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# Optimization of Green Synthesis Biopolymer Cellulose Using *Acetobacter xylinum* From Whey as Media of Bacteria

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**Abstract**

*Utilizing waste into a more valuable commodity was one of the objectives of that research. Waste which initially became an environmental pollutant factor could be processed into more useful products. Tofu liquid waste was referred to whey was a by-product of tofu production. Whey was waste that still contains organic materials such as protein, fat, carbohydrates and minerals. That whey was used as a growth medium for Acetobacter xylinum bacteria and produces cellulose biopolymer as a result of carbohydrate fermentation. The process of optimizing carbohydrate fermentation by Acetobacter xylinum bacteria was carried out by varying the ratio of the amount of whey to coconut water, the acidity level of the media, and the fermentation time. The research results showed that the biosynthesis of cellulose biopolymer was obtained from a ratio of whey concentration to coconut water of 1:1, variations in acidity levels showed that the optimum fermentation conditions occurred at pH 4, and the optimal amount of cellulose biopolymer was obtained on day 21, namely with the highest percent yield value. The resulting biocellulose was characterized using FTIR, XRD, and SEM. The characterization results show that biocellulose is well formed with a fiber surface shape and particle size in the range of 50-200 nm*

**Keywords:** *Optimation, Green Synthesis, Biopolymer Cellulose, Acetobacter xylinum*



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## INTRODUCTION

Cellulose was a natural polymer that can be obtained in various ways, such as using chemical principles in the form of pyrolysis and through a fermentation process using bacteria (Irham et al., 2020; Marpongahtun et al., 2018; Purba et al., 2023). Cellulose or modified cellulose composite is currently being developed as a multi-purpose material, especially in the health sector, such as as an antibacterial, food packaging material, biosensor, wound medicine, and as an absorbent (Dincă et al., 2020; Hassan et al., 2017; Wang et al., 2019a).

Tofu liquid waste or whey was one of the tofu production wastes that was not utilized and causes environmental pollution including water, air and soil pollution. From several research results, tofu liquid waste causes an increase in TSS, COD, BOD content, and decreases DO content in water. That results in the death of aquatic biota such as fish in extreme concentration conditions (Nurhasmawaty, 2020; Pagoray et al., 2021; Pradana et al., 2018). Utilizing whey was very important as a way to reduce environmental pollution levels. Whey waste from various research results shows that that waste still contains nutrients in the form of organic materials such as carbohydrates, proteins, fats and minerals which can be used as a growth medium for biocellulose-forming bacteria such as *Acetobacter xylinum* (Chua & Liu, 2019).

The use of *Acetobacter xylinum* bacteria was an important point in converting waste into products of higher economic value, namely by forming BC (Bacteria Cellulose) was often known as biocellulose. This method was a very environmentally friendly method because it did not produce hazardous waste and also does not require high energy and low production costs (Esa et al., 2014). The resulting biocellulose was non-toxic, biodegradable, has a high water content and is easy to modify because it was rich in -OH hydroxy groups (Hassan et al., 2017). This is what causes the development of research on biocellulose to be very massive, especially in the health sector. The application of a material as an antioxidant, antibacterial, anticancer and antidiabetic was an interesting topic in health research (Fransiska, 2021; Hasan et al., 2023; Purba et al., 2020).

Optimization and validation are steps that need to be carried out in various scientific research to produce conditions for an optimal and valid method (Purba et al., 2018; Purba et al., 2023). The aim of this research is to determine the optimization process for the formation of biocellulose through the fermentation process by Acetobacter xylinum bacteria with variations in whey and coconut water concentrations, media acidity, and fermentation time.

## METHOD

### Materials

The tools used in this research are media containers, ovens, incubators, autoclaves, bunsens, sieves, trays, Erlenmeyer, magnetic stirrers, stirring rods, analytical balances, The materials used are tofu liquid waste, coconut water waste, granulated sugar, ZA (Zwavelzure ammonium), 5% NaOH, glacial acetic acid, distilled water.

