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The Potential Use of Papaya Juice as Fermentation Medium for Bacterial Cellulose Production by *Acetobacter xylinum* 0416

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ABSTRACT

Bacterial cellulose had been proven to be a very versatile natural polymer produced by bacteria. One of the major constraints in producing bacterial cellulose is the high cost of fermentation medium. This study examines the potential use of papaya juice as a low-cost fermentation medium for the production of bacterial cellulose. The fermentation of *Acetobacter xylinum* 0416 was carried out under static fermentation with an initial pH of 5.5, a temperature of 28±1°C and a fermentation period of 5 days using different juice extracted from ripe and unripe papaya. The highest production of bacterial cellulose was detected in ripe papaya pulp juice with a total weight of 35.37 g/l. Juice obtained from various parts of the fruit produced bacterial cellulose between 3.33 g/l and 16.10 g/l. By referring to the standard medium (Hestrin-Schramm (HS) medium), ripe papaya pulp juice shows the highest potential to be used as an alternative for the production of bacterial cellulose. This is due to its high glucose concentration that provide suitable conditions for *A.xylinum* 0416 to grow and produce bacterial cellulose.

Keywords: *Acetobacter xylinum* 0416, bacterial cellulose, fermentation medium, papaya juice

INTRODUCTION

The most abundant natural polymer on Earth is cellulose, which is mostly produced by vascular plants, algae and other species of bacteria. With the increasing need for sustainable biofuel and bioproducts, the demand for plant cellulose has continued to rise (Ernsting, 2012). Typically, plant cellulose can be obtained from lignocellulosic resources or biomass, such as

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forestry materials and agricultural residues. Cellulose is embedded in the complex matrix of the lignocellulosic structure and thus, it is difficult to separate it from the biomass. An alternative resource for plant cellulose is bacterial cellulose (BC) (Brown, 2004), which are easily separated compared with its plant cellulose counterparts.

The molecular formula of bacterial cellulose $(C_6H_{10}O_5)n$ is similar with plant cellulose, but its physical and chemical features are different. The BC has unique mechanical and structural properties such as high degree of crystallinity and polymerisation. It is also purer than the fibrous polymer obtained from plant sources in which the cellulose fibrils are embedded with lignin, hemicellulose and waxy aromatic substances (Ross et al., 1991). Additionally, the thickness of cellulose fibrils for bacterial cellulose is between 0.1 μm and 10 μm, which is one hundred times thinner than cellulose fibrils obtained from plant cellulose (Gayathry & Gopalaswamy, 2014).

Due to the numerous potential applications of bacterial cellulose, it must be efficient, cost-effective and produced in high quantities to meet commercial demands. To ensure favourable yields and to lower costs, attention needs to be given to the species and genetic modification of the bacteria used, type and composition of fermentation medium and the type of reactor for the production process (Shi et al., 2014).

Previously, most of the BC was produced from a high cost defined medium such as Hestrin-Schramm (HS) medium containing various types of chemicals such as glucose, yeast extract, ammonium sulphate, peptone and other additional synthetic nutrients (Zahan et al., 2014a). Hungund et al. (2013) revealed that various fruits juice including pineapple, pomegranate, muskmelon, watermelon, tomato, orange, molasses, sugarcane and coconut water were used as alternative carbon sources for BC production. Fruit juice alone as a carbon source was capable of producing a high yield of BC instead of using a high cost defined medium (Zahan et al., 2014b).

Although fruit juices are known to be an efficient fermentation medium to produce BC, the use of papaya juice in particular for this purpose is still limited. Papaya or *Carica papaya* belongs to the genus Carica, found abundantly in Malaysia. Papaya is an excellent source of carbohydrate, vitamins and minerals. Among the carbohydrates, sugars are the principle constituents of papaya with a total content of 48.3% sucrose, 29.8% glucose and 2% fructose (Chan & Kwok, 1975; Gomez et al., 2006). According to Oloyede (2005) and Wall (2006), papaya contains sodium, potassium, magnesium, calcium, iron and other vitamins which encourage growth of bacterium.

Thus, this research aims to study the potential of papaya juice obtained from various parts of the fruit (pulp, peel and seed) to become a low cost medium for the production of bacterial cellulose by fermentation of *Acetobacter xylinum* 0416. Thus, no parts of the perishable fruit are wasted (Rohani et al., 1997).

