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KINETIC AND TECHNO-ECONOMIC EVALUATION OF BACTERIAL CELLULOSE PRODUCTION FROM PAPAYA PEEL

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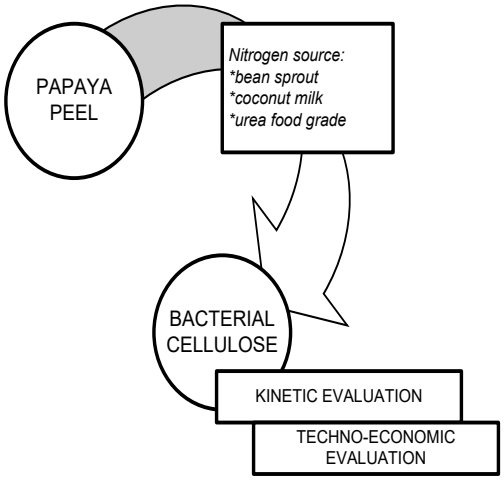
Graphical abstract



Abstract

Papaya peel is a fruit waste that was usually dumped into the environment. One way to utilize papaya peel waste was to convert it into a fermented product like bacterial cellulose. In this study, the effect of various bacterial cellulose nitrogen sources such as bean sprouts, coconut milk and urea food grade were used to produce bacterial cellulose from papaya peel. In addition, the determination of the bacterial cellulose kinetic parameters and techno-economic analysis were also evaluated. The results from this research showed that urea food grade as nitrogen source produced highest bacterial cellulose. Longer fermentation time produced higher bacterial cellulose and lower water content in bacterial cellulose production. From kinetic model optimization with bacterial cellulose data, kinetic parameters such as maximum specific growth rate (μmax), monod constant (Ks), cell death rate constant (Kd), and cell maintenance constant (m) were 0.06 (day^{-1}), 1.25 (g/L), 0.117 (day^{-1}), and 0.568 (day^{-1}). Techno-economic evaluation showed that bacterial cellulose production with recycle medium stage produced high profitability. Profitability parameters value such as return of investment (ROI), payback period (PBP), net present value (NPV), and internal rate of return (IRR) were 75.92%, 1.01 years, US$ 1,839,257,209, and 76.94%. This research showed that higher bacterial cellulose yield can be produced from papaya peel waste and urea food grade. Techno-economic simulations showed that large-scale production of bacterial cellulose from papaya peel waste can be profitable by recycle the fermentation medium.

Keywords: bacterial cellulose, kinetic, papaya, techno-economic

1.0 INTRODUCTION

Papaya plant is one of the many plants found in Indonesia. Papaya, Carica papaya L., is a herbaceous fruiting plant from the Caricaceae family. Papaya fruit is a fruit that has a good taste, high water content, and beneficial for human health [1]. Fresh papaya fruit has a pungent odor, high vitamins (vitamins A and C), and high fiber [2]. Papaya fruit which usually grows in tropical region not only used as food but also used as cosmetics [3].

Papaya in its utilization produces some waste. Papaya parts such as skin, pulp, seeds, stems and leaves contain protein, vitamins, and various photochemical compounds [2]. Papaya peel and seed are waste from papaya processing step which are 20 – 25% from papaya fruit mass [4]. Papaya peel is biodegradable due to its higher fiber content, saccharide, mineral and protein [5]. Papaya waste with high sugar content has potential for natural biodegradation during the hydrolysis and fermentation process by anaerobic digestion microorganisms [6].

Papaya waste can make environmental pollution [7]. Papaya peel in the form of paste contain fat, protein,

carbohydrates, and ash were 2.2%, 5.3%, 64.6% and 7.5% [8]. In addition, 100 grams of papaya peel flour contain minerals such as calcium, phosphorus, zinc, potassium, magnesium, sodium and iron were 18 mg, 221 mg, 1 mg, 516 mg, 19 mg, 9 mg and 0.6 mg [9]. Therefore, several studies have been conducted to utilize papaya peel waste such as animal feed [10][11], sources of pectin [12] and skin and hair care products [1].

Bacterial cellulose (BC) is a polymer that can be produced from various bacteria such as Acetobacter, Rhizobium, Agrobacterium, Aerobacter and so on [13], [14]. *Gluconacetobacter xylinus* or better known as *Acetobacter xylinum* most often used to produce bacterial cellulose because higher bacterial cellulose production and more economical. In the fermentation process, these bacteria can convert 108 sugar molecules/hour into cellulose [14].

