Optimization of Bacterial Cellulose Production from Pineapple Waste: Effect of Temperature, pH and Concentration

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Abstract

Bacterial cellulose is a type of biopolymer produced by *Acetobacter xylinum* in high purity, high water holding capacity, good mechanical strength, elasticity and high crystallinity. In this research, pineapple waste is used as the carbon sources for the synthesis of bacterial cellulose. The objective of this study is to investigate the effect of temperature, pH and concentration of pineapple waste in the production of bacterial cellulose by *Acetobacter xylinum*. Parameters investigated are varied from 40% to 100% for the concentration, while the temperature is between 28°C to 32°C and pH of 4.5 to 8.5. Besides, this study also aims to optimize the production of bacterial cellulose from pineapple waste by using response surface methodology (RSM) based on the central composite design (CCD). The known value of the parameters is estimated earlier based on one factor at that time (OFAT). The results obtained from the OFAT showed the optimum condition is at pH 5.50, temperature 30°C and concentration of pineapple waste is 80 %, where the amount of bacterial cellulose dry weight is 3.3948g. According to the RSM result, the optimal conditions for bacterial cellulose were pH 5.15, temperature 30.51°C and concentration of pineapple waste is 83.32%. By using these optimal conditions, 3.4368g of bacterial cellulose is produced. The existence of bacterial cellulose is proven by Fourier Transform Infrared (FT-IR) Spectroscopy analysis based on the appearance of absorbance peak which are C-C bonding, C-O bonding, C-OH bonding and C-O-C bonding. In short, the data presented in this paper showed that pineapple waste has a great potential to use as the carbon source in production of bacterial cellulose

***Keywords*:** bacterial cellulose, *Acetobacter xylinum*, pineapple waste

I. INTRODUCTION

Cellulose is the most abundance polymer 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 have 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*. Over a century ago, this organism and its produce were first identified and characterized (Iguchi and Yamanaka, 1997).

Bacterial cellulose is a polymer produced by *Acetobacter xylinum* in presence of glucose. Bacterial cellulose has high purity cellulose where it free from lignin and hemicelluloses differ from plant cellulose that has low purity cellulose and containing lignin and hemicelluloses (Klemn *et al*., 2001). There are several aspect that differentiate bacterial cellulose with plant cellulose which are bacterial cellulose has unique characteristic including good mechanical strength, high water absorption capacity, high crystallinity, ultra-fine and highly pure fibre network structure that caused bacterial cellulose is 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 microorganism such as *Acetobacter xylinum*. In the synthesis process, bacteria that can produce highest cellulose amount is *Acetobacter xylinum.* It produced cellulose in the form of extracellular pellicle composed of ribbons, *Achromobacter, Aerobacter, Alcaligenes* produce cellulose in fibrils form while *Agrobacterium* and *Rhizobium* produces cellulose in the form of short fibril (Barbara *et al*., 2008). The third and fourth method are by the first enzymatic in-vitro synthesis starting from cellobiosyl fluoride and the first chemosynthesis from glucose by ring opening polymerization of benzylated and pivaloylated derivatives (Klemn *et al*., 2001)

Cellulose is the main part in the cell wall plant and act as protective and coating, whereas plant cellulose plays a structural role in plant (Bielecki *et al* 2000). However cellulose is obtained from the plant is not pure because it have lignin and hemicellulose, so it is difficult to purify the cellulose from them through 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 research 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 produced by bacteria that has a unique physical and chemical properties with cellulose that produced from plant (Prashant *et al*., 2008).

Bacterial cellulose also has disadvantages that need to encounter although bacterial cellulose has a unique characteristic than the plant cellulose. The main problem is the price for the sugar as a substrate is very expensive but low in production. Using the 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 utilizes as substrates to produce cellulose (Akihiro *et al*., 2008).

2. METHODOLOGY

2.1 Experimental Procedures

2.1.1 Preparation of Inoculum

250mL of conical flask is filled with 200mL of deionized water, 4g glucose, 1g peptone, 1g yeast extract, 0.54g disodium phosphate and 0.23g citric acid. Then, it is sterilized in autoclave. After that, 20mL stock culture of *Acetobacter Xylinum sp* is added into the conical flask. The culture broth was incubated at 30°C for 3 days. After 3 days, the inoculums is ready for the next fermentation process

2.1.2 Preparation of Pineapple Waste Juice

300g of pineapple residue is weighted. The pineapple residue collected was crushed and blend with 200 mL of water using blender. After that, pineapple residue was filtered with a filter cloth to separate the pineapple waste juice and then it was sterilized using an autoclave.

