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Optimization of Bacterial Cellulose Production from Pineapple Waste using Different Fermentation Method

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Bacterial cellulose or *Nata* is produced using pineapple waste as substrate for the fermentation process by A*cetobacter xylinum*. This study was aimed to optimize the production of bacterial cellulose in static and agitated condition. Production of bacterial cellulose in agitated condition has been performed at different rpm, with and without adding micro particles. The fermentation conditions were set at pH 5.0 and 28 °C. The highest bacterial cellulose formation is obtained from agitated condition with additional micro particles at 120 rpm whereas the bacterial cellulose weight is increased by 70.23 % compared to the static condition. The addition of microparticles helped in increasing the bacterial cellulose formation by 15.19 %. From the optimization using Design Expert®, yeast extracts and potassium dihydrogen phosphate (KH_2PO_4) are the most significant factors affecting the bacterial cellulose production. From the results, the optimum formulation for bacterial cellulose production from pineapple waste as medium were yeast extract 6 g, sucrose 20 g, bactopepton 1.49 g and KH_2PO_4 1.08 g for 176.47 g (wet weight) of bacterial cellulose production.

1. Introduction

Bacterial cellulose is also known as Nata and it is a commercial product in many South-East Asia countries. It can be produced using variety of substrate as fermentation medium such as coconut water and fruit juices. The identified organism exists and responsible for the production of bacterial cellulose is *Acetobacter xylinum*. It can produce cellulose on the surface of the suitable medium (Verschuren, 1999). Recently, researchers start to give attention to natural biopolymer such as for its sustainability, low cost and environmentally friendly (Noor et al., 2018). Therefore, bacterial cellulose started to be used to replace plant cellulose. Its green production process makes bacterial cellulose as a good alternative to substitute plant cellulose which need multi purification process involving harsh chemical and time consuming (Pa'e et al., 2019). Up to the present time, static culture fermentation has been used for production of bacterial cellulose where cellulose layer being formed at air-liquid interface of the fermentation vessel. This method consumed large space to provide larger surface area for fermentation which is impractical for industrial scale production (Okiyama et al., 1992). Researcher start to develop economical mass production system based on agitated culture fermentation. Yet, harsh environment created by rotation or shaking speed can disturb the fermentation process and decrease the cellulose production (Zahan et al., 2014). Bacterial cellulose produced by *Acetobacter* species especially *Acetobacter xylinum* displays unique properties, including good mechanical strength (Pa'e et al., 2013), high water absorption capacity (Hokkanen et al., 2016), high crystallinity and an ultra-fine and highly pure fibre network structure (Klemm et al., 2001). It is expected to be a new biochemical commodity with diverse applications, if its mass production process could be improved (Tsuchida and Yoshinaga, 1997). Arjmandi et al. (2017) has reported the production of bacterial cellulose nanowhiskers reinforced PLA nanocomposites that has improved the tensile strength of the material.

Pineapple waste is used in this research because a huge amount of pineapple wastes being thrown away every year could be used via a sustainable development concept by turning it from waste to wealth. Furthermore, burning for decomposition of this huge biomass waste may increase the carbon footprints and

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lead to the greenhouse effect (Mansor et al., 2018). In general, a nutritionally rich medium supports good bacterial cellulose production of *Acetobacter xylinum*. Several authors have reported the production of bacterial cellulose in complex medium (Son et al., 2003) and the influences of adding microparticles in various speed of agitated culture (Vandamme et al., 1997). When compared with complex medium, synthetic medium has many advantages including enhanced process consistency, better control and monitoring. However, the used of complex medium especially from waste help to reduce production cost at the same time promote zero waste. On this view point, optimization on the complex medium from waste is important in order to minimize the production cost by limiting carbon source n micronutrients added into medium. This study aimed to optimize the production of bacterial cellulose from pineapple waste using different fermentation methods. Five factors were investigated using two levels factorial design in order to determine the factors that affected on bacterial cellulose production using pineapple waste as substrate. The five factors used for optimization variables were yeast extract, sucrose, bactopepton, potassium dihydrogen phosphate (KH_2PO_4) and magnesium sulfate ($MgSO_4$). The effects of these factors towards bacterial cellulose production were determined based on the wet weight of cellulose produced during the fermentation.