Bacterial cellulose is a biotechnology product that was produced on the surface between air and fermented liquid at 25-30 ^{o}C and pH range around 4-7. Bacterial cellulose production method can be carried out statically, agitated, and using bioreactor [15]. The static method was the most widely used method because it was the simplest and most common method for bacterial cellulose production on a large scale. Compared to other fermentation methods, static fermentation is cheaper because there is no stirring or other additional equipment. In contrast to cellulose from plants, cellulose from bacteria does not contain lignin and hemicellulose components. According to its structure, bacterial cellulose has a three-dimensional structure with (1-4)-glycosidic bonds, crystallinity reaches more than 80%, and high water content around 99% [14]. In Asia, bacterial cellulose is better known as a food product under the name nata de coco [13]. Besides that, bacterial cellulose can also be used in various applications such as in medical application [13], [16], cosmetic [17], and composite materials [18].

Nitrogen source has an important role in the growth of *Acetobacter xylinum* bacteria to produce bacterial cellulose. Nitrogen has role in protein formation for *Acetobacter xylinum* [15],[19]. In bacterial cellulose production, protein can accelerated cell growth and produced enzymes by Acetobacter xylinum [19]. In this research, nitrogen source that we used were bean sprouts, coconut milk, and urea food grade because these three ingredients have high nitrogen content, easy to find and affordable. Coconut milk is a thick white liquid extracted from coconut flesh [20]. While bean sprouts are obtained from green beans which every 1 kg of green bean can produce 5 kg of bean sprout [21].

Reaction kinetic is the study of reaction mechanism. Reaction kinetic parameters are needed to determine the reaction rate and reactor design. Several studies have been conducted to evaluate the reaction kinetic and bacterial cellulose production model. Hornung (2010) evaluated optimization of bacterial cellulose production on the culture surface and made bacterial cellulose production models [22]. Taylor (1999) investigated kinetic study of bacterial cellulose production in batches [23]. Aydin and Aksoy (2015) investigated kinetic modeling of bacterial cellulose production by Gluconacetobacter hansenii P2A [24]. Sulaiman et al (2018) monitored bacterial cellulose production by Acetobacter xylinum 0416 with Fuzzy Logic simulation [25]. Budhiono et al (1999) investigated kinetic of bacterial cellulose formation in nata-de-coco culture system [26].

Kinetic data of bacterial cellulose production is required to design the reactor. Kinetic data determines how much bacterial cellulose was formed and can affect the economics of bacterial cellulose production. Therefore, techno-economic evaluation of bacterial cellulose production based on kinetic data needs to be done. Research on techno-economic of bacterial cellulose production has not been done much. Dourado et al (2016) have simulated industrial-scale production of bacterial cellulose from beet molasses using SuperPro Designer [27]. The research showed profit value of bacterial cellulose production around US$ 3,301,863 with 4 years payout period.

In this study, bacterial cellulose from papaya peel will be compared using various nitrogen sources such as bean sprouts, coconut milk and urea food grade. Bacterial cellulose production will be evaluated for kinetics and techno-economic analysis.

2.0 METHODOLOGY

2.1. Bacterial Cellulose Production Using Various Nitrogen Sources

750 grams papaya peel was weighed then blended it with distilled water in a ratio of 1:2 (1500 mL water). Strain the papaya peel mixture using a filter cloth while squeezing and take 500 mL of filtrate to boil it on the stove. Next, added 10% of sugar (50 grams) and variation of 1% nitrogen source in the form of urea, coconut milk, and bean sprouts while stirring to dissolve sugar and nitrogen source completely. After that, the fermentation medium was cooled and poured into a sterilized fermentation bowl. In the fermentation medium, added 10% (v/v) *Acetobacter xylinum* (50 mL) and cover with a cloth. Bacterial cellulose static fermentation was carried out for 7 days and 10 days at room temperature.

2.2 Bacterial Cellulose Weight Measurement

Bacterial cellulose rinsed with distilled water. Soaked BC using 200 mL of 2M NaOH solution for 2 hours to remove bacteria. Next, washed BC using clean water until the pH was neutral. Dry the bacterial cellulose with tissue paper and weighed.

