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by Rahmayetty Rahmayetty_2

The Effect of *Acetobacter xylinum* Concentration to Bacterial Cellulose Production Using Waste Water of Palm Flour Industry as Fermentation Medium

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Abstract. Bacterial cellulose (BC) is produce extracellularly by acetic acid bacteria through oxidative fermentation. Utilization of waste water of palm flour industry as a medium for bacterial cellulose production is a new breakthrough, because the palm flour industry is quite large in Banten and an effort to reduce environmental pollution due to the waste. The purpose of this study was to determine the effect of *Acetobacter xylinum* concentration to the bacterial cellulose production. In this study, it was found that the concentration of *Acetobacter xylinum* greatly affects the bacterial cellulose produced. The highest yield of bacterial cellulose was 60.7% at 15% *Acetobacter xylinum* concentration.

Keywords: Acetobacter xylinum · bacterial cellulose · coconut water · palm flour · waste water

1 Introduction

The palm flour industry is an industry that processes palm tree trunks into palm flour. In the production process, this industry produces by-products in the form of solid and liquid waste. Currently, solid waste is widely used by the community as animal feed, while waste water is directly discharged into water bodies without being processed first, causing environmental pollution. Waste water of palm flour industry still contains quite high organic and inorganic compounds with chemical oxygen demand (COD) values of 4231 mg/L, free ammonia 24.82 mg/L, nitrate 1.18 mg/L, dissolved solids 2410 mg/L, and ratio of C/N is 15 [1, 2]. One of the efforts to reduce environmental pollution due to palm flour industry waste water is to use it to be used as a useful product. Based on the

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composition of palm flour waste water which contains carbon and nitrogen which is still quite high. It can be utilized as raw material for the manufacture of cellulose polymers through the fermentation process.

Cellulose is a natural polymer that is biodegradable, biocompatible, non-toxic, and can be an alternative to plastic packaging which is still the main source of pollution on earth.

In general, cellulose produce traditionally extracted from plants or their wastes. Increasing demand on derivatives of plant cellulose had increased wood consumption as raw material, causing deforestation and global environmental issue [3]. Sources of cellulose apart from plants and their waste, it can also come from algae and bacteria. Cellulose derived from bacteria is called bacterial cellulose. Bacterial cellulose has a higher purity than plant cellulose because it does not mix with lignin, pectin and hemicellulose [4]. In addition, bacterial cellulose also has a finer fiber size of up to about one hundred times [5], a denser and neater interwoven fiber and a much higher tensile strength than plant cellulose [4, 6]. Bacterial cellulose $(C_6H_{10}O_5)n$ is composed of cellulose fibers from glucose molecules, in the form of a thin gel-shaped layer or fine threads bound by microfibrils having a diameter of about 2–4 nm. Its chemical structure is the same as cellulose from plants [7]. Bacterial cellulose has a network structure composed of very fine cellulose fibers with a diameter of 100 nm and a length of about 100 m [8]. In addition, bacterial cellulose is biodegradable, non-toxic, antimicrobial, crystallinity and high tensile strength, heat resistance and good biocompatibility [4, 9]. Based on the superior properties of bacterial cellulose, this biopolymer has the potential to be developed into packaging materials.

Bacterial cellulose is produced extracellularly by acetic acid bacteria through oxidative fermentation, such as nata-de-coco, a fermentation product of coconut water by *Acetobacter xylinum*. In order to find alternative source of material for bacterial cellulose, many research have focused on industrial by-product as potential medium. Many studies have reported the waste water from industries as beneficial carbon source for bacteria cellulose production. The production of bacterial cellulose using waste industry as fermentation medium has begun to be developed, such as sago by-product using *Beijerinkia fluminensis* and *Gluconacetobacter xylinus* [10], sago waste water using *Acetobacter xylinum* [11] and molasses using *Komagataeibacter rhaeticus* [12]. Some bacteria that can produce cellulose are *Gluconacetobacter, Acetobacter, Agrobacterium, Aerobacterium, Azotobacter, Rhizpbium, Sarcina*, and *Salmonella* [11]. Based on differences in bacterial strains, it was found that *Acetobacter xylium*, also known as *Gluconacetobacter xylinus*, has been shown to have the highest rate of bacterial cellulose production [13]. *Acetobacter xylinum* grows well in medium that has a pH of 3 to 4. If the pH is more than four or less than 3, the fermentation process will not be able to run optimally. The optimum temperature for its growth is at room temperature (28–31 °C) [14].

Based on the literature study, there has been no research reporting on the isolation of bacterial cellulose using waste water from palm flour industry as fermentation medium. The production of bacterial cellulose using palm flour waste water medium is a new breakthrough, because there it is quite a lot of palm flour industry in the Banten area and can be an effort to reduce environmental pollution.

2 Methods

2.1 Making Acetobacterium Xylinum Starter

Waste water of palm flour industry is precipitated and filtered with gauze and 1000 ml was taken and then brought to a boil. After boiling, add 10 ml of glacial acetic acid, 5 g of ammonium and 100 g of glucose. The solution was stirred until completely mixed. Then the solution was put into glass bottles of 500 ml and covered with sterilized paper. Once cool, add 10 ml of Acetobacter xylinum in the solution. Incubation of the starter solution for 14 days can then be used in the synthesis of bacterial cellulose.

