ENZYMATIC SYNTHESIS OF CELLULOSE PROPIONATE AND ITS POTENCY AS RAW MATERIAL FOR MEMBRANE

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ABTSRACT

Modification of bacterial cellulose through enzymatic esterification between cellulose and propionic acid has been carried out to produce cellulose propionate as a raw material of dialysis membrane. The research aimed to characterize of cellulose propionate and assess its potency as raw material for a membrane. The esterification was performed for 8 hours at 50 ºC with cellulose and propionic acid in the mass ratio of 1:5. The physical-chemical properties of resulted cellulose propionate were determined for their functional group using FTIR, crystallinity index by XRD, swelling index by gravimetric method, specific gravity, maximum pore size diameter and membrane thickness. The cellulose propionate showed peak absorbance at the wave number of 1743 cm-1 from C=O absorption and 1108 cm-1 and 1037 cm-1 from C-O absorption with the degree of crystallinity of 61.56% and density of 1.39 g/cm3. Cellulose propionate membrane has a maximum pore size of 2.25 ± 0.04 µm and thickness of 0.029 ±0.001mm, the swelling index of 153%. Diffusion equilibrium of uric acid was 3 hours at average diffusion rate of 1.48ppm/h.

Keywords : *Bacterial cellulose, cellulose propionate, characteristic, diffusion rate*

1. INTRODUCTION

Generally, membrane is defined as selective barrier between two phases, feed and permeate. Separation process occurs because of material transportation between feed phase to permeate phase derived of driven force. The driven force is gradient of temperature, concentration or electric charge.

Membrane can be made from cellulose and its derivates derived from plants or bacteria. Bacterial cellulose made from fermented coconut water by *Gluconaceto bacterxylinus* has similar chemical composition with plant cellulose, different in three dimension structure and degree of polymeration (DP=2000-6000) and its physical chemical properties.

Modification of bacterial cellulose can be done through esterification which reacting bacterial cellulose and carboxylic acid in presence of catalyst. There are three types of catalysts, acid, base and enzyme. Lipase is enzyme used as biocatalyst in esterification reaction, because enzyme has specific reactivity to certain substrates. The lipase is used in the form of immobile to separate the product and enzyme at the end of reaction, moreover the enzyme can be reused. Lipase activity is affected by temperature and pH. Optimum temperature of *Mucormiehei* lipase activity is 50ºC.

Cellulose propionate is ester of bacterial cellulose that produced by reacting bacterial cellulose and propionic acid in the optimum ratio of 1:5 with immobilized lipase and n-buthanol for 18 hours. The cellulose propionate can be applied as raw material membrane, however, it has to be characterized. Uric acid is used as feed analytic for dialysis membrane because uric acid exists in blood or urine. Uric acid in normal serum is 15-60 ppm in female and 25-70 ppm in male.

This research will characterize cellulose propionate and its application for dialysis membrane for glucose and uric acid. Dialysis process used 30 ppm uric in the range of 1-5 hours. The dialysate was determined for uric acid by conductivity and glucose by UV-Vis spectrophotometry.

2. MATERIALS AND METHODS

2.1. Preparation of cellulose propionate

Bacterial cellulose, *Nata de coco* powder of 100-200 mesh in sizes was weighed 1.0g, added with 5.0g of propionic acid 5.0g and

1 g of immobilized lipase. The mixture was added 100mL of n-butanol, then incubated for 18hours at 50ºC. Enzyme was separated from product andthe cellulose propionate was washed with ethanol until propionic acid free and then dried in an oven at 50 ºC. The resulted ester was identified by *Fourier Transformed-Infra Red* (*FT-IR*) spectrophometer, crystalline index by X-Rays diffraction (XRD) and the cellulose propionate membrane was measured its porous diameter by bubble point.

2.2. Preparation of cellulose propionate membrane

Membrane of cellulose propionate was made by sintering method. 5.0 g of cellulose propionate was immersed in 100mL acetone : water (1:1), put on orbital shaker for 72 hours at ambient temperature for swelling process. The mixture was allowed to stand for 1 hour and then filtered. The filtrate was moved into template of 2 cm in diameter. The cellulose propionate was pressed using hydraulic pressure at temperature 80 – 110 °C for 3 minutes.Finally, the resulted membranes were chosen in similar thickness and porous diameter.

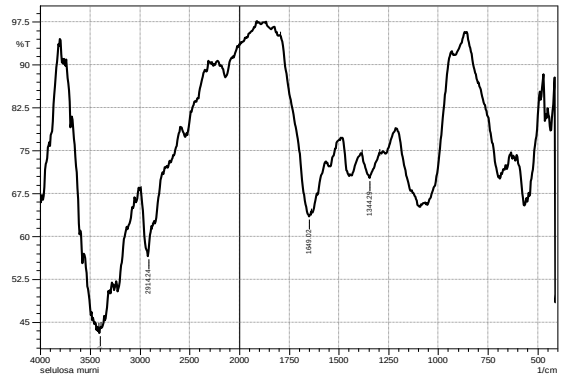
2.3. Dialysis of uric acid

Membranes of cellulose propionate were placed between two solution vessels which combined with silicon rubber. Both vessels were filled with liquid, one vessel was filled with water, while another vessel was filled with 100ppm of glucose solution. Sampling of resulted dialysis were carried out every hours for1, 2, 3, 4, 5, 6 hours. After reaching of equilibrium time, the uric acid in both vessel were measured byconductivity meter. The ability of cellulose propionate to diffuse uric acid was conducted by comparing concentration of uric acid in feed and permeate. The diffuse rate was calculated based Fick's Law.