2.3 Yield Analysis

Bacterial cellulose formed from fermentation was drained for 10 minutes. Bacterial cellulose was weighed then calculated the yield based on formula

$$Yield = \frac{bacterial\ cellulose\ weight\ (gram)}{fermentation\ medium\ weight\ (gram)}\ x\ 100\% \quad (1)$$

2.4 Water Content Analysis

Bacterial cellulose was dried with tissue paper and weighed as initial weight. After that, placed BC in the oven for 3-4 hours at a temperature of 100-105^{o}C until the weight was constant. The sample was then removed from the oven, put in a desiccator, and weighed as final weight. The water weight loss as a percentage of water content in BC was calculated based on formula

$$Water\ content = \frac{BC\ initial\ \ weig\,ht\ (g) - BC\ final\ \ weig\,ht\ (g)}{BC\ initial\ \ weig\,ht\ (g)}\ x\ 100\% \quad (2)$$

2.5 Determination of Reaction Kinetic in Bacterial Cellulose Production

2.5.1 Kinetic Experiment of Bacterial Cellulose Production

300 grams of papaya peel was weighed and blended it with distilled water in a ratio of 1:3 (900 mL water). The papaya peel solution was then filtered with a cloth to get 500 ml of papaya peel juice. Next, add 10% of sugar (50 grams) and 1% of nitrogen source into the solution. The nitrogen source used in kinetic experiment was taken from the nitrogen source that produced the highest bacterial cellulose. The solution was stirred until homogeneous and adjusted the pH of the solution with acetic acid to pH 5.0 using a pH meter. The solution (fermentation medium) was then heated using a stove until boiling. Then pour 500 ml of solution into a sterilized fermentation bowl and cooled. Added 10% (v/v) *Acetobacter xylinum* into the fermentation medium and cover with a cloth. Bacterial cellulose fermentation was done at room temperature for 7, 10, and 13 days. Duplicate kinetic experiments of bacterial cellulose production was done.

2.6 Determination of Bacterial Cellulose Production Kinetic Model

Reaction kinetic of bacterial cellulose production was adapted from fermentation kinetics of Fogler (2006) [28], with the following reaction scheme for the bacterial cellulose production process.

$$\text{Glucose} \xrightarrow{\text{Cell}} \text{Cell} + \text{Bacterial Cellulose} + \text{Glucose}_{(\text{residue})} \quad (3)$$

The reaction equation for kinetic model can be divided for each component in batch fermentation process. The kinetic equation of bacterial cellulose production was divided into kinetics equation of cell formation, product formation and residual substrate with the modification of no product inhibitor according to the following equation:

Kinetic of cell formation:

$$\frac{dC_C}{dt} = \left\{\left(\mu_{\max}\frac{Cc\,.Cs}{ks+Cs} - \right) kd.Cc\right\} \quad (4)$$

Kinetic of product formation (Bacterial Cellulose):

$$\frac{dCp}{dt} = \left\{Y_{\frac{P}{C}}.\left(\mu_{\max}\frac{Cc\,.Cs}{ks+Cs}\right)\right\} \quad (5)$$

Kinetics of residual substrate (Glucose):

$$\frac{dC_s}{dt} = \left\{Y_{\frac{S}{C}}.\left(\mu_{\max}\frac{Cc\,.Cs}{ks+Cs}\right) - m.Cc\right\} \quad (6)$$

The value of $Y_{P/C}$ and $Y_{S/C}$ was calculated according to Fogler (2006) with the following equation:

$$Y_{P/C} = \frac{mass\ of\ product\ formed}{mass\ of\ cell\ formed} \quad (7)$$

$$Y_{S/C} = \frac{mass\ of\ substrate\ consumed}{mass\ of\ cell\ formed} \quad (8)$$

2.7 Calculation of Kinetic Parameter Constants

Determination of kinetic constant for bacterial cellulose production was obtained using the MATLAB software simulation by entering weight of bacterial cellulose produced from kinetic experiment. Kinetic parameters from kinetic equation of cell formation, product formation and residual substrate were determined such as maximum specific growth rate (μmax), monod constant (Ks), cell death rate constant (Kd) and cell maintenance constant (m). The reaction constant was predicted by minimizing the sum of square error between experimental data on bacterial cellulose weight and the kinetic model according to the formula:

$$SS = \sum_i \sum_j ([j]_{i\,exp} - [j]_{i\,predicted})^2 \quad (9)$$

The value of sum of square error can be determined by optimizing fminsearch (Nedler – Mead Method) using MATLAB software.

