



Fabrication of Biomembrane from Banana Stem for Lead Removal

Afianti Sulastri* and Lena Rahmidar

Program Studi Keperawatan, Fakultas Pendidikan Olah Raga dan Kesehatan,
Universitas Pendidikan Indonesia
Jl. Dr. Setiabudhi No.229 Bandung 40154, Indonesia

*Corresponding author email: afiantisulastri@upi.edu

ABSTRACT

Heavy metal (i.e. lead (Pb)) is one of the environmental issues recently due to its danger for human health. Therefore, strategy for removing Pb from waste water treatment is important. One of the prospective methods to remove Pb is membrane biofilter. Here, the purpose of this study was to prepare the membrane biofilter for Pb removal process. In this study, membrane biofilter was produced from banana stem. Banana stem was selected because of its abundant availability in Indonesia. And, for somewhat, this banana stem can be environmental problems (become waste) since Indonesia is one of the top producers in the world. In short of the experimental procedure, we conducted three steps of experiments: (1) Preparation of microbial cellulose using *Acetobacter xylinum* using banana stem for a main source; (2) Synthesis of cellulose acetate; and (3) Preparation of biomembrane from obtained cellulose acetate. To produce membrane biofilter, the cellulose acetate was dissolved into dichloromethane to form a dope solution. Then, the doped solution was printed in Petri dish. Some biomembrane properties were characterized for identification, i.e. infrared spectra, electron microscope, and elemental analysis. Experimental results showed that we succeeded to prepare biomembrane with a pore size of 5 μm . The filtration efficiency of our prepared membrane was 93.7% of Pb when using Pb with a concentration of 10 ppm.

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ARTICLE INFO

Article History:

Submitted/ Received 2 Jan 2016

First Revised 18 Feb 2016

Accepted 23 Feb 2016

First Available online 29 Mar 2016

Publication Date 01 Apr 2016

Keyword:

Biomembrane

Banana stem

Lead

Heavy metal filter

1. INTRODUCTION

Environmental problems of developing countries (such as Indonesia) are not only the product of affluence from industries but also product of poverty, socioeconomy, and non-education people. (Walter & Ugelow, 1979). One of the elemental problems that should be considered is lead (Pb). Pb is very dangerous for human health. (Demayo *et al.*, 1982). Therefore, strategy for removing Pb from waste water treatment is important.

Many methods have been suggested as a problem solver for waste water treatment. (Sucahya *et al.*, 2016) One of the prospective methods to remove waste element in the waste water treatment is a membrane biofilter. Usually, cellulose fibers are used for membrane biofilter (Giorno & Drioli, 2000). This is because this material has many advantages, such as abundantly available, low weight, biodegradable, cheaper, renewable, low abrasive nature, interesting specific properties, since these are waste biomass, and exhibit good mechanical properties. However, cellulose fibers also have some disadvantages, such as moisture absorption, quality variations, low thermal stability, and poor compatibility with the hydrophobic polymer matrix (Kalia *et al.*, 2011).

One of the cellulose sources is the domestic waste relating to fruit and vegetable. (Anwar *et al.*, 2016) For example, banana stems can be used as a cellulose source. Banana stem is largely available. Thus, this tends to be a waste problem. Li *et al.* showed that banana stem contained 39.12% of cellulose and 72.71% holocellulose, in which the holocellulose contained 71.76% of glucose (as the predominant monomer), followed by xylose (11.20%), arabinose (7.34%), galactose (2.02%), mannose (0.58%), and galacturonic acid (7.09%). (Li *et al.*, 2010)

In 2010, Indonesia ranked 6th in the world in both production quantity and value of bananas. Banana represents 35% of tropical fruit production by volume in Indonesia. East Java accounts for 15.3% of the total area harvested to bananas and 16% of production. (see <http://unpan1.un.org/intradoc/groups/public/documents/apcity/unpan037535.pdf>) In general, banana stem is an abundant natural resource in subtropical and tropical regions and has potential for providing profitable products. After harvesting banana bunches from the trees, a large amount of waste biomass remains include the stems. It's become organic waste and cause environmental pollution. (Saba *et al.*, 2015) Therefore, usage of the waste banana-stem will be significantly beneficial to the environment and bring additional profits to farmers. In health application, usage of biomembrane in water filtration, typically for drinking water, it should be safer for living body than the synthetic polymer membrane.

Here, the purpose of this study was to prepare the membrane biofilter for Pb removal process. In this study, membrane biofilter was produced from banana stem. Banana stem was selected because of its abundant availability in Indonesia. And, for somewhat, this banana stem can be environmental problems (become waste) since Indonesia is one of the top producers in the world. (Chiaki *et al.*, 2015) In short of the experimental procedure, we conducted three steps of experiments: (1) Preparation of microbial cellulose using *Acetobacter xylinum* using banana stem for a main source; (2) Synthesis of cellulose acetate; and (3) Preparation of biomembrane from obtained cellulose acetate. To produce membrane biofilter, the cellulose acetate was dissolved into dichloromethane to form a dope solution. Then, the doped solution was printed in Petri dish. Some of

biomembrane properties were characterized, i.e: infrared spectra, electron microscope, and elemental analysis. Experimental results showed that we succeeded to prepare biomembrane with a pore size of 5 micrometers. The filtration efficiency of our prepared membrane was 93.7% of Pb when using Pb with a concentration of 10 ppm.