3. RESULTS AND DISCUSSION

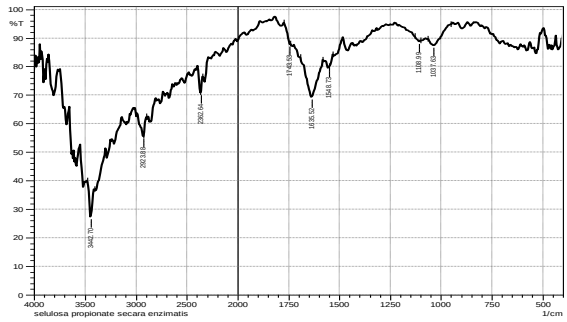
3.1. Characterization of cellulose propionate

FTIR spectra for both bacterial cellulose and cellulose propionate are depicted in Figures 1 and 2. The results of esterification can be distinguished by the presence of absorption peak at wave number of 3400 cm^{-1} for functional group - OH and 1730 cm^{-1} for functional group - C=O ester. Bacterial cellulose showed a strong absorption peak at 3400 cm^{-1} assigned to- OH stretching, while the cellulose propionate showed sharper absorption peak at 3400 cm^{-1}. This explained that functional group-OH has been reacted and decreased. In addition, specific absorption peaks of cellulose propionate were observed at 1743 cm^{-1} for –C=O stretching , 1108 cm^{-1} and 1037 cm^{-1} for –C-O stretching.



Wave number (cm^{-1})

Figure 1. FTIR Spectrum of Bacterial cellulose



Wave number (cm^{-1})

Figure 2. FTIR spectrum of Cellulose propionate

The powder –x ray diffraction pattern for both bacterial cellulose and cellulose propionate are shown in Figure 3. The crystallinity index of cellulose propionate was 61.56% and bacterial cellulose was 57%. This value is obtained based on calculation of mass of crystalline region divided by total mass of whole difractogram region at angle (2Ɵ) from 0-32[7]. The crystallinity index shows the ordered structure of cellulose which influence

by hydrogen bonding[8]. The increasing crystallinity index of cellulose propionate is caused by interaction between carbonyl group and hydroxyl groups to form stronger hydrogen bond, compared to intermolecular hydrogen bond among hydroxyl groups of bacterial cellulose. The increase of hydrogen bonding of the polymer chain can increase its crystallinity.

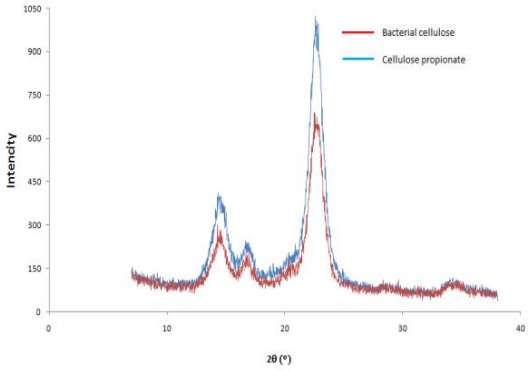


Figure 3. XRD of Bacterial cellulose and cellulose propionate

Density of cellulose propionate and bacterial cellulose were 1,39 g/cm^{3}and1,56 g/cm^{3}respectively.The lower density value showed the ordered structure of cellulose propionate higher than bacterial cellulose. This results showed that the density is not only influence by chain structure density, it is also affected by morphology of chain rearrangement derived of the presence of branch chain interaction of alkyl group and main chain.

Structure density shows solid density influence by solid and porous volume facing with liquid or inter chain. Bacterial cellulose has higher amorphous region than cellulose propionate. In amorphous region, disordered chain is easier formed because of the chain can be bent or folded yielding in some porous. The more porous formation can cause the increase of chain volume and higher density.

Membrane of cellulose propionate is produced through sintering method of swell ester powder in appropriate solvent that is mixture of acetone and water (1:1). The thickness of membrane is measured prior to application as dialysis membrane for uric acid. The membrane thickness can affect the diffusion rate of uric acid during dialysis process. Therefore, the dialysis process requires similar thickness of membrane. The thickness of cellulose propionate membrane is 0,029 ± 0,001 mm. The low standard deviation shows that the resulting membrane is uniform in thickness.