2.8 Economic Evaluation Method

The economic evaluation of the bacterial cellulose production process was carried out using SuperPro Designer 10 simulation software. The dependent variables generated in this simulation included return of investment (ROI), payback period (PBP), net present value (NPV), and internal rate of return (IRR). In addition, several constant variables used in calculating the economic simulation include:

- The currency used in this economic simulation was United States Dollars.
- The plant will be built for two years with will 20 years for plant operation.
- The total investment cost will be obtained by loan with 10% compound interest per year.
- The year for cost analysis was 2024.
- The plant capacity was 165 tons of papaya peel/hour.
- MACRS method was used for depreciation method with 15 years depreciation period.
- Minimum Acceptable Rate of Return was 11%.
- 25% tax was used
- The price of bacterial cellulose was US$ 1000/ton.

Total Capital Investment (TCI) and Total Operating Cost can be calculated using formulas:

- Total Capital Investment = Fixed Capital Investment + Startup Cost + Working Capital (10)
- Fixed Capital Investment (FCI) = 1.2 x Equipment Cost (11)
- Startup Cost = 5% x Fixed Capital Investment (12)
- TOC = Raw Material Cost + Labor-Dependent Cost + Facility-Dependent Cost + Laboratory/Quality Control Cost + Waste Treatment Cost (13)

Equipment Cost, Working Capital, Raw Material Cost, Labor-Dependent Cost, Facility-Dependent Cost, Laboratory/Quality Control Cost, and Waste Treatment Cost were calculated from SuperPro Designer simulation. Profitability Analysis such as

Return On Investment (ROI), Payback Period (PBP), The Net Present Value (NPV), and Internal Rate of Return (IRR) were calculated. Return On Investment (ROI) was defined as percent ratio of average profit (Np) to Total Capital Investment (TCI). Payback Period (PBP) identified as the project time required for payback, and defined as the ratio of Fixed Capital Investment (FCI) to Annual Cash Flow (Aj). The Net Present Value (NPV) is the total of the present worth of all cash flws minus the present worth of all capital investments, and Internal Rate of Return (IRR) is discount rate which make NPV is zero. The formula and calculation method of NPV and IRR was based on Peters et al (2003) [29].

3.0 RESULTS AND DISCUSSION

3.1 Yield Analysis

Based on Table 1, bacterial cellulose production with various nitrogen sources (bean sprouts, coconut milk, and urea) and fermentation time (7 and 10 days) produced different results. Urea food grade showed the highest bacterial cellulose production. Meanwhile, bean sprouts produced the lowest bacterial cellulose production.

Yield was used to determine the percentage of product. Factors that affect the yield of BC were nutritional factors such as carbon and nitrogen sources. Carbon and nitrogen ware needed by *Acetobacter xylinum* to cellulose synthesis. Based on Figure 1, The highest 7-day fermented BC yield was found in urea food grade sample with 70.23%, and the lowest yield was found in coconut milk samples with 12.14%. Meanwhile, BC yield that used bean sprouts was 16.90%. This was related with the nitrogen content in each nitrogen source. Media with the highest nitrogen content will produce the highest yield. Urea had the highest nitrogen content around 46% [30], so that BC yield produced was the highest among others. On the other side, nitrogen content in bean sprouts and coconut milk were 21% [31] and 5% [32]. Because the nitrogen content in bean sprouts was higher than coconut milk, the BC yield in bean sprouts was higher than in coconut milk. 10-days fermentation time for BC production was the highest using urea food grade with 97.48%. The lowest BC yield was found in the bean sprout sampel with 37.25%. While the sample with coconut milk produced 50.51% BC yield. Same as in the 7-day fermentation, media with high nitrogen content produced high yield.

In addition to nitrogen sources, fermentation time also affects the amount of BC production. Based on Figure 2, different yields were produced among the same nitrogen sources but different fermentation time. Within 10 days, *Acetobacter xylinum* can grow enough to form cellulose fibers. The optimum condition for BC fermentation time was between 5-14 days [33]. If BC was fermented for more than 14 days, it can cause death or cannibalism in bacteria. It was happened because the nutrient content in the fermentation media was no longer sufficient to form BC. If this condition was continued to happened, the media will clot and turn into black due to the remnants of dead bacteria [34].

3.2 Water Content Analysis

The results of water content in the BC production were presented in Figure 2. Based on Figure 2, bean sprouts sample had the highest water content of 97.93%. Coconut milk sample produced water content of 93.96%. Meanwhile, the urea food grade sample produced the lowest water content of 82.50%. The lowest water content in BC was the best quality BC because BC with the lowest water content indicated that the fibers in BC were tightly packed, so that the cellulose membrane was denser and stronger than BC with high water content [35].