2. MATERIALS AND METHODS

The materials used in this study are banana stem (obtained from Bandung, Indonesia), coconut water (obtained from Bandung, Indonesia), commercial pure sugar, urea (Technical grade, Bratachem Co., Indonesia), ammonium sulphate (Technical grade, Bratachem Co., Indonesia), sodium hydroxide (Technical grade, Bratachem Co., Indonesia), glacial acetic acid (Analytical grade, Merck, Germany), sulfuric acid (Analytical grade, Merck, Germany), ethanol 70% (Technical grade, Bratachem Co., Indonesia), lead nitrate (Analytical grade, Merck, Germany), dichloromethane (Technical grade, Bratachem Co., Indonesia), 2-propanol (Technical grade, Bratachem Co., Indonesia), filter paper (11 micron, Whatmann, US) and distilled water. We also used *Acetobacter xylinum* for growing cellulose, which was purchased in Bandung, Indonesia.

In the experimental procedure, we used six steps of processes. In short, the step can be described in the following:

- (1) Rejuvenation of starter of *Acetobacter xylinum*;
- (2) Synthesis of microbial cellulose from banana stem;
- (3) Purification of cellulose;
- (4) Synthesis of cellulose acetate;
- (5) Preparation and fabrication of membrane;
- (6) Characterization of microbial cellulose, cellulose acetate, and the membrane.

2.1. Starter rejuvenation of *Acetobacter xylinum*

Sterilized bottle was used as a new medium for the rejuvenation of bacteria. The bottle was sterilized by autoclaved for 15 minutes at temperature of 121°C. Then, a total of 3500 mL of coconut filtrate (nata de coco) was poured in small pot and boiled for \pm 20 minutes. Next, we added 262.5 grams of sugar and 17.5 grams of urea. The pH of the mixed solution was adjusted by adding acetic acid until pH range of between 3 and 4. After cooling solution into the room temperature, we put 450 mL of nata de coco (as a new media) into the sterilized bottle. Then, we added 90 mL of *Acetobacter xylinum* starter and incubated at a room temperature (25°C) for four days.

2.2. Synthesis of microbial cellulose from banana stems

Banana stems were washed, cut into small pieces, blended, and then filtered to get a liquid concentrate. About 600 mL of concentrate was then heated up to boil (temperature of 100°C). Next, we added 60 grams of sugar and 15 grams of ammonium sulfate. The mixed solution was then stirred, and the pH was adjusted to pH 4 by adding acetic acid. After cooling to room temperature, we performed some variations of starter *Acetobacter xylinum* amount of 200, 150, and 100 mL, which is added to each 50 mL concentrate on the bottle. The solution was incubated at room temperature (25°C) for nine days.

2.3. Purification of microbial cellulose

Nata (resulted from microbial cellulose with 0.5 cm in thickness) were soaked in 50 mL of sodium hydroxide solution for 24 hours and covered with aluminum foil. Soaking process was continued using 50 mL of acetic acid for 24 hours and covered with aluminum foil. Finally, the microbial cellulose is washed by distilled water \pm 5

times, and then dried in oven with a temperature of 48°C for six hours.

2.4. Synthesis of cellulose acetate

The synthesis of cellulose acetate consisted of three stages, including: swelling, acetylation, and hydrolysis. Each sample, containing of 1 gram of microbial cellulose, was diluted with acetic acid glacial with amount of 8 mL, 16 mL, and 38 mL for 24 hours on 100 mL beaker glass. Then, we continued the process by adding 1 mL of sulfuric acid in each beaker glass during 24 hours on acetylation stage. At hydrolysis stage, we added about 6.3 mL of distilled water and then stirred them for an hour.

2.5. Preparation and fabrication of cellulose acetate membrane

The clump that results from the hydrolysis reaction of cellulose acetate was filtered, washed several times using distilled water and ethanol, and dried at a temperature of 45°C for 4 hours. Then, we continued mixing process by adding 10 mL of dichlorometane to get dope solution.

When forming the membrane, we used polyester as supporting layers on Petri dish. Then, we spread out the polyester and then shaped the cellulose acetate dope over the Petri dish to form film. The film was then let until smooth by leaving the dish for about 2 days (depending on dry mass). The membrane formed in this process should be washed several times by running water to remove all solvents and additives.

2.6. Membrane characterization

To ensure the results, we analyzed the samples using the following characterizations: Fourier Transform InfraRed (FTIR; Shimadzu Corp., Japan), Scanning Electron Microscope Analysis, Atomic Absorption Spectroscopy (AAS).

3. RESULTS AND DISCUSSION

3.1. Starter rejuvenation of *Acetobacter xylinum*

The rejuvenation aims to reactivate bacteria that have been saved long. The rejuvenation of the starter was done by using coconut water, sugar, urea and *Acetobacter xylinum* starter. In this study, coconut water was used for the medium growth because it contains many nutrients that are necessary by bacteria. (Requirement, 1985) The coconut water was cleaned from contaminants through filtration.

