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Research paper

Simulation of Bacterial Cellulose by *Acetobacter Xylinum* 0416 Using Superpro Designer®

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Abstract

Bacterial cellulose (BC) is a non-toxic biopolymer that is used as a raw material in food, textile and medical industries. The production cost of BC comprises the price of labor, raw materials, taxes and manufacturing supplies. In BC manufacturing, the fermentation media culture comprises between 50-65% from the total production rate. To reduce the production cost, other types of fermentation media culture should be studied, and mature coconut water is chosen in this research. Mature coconut water is considered as waste and it is estimated to be discarded 5.3 m^3/day mainly in the coconut processing industry. In this research study, mature coconut water is used as a fermentation medium because it helps to reduce the price, easy to handle and found easily in the country. The optimization study was used in order to produce BC in large amount with a minimum fee. To conduct the optimization study, the pH of mature coconut water was adjusted to pH 4, 5 and 6 by using acetic acid (CH_3COOH) or sodium hydroxide (NaOH) with incubation temperature of 32°C. The result indicates pH 4 produces the highest harvested BC with 2.277g dry weight. By using this result, pH 4 was chosen to be used as a media in the fermentation process. For industrial purposes, BC production must be carried out in the industrial scale. Before real industrial scale is conducted, process simulation should be done to simulate several processes and produce the desired end product at low cost, low energy and good product specifications. The simulation software named as *SuperproDesigner*® was used to select the unit operation of a plant, to observe the total BC production and also the cost of BC production. This simulation was used to run three selected process alternatives named as alternative 1(Design by Prades et al.); 2 (Design by Norliza et al.) and 3(Design by Kerala Institute). After conducted the simulation, alternative 2 shown the cheapest BC production which is RM 170/kilogram compared to alternative 1 and 3.

Keywords: *Bacterial cellulose, mature coconut water; synthesis, simulation, Superpro Designer®*

1. Introduction

Bacterial cellulose (BC) is a biopolymer that has the same molecular formula $(C_6H_{10}O_5)_n$ with plant cellulose but different in chemical and physical characteristics. BC is preferred than plant cellulose because it has high purity, polymerization and also crystallization. In addition, BC also has high tensile strength and high-water holding capacity compared to plant cellulose [2]. Several types of bacteria such as Sarcina, Agrobacterium dan Rhizobium have the ability to synthesize BC. However, only Acetobacter can produce BC in a large amount and enough for the industrial used [3]. Mature coconut water is a waste mainly in coconut processing industry. The mature coconut water contains varieties of carbon source that can be used as substrate especially as fermentation media. The disposal of mature coconut water will give impact to the environment especially on river pollution. To curb this situation, the mature coconut water should be collected and processed to produce more valuable products. Plant design determines the workflow, equipment requirements, and the implementation of the specific process [4]. There are various types of commercial software used to simulate or design the industrial scale processes such as AspenPlus®, Hysys®, Pro/II®, WinSim®, ProMax®, UniSim®, SuperoPro Designer®, SulSim®,and much more [1]. SuperoPro Designer® from Intelligen Inc. was used in this research study because it is very useful for special unit operation especially for biological operations. It can handle batch and continuous processes of biological processes as good as others commercial software and can estimate cost for equipment and plant operation cost.

The objectives of this paper are to propose alternative design for production of Bacterial cellulose (BC) and to find the most economic costing by using simulation of SuperoPro Designer®.

2. Process description in laboratory scale

2.1. Fermentation media

Mature coconut water was chosen as the fermentation media because it contains varieties of carbon source that can be used as a substrate for the growth of *A.xylinum* 0416.The fermentation media contained 100 mL filtered coconut water, 8 g glucose and 0.5 g ammonium

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sulphate. Then, the pH was adjusted to 4.5 by using 1.0 M of sodium hydroxide (NaOH) and the media was then sterilized at 121 °C for 15 minutes to avoid contamination and cooled to 28 °C before it is used for *A.xylinum* 0416 inoculation.

2.2. *Acetobacter xylinum 0416* inoculation

10mL of *A.xylinum* 0416 was added to the sterilized media by using aseptic technique. The sterilized media contains 100 mL filtered coconut water, 8 g glucose, and 0.5 g ammonium sulfate then incubated for three days with maintain temperature at 30 ^{0}C. This culture must undergo subculture once in two weeks to ensure the presence of bacteria in the media.

2.3. *Acetobacter xylinum 0416* inoculation

Fermentation process was carried out for 7 days in a static incubator with pH 4, 5 and 6 at 32 °C. After the fermentation process, the gel-like pellicle will be observed at the surface of the media.