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**Optimization of Concentration Variations**

This study used varying concentrations of whey and coconut water with a ratio of 1:1, 1:3, and 3:1 respectively. The comparison carried out was a volume comparison with a total media volume of 1 L. The 1:1 comparison was carried out by adding 500 mL of each volume of whey with coconut water. The 1:2 ratio was carried out by mixing 250 mL of whey media and 250 mL of coconut water. 750 mL. A 3:1 ratio was done by mixing 750 mL of whey and 250 mL of coconut water. The procedure for making media was carried out with constant additions for all variations, namely 5 g of ZA and 25 g of sucrose per one liter of media.

**Optimization of Acidity Variations**

The media that has been obtained from varying concentrations was then adjusted for bacterial growth media by adding glacial acetic acid with pH variations, namely 2,3,4,5 and 6. The addition of glacial acetic acid solution was carried out on media that has been sterilized and measured using a pH meter to control acidity of the medium.

**Optimization of Fermentation Time Variations**

The fermentation time carried out was the time required for Acetobacter xylinum bacteria to produce the largest amount of biocellulose (measured by % yield). The time variation carried out was up to day 21 starting from day 1. The time measurement process was carried out by providing media with the optimization results of stage 1, namely variations in concentration and stage 2, namely variations in acidity and calculating the weight of the biocellulose formed. At this stage the same thing was done in adding ZA and Sucrose and the number of bacteria used was fixed.

**Characterization**

The resulting biocellulose was then purified until the biocellulose was clean and odorless. The resulting biocellulose was then soaked in 5% NaOH for 2 hours. The soaking results are then washed again with distilled water until biocellulose was obtained in neutral conditions or pH 7. The biocellulose was then dried and characterized.

## RESULTS AND DISCUSSION

**Optimization of Concentration Variations**

Biocellulose produced from this variation was carried out by calculating the percent yield of the biocellulose produced. Calculation of percent yield was carried out by comparing dry biocellulose with the mass of the media used. The research data was presented in Figure 1 and Table 1 below.

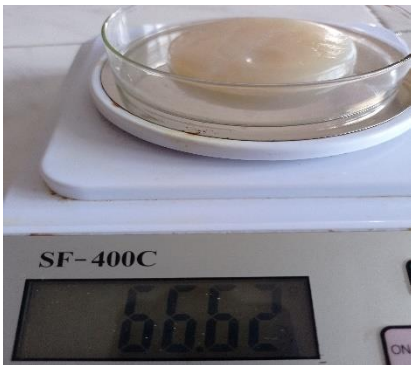


**Figure 1.** Biocelulose from whey

**Table 1.** Percentage Yield of Biocellulose

| ***Whey:* Coconut water** | **Volume of Media (mL)** | **Volume of Bacteria (mL)** | **Wet mass (g)** | **Diameter Thickness (cm)** | **Dry mass (g)** | **Percentage yield (%)** |
|---|---|---|---|---|---|---|
| 1:1 | 250 | 30 | 31 | 4;3.5; 3.6; 3.7 | 2.1 | 0.75 |
| 1:2 | 250 | 30 | 20 | 4; 3; 3,1; 3,2 | 1.5 | 0.53 |
| 2:1 | 250 | 30 | 24 | 3; 3; 3,2; 3,1 | 1.8 | 0.64 |

**Optimization of Acidity Variations**

The data obtained from the biocellulose produced in this variation showed that at acidic pH 2 biocellulose was unformed. However, at pH 3, 4, 5 and 6 there was a layer of biocellulose at the top of the media container. The results of this data were obtained on the 7th day of harvest. In table 2 below, data was obtained that the optimum pH was at pH 4. The unformated of biocellulose at pH 2 was caused by the fermentation process not occurring by *Acetobacter xylinum* and at this pH the bacteria tend to be inactive or dead. Under conditions of media acidity at pH 4, the fermentation process occurs under optimum conditions. At this pH, based on the results of previous research, it shows that metabolism in *Acetobacter xylinum* runs optimally so that the production of biocellulose, which was an excretion product from these bacteria was in maximum quantities (Wang et al., 2019a).