2. Methodology

2.1 Materials

The stock culture *of Acetobacter xylinum* used in this study was supplied by MARDI, Serdang, Selangor. It was stored at temperature below 40 °C to maintain viability. 10 % of stock culture used for each 50 ml medium. Pineapple wastes are in the form of juice extracted from pineapple core, the peeling skin and the pineapple crown. The pineapple wastes were obtained from pineapple processing factories in Johor, Malaysia. All the chemicals were obtained from local supplier (BDH Laboratory Supplies).

2.2 Fermentation

The static culture was studied based on different fermentation duration on 4, 8, 10, and 13 d at 28 °C. The agitated conditions were studied at different rotation speed of 80, 120, and 160 rpm in an incubator shaker for 4 d at 28 °C. The glass beads of the size 2 ±1 mm diameter was added into each medium where the medium without addition of micro particles act as control. Medium were prepared with concentration ratio of pineapple waste to distilled water of 1:1. Each prepared medium used the same amount of chemical reagents. The chemicals were stirred until dissolved and the pH of medium was adjusted to pH 5. The medium was put in the autoclave at 121 °C for 15 min. After the medium reached the room temperature, 10 % of the starter culture was transferred into the medium. The solution was mixed apparently and put aside for fermentation to occur.

2.3 Determination of wet weight and dry weight cellulose

The harvested bacterial cellulose was washed with NaOH solution 2 % (w/v) to elude excessive fermentation liquid and bacteria cells and then washed again with distilled water. The clean wet cellulose weight was recorded and dried in the oven (Memmert, UF 55) at 60 °C for 24 h until the moisture content achieved less than 8 % and then the cellulose weight was recorded again to get the dry weight (Zahan et al., 2013).

2.4 Determination of *glucose content*

10 mL of sample was taken and centrifuged at 5000 rpm for 3 min to separate the cellulose from other solutes. 3 mL of supernatants were added with 3 mL of 3,5-dinitrocylicsilic (DNS) and centrifuged. The mixed was put in the water bath at 90 °C for 15 min. The mixture was cooled to ambient temperature then 1.0 mL of potassium natrium tartarate was added into the solution to stabilize the colour. The optical density (OD) value at 550 nm wavelength was recorded as triplicate via UV- vis spectrophotometer with <1.5 nm limiting resolution (Agilent, Varian Carry 50).

2.5 Optimization process

The best selected medium culture from the experiment was optimized and analysed virtually using suitable chemical engineering software Design Expert® (2007) for two level factorial designs.

3. Results and discussion

3.1 Fermentation in static culture

Bacterial cellulose yield for static culture fermentation was shown in Figure 1. The results show increased of bacterial cellulose production with fermentation day. The measurement of glucose at 550 nm using spectrophotometer shows that the medium has high concentration of glucose before inoculating the medium with *Acetobacter xylinum*. During exponential phase, the bacteria consumed the glucose to produce energy

and bacterial cellulose. Consequently, glucose concentration decreased drastically during exponential phase. It shows that cellulose weight increased with the fermentation time. The highest cellulose weight is collected at day 13.

