

Effect of Sugar, Ammonium Sulfate and Magnesium Sulfate as Supplementary Nutrients in Coconut Water Fermented by *Acetobacter xylinum* to Produce Biocellulose Membranes

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ABSTRACT

Biocellulose can be made by fermentation of coconut water by *Acetobacter xylinum*. Microorganism. Several developed countries have been starting to research the use of biocellulose as a bio-cellulose that is easily broken down in the world of plastics or membranes, such as edible films and biocellulose membranes. In this study, the biocellulose produced from fermenting at room temperature for 15 days using nutrient-enriched coconut water was converted into cellulose membranes. The treatments in the fermentation process were variations of sugar (10%, 11%, 12%), variations in nitrogen sources using ammonium sulfate (0.3%, 0.4%, 0.5%) and variations in mineral sources using MgSO₄ (0.10 %, 0.11%, 0.12%). The optimum results obtained in the experimental volume of 1 liter were in the condition of 12% sugar media, 0.5% ammonium sulfate and 0.12% MgSO₄ where the biocellulose results had a thickness of 1.7 cm, 63.50% yield and 4.41 fiber content. %. Conversion of biocellulose into a biocellulose membrane through pressing and drying The test results of the biocellulose membrane had a rejection coefficient of 63.24% and a flux value of 15.28 lt.m⁻².hr⁻¹

Key words: coconut water, biocellulose, membrane, fermentation, *Acetobacter Xylinum*

1. INTRODUCTION

Natural disasters, especially floods and landslides that often occur in Indonesia, are alleged to be one of the negative impacts of sporadic, enormous and continuous deforestation. In order to avoid a wider and more severe disaster, the Government is currently limiting deforestation.

One of the products from forest products is cellulose. With forest logging restrictions, natural cellulose production from wood will also be limited. For this reason, one way to meet the needs of cellulose is by using bioprocess technology that utilizes microbes to produce cellulose, known as bacterial cellulose.

On the other hand, Indonesia is one of the most coconut producing countries because the soil and climate are suitable for this versatile crop, especially in coastal areas and coastal lands or swamps. None of the

parts of the coconut are useless, including coconut water. Currently coconut water has not been used optimally by the community or by industries that use coconut as raw material. Until now, coconut water is widely used in the manufacture of nata de coco or biocellulose which is fermented by *Acetobacter xylinum*. The name nata de coco is better known by the trade name as a complementary food / drink and is popular in the community.

Coconut water, with the help of microorganisms (bacteria), can be used into various products such as alcohol, vinegar and nata de coco because coconut water tastes sweet and contains 4% minerals, 2% sugar and water (Palungkun, 1993 in Margaretty, et al. al., 2001).

Coconut water is a very suitable medium for the growth of various types of microorganisms.

Therefore, the fermentation process is a stage that cannot be ruled out. Fermented coconut water will contain various types of bacteria such as *Sacharomyces* and *Acetobacter*, because fermented coconut water contains a lot of acid, the bacteria that grows faster and is more abundant is *Acetobacter xylinum*.

The composition of coconut water contains a lot of minerals and vitamins so that it can be used as a good medium for microbial growth, assisted by the sugar contained in coconut water, it will easily ferment into alcohol and acetic acid.

Meanwhile, the name biocellulose is still foreign to society and seems more scientific. Biocellulose itself can be defined as cellulose which is produced through biological processes in the sense that it uses microorganisms (microorganisms).

Cellulose is a fibrous, clayey, water insoluble compound found in the protective cell walls of plants. Cellulose cannot be hydrolyzed by α -amylase. D-glucose units contained in cellulose cannot be digested by all higher organisms (Margaretty, E. et al., 2001).

In other words, biocellulose is a cellulose polymer whose formation is carried out by the *Acetobacter xylinum* bacteria from glucose in the media from water / fruit juice through cooking. So far, the fruit that has often been used as a medium for the breeding of *Acetobacter xylinum* bacteria to produce biocellulose include coconut water, tofu waste water, pineapple waste water, guava fruit water, melon water and so on.

