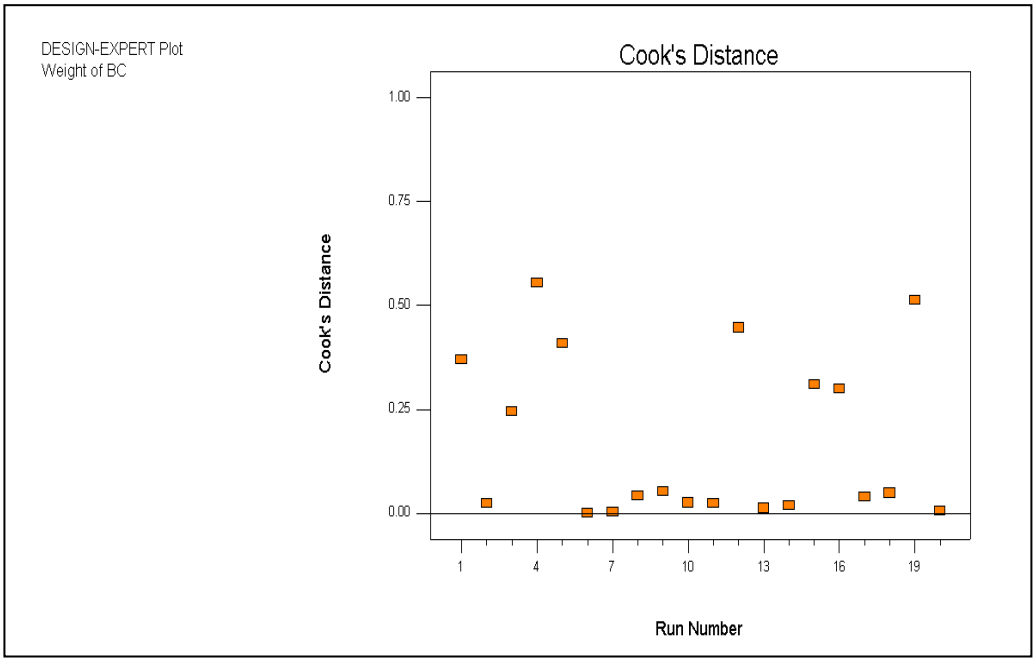


Figure B6: Graph of the Cook's distance of Bacterial Cellulose production

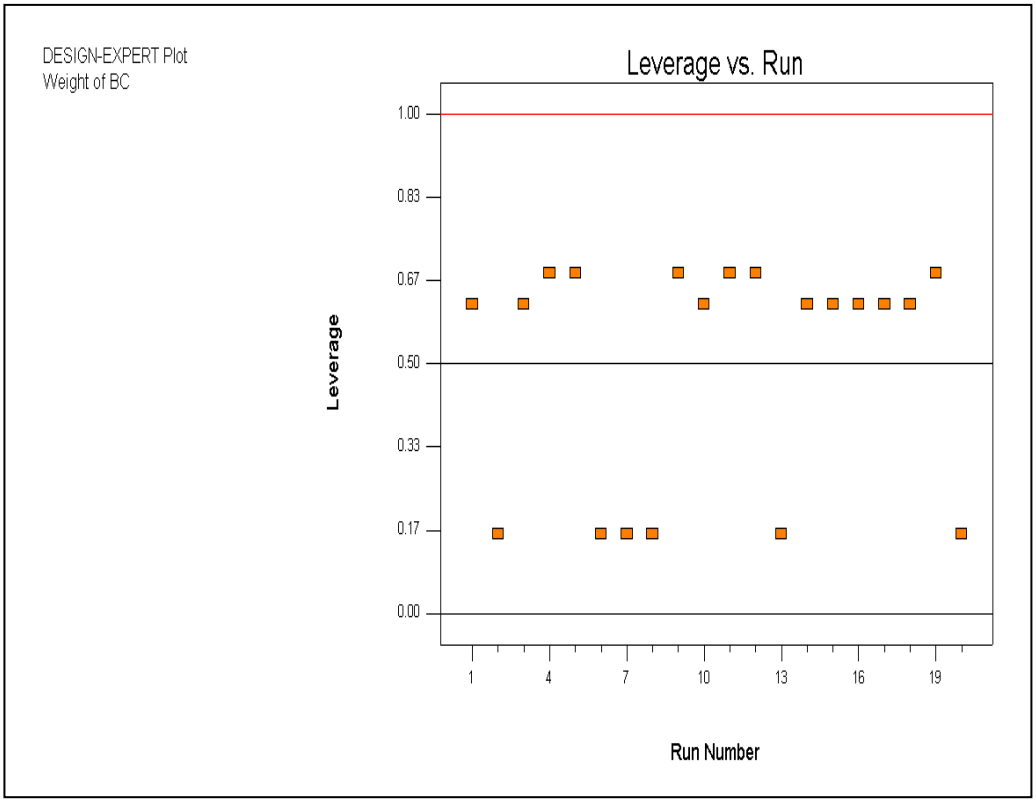