Fermentation time also affected the water content of bacterial cellulose. Longer fermentation time produced lower water content in BC. Fermentation time affected the amount of fiber produced by *Acetobacter xylinum*, longer fermentation time produced more fibers which caused cellulose membrane layer denser. *Acetobacter xylinum* are able to break down sugar into a layer of cellulose. Cellulose tissue that obtained from the metabolism of *Acetobacter xylinum* is able to absorb water. Longer fermentation time made cellulose thicker, thereby reducing the water content trapped in BC [35].

Table 1 Bacterial cellulose production with various nitrogen source

Nitrogen Source	Fermentation Time (days)	Fermentation Medium Weight (gram)	Bacterial Cellulose Weight (gram)
Bean sprouts	7	225.91	38.19
	10	228.35	85.06
Coconut milk	7	193.56	23.50
	10	222.50	112.39
Urea food grade	7	122.39	85.96
	10	228.33	222.58

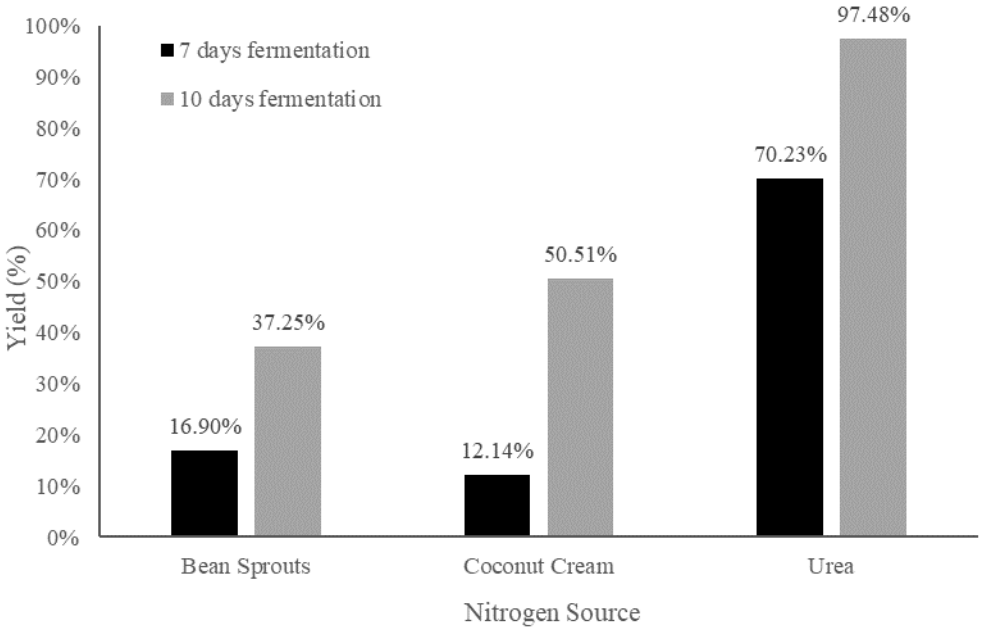


Figure 1 Bacterial cellulose yield

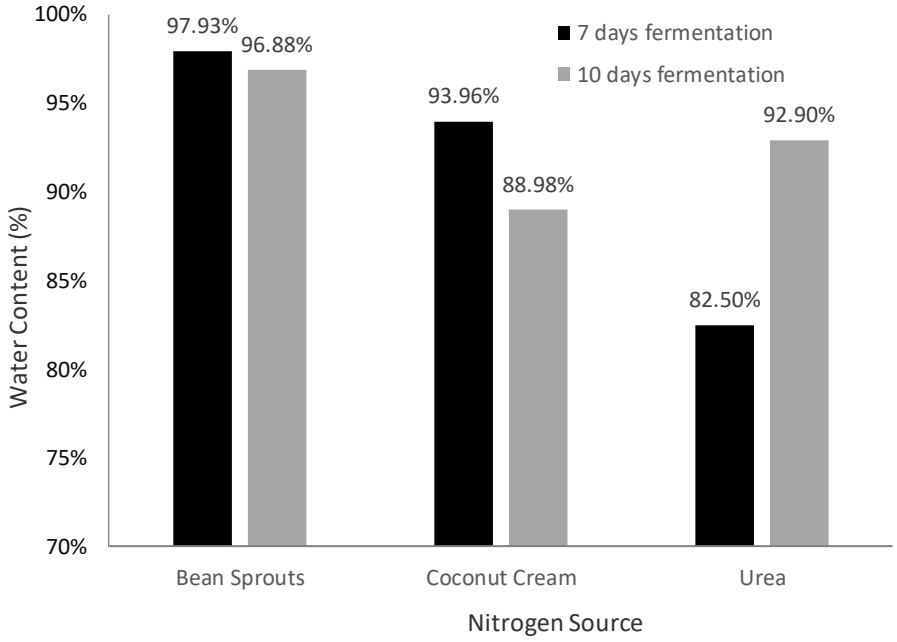


Figure 2 Water content of bacterial cellulose

3.3 Bacterial Cellulose Production for Kinetic Evaluation

Based on the result of nitrogen source variations, Urea was the best nitrogen source in producing bacterial cellulose. Therefore, urea was used for bacterial cellulose production for kinetic evaluation. The results of kinetic experiment for bacterial cellulose production was showed in Figure 3.

Based on Figure 3, the weight of bacterial cellulose showed on the 7, 10, and 13 days of fermentation were 22.77 gram, 41.39 gram, and 53.95 gram which showed the longer fermentation time produced more bacterial cellulose.