**Table 2.** Percentage yield of biocellulose

| **pH** | **Volume of Media (mL)** | **Volume of Bacteria (mL)** | **Wet mass (g)** | **Diameter of Thickness (cm)** | **Dry mass (g)** | **Percentage of Yield (%)** |
|---|---|---|---|---|---|---|
| 2 | 250 | 30 | - | - | - | - |
| 3 | 250 | 30 | 25 | 2.5; 3.6; 2; 2.4 | 1.5 | 0.5 |
| 4 | 250 | 30 | 38 | 4;3.5; 3.6; 3.7 | 2.6 | 0.93 |
| 5 | 250 | 30 | 31 | 4; 3; 3,1; 3,2 | 2.2 | 0.78 |
| 6 | 250 | 30 | 27 | 3; 3; 3,2; 3,1 | 1.9 | 0.67 |

**Optimization of Fermentation Time Variations**

Fermentation time was measured up to week 3 or day 21. From week 2 or day 14 the mass produced was stable until week 3 or day 21. Data on the percent yield obtained from the research results was shown in following Table 3.

**Table 3.** Percentage Yield of Bioccelulose

| Days | Volume of Media (mL) | Volume of Bacteria (mL) | Wet mass (g) | Diameter of Thickness (cm) | Dry mass(g) | Percentage Yeild (%) |
|---|---|---|---|---|---|---|
| 1 | 250 | 30 | - | - | - | - |
| 2 | 250 | 30 | - | - | - | - |
| 3 | 250 | 30 | 5 | 0.5 | 0.4 | 0.1 |
| 4 | 250 | 30 | 10 | 0.8 | 0.9 | 0.32 |
| 5 | 250 | 30 | 16 | 1.2 | 1.3 | 0.46 |
| 6 | 250 | 30 | 30 | 2.6 | 2.1 | 0.84 |
| 7 | 250 | 30 | 38 | 2.8 | 2.7 | 0.96 |
| 8 | 250 | 30 | 40 | 2.8 | 2.8 | 1.0 |
| 9 | 250 | 30 | 42 | 2.9 | 2.9 | 1.04 |
| 10 | 250 | 30 | 44 | 3.0 | 2.9 | 1.04 |
| 11 | 250 | 30 | 47 | 3.1 | 3.2 | 1.14 |
| 12 | 250 | 30 | 48 | 3.5 | 3.2 | 1.14 |
| 13 | 250 | 30 | 50 | 3.5 | 3.4 | 1.21 |
| 14 | 250 | 30 | 51 | 3.5 | 3.5 | 1.25 |
| 15 | 250 | 30 | 51 | 3.6 | 3.4 | 1.21 |
| 16 | 250 | 30 | 52 | 3.5 | 3.5 | 1.25 |
| 17 | 250 | 30 | 52 | 3.6 | 3.5 | 1.25 |
| 18 | 250 | 30 | 52 | 3.6 | 3.5 | 1.25 |
| 19 | 250 | 30 | 52 | 3.6 | 3.6 | 1.28 |
| 20 | 250 | 30 | 52 | 3.6 | 3.6 | 1.28 |
| 21 | 250 | 30 | 52 | 3.6 | 3.6 | 1.28 |

The data in table 3 shows that on the first and second days there was no microorganism activity or fermentation process by Acetobacter xylinum bacteria. This causes no biocellulose to form in the media container. However, on the 3rd day onwards, cellulose bacteria are formed in a gel condition, initially reaching a solid state. The longer the fermentation time, the denser the form of biocellulose produced.