Figure B7: Leverage versus run values of Bacterial Cellulose production

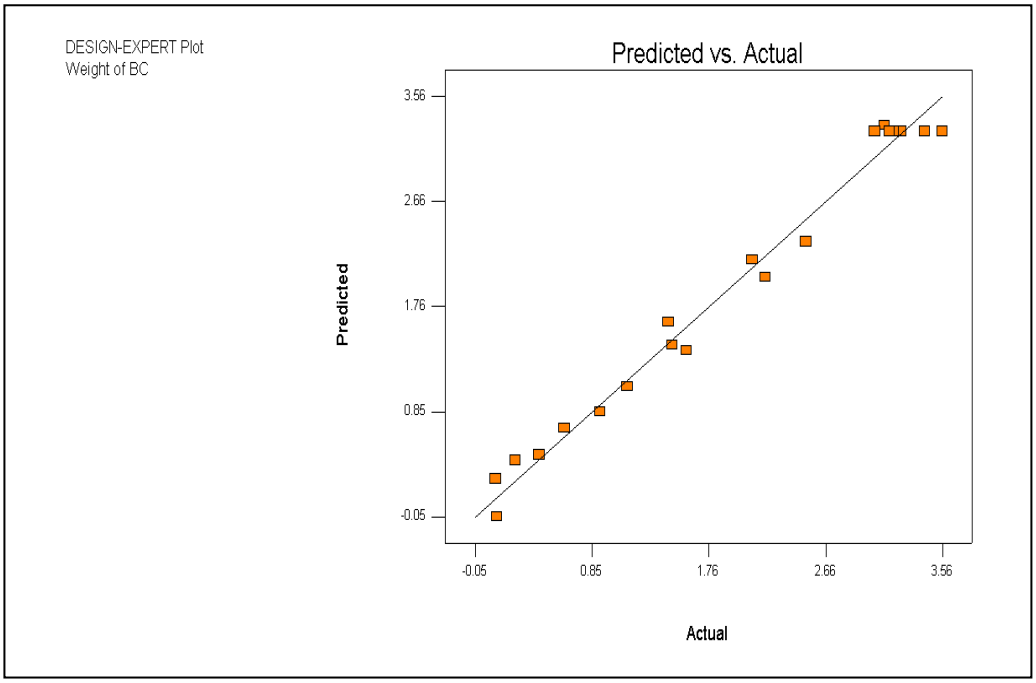


Figure B8: Graph of predicted versus actual values of Bacterial Cellulose production.