2.1.3 Synthesis of Bacterial Cellulose

250mL conical flask is filled with 100mL of fermentation medium consist of 60mL of distilled water, 4g of yeast, 45 g of peptone, 1.035g of citric acid, 2.43g of disodium phosphate and 0.45g of magnesium sulphate. 40mL of pineapple waste juice with concentration of 40% is added to the medium. Then, the fermentation medium is sterilized by using autoclave. After that, 10mL of the inoculums is transferred to the medium. The medium was incubated at 30°C for 5 days. After 5 days of incubation, the pellicles formed at the surface layer are treated with 1% sodium hydroxide for 30 minutes at temperature 90°C then it is rained with distilled water. Lastly, weight of the pellicles is measured. All the steps are repeated for different incubation temperature of 28°C,29°C,30°C,31°C,32°C, pH medium of 4.5,5.5,6.5,7.5,8.5 and concentration of pineapple waste juice of 40%, 50%, 60%, 70%, 80%, 90% and 100% .

2.1.4 Bacterial Cellulose Analysis

The cleaned bacterial cellulose is dried at 60°C for 5 hour to remove the water content. After drying for 5 hours, the dried bacterial cellulose is analyzed using FTIR. After that, the graph for FTIR is collected and studied.

3.0 RESULTS & DISCUSSION

3.1 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, anti-symmetric bridge stretching of C–O–C, C-C stretching at C_6 (Sun *et al*., 2008), H bond in OH group, aliphatic OH group (Guo et al., 2008), C-O stretching at C_3 and C-O stretching are the chemical bonding that present in cellulose molecular structure. Figure 3.1 show the absorbance peak at 3744.97cm^{-1}, 3615.08cm^{-1}, 3410.62cm^{-1}, 3332.89cm^{-1} and 3114.28cm^{-1} that is originated from the OH stretching. This result had been proven from the previous study by Parmjit, *et al*., 2008 where the peaks that appears near in range of 3853 -3256 cm^{-1} is hydroxyl functional group. The absorbance peak at 1393.77cm^{-1} is also showed and according to the Sun et al., 2008, several bands typical for bacterial cellulose were shown in region of 1500-1235cm^{-1} due to in plane bending vibration of CH_2, CH, OH groups. In addition, absorbance peak at 1393.77 cm^{-1} also represent the strong bond in bacterial cellulose due to C-O symmetric stretching (Esin *et al*., 2011). From the figure, absorbance peak also

appeared at 1076.39 cm^{-1}. The strong band in bacterial cellulose that appears near 1081cm^{-1} is representative for the C-O-C asymmetric stretching (Sun *et al*., 2008). Besides, there is also absorbance that appears at 1612.90cm^{-1}. This absorbance peak attributing to the bending mode of the absorbed water in the cellulose.

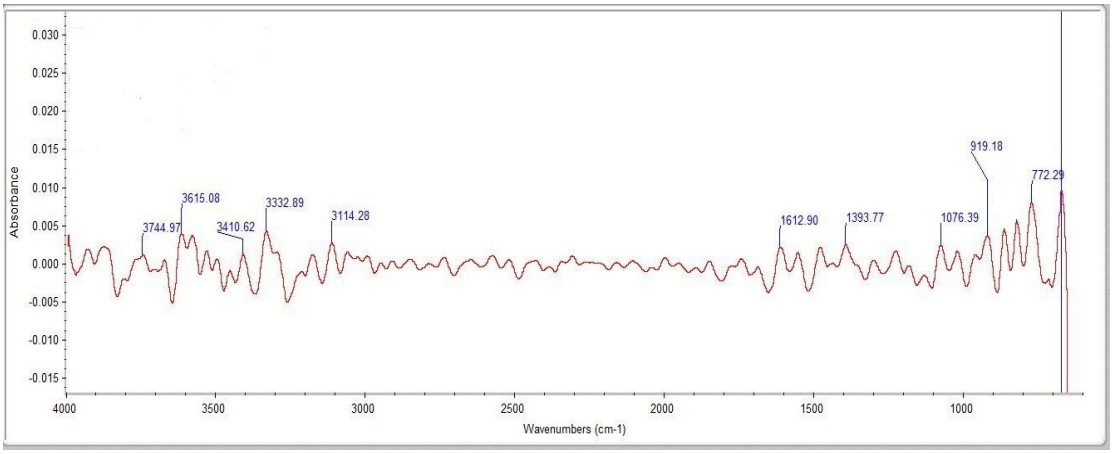

Figure 3.1: FT-IR spectra for bacterial cellulose (cm^{-1})