The nutritions that needed for bacterial growth were provided by sucrose (as a source of carbon) and urea (as a source of nitrogen). Boiling and stirring were conducted to provide the mixing and to speed up the substance dilution process. This mixing process was also used to kill bacterial cells that are not desired so that the fermentation process. Therefore, the process will free of contaminant. Chilling is needed so that the bacterial starter can grow well because the optimum temperature for the growth of *Acetobacter xylinum* is 25°-27°C. (Gillis *et al.*, 1989) In addition, *Acetobacter xylinum* grow well on pH range 3-4 so we need to set the pH in addition to inhibit the growth of other bacteria. (Gullo & Giudici, 2008). After the fermentation media was ready, then we added bacteria into it for the rejuvenation. Fermentation was done in the sterilized bottle. Rejuvenation process will be successful if only there was formed a white thin layer on the top of the solution. In this study, a white layer is formed on the fourth day, which can be used directly for making the microbial cellulose.

3.2. Synthesis of microbial cellulose from banana stem

Similarly, as the bacteria rejuvenation procedures, the synthesis of microbial cellulose from banana stem used an amount of sugar, ammonium sulfate, and *Acetobacter xylinum* bacteria. In the first stage, banana stems should be cleaned and washed. Then, we cut the banana stem into small pieces in order to facilitate the blender process. After that, we took its liquid concentrate. The concentrate of banana stem will be used as a medium, because it contains cellulose, tannin, lignin, hemicellulose and other nutrients that can be utilized by bacteria. (Das & Singh, 2004) Furthermore, the addition of sugar as an energy and carbon source that was needed by bacteria to construct protoplasm (Russell & Cook, 1995), as well as ammonium sulfate for nitrogen source (Gamborg *et al.*, 1968). Nitrogen is necessary for the formation of proteins that are important in cell growth and the formation of the enzyme (Stark *et al.*, 1998).

Boiling and stirring were done in order to speed up the dilution process of ingredients. In addition, the aim of boiling is also to kill bacteria that are not desired which can interfere in the making process of cellulose. (Mistry, 2001) Chilling process was done in order to maintain *Acetobacter xylinum* starter do not let it die due to high temperatures. Adjusting pH was done on the range 3-4 by adding acetic acid as buffer pH 4. This is intended to provide an environment that supports the growth of *Acetobacter xylinum* bacteria but inhibits the others that are not desired. In addition, acetic acid is also useful as a substrate for *Acetobacter xylinum*. (Effendi *et al.*, 2015) After the media of banana stem concentrate was ready, added *Acetobacter xylinum* starter with some volume variations (i.e. 100 mL, 150 mL, and 200 mL) in order to compare the microbial cellulose that will be formed.

The incubation process was done in the closed room in order to avoid from sunlight so that the growth of microbial cellulose can be effective. The process will produce a white clumps called nata with thickness about 0.10 - 0.50 cm. In this study, the cellulose formed on the ninth day with the thickness of 0.5 cm.

Acetobacter xylinum was experienced some cell growth phases (i.e. adaptation phase, early growth phase, exponential growth phase, slow growth phase, growth phase and the death phase). (Mota *et al.*, 2013) During 0-24 hours, adaptation phase in progress where the growth of nata has not occurred yet and the starter bacteria will do the adaptation to new media. The early growth phase begins with the cell division in low speed and progress in only a few hours. The exponential phase progressed in 1-5 days. In this phase the bacteria produce enzymes called extracellular polymerase as much to arrange glucose polymer become cellulose. This was a phase that determined the rate of *Acetobacter xylinum* strain in produce the nata. When the balance between grow and dead cells have obtained, more nata was produced. This phase occurred on the ninth day of the incubation period.

Figure 1 shows the photograph images of microbial cellulose. Based on Figure 1, it shows that volume variations of the starter can also affect to the formation of cellulose that produced. More starter added to the media, the growth of bacteria will be more optimal. This performance can be seen from the thickness of formed nata. The addition of 200 mL starter bacteria was produced nata whose thickness of 0.5 cm with a smooth and clear white structure instead of other variations in 100 and 150 mL starter. This could be happened because of the quantity bacteria starter affect to the production of cellulose that resulted in a cellulose acetate membrane. The more volume is added, the greater production of

cellulase resulted. Because its pore size distribution is more spread and smooth, the pore size can be denser.

During the ongoing metabolism of *Acetobacter xylinum*, the monomers of

cellulose (resulted from the bacteria secretion) continued to bind each other forming layers that continue to thicken ones. The more secretion of *Acetobacter xylinum*, the thicker of cellulose produced from the fermentation process.



Figure 1. The photograph images of microbial cellulose

In the metabolism process, the cellulose membrane is formed due to activity of *Acetobacter xylinum* toward sugar. Thus, we can conclude in the following. Carbohydrates in the medium is broken down into glucose, which then binds with fatty acids (Guanosine triphosphate) to form precursors of cellulose marker by selulosa sintetase enzyme. Next, we released into the environment forming the braid of celluloses on the medium surface. During the carbohydrates metabolism, glycolysis (which begins with the conversion of glucose to glucose-6-phosphate) occurred and ended with the formation of pyruvic acid). Glucose-6-phosphate, which is formed in this process, is used to produce cellulose by *Acetobacter xylinum*.

3.3. Purification of Microbial Cellulose

Microbial cellulose purification process is done by soaking the nata to NaOH solution. Soaking with NaOH aims to separate cellulose from lignin or other compounds, wherein the non cellulose components will hinder the hydrogen bonding between the cellulose molecular chains. Reaction with NaOH solution causes the lignin molecules degraded as resulted due to termination of aryl-ether bond, carbon-carbon, aryl-aryl and alkyl-alkyl so that the lignin dissolved. The presence of lignin in the compound is characterized by emergence of dark yellow solution. The reactions are shown in **Figure 2**.