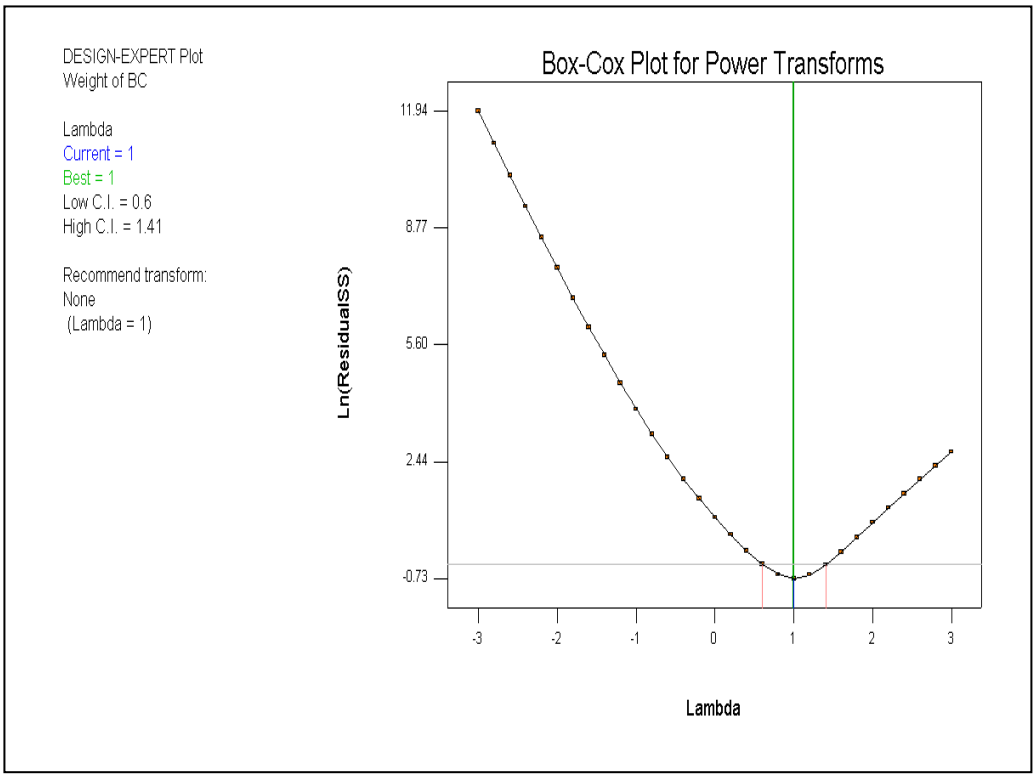
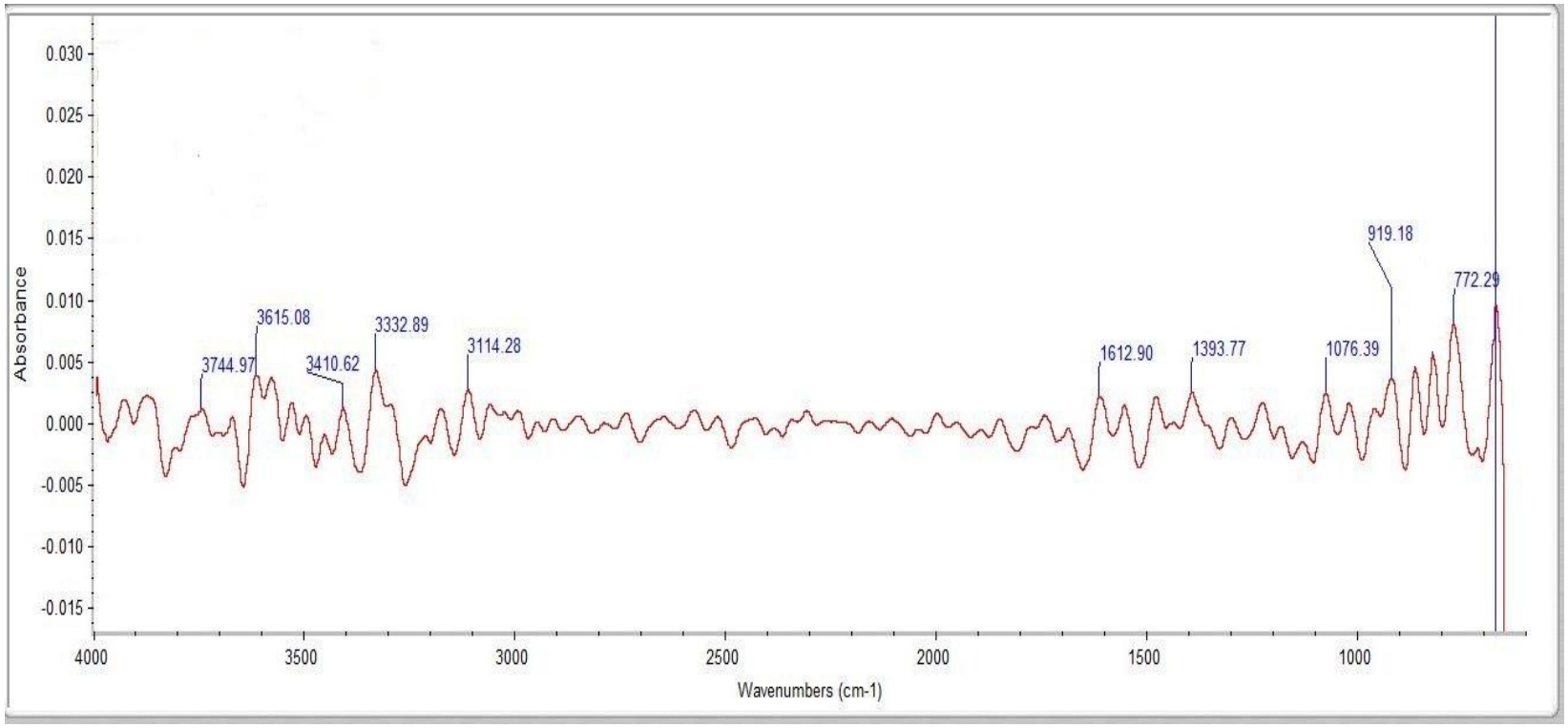


Figure B9: Plot of the Box- Cox for power transforms.

APPENDIX C

FOURIER TRANSFORM SERIES (FT-IR) SPECTROSCOPY



FT-IR spectra for bacterial cellulose (cm^{-1}).