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Research Article

Optimization Production and Characterization of Bacterial Cellulose from Cornhusk

Optimasi Produksi dan Karakterisasi Selulosa Bakteri dari Klobot Jagung

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Abstract

Cornhusks are agricultural wastes with low economic value that will cause environmental pollution if not appropriately handled. Cornhusk waste can be processed as raw material for bacterial cellulose (nata) since it contains 44% cellulose. This study aims to optimize bacterial cellulose production from cornhusks and determine the effect of cornhusk mass and fermentation duration on the characteristics of the nata produced. The primary process for producing bacterial cellulose from cornhusks was fermentation by *Acetobacter xylinum*. The nata characterization carried out in this study includes thickness, yield, crude fiber, and moisture content, as well as statistical analysis to determine whether there was significant effect of variations in cornhusk mass and fermentation duration on bacterial cellulose production. Based on the results of optimizing the production of nata from cornhusks, the optimal mass of cornhusks was of 25 grams with fermentation duration of 17 days. Based on the characterization and data analysis results, variation on the cornhusks mass and duration of the fermentation had a significant effect on fiber content, yield, and tensile strength of bacterial cellulose from cornhusks. On the other hand, the variations on cornhusks mass and the duration of fermentation did not significantly affect the moisture content and thickness of bacterial cellulose from cornhusks.

Keywords: cornhusk; fermentation; nata; optimization

Abstrak

Klobot jagung merupakan limbah pertanian yang bernilai ekonomis rendah dan akan menyebabkan pencemaran lingkungan jika tidak ditangani dengan tepat. Limbah kulit jagung dapat diolah sebagai bahan baku selulosa bakteri (nata) karena mengandung 44% selulosa. Penelitian ini bertujuan untuk mengoptimalkan produksi selulosa bakteri dari klobot jagung dan untuk mengetahui pengaruh massa klobot jagung dan lama fermentasi terhadap karakteristik nata yang dihasilkan. Proses utama dalam memproduksi selulosa bakteri dari klobot jagung adalah fermentasi dengan Acetobacter xylinum. Karakterisasi nata yang dilakukan pada penelitian ini meliputi ketebalan, rendemen, serat kasar, dan kadar air, serta analisis statistik untuk mengetahui ada tidaknya pengaruh variasi massa kelobot jagung dan lama fermentasi terhadap produksi selulosa bakteri atau nata. Berdasarkan hasil optimasi produksi nata dari klobot jagung diperoleh massa klobot jagung yang optimal adalah 25 gram dengan lama fermentasi 17 hari. Berdasarkan hasil karakterisasi dan analisis data, variasi massa klobot jagung dan lama fermentasi berpengaruh nyata terhadap kadar serat dan rendemen selulosa bakteri dari klobot jagung. Sedangkan variasi massa klobot jagung dan lama fermentasi tidak berpengaruh nyata terhadap kadar air dan ketebalan selulosa bakteri yang dihasilkan.

Katakunci: *kulit jagung; fermentasi; nata; pengoptimalan*

1. Introduction

The most abundant natural polymer in the world is cellulose. Cellulose obtained from the synthetic process of acetic acid bacteria is commonly known as bacterial cellulose [1,2]. Bacterial cellulose is a nanomaterial produced by various strains of Acetobacter species including Pseudomonas, Achrobacter, Alcaligene, Aerobacter, and Azotobacter [3]. Bacterial cellulose or natural hydrogels have better properties than hydrogels produced from synthetic polymers. For instance, bacterial cellulose shows high water content (98-99%), good liquid absorption, wet strength, high chemical purity and can be safely sterilized without changing its structure and properties in the slightest [4].

Bacterial Cellulose (BC) has always attracted the interest of scientists because it has a high level of purity, biodegradability, biocompatibility, and ease of polymerization [5,6]. BC can be applied to engineering skin tissue and bone, to barrier technology and electricity, to electrochemistry, and to sensing applications [7–11]. Although BC has excellent potential, its high production costs limit its industrial-scale applications.