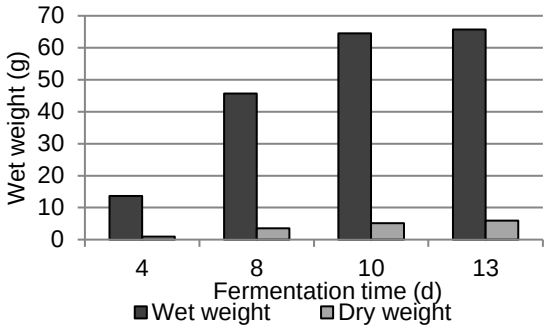

Figure 1: The weight of bacterial cellulose produced versus fermentation days from static culture

3.2 Fermentation in agitated culture

Figure 2a shows the different shaking speed in the incubator shaker at 28 °C for 4 d of fermentation where 120 rpm presented the highest yield. Agitated culture provides better aeration which helps *Acetobacter. xylinum* to grow rapidly. However, the produced cellulose appears in slurry form which is different compared to well organize cellulose fibrils from static culture. Cellulose yield decreased at higher rpm of 160 and above. This may due to development of harsh condition which lead to adaptation difficulty of *Acetobacter xylinum*. Moreover, the original habitat of this bacteria is in static condition (Okiyama et al., 1992) for example in rotten fruits.

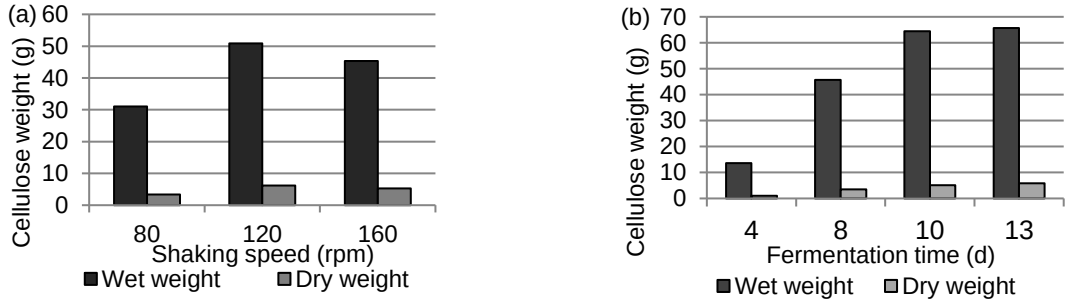

Figure 2: (a) Bacterial cellulose production at different shaking speeds in shaken culture (b) Bacterial cellulose production at different shaking speed with glass beads

3.3 Fermentation in agitated culture with micro particles

The effect of microparticles addition to cellulose yield was shown in Figure 2b. Bacterial cellulose synthesis has occurred more rapidly due to larger static surface area for *Acetobacter xylinum* attachment provide by the glass beads. At the same time, agitated condition supply higher dissolved oxygen in the medium hence increasing the cellulose formation. The beads act as sites for attachment in the medium. Therefore, it can enhance cellulose synthesis as reported by Vandamme et al. (1997).

3.4 Comparison of culture condition

The comparison of bacterial cellulose production from different medium culture conditions is shown in Figure 3. Production of bacterial cellulose is better in agitated culture compared to static culture condition due to better dissolved oxygen supplied for bacterial growth at certain rpm. It shows 73.17 % yield increment compared to yield from static condition in similar composition of nutrient medium. Interestingly, by adding micro particles in agitated culture at optimum shaking speed 120 rpm, the produced bacterial cellulose weight is 15.19 % higher than that in shaken culture without micro particles added. This phenomenon may happen due to the development of an oxygen limiting biofilm around the micro carrier (glass beads) resulting in a favoured shift of glucose incorporation into cellulose, rather than oxidation into (keto) gluconic acids.

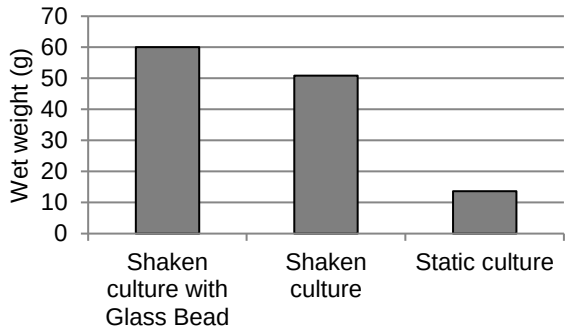

Figure 3: The comparison of the cellulose weight in different culture conditions after 4 d at 28 °C.