MATERIALS AND METHODS

Preparation of inoculum *A.xylinum* 0416 using papaya juice

Local ripe papaya was peeled and washed. About 200 g of its pulp was weighed and blended with 200 ml of distilled water. The juice was filtered using a filter cloth to obtain 200 ml of clear juice (Mehtab & Paul, 2014) and transferred to a 250 ml schott bottle. The pH of the juice was adjusted to 5.5 with 2 M sodium hydroxide (NaOH) or 2 M hydrochloric acid (HCl) before it was sterilised at 121°C for 15 minutes. After it was cooled to 28±1°C, 10% (v/v) of *A.xylinum* 0416 (obtained from Biotechnology Research Centre, MARDI, Serdang) was added to the juice aseptically in a laminar flow. The inoculum was mixed by shaking the bottle gently and slowly before it was incubated at 28±1°C for three days (Zahan et al., 2014b).

Preparation of inoculum *A.xylinum* 0416 using standard medium (Hestrin & Schramm (HS) medium)

200 ml of HS medium containing 0.5% (w/v) yeast extract, 0.5% (w/v) peptone water, 2% (w/v) D-glucose, 0.115% (w/v) citric acid ($C_6H_8O_7$) and 0.27% (w/v) disodium hydrogen phosphate (Na_2HPO_4) were mixed homogeneously (Shi et al., 2013). The mixture was transferred to a 250 ml schott bottle, and its pH adjusted to 5.5 with 2 M sodium hydroxide (NaOH) or 2 M hydrochloric acid (HCl). The mixture was sterilised at 121°C for 15 minutes. After it was cooled to 28±1°C, 10% (v/v) of *A.xylinum* 0416 was added to the mixture aseptically in a laminar flow. The inoculum was mixed by manually shaking the bottle gently and slowly before it was incubated at 28±1°C for three days (Zahan et al., 2014b).

Preparation of fermentation medium and synthesis of bacterial cellulose

300 ml of fermentation medium was prepared for HS medium and different parts of papaya (ripe papaya pulp, ripe papaya peel, unripe papaya pulp, unripe papaya peel and papaya seed) as mention in previous section. The juices and HS medium were transferred to a different 500 ml schott bottle, and the pH was adjusted to 5.5 with 2 M sodium hydroxide (NaOH) or 2 M hydrochloric acid (HCl). Then the fermentation medium was sterilised at 121°C for 15 min. After it was cooled to 28±1°C, the fermentation medium was transferred to a sterilised 1 L plastic container aseptically in a laminar flow. Then, 10% (v/v) of *A.xylinum* 0416 inoculum was aseptically poured into the fermentation medium. Finally, the fermentation medium was incubated at 28±1°C for five days. 10 ml of the fermentation medium was sampled every 24 hours. The steps were repeated twice for each type of fermentation medium (Pa'e et al., 2011).

Measurement of bacterial cellulose weight

The BC was later harvested and washed with boiling water at 100°C for 60 minutes. The BC was rinsed again with plenty of water (until pH became 7.0). Excess water on the

surface of the BC was wiped with tissue papers and the total weight were recorded using an electronic balance until a constant value was obtained (Shi et al., 2013).

Determination of *A.xylinum* 0416 growth.

Two dried and clean 1.5 ml microcentrifuge tubes were weighed. Then 10 ml of fermentation medium sample was mixed by manually shaking the bottle gently and slowly. After that, 1.5 ml of fermentation medium sample was pipetted into each tube. The tubes were centrifuged at 3000 rpm for 20 min. Then, the supernatant was decanted, and the tubes were placed in an oven at 90°C for 24 hr. After that, the tubes were weighed to obtain the cell dried weight (Banerjee et al., 1993). The cell dried weight (CDW) was calculated using Eq. 1:

$$\text{CDW (g/l)} = \frac{\text{weight of tube and dried cells, (g) - weight of empty tube, (g)}}{\text{sample volume, (ml)}} \times 1000 \quad [1]$$

Determination of glucose concentration.

A 10 ml of fermentation medium sample was mixed by manually shaking the bottle gently and slowly. After that, 1.5 ml of sample was pipetted into a cuvette. The glucose concentration was detected using a biochemistry analyser (YSI 2700D, USA) (Zahan et al., 2015a).

Analysis of pH

The electrode was rinsed using a distilled water. Then, the electrode was immersed in a universal bottle containing 10 ml of fermentation medium sample obtain at the initial and final day of the fermentation process. The pH values were recorded when the reading was stable.