Kurniawan et al. [12] had created a new BC from chayote fruit and bamboo shoots. The BC has excellent mechanical properties such as tensile strength, elongation and water absorption capacity. Despite this fact, there have been efforts to evaluate the possibility of utilizing other agricultural wastes for carbon sources in BC production, including corn products, coffee cherry husk (CCH), date fruits, and banana peel. The findings were parallel with research evidence showing that corn steep liquor was rich in nutrients such as carbon and nitrogen, which supplied organic content during BC production [5,13].

Sulistiyana [14] also found that light yellow corn extract can be used as an ingredient for making nata de corn with the optimum condition for 14 days of fermentation. The characterization of nata de corn from light yellow corn substrate includes the yield of 46.82%, the water content of 93.13%, and fiber content of 1.31%. This value had met the quality standards of nata according to SNI No. 01-4317-1996. Among the substrates that have been widely used in previous studies, the use of agricultural waste biomass can be an alternative for the use of food ingredients that will disrupt food security.

That is why, active research investigating the cost-effectiveness of BC synthesis from different waste products has been conducted and elaborated. Many agricultural wastes are rich in carbon and nitrogen content; therefore, utilizing them as substrates can produce high concentrations of microbial cellulose by optimizing the culture conditions [15].

One of the agricultural waste biomasses that can be used as a substrate is cornhusks. Cornhusk is an abundant agricultural waste and is widely used as a raw material for handicrafts, bio-ethanol production, pulp alternatives, etc. The cornhusk is the part of the plant that protects the corn kernels, is bright green when young, and dries on the trees as it ages. Communities commonly use cornhusk waste as animal feed, but the usage is still limited. Because of their limited use, cornhusks still have little economic value.

Therefore in the present work, it is necessary to find out if cornhusks could be used as raw material to manufacture bacterial cellulose and to determine the optimal conditions required for the production of bacterial cellulose in terms of the results of the characterization of its physical and chemical properties.

2. Research Methods

2.1 Materials

Materials used in this research were cornhusk (obtained from the traditional market Landungsari-Malang), *Acetobacter xylinum* starter in liquid medium of coconut water (from Laboratory of Process Engineering, Agricultural Industry Technology Study Program, UNITRI), pro analysis urea, pro analysis glucose, glacial acetic acid (Merck 100%), and aquadest. Characterization tests were done at Laboratory of Chemistry - UNITRI and at Laboratory of Animal Husbandry - UMM.

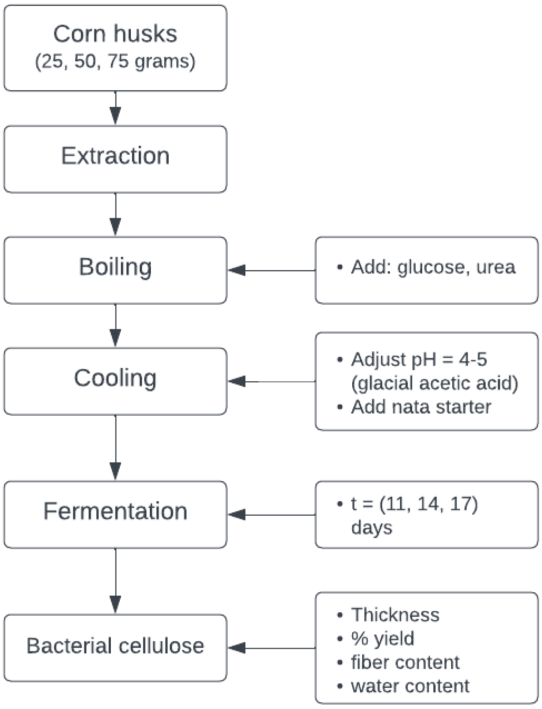

Figure 1. Research Method Flowchart

2.2 BC Production from Cornhusk Extract

Raw materials (cornhusks) were weighed as much as 25, 50, and 75 grams and then was blended with 1 liter of distilled water. Hot maceration was done after the cornhusk was boiled and filtered to get the cornhusk extract. Then, 100 grams of glucose and 4 grams urea were added and stirred while brought to ~~a~~ boil. The mixture was then adjusted to pH 4 by adding glacial acetic acid. When pH 4 has been reached, the mixtures of 250-300 mL (each) were poured into a sterile plastic box containers and then for every 100 mL of the mixture was added 20 ml of nata starter. All of them were stored for fermentation at room temperature with variation of fermentation duration of 11, 14, and 17 days. The same procedures were applied for each cornhusk mass variation. BC/nata were harvested and cleaned using running water and sterilized by soaking it in hot water.