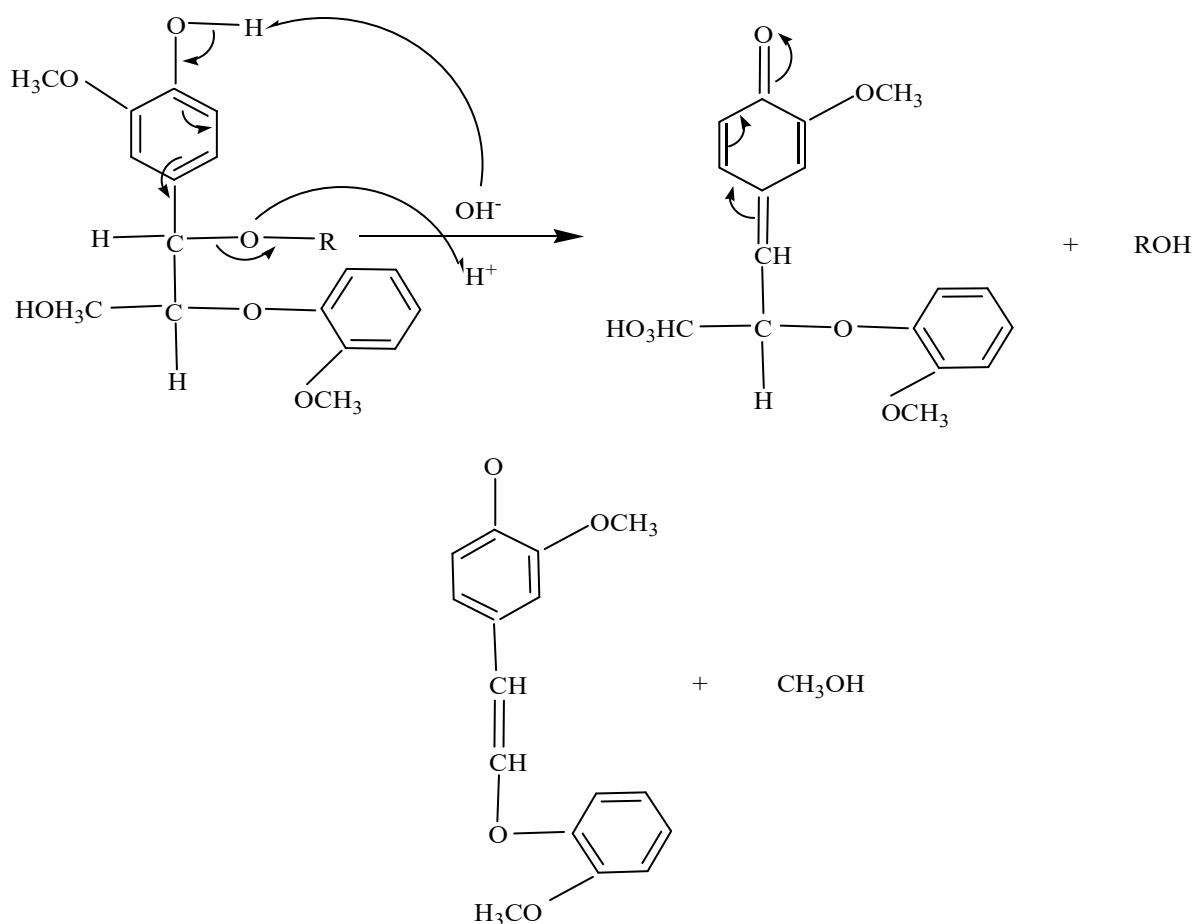


Figure 2. The degradation of lignin. Figure was adopted from reference (Crawford & Crawford, 1980)

Explanation about the degradation is explained by Crawford group. (Crawford & Crawford, 1980) In short, the lignin degradation was initiated by the attack of H atoms bonded to the OH phenolic group by hydroxyl ion (OH) of NaOH. The H atoms in the molecule become acidic because of O atoms have a large electronegativity. More electronegative O atoms will pull the electrons in the H atoms, so that H atoms partially positively charged ($\delta +$) and easily separated into H⁺ ions. Acidity is also influenced by the resonance effect of the alkyl group in the para position, so that the phenolic H atoms in the group will be more acidic. The next reaction is termination of aryl-ether and a carbon-carbon bonds generating fragments which soluble in NaOH. The soaking with NaOH resulted nata

with dark yellow in yellow NaOH solution. Soaking is continued using acetic acid until the yellowish-white nata produced. The purpose of this immersion is to neutralize the content of non-cellulose which still present in the microbial cellulose. The amount of soluble lignin can be indicated from the bright color of the solution.

The differences of microbial cellulose before and after its lignin being removed are shown in **Figure 3**. After being soaked with acetic acid, microbial cellulose soaked with distilled water. Thus, nata and the solution became white and clear. Soaking with distilled water is aimed to eliminate the sour odor and reduce acid content. (Browne, 1919) Then, we dried and proceeded to manufacture cellulose acetate.