Statistical Analysis

All data were analysed with one way analysis of variance (ANOVA), and mean values were compared at $P<0.05$ significant level test using Microsoft Excel 2010.

RESULTS AND DISCUSSIONS

Production of bacterial cellulose by *A.xylinum* 0416 in different parts of papaya juice and HS medium were evaluated by the total weight of bacterial cellulose produced after 5 days of fermentation. Figure 1 shows that the highest yield of bacterial cellulose was produced in ripe papaya pulp medium (35.37 g/l) and followed by HS medium (35.2 g/l). Result also shown that fermentation of *A.xylinum* 0416 in other parts of papaya juice medium also manages to produce the bacterial cellulose but in different amount; ripe papaya peel (16.1 g/l), unripe papaya pulp (15.93 g/l), unripe papaya peel (5.2 g/l) and papaya seed (3.33 g/l). By comparing with the standard medium (HS medium), ripe papaya pulp medium produced a slightly higher amount of bacterial cellulose which shows a potential to be used as an alternative and low cost fermentation medium for the production of bacterial cellulose. While, the other parts of papaya fruits such as peel and seed also can be utilized for production of bacterial cellulose instead of being disposed.

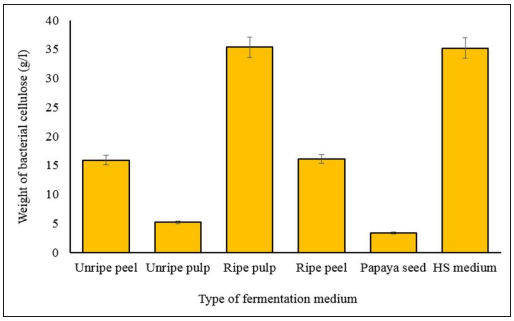

Figure 1. Weight of bacterial cellulose produced in different type of fermentation medium

The analysis of variance (ANOVA) show the calculated F_{value} (184.22) was higher compared with F_{crit} (4.39) and a very low probability value ($P \leq 0.00000177$). Therefore, the juice obtained from different parts of papaya to produce bacterial cellulose by *A.xylinum* 0416 fermentation was statistically proven.

Production of BC is higher when the nutrients in the fermentation medium such as carbon sources is high. The example of carbon sources are glucose, fructose, sucrose and maltose but in this research, the focus is on glucose as primary carbon sources. Glucose was recommended as the ideal carbon source for BC production since most of the bacterial cellulose synthesis are done in glucose culture medium (Santos et al., 2013; Zahan et al., 2014a). Figure 2 shows the highest glucose concentration was 22.4 g/l and 21.3 g/l in ripe papaya pulp medium and HS medium respectively. The lowest glucose concentration was 2.07 g/l in papaya seed medium. The result shows different types of fermentation medium have different levels of glucose concentration. This finding is consistent with that of Chan and Kwok (1975), Gomez et al., (2006) and Duke (1996) which stated that the highest carbon sources present in ripe papaya pulp was glucose.

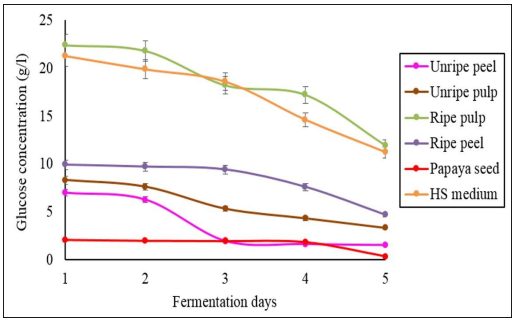

Figure 2. Glucose concentration in different type of fermentation medium

Bungay and Serafica (1999) stated that increasing the glucose concentration above 25 g/l does not correspond to an increase in bacterial cellulose production during the 5 days of the fermentation process, while an inadequate amount of glucose, namely 5 g/l, can inhibit BC production. Therefore, in order to maximise the amount of bacterial cellulose produced, a certain amount of glucose should be maintained during the fermentation process. Based on this research, it is suggested glucose concentration is maintained between 5 g/l and 25 g/l. The results also indicate represent that all types of fermentation medium are suitable for BC production except for papaya seed.