A. xylinum can convert about 19% sugar into cellulose when grown on a medium containing sugar. The cellulose that is formed in the medium is in the form of threads which together with the slimy polysaccharides form a network that continues to thicken into a cellulose layer. Cellulose forms a lumpy mass on the surface of the medium. The cell receives glucose molecules joined by fat to form supports found in the cell membrane and then comes out with the enzymes that combine the remaining hexose into fibers. This fiber formation is seen by the growth of an area on the surface of the medium. The fat is then reabsorbed by microorganism cells (Kenneth, 1964 in Haliwat, 1997).

The biopolymer from the pellicle / nata of these fruits, known as biocellulose, has begun to be noticed by scientists since it was discovered that this material has good mechanical properties. In Japan, biocellulose is made into flexible display materials that are thin, strong, heat resistant, light weight, and flexible. The materials are expected to be used as flexible mobile displays, e-books, e-newspapers, e-posters, and other new products.

Indrarti et al. (1998) have utilized biocellulose for acoustic membranes as a loud speaker diaphragm, improved mechanical properties using alkaline (NaOH) and / or NaClO solutions, and after being tested the sound velocity has a sound velocity of 4522.67 m / sec and has Young's modulus of up to 23.5 GPa. From their

research, they think that the loudspeaker membrane from biocellulose is more prospective than the membrane made from cellulose paper. However, it is not known the stability of the loud speaker diaphragm.

Bacterial cellulose or biocellulose is chemically pure, free from lignin and hemicellulose, has fine fiber tissue, its isolation and purification are relatively simple and its products can be degraded biologically so that it does not pollute the environment. Alternative uses include raw materials for high-quality paper production, low-calorie foods and ultrafiltration membranes.

In recent years researchers have begun to develop the production of cellulose by bacteria called microbial cellulose or bacterial cellulose. Bacterial cellulose is free from contamination of other polysaccharides, and its isolation and purification does not require energy or complicated chemical processes. Matsuoka et al. (1996) suggested that the *Acetobacter xylinum* bacteria was able to produce bacterial cellulose which had unique physical properties, including ultra-fine fiber networks, high purity and crystallinity, so that this bacterial cellulose had the prospect of being developed as raw material for various industries in the future.

Microbes that are known to produce cellulose are bacteria of the genus *Acetobacter*. Cellulose produced by *Acetobacter* bacteria is called bacterial cellulose.. These microbes are usually found in ripe fruits which can be isolated and selected.

In producing cellulose by *Acetobacter xylinum*, there are several factors that influence the yield, including the degree of acidity, nitrogen source, type of substrate and substrate concentration. Currently coconut water is widely used as a fermentation medium for *Acetobacter*.

Besides that, the use of synthetic medium is also developed to avoid dependence on natural media supplies such as coconut water. One of the known biocellulose products from coconut water is nata de coco. However, to produce nata de coco, there is no need to add minerals, and the nitrogen source and its products are not dried for consumption.

This research will produce bio-cellulose (bacterial cellulose) from fermented coconut water and its modification enriched with a combination of minerals and nitrogen. Then the product is dried in a vacuum oven to obtain dry biocellulose such as membrane biocelulosa. Biocelulose membrane can be applied in many industries, especially in the filter or ultra-filtration process. The membrane is a thin, porous layer that has barrier properties against a material. The membrane has selective transfer properties, which means that material that is larger than the pore size of the membrane will be retained and material with smaller sizes can pass through the membrane.

The aim of the research was to produce biocellulose (bacterial cellulose) by means of silent

fermentation by *Acetobacter xylinum* in coconut water and the combination medium with a variety of sugars, nitrogen and minerals added to the media in order to obtain products that meet quality standards. Furthermore, the biocellulose is processed downstream into biocellulose membranes and its performance is tested.

2. RESEARCH METHODOLOGY

2.1. Materials and Tools

The raw materials used were the microbe *Acetobacter xylinum*, nata seed liquid, coconut water. The chemicals used are buffer solutions of sodium phosphate, glucose, bakto-peptone, yeast extract, Na_2PO_4 , citric acid, glacial acetic acid, dimethyl formamid, nystatin and griseofulvin, sugar, glucose, HCl solution, NaOH solution, ethanol, distilled water, tap water, cotton, paper, tissue, label paper, aluminum foil and heat-resistant plastic.