2.2 Production of Bacterial Cellulose

Fermentation of BC production was conducted in static batch condition. Five hundred ml of palm liquid waste was sterilized at 100 °C. Ammonium phosphate (ZA-food grade) of 5 g and sucrose of 100 g were added to the culture medium. The acidity was adjusted to medium pH of 4 using glacial acetic acid. The culture medium was cooled at room temperature, after that the inoculum of *Acetobacter xylinum* bacteria was added to the culture medium with variations of concentration of 5, 10, 15 and 20% v/v and incubated statically at room temperature for 14 days. The pellicles produced were harvested after 16 of incubation days and washed with distilled water to remove medium components. Afterwards, it was immersed into 2% NaOH at 80 °C for 60 min to eliminate bacterial cells. Finally, the pellicles were washed extensively with distillate water up to pH of 7. It was then dried at 105 °C for 24 h to obtained BC film. BC film was grided to obtained BC powder.

2.3 Characterization of Bacterial Cellulose

Analyze the number of bacteria using the total plate count (TPC). This analysis was done by growing living microorganism cells on agar media, so that microorganisms will multiply and form colonies that can be seen directly and counted. The Infrared (IR) Spectra of the bacterial cellulose acetate samples was determined by using Thermo Scientific type Nicolet iS5 FTIR spectrometer. The samples were prepared by the KBr-disk method. Test the thickness of the nata using a ruler. Calculation of yield by weighing the product obtained with the waste used and entering it into the equation:

$$yield = \frac{weight\ of\ \ cellulose\ produced\ (gr)}{total\ weight\ of\ \ fermentation\ medium\ (gr)} \times 100\%$$

3 Results and Discussion

3.1 Acetobacter Xylinum Growth During Starter Formation

The stage of making *Acetobacter xylinum* starter is the stage for developing bacteria and adapting it to the given substrate medium. The increase in the number of bacteria each time was calculated using the Total Plate Count (TPC) tool.

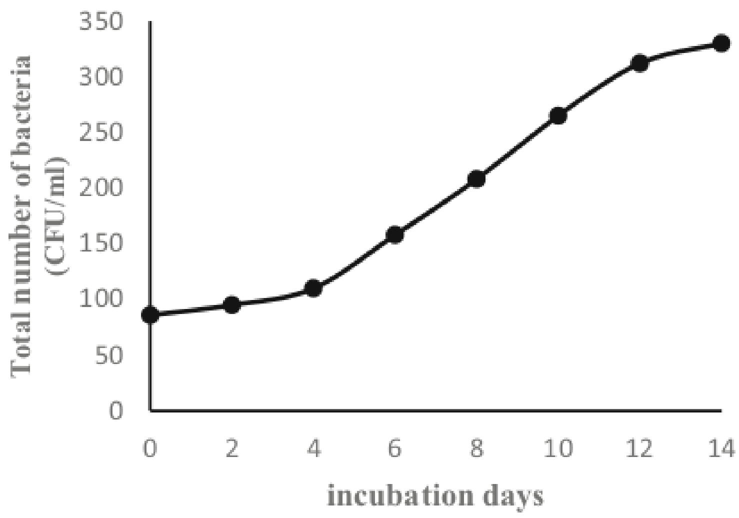

Fig. 1. *Acetobacter xylinum* during starter formation

Acetobacter xylinum is a bacterium capable of producing cellulose from glucose sources through a fermentation process. In the batch process, the data generated by TPC (Total Plate Count) analysis showed that the total *Acetobacter xylinum* increased with the length of acclimatization time, as shown in Fig. 1.

In the first four days the bacteria experienced a lag or adaptation phase, which was seen with an increase in the number of bacteria that was not too significant. The exponential phase (fast growth) begins to occur after the fourth day. This increase is influenced by the amount of nutrients contained in the palm liquid waste which is sufficient and the condition of the bacteria that have adapted to the media. Bacterial cells are able to grow and divide exponentially to the maximum number which is influenced by environmental conditions and nutrients in the media. Microbial growth is closely related to the growth media available for microbial growth in it. The growth rate of *Acetobacter xylinum* during the acclimatization process in the manufacture of starters can be calculated by the equation:

$$\mu_{net} = \frac{\ln X_2 - lnX_1}{t_2 - t_1} = \frac{\ln 348 - \ln 158}{14 - 6} = 0,098day^{-1}$$

From the calculation results, the net growth rate of *Acetobacter xylinum* was 0.098 day^{-1}.

3.2 Isolation of Bacterial Cellulose

Bacterial cellulose was isolated from palm flour waste water with varying concentrations of *Acetobacter xylinum.* The growth of bacterial cellulose during the fermentation process can be seen in Fig. 2.

Variations in the concentration of *Acetobacter xylinum* greatly determine the amount of bacterial cellulose formed. The concentration of *Acetobacter xylinum* which produced the highest bacterial cellulose in this study was 15% with a thickness of 2.1 cm of bacterial cellulose.