Figure 2 also shows, as fermentation period (days) increases, the glucose concentration (g/l) decreases for all types of fermentation medium because the glucose has been used by *A.xylinum* 0416 for BC formation and its growth. Figure 3 shows

that growth of *A.xylinum* 0416 increases as fermentation period increases where the population growth in a closed system follows the "standard growth curve". The lag, exponential and stationary phase for *A.xylinum* 0416 in a different type of fermentation medium can be determined. The highest growth of *A.xylinum* 0416 was in ripe papaya pulp medium and HS medium that showed the exponential phase began from the first day until the fifth day of fermentation. While the lowest growth of *A.xylinum* 0416 was recorded in papaya seed medium where the exponential phase occurred from the first day until the third day of fermentation before proceeding to the stationary phase. These results were consistent with the analysis of glucose concentration discussed earlier which proves that when the glucose consumption is high, the growth of *A.xylinum* 0416 is also high.

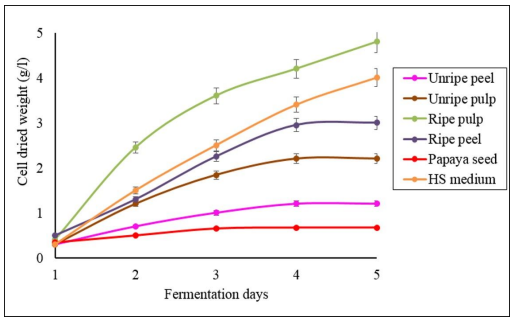

Figure 3. Growth of *A.xylinum* 0416 in different types of fermentation medium

The analysis of variance (ANOVA) showed the calculated F_{value} (3.66) was higher compared with the F_{crit} (2.62) and a very low probability value ($P \leq 0.01323$). Therefore, juice from different parts of the papaya for the growth of *A.xylinum* 0416 was statistically proven.

A.xylinum 0416 reproduction is limited by the availability of nutrients in the fermentation medium. Each *A.xylinum* 0416 cell divided into two, and will continue to do so until it runs out of nutrients. During the exponential phase, a rapid growth of *A.xylinum* 0416 occurs due to suitable conditions. In this phase, the consumption of glucose, and BC production rate is high (Zahan et al., 2015a). The stationary phase shows a linear growth of *A.xylinum* 0416 during fermentation process where the consumption of glucose and bacterial cellulose production is constant. Through this research, there is no death phase for *A.xylinum* 0416 during the 5-day fermentation period. Thus, results indicated that ripe papaya pulp medium can serve as a suitable fermentation medium for the growth of *A.xylinum* 0416 due to the high amount of glucose and supported by other natural elements present.

During the exponential phase of *A.xylinum* 0416 growth, the glucose consumption was high and at the same time, the formation of acetic acid as its by-products is also high (Zahan et al., 2015b). Consequently, there is an increase in acidity levels in the fermentation medium that may suppress the growth of *A.xylinum* 0416 and also the BC production (Vandamme et al., 1998). Figure 4 shows the decreasing trend for the pH for each type of fermentation medium between 3% and 18%. But the pH for each type of fermentation medium is still

in the optimum range. Chawla et al. (2008) reported that the optimum pH of the medium for BC production in the range of 4.0 to 6.0, and the yield decreases when it is below 4.0. Thus, it is important to ensure that the pH is controlled during the fermentation process to reduce the possibility of bacterial inhibition which can affect yield.

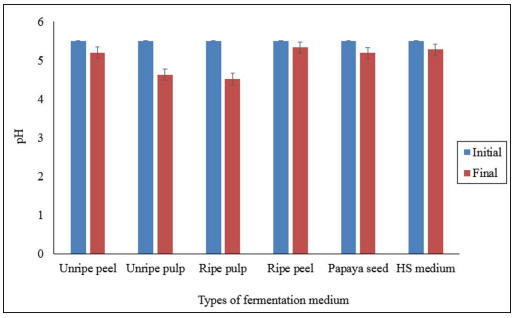

Figure 4. pH for different type of fermentation medium

CONCLUSION

The results show that the production of BC by fermenting *A.xylinum* 0416 in ripe papaya pulp medium was slightly higher than using the HS medium, thus proving that ripe papaya pulp medium can be used as a low-cost medium of fermentation in the production of BC. In addition, the other parts of papaya showed a good potential to produce BC. In conclusion, the growth of *A.xylinum* 0416 is the highest in ripe papaya pulp medium due to its highest glucose concentration and suitable pH .

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