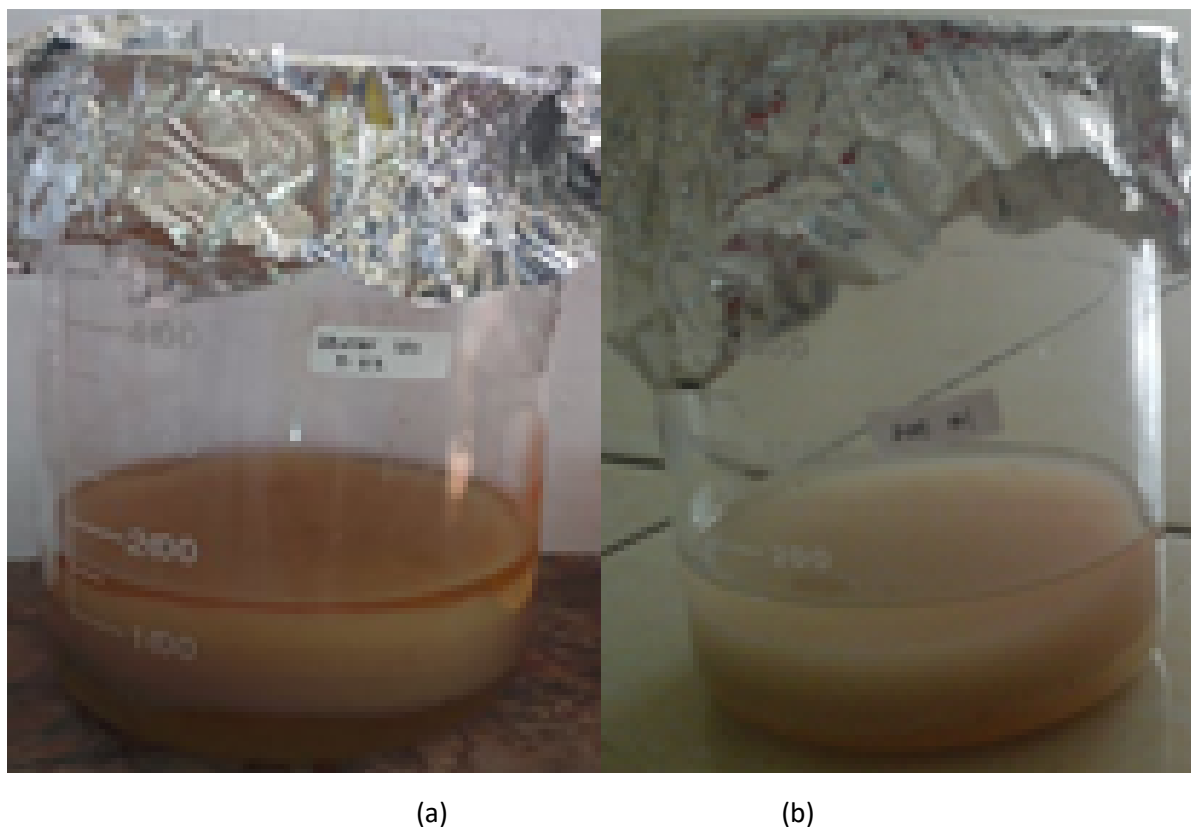


Figure 3. The photograph images of Soaking Nata with (a) NaOH and (b) Acetic

3.4. Synthesis of cellulose acetate

Cellulose acetate manufacture consists of three stages, i.e: swelling, acetylation, and hydrolysis. Swelling phase aims to activate pulp, using concentrated acetic acid. The variations of acetic acid with the most clumps were generated on the variation of 1:32. Stirring is done to increase the surface contact area of cellulose acetate which will enhance the reactivity of cellulose acetate toward acetylation reaction.

The addition of glacial sulfuric acid in the reaction serve as a catalyst. Acetylation

process also aims to minimize the pore size, since the average pore size will be smaller in line with the increasing of product reaction. Acetylation long periode can cause the cellulose and cellulose acetate degraded, resulting in clumps down. Then added distilled water into acetylated cellulose. This phase is named hydrolysis stage that aims to degrade the glacial acetic acid into acetic acid. The final result of this precipitation are yellowish white clumps of cellulose acetate as seen on **Figure 4**.



(a)

(b)

(c)

Figure 4. Results of swelling stage with (a) glacial acetic acid; (b) acetylation by concentrated sulfuric acid; (c) hydrolysis by distilled water



Figure 5. Filtrate of cellulose acetate

3.5. Preparation of membrane

In the early preparation stages of cellulose acetate membranes, filtering the yellowish white clumps aims to separate the clumps from filtrate. Furthermore, washing with distilled water aims to reduce its smell and the acid content. The result can be seen on **Figure 5**.

In the shapping process of membrane, we used a polyester support layer. Cellulose acetate membrane with polyester support layer has a better strength than without it. The addition of 2-propanol coagulant aims to obtain a more dense membrane structure. Meanwhile, washing with running water aims to eliminate all residual solvent and additives.