3.5 Optimization process

Two levels factorial design was used to determine the optimum medium culture conditions to maximize the production of bacterial cellulose. Based on Table 1 and 2, there were 5 variables with 1 response involved, and these parameters were used in medium preparation for microbial cellulose production.

*Table 1: Variable for m**e**dium culture*

Factor	Factor name	Factor level
A	yeast extract	5 g(-1), 6 g(+1)
B	sucrose	4 g(-1), 20 g(+1)
C	bactopepton	0.8 g(-1), 2.0 g(+1)
D	KH_2PO_4	0.6 g(-1), 1.08 g(+1)
E	$MgSO_4$	0.05 g(-1), 0.1 g(+1)

Table 2: Optimization factor for shaken medium culture with additional micro particles

Run	Factor 1 Yeast Extract (g)	Factor 2 Sucrose (g)	Factor 3 Baktopepton (g)	Factor 4 KH_2PO_4 (g)	Factor 5 $MgSO_4$ (g)	Response 1 Wet Cellulose Weight (g)
1	5.00	4.00	2.00	1.08	0.05	107.6929
2	5.00	20.00	2.00	0.60	0.05	74.0116
3	6.00	4.00	2.00	0.60	0.10	127.4142
4	5.00	20.00	0.80	0.60	0.10	63.0673
5	6.00	20.00	2.00	1.08	0.10	160.9882
6	6.00	20.00	0.80	1.08	0.05	173.3074
7	6.00	4.00	0.80	0.60	0.05	148.6573
8	5.00	4.00	0.80	1.08	0.10	152.2127

Normal plot graph in Figure 4a showed the most significant factors to be investigated which were yeast extract, KH_2PO_4, sucrose and bactopepton. Straight line in Figure 4b indicated that bactopepton and sucrose did not give effect to cellulose yield. This may due to high sugar content in pineapple waste. Hence, in this case the added sucrose does not significantly affecting much in the cellulose production. Obviously, there are two factors that strongly affect the production of bacterial cellulose using pineapple waste as substrate which were yeast extract which contributes nitrogen source and potassium dihydrogen phosphate with similar function of nitrogen sources. These two factors demonstrate a strong linear correlation with cellulose yield. It can be said that the combination of natural nitrogen source (yeast extract) and synthetic nitrogen sources (KH_2PO_4) enhanced the production of bacterial cellulose as reported by Budhiono et al. (1999).

Figure 5 demonstrated the ramp model for each variable in optimization process. The results concluded that the amount of each variable for optimum production of bacterial cellulose will be as follow: yeast extract 6.0 g, sucrose 20.0 g, bactopepton 1.49 g, KH_2PO_4 1.08 g. Therefore, by using this value will provide maximum cellulose yield of 176.47 g. The optimization results also revealed that magnesium sulfate has nil effect in

cellulose yield and can be considered to be ignored in preparing the medium culture. This may due to magnesium contain in pineapple waste itself already enough to support the bacterial growth.