3.2 Synthesis of Bacterial Cellulose

3.2.1 Effect of Temperature

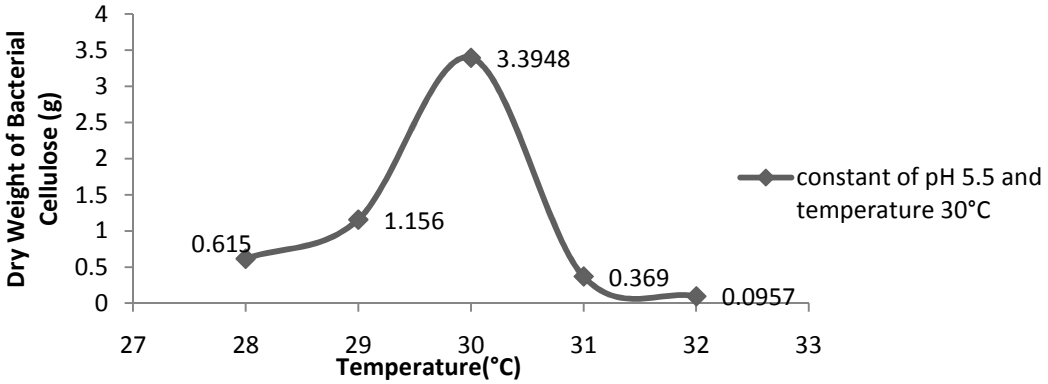

Figure 3.2: The effect of temperature

3.2.2 Effect of pH medium

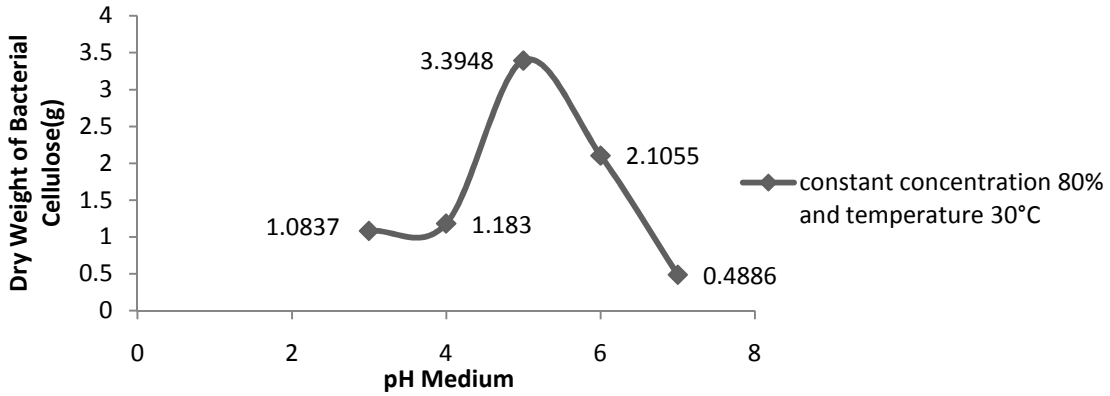

Figure 3.3: The effect of pH

3.2.3 Effect of Pineapple Waste Concentration

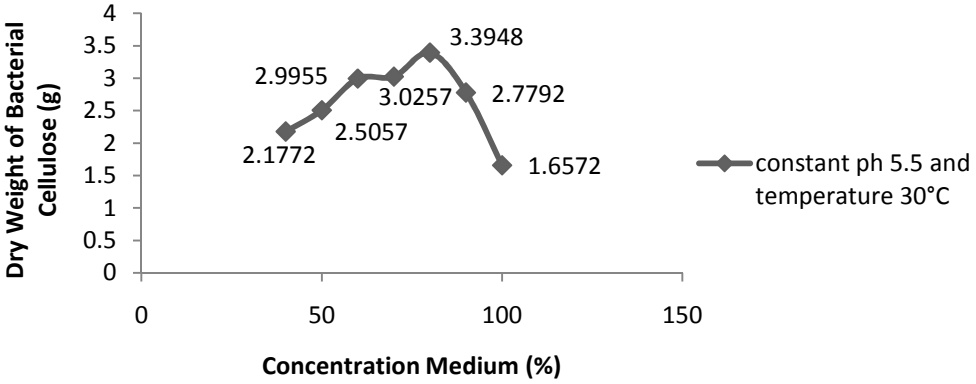

Figure 3.4: The effect of concentration of pineapple waste

Results from OFAT showed that the optimum temperature is 30°C, pH of 5.5 and concentration of pineapple waste is 80%. Thus, these factors is used for optimization study to obtain higher yield of bacterial cellulose by using RSM.