3.6. Fourier Transform InfraRed (FTIR) analysis

To prove the existence of cellulose acetate and determine what the functional groups contained in the membrane, we analyzed samples using the FTIR (See **Figures 6, 7, and 8**). Detailed explanation of the FTIR results is presented in the following

As shown in **Figure 6** that the results of the IR spectrum detected peaks at wavelengths of 1714.72 cm^{-1} , which indicated the presence of acetyl and ester chain carboxyl groups, as well as indicating the presence of lignin and hemicellulose. In addition, there is a peak at a wavelength of 1639.49 cm^{-1} , which indicated the presence of the C = C group on the aromatic ring of lignin. Hemicellulose can be seen from the presence of the peak at a wavelength of 1425.40 cm^{-1} . The three of groups showed the presence of hemicellulose and lignin. On the other hand, the results of IR spectrum show peaks in the

wavelength of 3348.42 and 2897.08 cm^{-1} indicated the presence of C-H and -OH groups, in which both of them are major group of cellulose.

Figure 7 shows the IR spectrum results of cellulose acetate and microbial cellulose. The results showed that cellulose acetate and microbial cellulose have significant differences. The FTIR of cellulose acetate showed a sharp peak at a wavelength of 1722.43 and 1267.23 cm^{-1} , which indicated carbonyl group C=O and C-O ester groups. Besides, the FTIR result of cellulose acetate also showed carbonyl functional group C = O and CO ester bond. These groups were significant for cellulose acetate, while these peaks in the microbial cellulose did not examine. This proves that the cellulose has turned into cellulose acetate. In addition, the FTIR spectrum of cellulose acetate widened sharply, specifically at a peak in the wavelength of 3433.29 cm^{-1} , which indicated the presence of hydroxyl groups (-OH).

As shown in **Figure 8**, identification result of functional groups in the ftir spectrum for bio membrane were oh stretch vibration in wavenumber at 3350.35 cm^{-1} and reinforced by the strain of C-C bonds in wave number at 1058.92 cm^{-1} . Then, wavenumber at 1232.51 cm^{-1} showed the presence of C-H bending vibration, and the wavenumber at 669.30 cm^{-1} indicated to the presence of C-H bending vibration. In addition, the absorption band at 1658.78 cm^{-1} wavelength indicated to the presence of C = O strain. Thus, the biomembrane has functional groups, including C=O, -COOH, and -OH, proving the presence of cellulose acetate through the existence of wavelength absorption.