From the optimization data it can be concluded that the best effect of varying concentrations is with a ratio of whey and coconut water of 1:1. The role of coconut water in this context is as a provider of nutrients to the Acetobacter xylinum bacteria, especially in providing the mineral content needed by the bacteria (Purba et al., 2023). Based on variations in acidity, data was obtained that at pH 4, the largest biosleulose (% yield) was obtained. That was in accordance with the previous statement that the Acetobacter xylinum bacteria works optimally under acidic pH conditions at pH 4 (Wang et al., 2019b). Meanwhile, variations in fermentation time can be seen from the second week to the third week when the biocellulose formed has a constant mass. That was because at that time the nutrient content in the media has run out or become thin so that the biocellulose layer no longer forms.

## Characterization

### FTIR (Fourier Transform Infra Red)

Analysis with FTIR shows that there are 3 specific wave numbers that indicate the presence of biocellulose functional groups, namely as follows:

1. Wavelenght number 2300-3700 $cm^{-1}$, was representative of –OH group
2. Wavelenght number 2800-2950 $cm^{-1}$, was representative of –CH group
3. Wavelenght number 1050 $cm^{-1}$, was representative of –C-O-C- group

The results of this analysis show that biocellulose has been formed. This is in accordance with the results of previous research that the range of the three types of prerequisites mentioned above are characteristics of biocellulose (Costa et al., 2017). The following are the results of Figure 2. The IR spectrum results of the sample show that the three characteristics above were presented.

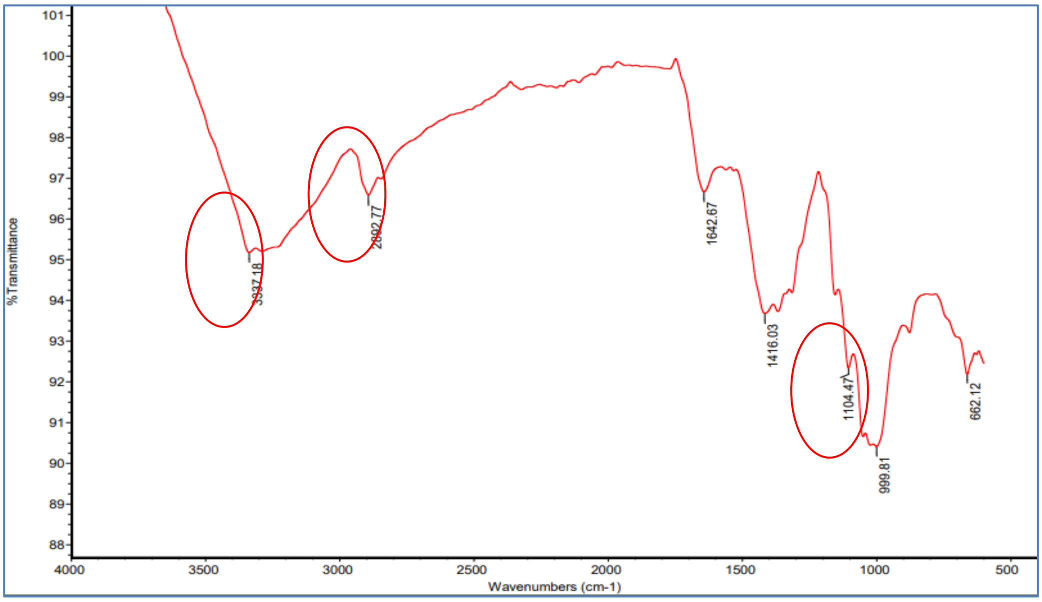


**Figure 2.** Spectrogram IR of Bioccelulose

### XRD (X-Ray Diffraction)

Diffractogram data can also determine whether biosleulose is formed or not. From the research data, it can be seen that the 2θ angle that was characteristic of biocellulose at 14° dan 26°. That was related with previous research which explains that the character of biosleulose can be seen from the diffractogram, namely at an angle 14°, 16°, dan 26° (Costa et al., 2017).