2.4. *Acetobacter xylinum 0416* inoculation

BC was taken out, soaked and boiled with diluted sodium hydroxide (NaOH) for 15 minutes. This process was performed to treat BC from unwanted substances and for purification purpose. Diluted NaOH was washed with distilled water to neutralize BC. Wet BC was harvested and then placed in the oven until the dried weight of BC became constant.

3. Plant design on the production of bacterial cellulose

Plant design is the drawing or modeling of the plant to maximize the effectiveness of the process. It can also perform smooth material and workflow in and out in plant. Plant design of BC relates with the production process in industry. The optimum temperature and pH were observed in a laboratory scale before implemented in the industrial scale.
In this study, the production of BC was observed in a laboratory scale first before being implemented into the SuperPro Designer® software. In this experiment, BC was conducted in the laboratory with optimization pH range between pH 4 to pH 6. The incubation temperature was set at 32 ^{0}C and placed in the incubator. During BC production process, BC was synthesis in a static fermentation media. BC by *A.xylinum* 0416 was produced through extracellular mechanism [5] and will further process will be conducted to produce desired end product in different industries.

Table 1 shows pH 4 (2.277 g) produced the highest BC dry weight compared to pH 5 and 6. Hence, pH 4 was chosen as the optimum pH to produce pH in industrial scale.

Table 1: The productions of BC dry weight

pH	BC Dry weight (g)
4.0	2.277
5.0	1.9510
6.0	1.5768

The optimization data (pH 4 and 32 ^{0}C) were simulated into the *SuperoPro Designer*® software. *SuperoPro Designer*® was used to select the process and due to its ability to be implemented in bio-based industry. There are three different processes with different unit operations called as alternatives 1, 2 and 3.
Figure 1 shows water, glucose, ammonium sulfate and mature coconut water were mixed into the blending tank. Mature coconut water was filtered before entering the sterilization process. The mixture was then left to cool in blending tank (V-101). After it is cooled down, the mixture was then transferred to the storage tank (V-102) for the fermentation process. The fermentation process was conducted for 10 days in simulation which was different from laboratory due to assumptions done in simulation, at 32 ^{0}C and pH 4. After 10 days of fermentation, BC was observed floating at the surface of the media. After that, the mixture was transferred to diafiltration (DF-101) to separate BC and waste from the media mixture. Lastly, BC undergo wash (WSH-101) and filtration (V-103) process for several times to form pure transparent BC (Prades et al. 2012).

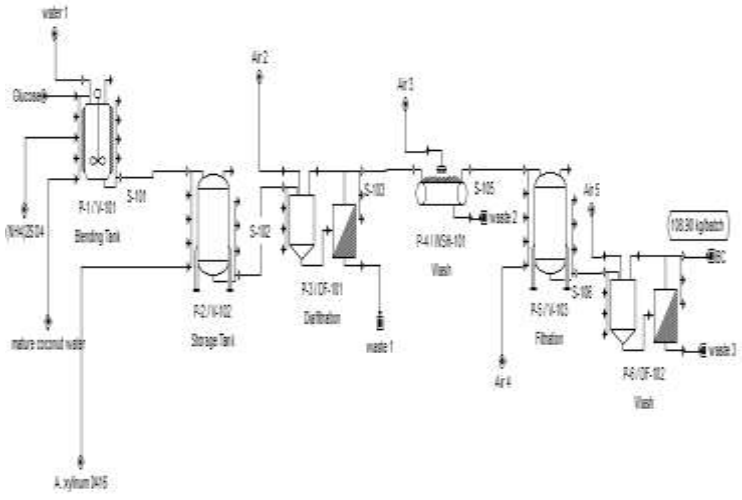


Fig 1: Alternative 1 [6]

Figure 2 shows the filtered and sterilize mature coconut water was inoculated in the storage tank (V-101) for 4 days with the presence of oxygen. The oxygen from air compressor (G-101) was then passed through the air filter (AF-101) to prevent contamination. The dissolved oxygen rate was maintained at 20 % by regulating the speed of the mixer and also air flow rate. The pH in the storage tank (V-101) was maintained at pH 4. After three days of inoculation, *A.xylinum* 0416 underwent fermentation process in storage tank (V-101) where the temperature was maintained at 32 ^{0}C and pH 4. After seven days of fermentation, floating white pellicle (BC) can be observed at the surface of the culture media. BC that was formed from fermentation tank (V-101) is then soaked in sodium hydroxide in storage tank 2 (V-102) for neutralization process. In this tank, host cell and other growth substances in BC was dissolved in NaOH. At this tank, sedimentation process occurred where BC was separated from other unwanted liquid by its density and it consists of cell debris, media, and BC. Cell debris is then removed by filtration (DF-101) at 30 °C and another dissolved particle was also filtered and removed. BC was then subsequently purified by using drying and freezing processes before it is stored as the end product [5].