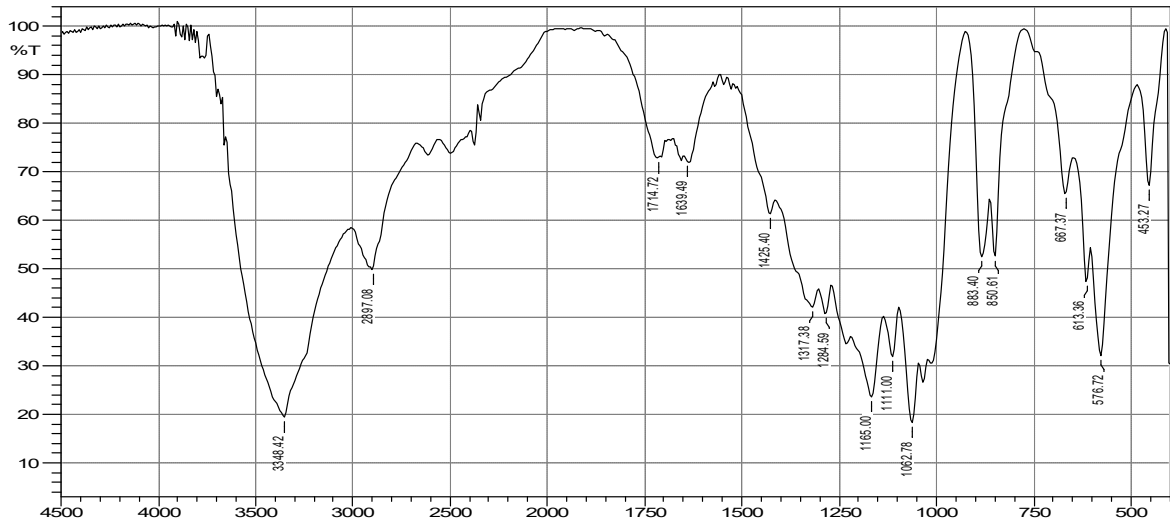


Figure 6. FTIR spectrum of microbial

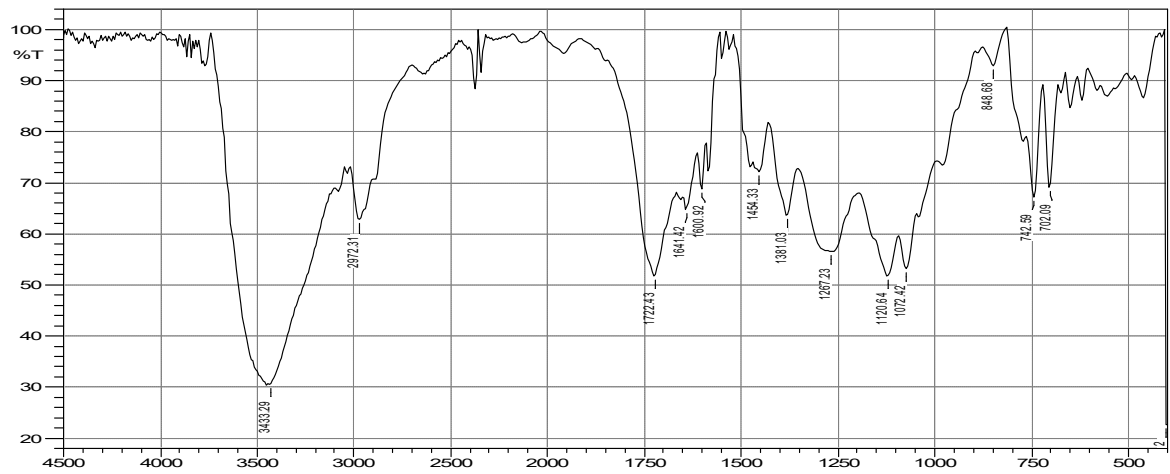


Figure 7. FTIR spectrum of cellulose

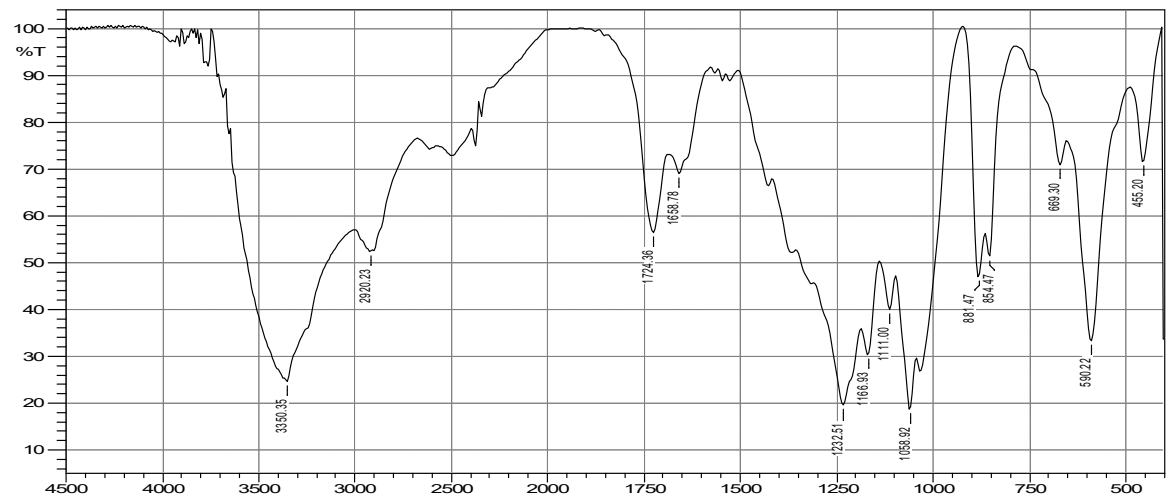


Figure 8. FTIR spectrum of biomembrane Acetate

At 1724.36, 1232.51, and 3350.35 cm^{-1} , there are different peaks and intensities. The three of spectrum showed a difference of absorption band significantly at wavenumber 1724.36 cm^{-1} , indicating a carbonyl group and the C=O absorption. This is due to the solvent influence factor that cause the cellulose bonded each other to form a very strong fiber.

3.7. SEM results

Morphology structure of the membrane can be analyzed as well as determine its pores size by using SEM (Scanning Electron Microscope) Analysis (See **Figure 9**). In this study, there are three membranes were analyzed using SEM, the microbial cellulose, cellulose acetate, and biomembrane. We found that the third cross-sectional surface of the membrane showed different results. Based on the SEM analysis, the diameter of the membrane pore size was 5 micrometers, in which this type of biomembrane can be categorized as a microfiltration membrane. By comparing the three membranes, the pores of biomembrane were denser than that of microbial cellulose and cellulose acetate. This is probably due to the influence of coagulant factors.

3.8. Pb metal separation using biomembrane

Determination of membrane selectivity towards heavy metals was performed by qualifying the membrane (see **Figure 10**). In this study, we used Pb as heavy metals representation. The membrane that has high selectivity is also considered to have a good selectivity. The determination

parameter expressed in coefficient rejection of Pb filtrat absorbtion that measured by AAS analysis.

Figure 10 shows a curve resulted from standardization of metal Pb solution. We found that the curve had the linear regression of 0.9976 with 5, 10, 15 ppm, 20, and 25 ppm Pb standard solution.

Figure 11 shows that the metal Pb in the filter. We found that the Pb was observed as suspended solids with solid white colour. The results of this screening will be analyzed using AAS analysis.

Table 1 shows result of AAS analysis. The sample consists of several feed concentrations, depending on the concentration of Pb solution. As shown in **Table 1**, the optimum concentration for Pb metal filtration is 10 ppm of feed concentration. This is due to other biomembrane feed concentration (i.e. 15 ppm, 20 ppm and 25 ppm) had been saturated. This is because the dissolved substances concentration had suspended on the membrane surface and accumulated later. The main reason can be described in the following. Pb concentration in upper side of membrane layer (between solution and the membrane surface) increased and followed by reduction of water permeability. This caused a clogging on the membrane surface which characterized by the downturn in filtration ability of the membrane toward Pb solution.

Curve of efficiency of Pb Metal Filtration can be seen on **Figure 12**. We found that the best performance of filtration is when performing biomembrane at 10 ppm of Pb metal concentration. The efficiency level in the maximum condition was about 93.70%.