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*** Basic Data Process ***

Group : Standard
Data : NanoSelulosa

\# Strongest 3 peaks

| no. | peak no. | 2Theta (deg) | d (A) | I/I1 | FWHM (deg) | Intensity (Counts) | Integrated Int (Counts) |
|---|---|---|---|---|---|---|---|
| 1 | 11 | 22.6600 | 3.92091 | 100 | 1.80000 | 20 | 1666 |
| 2 | 6 | 14.3300 | 6.17588 | 65 | 1.14000 | 13 | 850 |
| 3 | 18 | 43.8800 | 2.06164 | 60 | 0.56000 | 12 | 304 |

**Figure 3.** Difractogram of Bioccelulose

**SEM (Scanning Electron Microscope)**

Surface morphology analysis was carried out using SEM. The results of sample analysis show that the surface morphology of Biocellulose is in the form of fibers with particle sizes as follows:

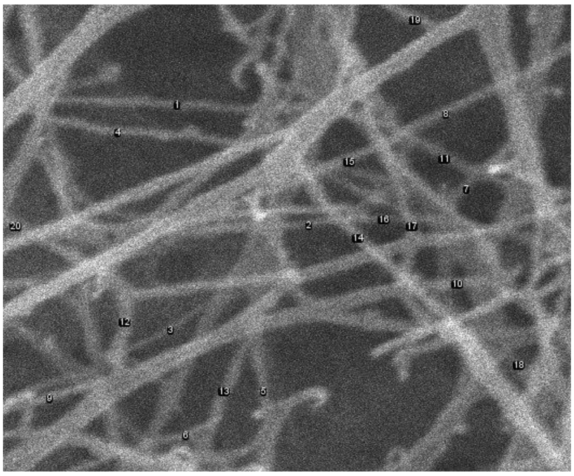

**Figure 4.** Structure of Biocellulose surface area

From the SEM data, the average particle size can be determined using ImageJ and Origin software. Data was obtained that the biocellulose particle size was 200 nm. That was in accordance with previous research that biocellulose has a size range of 10-300 nm. This is adjusted to the type of sample and preparation technique used (Lu & Jiang, 2014).

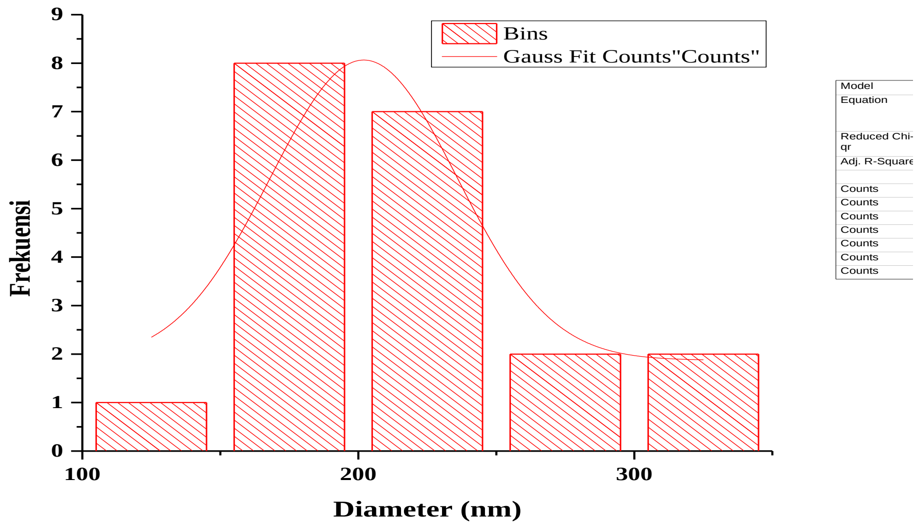


**Figure 5.** Biocellulose particle size

## CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that:

1. Tofu liquid waste can be used as a medium for forming nanocellulose with an optimal ratio of 1:1 with coconut water
2. Biocellulose was formed optimally in media conditions with an acidity level of pH 4
3. Biocellulose was formed maximally starting in the second week

## ACKNOWLEDGMENT

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