The tools used are measuring cups, beakers, pipettes, Erlenmeyer flasks, knives, test tubes, test tube racks, digital scales, Ose needles, magnetic stirrers, Bunsen burners, Petri dishes, autoclaves, refrigerators, thermometers, spectrophotometers (UVI / VIS), incubator, microscope, pH-meter, tray / tray, sugar analyzer, bottles.

2.2. Experimental design

The experimental design in this study were: 1) Experiment with coconut water media and various sugars (8, 10%, 12%), 2). Variation of nitrogen (ZA): 0.3%, 0.4%, 0.5%, and 3). MgSO_4 mineral variation (0.10%, 0.11%, 0.12%).

2.3. Biocellulose production

The medium used for the production of bacterial cellulose was the selected medium from the research stage in H.2.2. The fermentation that is carried out is static fermentation. One full loop of bacterial cell colonies was inoculated on 100 ml of selected medium in 500 ml Erlenmeyer and propagated for 4 days. After the inoculum is propagated by propagation, a fermentation process is carried out for the production of bacterial cellulose.

At the fermentation stage (Figure 3), the inoculum concentration, the added acid concentration and the combination of nitrogen sources and mineral sources were varied, each of 3 levels. After that the mixture of inoculums was incubated for 7 days at 30oC by means of silent fermentation.

Harvesting is done by taking the cellulose layer that forms on the surface of the medium. Harvesting is done by separating the water and the pellicle. The pellicle is washed in water repeatedly and soaked for a while until the acid level is very low. The pellicle is dried in a vacuum oven at 70 oC for several days until the moisture content stabilizes. Dry pellicle (cellulose

layer) analysis was performed on dry weight (mass), moisture content, color, and tear resistance.

The remaining liquid media analyzed were the residual sugar content, nitrogen and mineral content to see if the media could be used again.

2.4. Making biocellulose nata membranes from coconut water

(Widyaningsih, S and Purwati. 2013) The form of coconut water biocellulose (nata de coco) which is fermented in the form of a gel is washed with running water for 24 hours. The gel was washed with 2% NaOH for 1 hour at a temperature of 80-90 0C. Finally, wash it again with water until the pH is neutral. The purified gel was then pressed with a hand-press to obtain the nata de coco membrane. The membrane / film obtained was then dried at room temperature ($\pm 27\text{-}30$ 0C) and a dry nata de coco membrane was obtained. Membrane testing is applied to filtering / purifying tap water (PAM) using a pressure of 2 bar. Furthermore, the flux value and rejection coefficient are calculated.

2.5. Cellulose biomembrane analysis

The quality of the cellulosic biomembrane was measured for thickness, yield, fiber content, flux value, and the rejection coefficient of the membrane.

3. RESULTS AND DISCUSSION

3.1. Effect of sugar levels on the quality of biocellulose

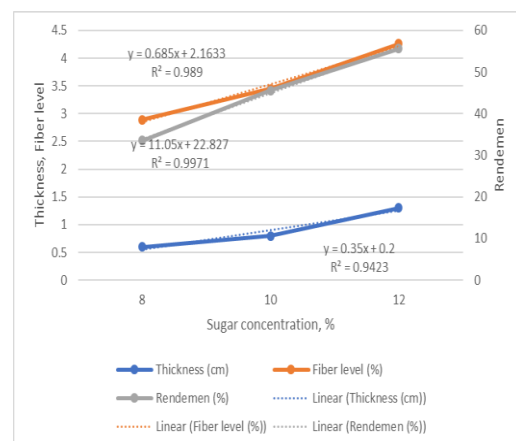


Figure 3.1. Effect of sugar levels on the quality of biocellulose

From the results of silent fermentation for 15 days at room temperature with variations in sugar content of 8%, 10% and 12%, it was obtained biocellulose with thickness, fiber content and yield. As presented in Figure 3.1. It can be seen that the higher the sugar concentration in the range of 8% -12%, the parameter values of thickness, fiber content and yield also increase. Quantitatively, the highest parameter value was obtained at a sugar concentration of 12%

where the biocellulose was obtained with a thickness of 1.3 cm, a fiber content of 4.26% and a yield of 55.63%.