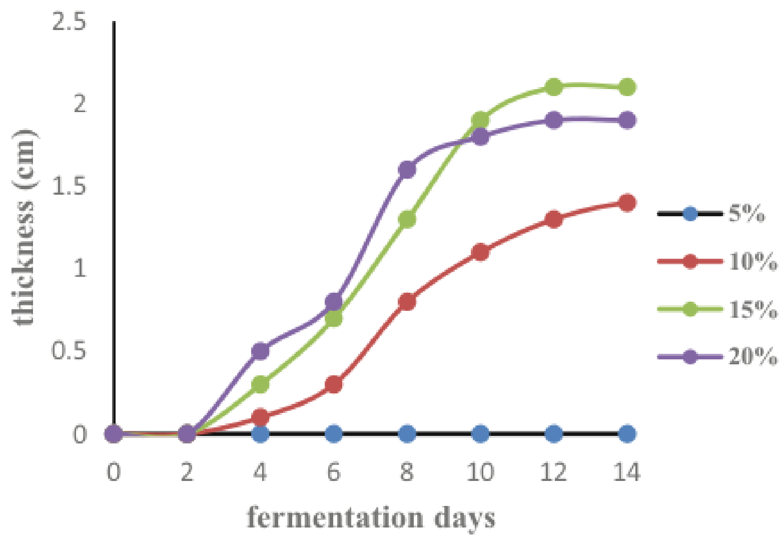

Fig. 2. Thickness of bacterial cellulose with various concentrations of Acetobacterium xylinum

Table 1. Bacterial cellulose yield

Acetobacter xylinum concentration	Thickness of bacterial cellulose (cm)	Yield (%)
5%	0	0
10%	1.4	31.66
15%	2.1	50.56
20%	1.9	39.72

Fig. 3. Bacterial cellulose with concentration of *Acetobacter xylinum* (a) 15%; (b) 20%

The yield of bacterial cellulose in the waste water medium of the palm flour industry is proportional to the thickness of the product. The highest yield was obtained at 15% *Acetobacterium xylinum* concentration. The resulting yield is listed in Table 1.

3.3 Visual Form of Bacterial Cellulose from Palm Liquid Waste

Bacterial cellulose from fermented palm effluent using Acetobacter xylinum 15% and 20% is shown in Fig. 3.

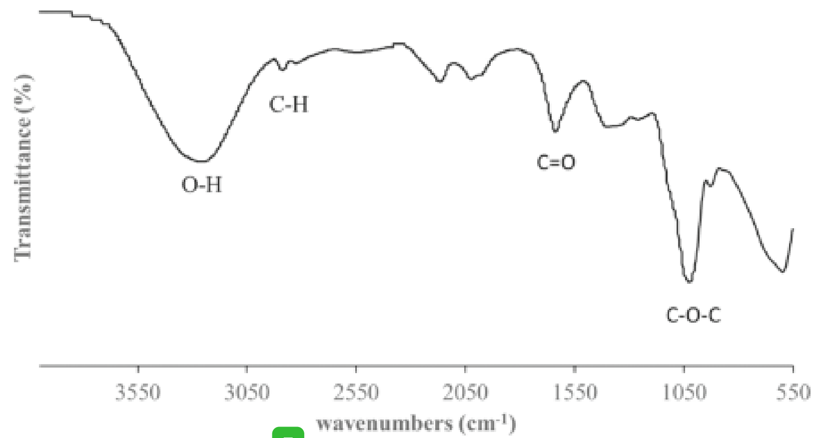

Fig. 4. The FTIR Spectrum of bacterial cellulose with Acetobacter xylinum 15%

The bacterial cellulose texture produced in the liquid waste medium of the palm flour industry with various concentrations of *Acetobacter xylinum* 15 and 20% had the same elasticity but with different colors. Bacterial cellulose using *Acetobacter xylinum* 15% looks light yellow while that with *Acetobacter xylinum* 20% is light brown.

3.4 FTIR Characterization

The functional groups of bacterial cellulose were analyzed using FTIR technique. The bacterial celluose-produced functional groups from the fermentation process is shown in Fig. 4.

Figure 4 shows that bacterial cellulose has four main peaks located in the wave number range of 1026; 1637; 2889, and 3259 cm^{-1}. The wave number in the range of 1026 cm^{-1} is the C-O-C functional group [15], the wave number in the range of 3259 cm^{-1} is the -OH functional group [16], while the range in the wave number is 2889 cm^{-1} indicates the presence of a C-H functional group which is the main bond in cellulose [15, 17] and 1637 cm^{-1} is carboxylic acid ($C = O$) [18]. The two bonds are bonded to each other and cause the formation of a hexagonal structure of carbon atoms.

4 Conclusion

Palm flour industrial wastewater has the potential to be developed as a medium for bacterial cellulose production. The concentration of Acetobacter xylinum in the fermentation of waste water from the flour industry greatly affects the bacterial cellulose produced. The highest bacterial cellulose obtained was 60.7% at 15% Acetobacter xylinum concentration.

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