Table 3.1: 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					

Table 3.1 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 provides 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 R^2 corrected the R^2 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 R^2 may be noticeable smaller than R^2. This should be the caution signal as too many terms were present in the model. The plot of predicted versus experimental dry weight of bacterial cellulose are shown in Figure 3.5 with R^2=0.9831, thus indicating an excellent adequacy of the proposed model. In Table 3.1, 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.

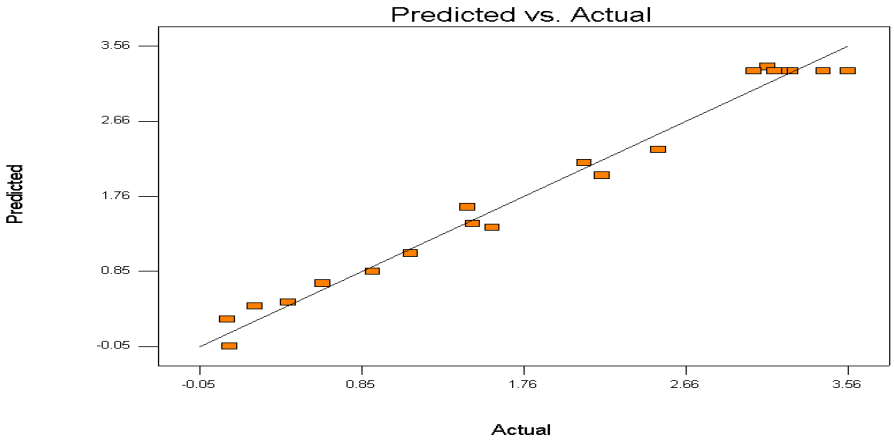

Figure 3.5: Plot of predicted versus experimental data for dry weight of Bacterial Cellulose.

In order to investigate the effects of the parameters on the dry weight of bacterial cellulose, the response surface methodology is used and the tree-dimensional plot is drawn. Figure 3.6, 3.7 and 3.8 shows the response surface plot for the parameters studied. Temperature, pH and concentration of pineapple waste medium gave the significant effect to the production of bacterial cellulose. From the figure 3.6, 3.7 and 3.8, the result indicated within the range of 5.25-5.75 for pH, temperature of 29.5°C -30.5 °C and 75%-85% of concentration, the yield of bacterial cellulose is increased. After this range, the production is slightly decreased. The maximum production of 3.48818g of bacterial cellulose is obtained when pH of 5.15, 30.51°C of temperature and 83.32% of concentration is used.

Based on report by Chawla *et al*., 2008, *Acetobacter xylinum* is active to synthesize bacterial cellulose during acidic condition because it is a type of acetic acid microbe that needs acidic condition for growth. The data indicated that as the pH outside from the optimum pH range 5.25-5.75 resulted decrease in bacterial cellulose production. This showed that more gluconic acid is formed in range of pH 5.00-5.25. 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 and gluconic acid is not beneficial for overall bacterial cellulose productivity.

Based on report by Jonas and Farah, 1998 the optimal growth temperature for bacterial cellulose production is 25°C - 30°C, although most researchers observed that 30^{O}C is the best temperature for cellulose yield. Figure 3.6 and 3.8 revealed that the highest value of bacterial cellulose yield is at temperature of 29.50°C -30.50°C and the optimum temperature is 30°C. According to the report by Chawla *et al* 2008, the conversion of glucose to cellulose is regulated by a multi step carbon metabolism pathway involving a large number of both individual enzymes and complexes of catalytic and regulatory proteins. For the temperature range 30.50°C to 31°C, the bacterial cellulose yield is decreased because of the enzyme cannot perform well when the temperature is over than optimum temperature. Suitable temperature is between 28°C to 30°C for the enzyme to work at optimum performance.