When viewed from the trend, the relationship curve of sugar concentration to the thickness of the biocellulose, to the fiber content, and to the yield respectively shows a linear curve and the overall confidence level is above 90%. The correlation curve between sugar concentration and the thickness of the biocellulose shows the linear equation model $Y = 0.35x + 0.2$ with a confidence level of $R^2 = 0.9423$. The thing that tends to be the same is that the relationship curve between sugar concentration and fiber content shows the linear equation model $y = 0.685x + 2.1633$ with a confidence level of $R^2 = 0.989$. Likewise, the correlation curve between sugar concentration and fiber content shows the linear equation model $y = 11.05x + 22,827$ with a confidence level of $R^2 = 0.9971$.

3.2. The effect of ammonium sulfate concentration on the quality of the biocellulose.

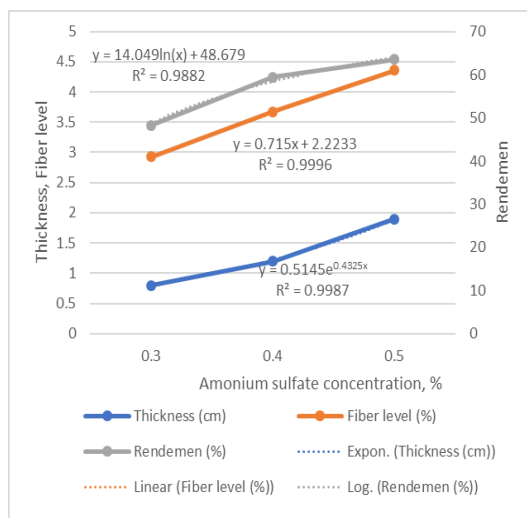


Figure 3.2. The effect of ammonium sulfate concentration on the quality of the biocellulose

From Figure 3.2, the results of silent fermentation for 15 days at room temperature with variations in the concentration of ammonium sulfate 0.3%, 0.4%, and 0.5% obtained biocellulose with thickness, fiber content and yield. As shown in Figure 3.2. It can be seen that the higher the concentration of ammonium sulfate in the range of 0.3% -0.5%, the parameter values of thickness, fiber content and yield also increased. Quantitatively, the highest parameter value was obtained at the concentration of ammonium sulfate 0.5% where the biocellulose was obtained with a thickness of 1.7 cm, a fiber content of 4.36% and 63.50% yield.

When viewed from the trend (trend), the correlation curve of the concentration of ammonium sulfate to the thickness of the biosellulose, shows an exponential curve shown by the equation model $y = 0.5145e^{0.4325x}$ and the confidence level $R^2 = 0.9987$. The correlation curve between ammonium sulfate

concentration and fiber content shows the linear equation model $y = 0.715x + 2.2233$ with a confidence level of $R^2 = 0.9996$. Meanwhile, the relationship between ammonium sulfate concentration and yield shows a logarithmic model with the equation $y = 14.049\ln(x) + 48,679$ with a confidence level of $R^2 = 0.9882$.

3.3. Effect of magnesium sulfate concentration on the quality of biocellulose

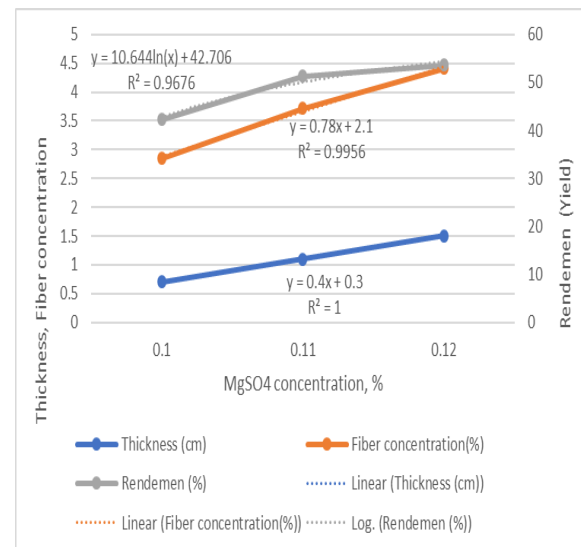


Figure 3.3. Effect of magnesium sulfate concentration on the quality of biocellulose

Figure 3.3 presents the quality of wet fermentation resulting from silent fermentation for 15 days at room temperature. Based on the experiment, through variations in the concentration of magnesium added in the fermentation medium were 0.10%, 0.11%, and 0.12%, the highest wet biocellulose results were obtained in the addition of 0.12% $MgSO_4$ concentration with a thickness of 1.5 cm, fiber content 4.41% and 53.62% yield.