3.4 Bacterial Cellulose Fermentation Kinetic Parameters

3.4.1 Determination of $Y_{P/C}$ and $Y_{S/C}$

The weight of bacterial cellulose production from 7, 10, and 13 days of fermentation were 22.77 gram, 41.39 gram, and 53.95 gram. The mass of new cells formed is a linear function of BC formed, so the mass of new cells formed was assumed to be equal to the weight of BC. The mass of the substrate consumed was the amount of glucose used in fermentation media which was 50 gram. Therefore, the values of $Y_{P/C}$ and $Y_{S/C}$ are 1 and 2.95.

The optimization of bacterial cellulose fermentation model and bacterial cellulose production from papaya peel in various fermentation time can be seen in Figure 4

Based on Figure 4, the optimization between the data and the model of bacterial cellulose production was showed using minimizing the sum of square error method. Sum of square error (SSE) is a method used to evaluate the error between model and data. SSE from this optimization was 4%. SSE was acceptable if it is less than 10% [36]. The result of optimization between data and kinetic model produced kinetic parameters of bacterial cellulose production. Kinetic parameters of bacterial cellulose production can be seen in Table 2. Based on Table 2, kinetic parameters of bacterial cellulose production such as maximum specific growth rate (μmax), monod constant (Ks), cell death rate constant (Kd) and cell maintenance constant (m) were 0.06 (day^{-1}), 1.25 (g/L), 0.117 (day^{-1}) and 0.568 (day^{-1}) using MATLAB optimization.