Swelling index indicates the ability of polymer to interact with solvent. Swelling index is measured to determine the membrane hydrophobicity. Based on the curve of immersing time to swelling index, that obtained linear regression equation :

$y=-0.0027x^4+ 0.0666x^3 - 0.5507x^2 + 1.6994x$

that can be calculated the swelling equilibrium time of 4.16 hours and swelling index of 153%. Swelling index of cellulose propionate is lower than the swelling index of bacterial cellulose 315%. That means cellulose propionate has more hydrophobicity than bacterial cellulose because of the ester group difficult to absorb water.

The maximum diameter porous of membrane is measured using bubble point method resulting in average of 2,25 ± 0,04 μm. Based on the size of diameter porous, the cellulose propionate membrane is included in microfiltration membrane. Microfiltration membrane has diameter porous size average of 0.1-10μm.

3.2. Diffusion rate of uric acid through dialysis using cellulose propionate membrane

The cellulose propionate is applied as dialysis membrane using uric acid model. Dialysis processis conducted for 1-5 hours to determine the ability of membrane to permeates uric acid molecules. Diffusion rate of uric acid is obtained from calculation based on equation from curve of dialysis time vs uric acid concentration. The diffusion rate of uric acid was 1.48ppm/h with equilibrium time 3.35 h. The average concentration of uric acid passed through cellulose propionate membrane is 1.48 ppm in one hour, however at the first hour of dialysis process, the uric acid in feed solution remained 12.49ppm, that has to be 28.52ppm. That means some uric acid is retained in membrane, because of the formation of hydrogen bonding between uric acid and membrane resulting in lower diffusion rate.

 P-ISSN:2356-3109 E-ISSN: 2356-3117

4. CONCLUSIONS

The cellulose propionate resulted from enzymatic esterification of bacterial cellulose and propionic acid has specific absorption peak at 1743cm^{-1} assigned to C=O ester stretching., 1108 cm^{-1} and 1037 cm^{-1} assigned for –C-O stretching, crystallinity index of 61.56%, and density of 1.39 g/cm^3. Cellulose propionate membrane has swelling index 153%, porous diameter (2,25 ± 0.04) μm, membrane thickness (0.029 ± 0.001) mm. Diffusion rate of uric acid using cellulose propionate membrane is 1.48ppm/h.

ACKNOWLEDGMENTS:

We are grateful to Ministry of Research, Technology and Higher Education, Republic Indonesia for financial support with contract No : 033/SP2H/LT/DRPM/II /2016. February 17th 2016

5. REFERENCES

A. KRYSTYNOWICZ & S. BIELECIK, 2001, *Biosynthesis of Bacterial Cellulose and itsPotential Application in The Different Industries*, Polish Biotechnology News.

AMALIYAH, S, PRASETYAWAN, S, MARDIANA.D. & ROOSDIANA A., 2016, Enzymatic Sythesis and Characterization of Cellulose Stearate Ester from Bacterial Cellulose and Steraric Acid Using Immobilized Lipase, *JurnalMolekul*, 256-264.

JI, KAIHUA, WANG. W., ZENG, B.,SIBIN CHEN1, ZHAO Q, YUEQING C, GUOQIANG LI, & TING MA, 2016, Bacterial Cellulose Synthesis Mechanism of Facultative AnaerobeEnterobacter sp. FY-07, *Scientific Report*, 1-12.

LAU, SIAW C., HONG G. L., MAHIRAN B., HAMID REZA, ASILAH A.T., NAY M. H., ALAGARSAMY P., CHI H. C., & YOSHITO A., 2014, Enhanced Biocatalytic Esterification with Lipase-Immobilized Chitosan/Graphene Oxide Beads,*Plos One*, 9, 1-10.

MULDER, M.1996, *Basic Principles of Membrane Technology*. Kluwer Academic Publishers, Drodrecht.

MAIUOLO J., FRANCESCA O., SANTO G., CAROLINA M. & VINCENZO M., 2016, Regulation of Uric Acid and Excretion, *International Journal of Cardiology*, 213, 8-14.

MORAWSKA, P.O., YUE W., ARVYDAS R., SLARA O.P., ALEXANDER L.K., RAMKUMAR S., NILS F., MARTIN F. SYBILLE A., ULLIRCH S.,PETER J.S., IFOR D.W.S. & GRAHAM A.T., 2015, Side-Chain Influence on the Mass Density and Refractive Index of Polyfluorenes and Star-Shaped Oligofluorene Truxenes, *Journal of Physical Chemsitry*, 22102-22107.

PURNAWAN, C., WIBOWO A.H & SAMIYATUN, 2012, Kajian Ikatan Hidrogen dan Kristalinitas Kitosan dalam ProsesAbsorbsi Ion Logam Perak (Ag), *Jurnal Molekul*, 121-129.

PABBY, ANIL K., SYED S.H. RIZVI D & S ASTRE A.M, 2008 Hanbook of Membrane Separation: Chemical, Pharmaceutical, Food and Biotechnological Application, CRC Press, Boca Raton.

ROOSDIANA, A., MARDIANA, D., SETIANINGSIH. T. & SURATMO, 2009, Characterization of Immobilized Lipase in Aluminosilicatefor Lactosyl Palmitate Synthesis, *Indonesian Journal of Chemistry*, 9, 201-205.