Further detection of the curve of the relationship between the concentration of magnesium sulfate and the thickness of the biosellulose, shows a linear curve shown by the equation model $y = 0.4x + 0.3$ with a confidence level of $R^2 = 1$. The same is shown by the curve of the relationship between the concentration of magnesium sulfate and fiber content which is expressed by linear equation $y = 0.78x + 2.1$ with a confidence level of $R^2 = 0.9956$. While the correlation curve between the concentration of magnesium sulfate and yield tends to be logarithmic as shown by the equation model $y = 10.644\ln(x) + 42.706$ with a confidence level of $R^2 = 0.9676$.

3.4. Test results of three samples of the biocellulosic membrane

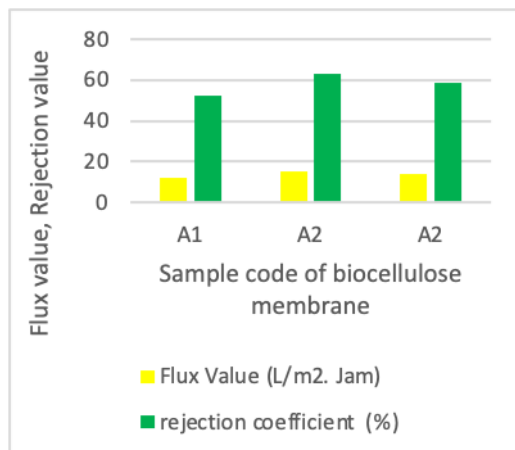


Figure 3.4. Flux value and rejection coefficient of biocellulose membrane samples

The rejection coefficient (R) is one of the parameters in determining the membrane performance where this value indicates the selectivity in escaping particles. If the particles in the feed solution can be completely retained, then R will be worth 100%, and if the feed solution can pass through the membrane freely then R will be worth 0% (Fauzia, 2018). Thus, it means that the higher the R value, the better the performance of the membrane because it can withstand suspensions or fine particles in the liquid.

Another important parameter in assessing membrane performance is the flux value where the flux value shows the permeate speed that can be passed by a membrane per unit area per unit time.

From the initial experiment, crude biocellulose was obtained, the downstream process was carried out including washing, soaking and boiling until the biocellulose was not acidic or neutral. Furthermore, the wet biocellulose is pressed (hand press) to reduce the moisture content. There were 12 samples of biocellulose which were further processed to be used as membrane biocellulose. Based on the experiment, there were three membranes with the best physical quality in terms of the rejection coefficient (R) and flux values, namely samples A1, A2, and A3. In fact, the flux and rejection coefficient values of the three samples were not significantly different. If examined more deeply, the membrane sample A2 has better test results where the flux value is 15.28 (L / m2. Hour) and the rejection coefficient is 63.24%. If the rejection coefficient (R) of a membrane is high, the membrane's performance is categorized as good.

4. CONCLUSION

4.1. Conclusion

Based on the results of this study it can be concluded that:

1. Coconut water can increase its added value into biocellulose by fermentation with the help of *Acetobacter xylinum* bacteria at room temperature and pH range 2-4 by adding sugars, protein and mineral sources.
2. Biocellulose can be processed downstream and then processed downstream to become membrane biocellulose.
3. In this study, the best wet biocellulose was obtained in 1000 ml coconut water media enriched with 12% sugar, 0.5% ammonium sulfate and 0.12% magnesium sulfate where the physical quality of the biocellulose obtained was 1.7 cm thick, yield 63.50% and 4.41% fiber content.
4. Conversion of biocellulose into biocellulose membrane by pressing and drying at room temperature for 14 days.
5. The test results of the biocellulose membrane which were applied in filtering / purifying tap water (PAM) using a pressure of 2 bar, had a rejection coefficient of 63.24% and a flux value of 15.28 lt.m-2.hr-1.