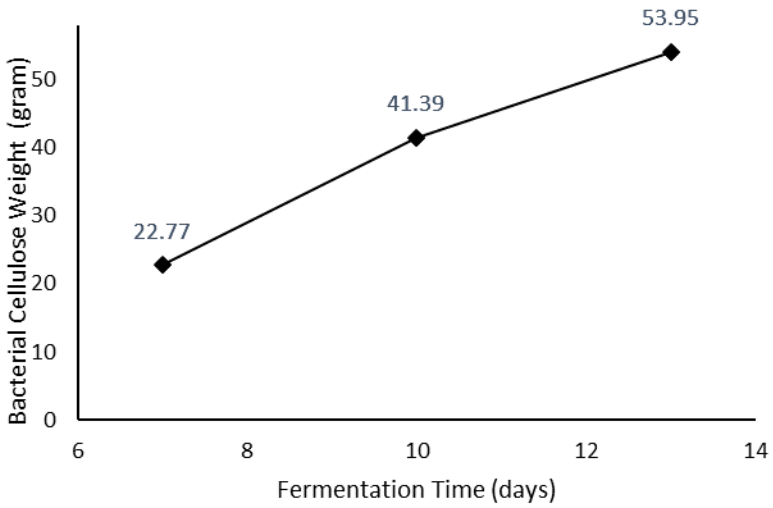


Figure 3 Kinetic experiment for bacterial cellulose production

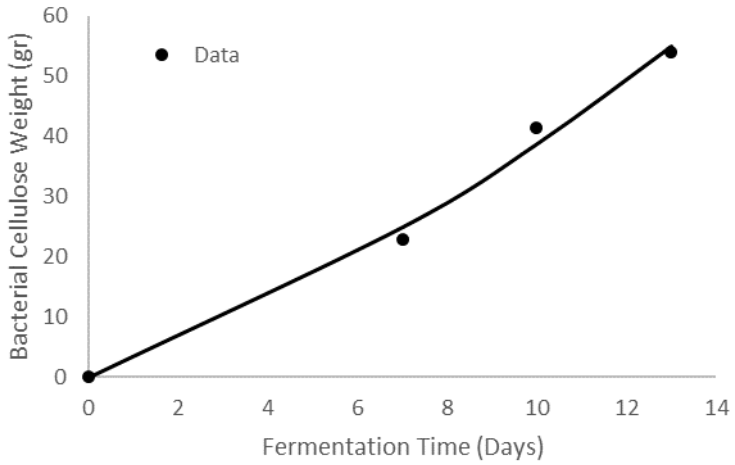


Figure 4 Optimization of bacterial cellulose fermentation model and bacterial cellulose production from papaya peel

Table 2 Kinetic parameters of bacterial cellulose production

Kinetic Parameters	Value
μmax	0.06 (day^{-1})
Ks	1.25 (gr/L)
Kd	0.117 (day^{-1})
m	0.568 (day^{-1})

3.5 Economic Evaluation of Bacterial Cellulose Production

Based on the SuperPro Designer 10 simulation, BC production was started by feeding 165 tons/hour of papaya peel waste into a grinder to reduce the particle size of papaya peel. Papaya peel then mixed with water in 1:3 ratio using mixer. Sugar (50 tons/hour), urea food grade (5 tons/hour), and acetic acid (0.5 tons/hour) were added. Next, all materials which were fermentation medium entered the sterilization unit to sterilize it using heat. The fermentation medium then entered the reactor. Air with composition of 79% nitrogen and 21% oxygen was added as oxygen source at 1 ton/hour. *Acetobacter xylinum* was also added to the reactor at 50 tons/hour. Kinetic parameters of bacterial cellulose production according to Table 2 were input into the reactor. After 10-days fermentation, reactor products were entered the washing unit. Bacterial cellulose production in this simulation was 157.25 tons/hour. Process flow diagram of bacterial cellulose production using SuperPro Designer 10 (Route-1) was shown in Figure 5.

Bacterial cellulose production in Figure 5 based on economic calculation in SuperPro Designer suffered a financial loss. This was due to the high cost of *Acetobacter xylinum*. Therefore, bacterial cellulose production process was evaluated by adding a recycle medium stage which could make bacterial cellulose production profitable. Recycle medium stage was P-8 in Figure 6. Bacterial cellulose production with recycle medium stage (Route-2) can be seen in Figure 6.

Bacterial cellulose production with recycle medium stage was a solution to reuse the remaining bacterial cellulose fermentation medium for *Acetobacter xylinum* starter [37]. Based on Ariyanti et al (2014), it was known that waste fermentation medium can be recycled to make Acetobacter xylinum starter so that it can reduce production costs [38]. Economic comparison of bacterial cellulose production with and without recycle medium can be seen in Table 3. Based on Table 3, Bacterial cellulose production with recycle medium stage (Route-2) was profitable with profitability parameters of ROI (%), PBP (years), NPV (US$), and IRR (%) were 75.92%, 1.01 years, US$ 1,839,257,209, and 76.94%.