2.3 Characterization of BC from Cornhusk

The next step was to characterize the physical properties (thickness and yield) and chemical properties (fiber and water content). The thickness of the cellulose produced by *A. xylinum* was measured using a vernier caliper at three different points. The yield of bacterial cellulose from cornhusks was determined based on the ratio between the weight of the nata and the weight of the medium. The determination of crude fiber content was based on SNI ISO 5498: 2015, while the determination of water content was done by using the thermogravimetric method according to SNI-01-2354.2-2006.

3. Result and Discussion

3.1 Optimization of Cornhusk BC Production

Figure 2 showed that the fiber content of nata was affected by the duration of fermentation and the mass of the cornhusk substrate. Among the three mass variations of cornhusks, the smallest mass of 25 grams actually had the highest nata fiber content compared to the others. Meanwhile, the duration of the 17-day fermentation had resulted in high fiber content for all three variations of cornhusk mass.

When compared to each fermentation duration, all of them had the same curve patterns; especially on the fiber content was decrease for the cornhusk mass of 25 grams to 75 grams. Therefore, the optimal condition in cornhusk bacterial cellulose production was one with the highest 25 grams fiber content and 17 days fermentation duration.

In the production of BC, fermentation duration had a determining effect on the formation of nata or BC. Too long fermentation would cause *A. xylinum* bacteria went to the death phase due to a lack of nutrients and depletion, causing cells to lose a lot of energy reserves. According to Putriana & Aminah [16], fermentation duration had caused bacteria growth slowing down due to reduced sugar levels and the emergence of acid as metabolite of the fermentation process. Types of incubation time in the production of nata were 6-12 days, 14 days, 16 days, and 21 days.

3.2 Characterization and Results of Statistical Analysis

The data on the characterization of BC from cornhusks with the variable in the cornhusks mass and the fermentation duration, including fiber content, moisture content, yield, and thickness are summarized in Table 1.

The factorial ANOVA test was carried out to test whether there were differences in the average for the mass of corn-husk, the duration of fermentation, and interactions between corn-husk mass and fermentation duration on the variables measured (fiber content, moisture content, yield, and thickness).

If the ANOVA factorial analysis results showed a significant difference, then a further test (if the treatment being compared was more than 2), namely the Tukey test, then a different notation would be given if the two treatments were in different subsets, which means that the two were significantly different. Meanwhile, the same notation would be given if they were in the same subset, which was not significantly different.

The highest average thickness score in treatment A2B2 (mass of cornhusks 50 gr, fermentation duration 14 days) was 0.8750, and the lowest average thickness score in treatment A3B1 (mass of cornhusks 75 gr, fermentation duration 11 days) was 0.3400. To see whether the difference in the mean between the treatment groups was significant or not, a factorial ANOVA analysis was carried out.

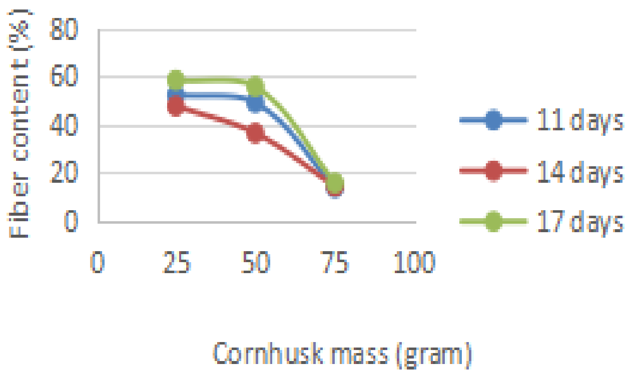

Figure 2. The correlation between cornhusk mass and fermentation duration to the fiber content