4.2. Suggestion

In order to obtain higher quality biocellulose and membrane biocellulose, both physically and chemically, it is recommended that further research be carried out as follows:

1. Variation in pH, nitrogen source, type of mineral and its composition in the enriched coconut water medium at stationary fermentation at the stage of making biocellulose.
2. Various methods of conversion of biocellulose to membrane biocellulose
3. Addition of tested parameters to determine the quality of biocellulose and membrane biocellulose

REFERENCES

- [1] Bhongsuwan, D. et al, Membrane Test Cell And Performance Test With Laboratory-Made And Commercial Membrane, Journal Science and Technology, Vol 24, <http://www2.psu.ac.th/PresidentOffice/EduService/Journal/24Membrane2002-pdf/26reverse-osmosis.pdf>, 2002
- [2] Fauzia, I, K., W.Maulina, M, Misto. 2018. Kajian Membran Nilon Sebagai Filter Pada Proses Penjernihan Nira Tebu. Spektra: Jurnal Fisika dan Aplikasinya. p-ISSN: 2541-3384, e-

- ISSN: 2541-3392 ,
<http://doi.org/10.21009/SPEKTRA>. Volume 3
Nomor 1, April 2018. DOI:
doi.org/10.21009/SPEKTRA.031.08.
- [3] Greenberg, D. M. 1967. *Metabolic Pathways*. Academic Press, New York and London.
- [4] Haliwat. 1997. *Isolasi dan Identifikasi Mikroorganisme yang Potensial Memproduksi Microbial Cellulose*. Skripsi. Jurusan Teknologi Industri Pertanian, Fateta, IPB.
- [5] Hestrin, S dan Schramm, M. 1954. Factors affecting production of cellulose at the air/liquid interface of a culture of *Acetobacter xylinum*. *Journal Gen. Microbiol.* 11: 123-129.
- [6] Indrarti et al, (1998) "Application of biocellulose as an acoustic membrane", *Indonesian Journal of Biotechnology*, pp 180-184
- [7] Judoamidjojo, R. M., E. Gumbira Sa'id, dan L. Hartoto. 1989. *Biokonversi*. Depdikbud Dirjen Dikti, PAU Biotek, IPB, Bogor.
- [8] Kamide, K., Y. Matsuda, H. Lijima, dan K. Okajima. 1990. Effect of culture conditions of acetic acid bacteria on cellulose biosynthesis. *British Polymer Journal*, 22: 167-171.
- [9] Margaretty, E., Amin J.M., dan Chodijah S. 2001. *Isolasi Dan Seleksi Bakteri Acetobacter Dari Buah-Buahan Untuk Memproduksi Bioselulosa*. Laporan penelitian. Politeknik Negeri Sriwijaya, Palembang.
- [10] Matsuoka, S., T. Ohe, dan N. Sakota. 1996. Production of cellulose from glucose by *Acetobacter xylinum*. *Journal of Fermentation and Bioengineering*, 75(1), 18-22.
- [11] Yoshino, T., T. Asakura, and K. Toda. 1996. Cellulose production by *Acetobacter pasteurianus* on silicone membrane. *Journal of Fermentation and Bioengineering*, 8(1), 32-36.
- [12] Widyaningsih, S dan Purwati. 2013. *Pemanfaatan Membran Nata De Coco Sebagai Media Filtrasi Untuk Rekoveri Minyak Jelantah*. *Jurnal Molekul*. 2013;8(1):20-30. ISSN: 1907-9761 (Print); 2503-0310 (Online). Publisher: Jenderal Soedirman University. Program Studi Kimia, Jurusan MIPA, Fakultas Sains dan Teknik UNSOED Email : sennysetiadi@yahoo.com.
- [13] Strathmann, H. 199 , —*Synthetic Membrans and Their Preparation*], *Handbook of industrial Membran Technology*, Editor: M.C.Porter, Noyes Pub, New Jersey, 1-60.
- [14] Shibazaki, H., S. Kuga, F. Onabe and M. Usuda, 1993, "Bacterial cellulose Membran as Separation Medium", *Journal of Applied Polymer Science*, 50, 965 - 969.