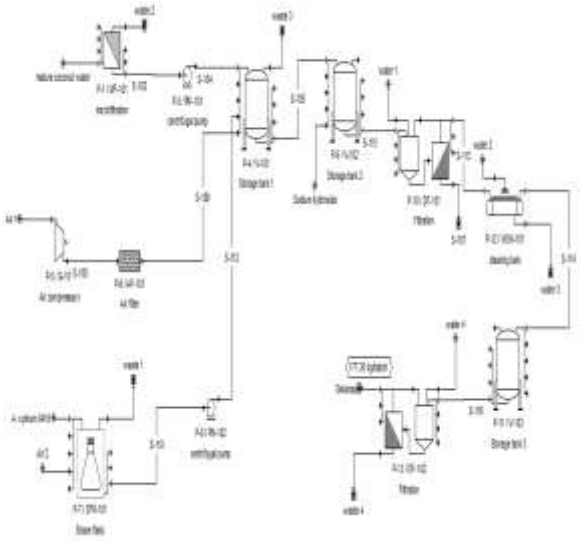


Fig. 2: Alternative 2 [5]

Figure 3 shows mature coconut water was filtered by using ultrafiltration (UF-101). After that, the mature coconut water was boiled and sterilized for 10 minutes and left to cool in storage tank 1 (V-101). *A.xylinum* 0416 and sugar were added in the sterilized mature coconut water and then transferred to the storage tank 2 (V-102). At this stage, the mixture was left for two to three weeks to allow the BC to grow. BC that was formed at the surface of the mixture was washed and harvested by filtration (DF-101) [7].

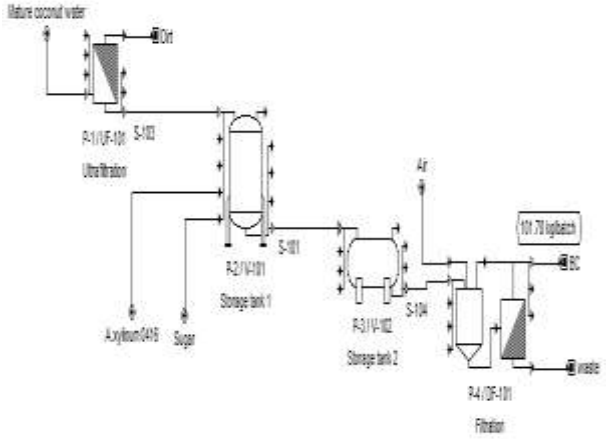


Fig. 3: Alternative 3 [7]

By using the *SuperPro Designer*® software, the cost and the wet weight of BC for alternative processes 1, 2 and 3 were obtained. The results obtained from the software were interpreted and inserted into Table 2. It was found out that alternative 2 is the cheapest cost for the BC production (RM 16,862,942/year) with RM 170/kilogram even though the total production cost (98700 kg/year) for alternative 2 is expensive compared to alternative 1 (RM4,868,136/year) and alternative 3 (RM4,146,497/year).

Table 2: Total operating cost

Alternative	Total unit operation	Total operation cost RM/year	Production of BC (kg/year)	BC cost (RM/BC.kg)
Alternative 1	5	4,868,136	3484	1397
Alternative 2	10	16,862,942	98700	170
Alternative 3	4	4,146,497	1818	2280

4. Conclusion

Mature coconut water was used as the fermentation media to produce BC by *A.xylinum* 0416 with different range of pH and days. In laboratory scale, media with pH 4 produce the highest production of dry BC which is 2.277g. Due to the high demand of BC, process simulation of BC in industrial scale with the cheapest price was conducted. In bioprocess industry, the process simulation is an important tool to improve, assess, design and estimate the product produced from the selected processes. It is also can represent the whole process, estimation of equipment size, cost analysis, waste minimization and treatment methods. For the purpose of reducing the costs, the selection of an operating unit of the plant is very important. By using SuperproDesigner® software, there are three processes alternatives have selected from the literature review. Among three processes, alternative 2 is the cheapest BC production cost which is RM 170/kg.

Acknowledgement

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