Table 1. BC Characterization Results Data

No	Cornhusk mass (gram)	Fermentation Duration (days)	Fiber Content (%)	Moisture content (%)	Yield (%)	Average Thickness (cm)
1	25	11	52.39	98.62	30.05	0.50
		14	49.18	98.64	37.27	0.65
		17	13.69	94.29	38.36	0.63
2	50	11	47.75	98.44	40.59	0.67
		14	36.45	97.97	53.08	0.87
		17	14.26	93.97	54.25	0.85
3	75	11	58.60	98.89	19.83	0.34
		14	55.88	98.90	23.12	0.44
		17	15.69	95.93	23.69	0.41

Table 2. The Anova Factorial Analysis Results of Average Thickness Parameter

Treatment	Average	SD	Notation	Average A (Mass)	Average B (Fermentation Duration)
A1B1	0.5000	0.35355	a	A1 (25 gram) = 0.5938 **ab**	B1 (11 days of fermentation) = 0.5033 **a**
A1B2	0.6500	0.28284	a		
A1B3	0.6313	0.14142	a		
A2B1	0.6700	0.14142	a	A2 (50 gram) = 0.8004 **b**	B2 (14 days of fermentation) = 0.6542 **a**
A2B2	0.8750	0.07071	a		
A2B3	0.8563	0.05657	a		
A3B1	0.3400	0.28284	a	A3 (75 gram) = 0.3967 **a**	B3 (17 days of fermentation) = 0.6333 **a**
A3B2	0.4375	0.14142	a		
A3B3	0.4125	0.11314	a		
F (p-value)					
Nata	6.014 (0.022)*				
Fermentation duration	0.986 (0.410)				
Nata * Fermentation duration	0.050 (0.994)				

From the results of the ANOVA factorial test in Table 2, it demonstated that:

a) There was a significant average difference based on treatment factor A (mass of cornhusks) on the variable mean thickness number measured, it can be seen from the p-value which is smaller than 0.050 (0.022 < 0.050). The highest average thickness of the 50 grams cornhusk mass was significantly different from the 75 grams cornhusk mass, but the 50 grams cornhusk mass was not significantly different from the 25 grams cornhusk mass.

b) There was an insignificant average difference based on treatment factor B (fermentation duration) to the measured average thickness number variable, it can be seen from the p-value which is greater than 0.050 (0.410 > 0.050). The average thickness of the average number of fermentation duration was not significantly different from the average number is not too much different.

c) There was an insignificant average difference based on the interaction of treatment factors A (mass of cornhusk) and B (fermentation duration) on the variable number of average thicknesses measured, it can be seen from the p-value which is greater than 0.050 (0.994 > 0.050).

The average thickness score on the interaction of cornhusk mass treatment and fermentation duration was not significantly different, as seen from the average number between treatments which was not much different.

The conclusions from the results of the factorial ANOVA test for all characterization parameters were:

a) The treatment of cornhusk mass and fermentation duration and their interactions had a significant effect on fiber content, with the significance value of the ANOVA test results being significant ($p<0.05$)
b) Fermentation duration had a significant effect on water content with the significance value of the ANOVA test results being significant ($p <0.05$). While the treatment of cornhusk mass and its interaction with fermentation duration did not significantly affect the water content with the significance value of the ANOVA test results was not significant ($p>0.05$).
c) Treatment of cornhusk mass and fermentation duration and their interactions had a significant effect on the yield, with the significance value of the ANOVA test results ($p<0.05$).
d) The treatment of cornhusk mass had a significant effect on water content with the significance value of the ANOVA test results ($p <0.05$). While the fermentation duration treatment and its interaction with the mass of cornhusk did not significantly affect the water content, the significance value of the ANOVA test results was not significant ($p> 0.05$).

4. Conclusion

As found in this research, the optimal corn-husk mass was 25 grams with fermentation duration of 17 days. Both corn-husk mass and fermentation duration were significantly affected fiber content and yield, but did not show significant effect to the moisture content and thickness of bacterial cellulose. The results of this study were expected to provides an overview of the optimal conditions in the production of bacterial cellulose from cornhusks and this research can serve as a ground point for the development of the manufacture of bacterial cellulose, especially in terms of the application of bacterial cellulose from cornhusks as a natural source of cellulose.

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