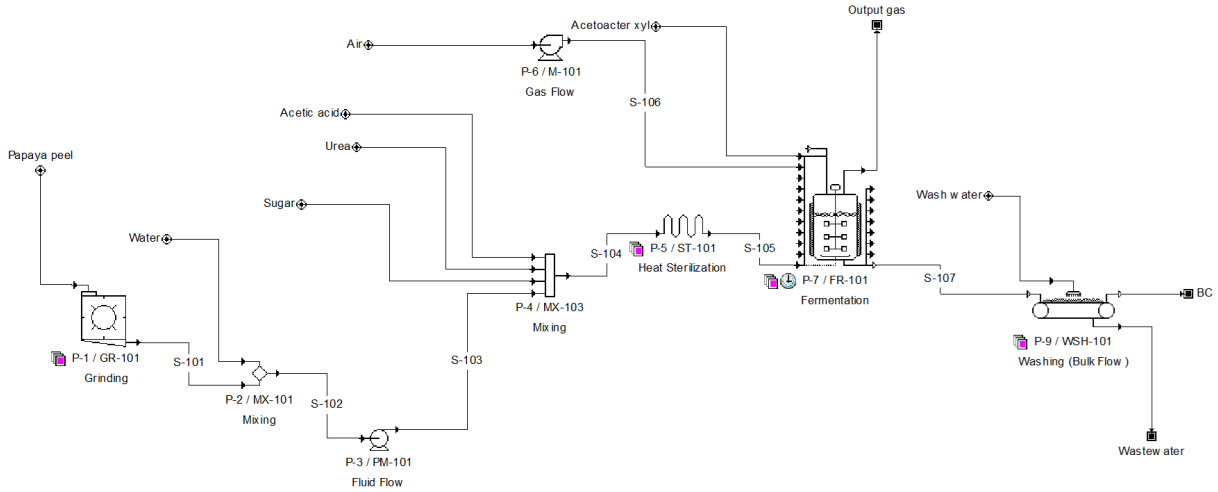


Figure 5 Simulation of bacterial cellulose production from papaya peel (Route-1)

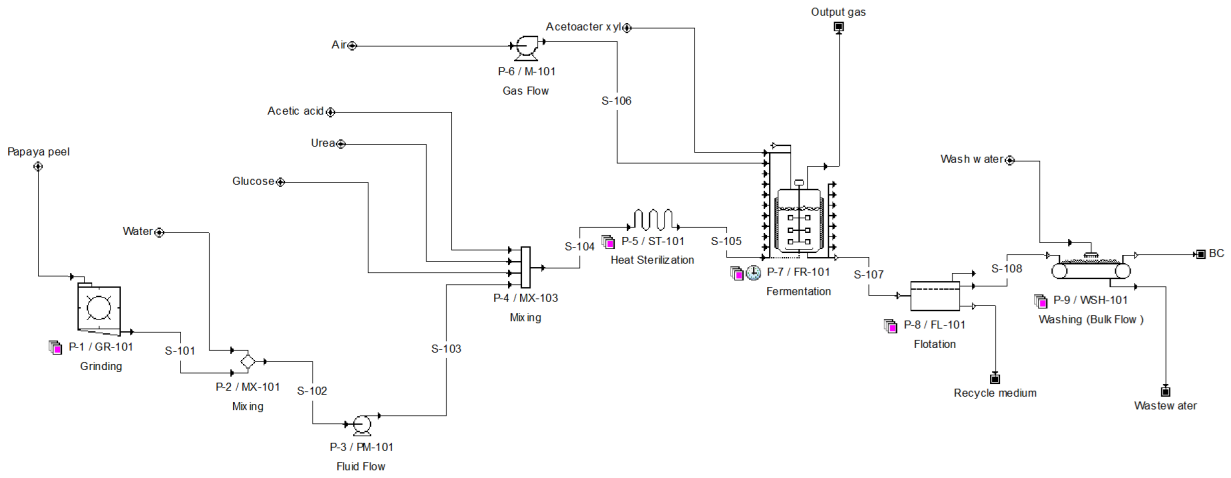


Figure 6 Simulation bacterial cellulose production from papaya peel with recycle medium stage (Route-2)

Table 3 Economic evaluation of bacterial cellulose production

Parameters	Route-1	Route-2
Total Capital Investment (TCI) (US$)	828,280,660	350,734,560
Total Revenue (US$/year)	1,556,478,000	1,276,525,800
Total Operating Cost (US$/year)	6,750,443,000	865,256,000
ROI (%)	-	75.92
PBP (years)	-	1.01
NPV (US$)	-32,532,645,932	1,839,257,209
IRR (%)	-	76.94

4.0 CONCLUSION

This research was evaluated the effect of various nitrogen sources such as bean sprouts, coconut milk and urea food grade on bacterial cellulose production from papaya peel and determining the kinetic and techno-economic parameters. The results showed that urea food grade as nitrogen source produced the highest bacterial cellulose. In addition, the fermentation time also affects the bacterial cellulose production which longer fermentation time produced higher bacterial cellulose and lower water content. Kinetics parameters of bacterial cellulose production in this research such as maximum specific growth rate (μmax), monod constant (Ks), cell death rate constant (Kd) and cell maintenance constant (m) were 0.06 (day^{-1}), 1.25 (g/L), 0.117 (day^{-1}) and 0.568 (day^{-1}). Techno-economic evaluation showed that bacterial cellulose production with recycle medium stage was profitable with profitability parameters such as ROI (%), PBP (years), NPV (US$), and IRR (%) were 75.92%, 1.01 years, US$ 1,839,257,209, and 76.94%.

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Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper

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