In the synthesis of bacterial cellulose, *Acetobacter xylinum* used the natural glucose present in the pineapple waste as carbon source. In the normal condition, *Acetobacter xylinum* produced thicker layer of bacterial cellulose by synthesizing more glucose resulting to higher yield of bacterial cellulose as shown in Figure 3.7 and 3.8. For other case, the data indicated that as the concentration outside the optimum concentration range 75%-85%, resulted decrease in bacteria cellulose production due more gluconic acetic or lactic acid formed when more glucose is synthesized during fermentation process. This side product will accumulate into the medium and decreases the pH level, thus decrease the bacterial cellulose production (Prashnant *et al*., 2008)

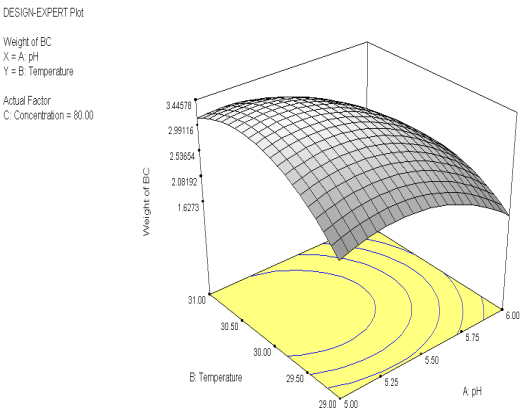

Figure 3.6: Temperature vs pH

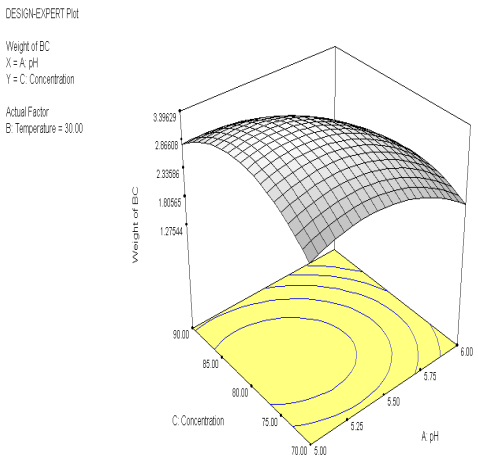

Figure 3.7: Concentration vs pH

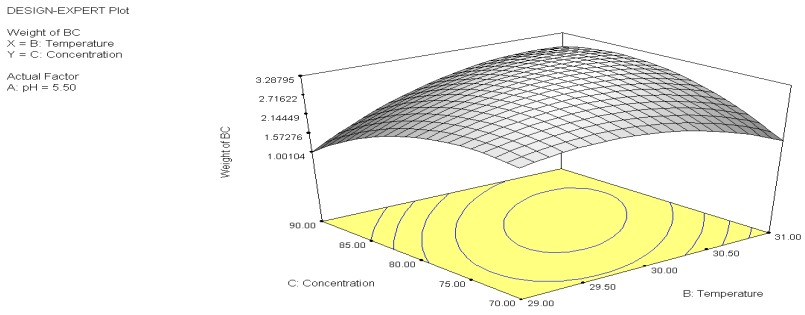

Figure 3.8: Concentration vs temperature.

3.3 Validation of the Model

The production of bacterial cellulose was successfully optimized and Table 3.2 shows the summary of the optimized conditions. In order to validate the adequacy of the model, a total of three verifications experiments for wet weight of bacterial cellulose responses are carried out under various fermentation conditions as shown in Table 3.2. The verification of the results was accomplished by carrying out the experiments under optimal condition of pH 5.15, temperature of 30.51^{o}C and concentration of 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 3.2 Validation of the data and models constructed for Bacterial Cellulose yield.

Condition	**After Optimization**			**Before Optimization**	
	Value	**Wet weight Bacterial cellulose (g)**		**Value**	**Wet 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

4.0 CONCLUSION AND RECOMMENDATION

As conclusion, the results obtained showed the optimum bacterial cellulose production is at temperature of 30.51^{o}C, pH of 5.15 and concentration of pineapple waste is 83.32% where the amount of bacterial cellulose produced is 3.4368g. Temperature, pH and concentration of pineapple used in this research are major parameters that gave major effect in the synthesis of bacterial cellulose. Besides that, this result also proved that pineapple waste that high in glucose content has great potential to be used for the synthesis of bacterial cellulose. Future works to scale up the production of bacterial cellulose are highly recommended.

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ACKNOWLEDGEMENT

Financial support from Universiti Malaysia Pahang is highly appreciated.