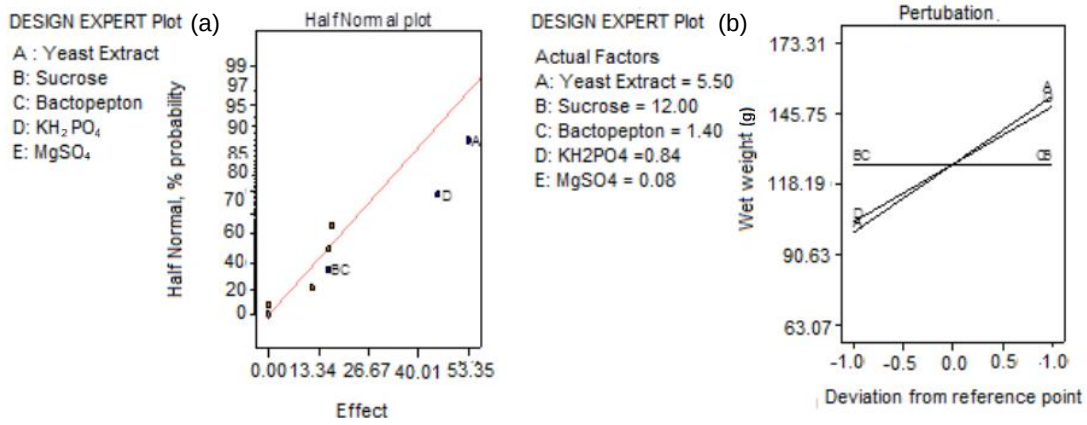

Figure 4: (a) Half normal plot and effect for wet cellulose weight (b) perturbation for wet cellulose weight

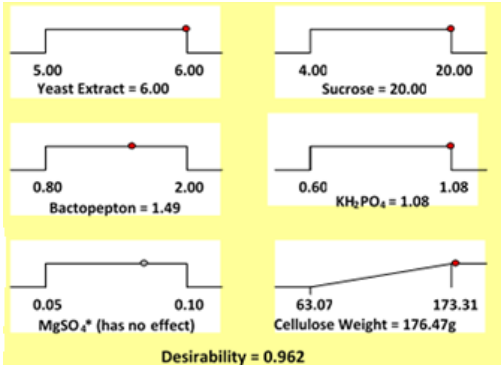

Figure 5: Ramps for each variable in optimization process

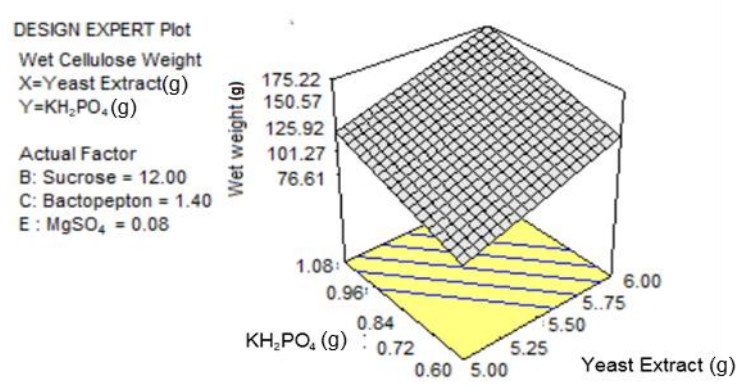

Figure 6: 3-D graph of wet cellulose yield as function of KH_2PO_4 (g) and yeast extract (g)

The 3-D graph as shown in Figure 6 is analysed effect of yeast extract and KH_2PO_4 to cellulose yield. These two variables were chosen since there are the most significant variables that affect the cellulose yield. The graph shows increased of cellulose yield proportional with the increased of both variables with the highest yield of 175.22 g in 4 d of (agitated culture with added micro particles) fermentation.

4. Conclusions

The pineapple waste has low acidity with high sugar concentration. These properties make pineapple waste suitable to be used as fermentation medium for bacterial cellulose production. The agitated culture resulted to higher cellulose yields compared to static fermentation. Interestingly, agitated culture with presence of micro particles increased the cellulose yield by 15.19 % higher. From the optimization process, yeast extract and KH_2PO_4 are the most significant factors that give effect to bacterial cellulose production using pineapple waste as fermentation medium. The output from the optimization process reported that optimum cellulose yield in this fermentation can be achieved with the used of these ingredients: yeast extract 6.0 g, sucrose 20.0 g, bactopepton 1.49 g, KH_2PO_4 1.08 g, which resulted to 176.47 g wet cellulose. From statistical analyses on design experiment, it can be concluded that magnesium sulfate is negligible in preparing medium culture for the bacterial cellulose production.

Acknowledgments

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