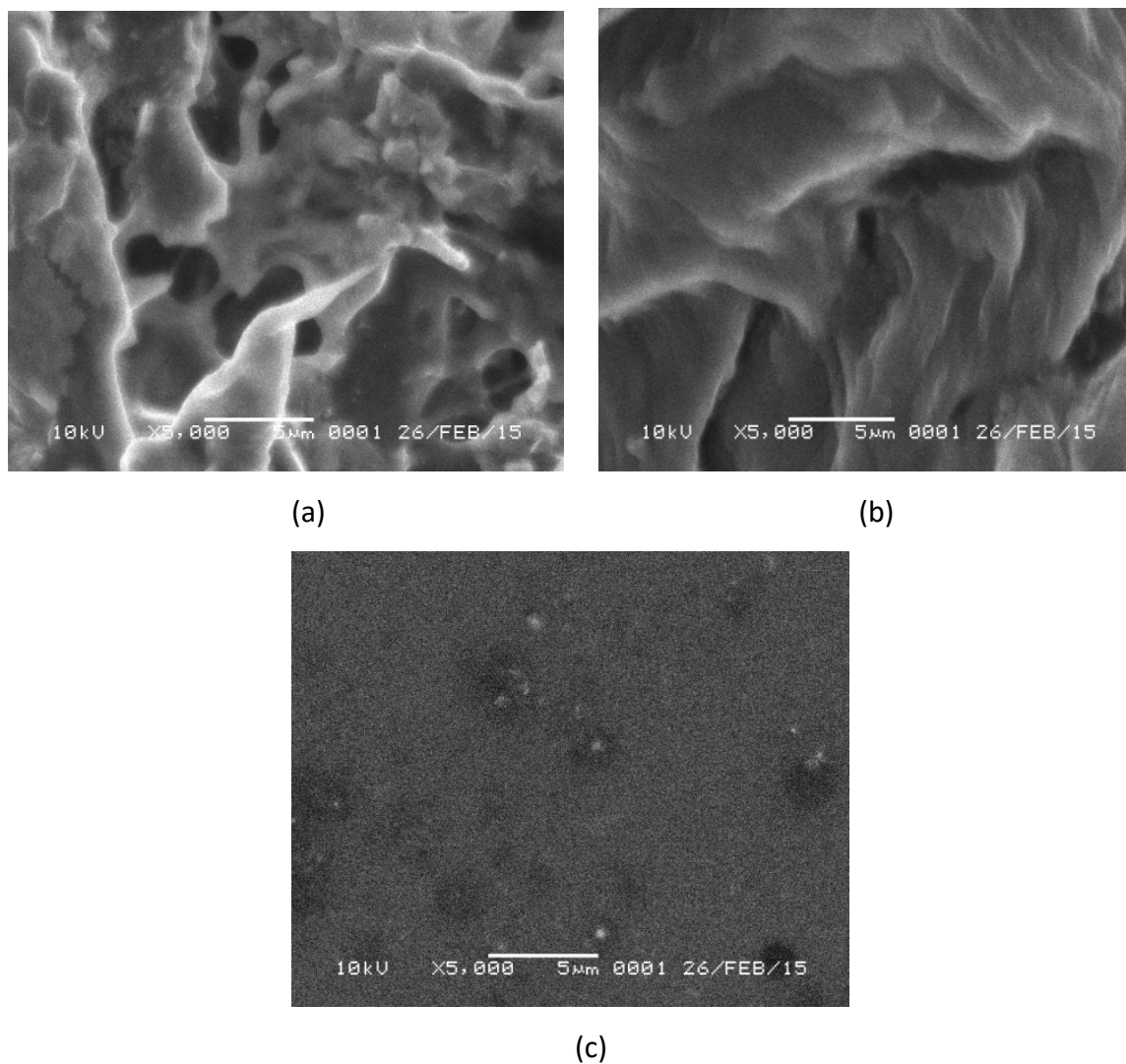


Figure 9. The SEM images of membrane surface with 5000 x magnification. Figures (a), (b), and (c) are the microbial cellulose, cellulose acetate, and biomembrane. acetate cellulose,

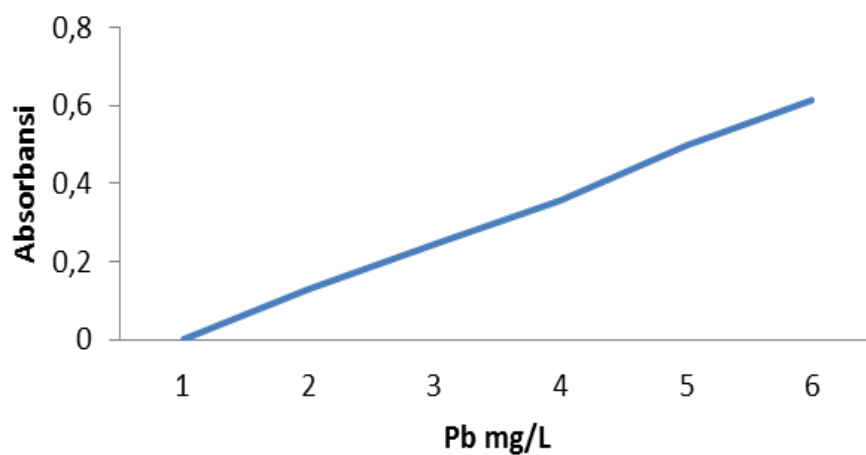


Figure 10. Standardization curve of Pb against absorption

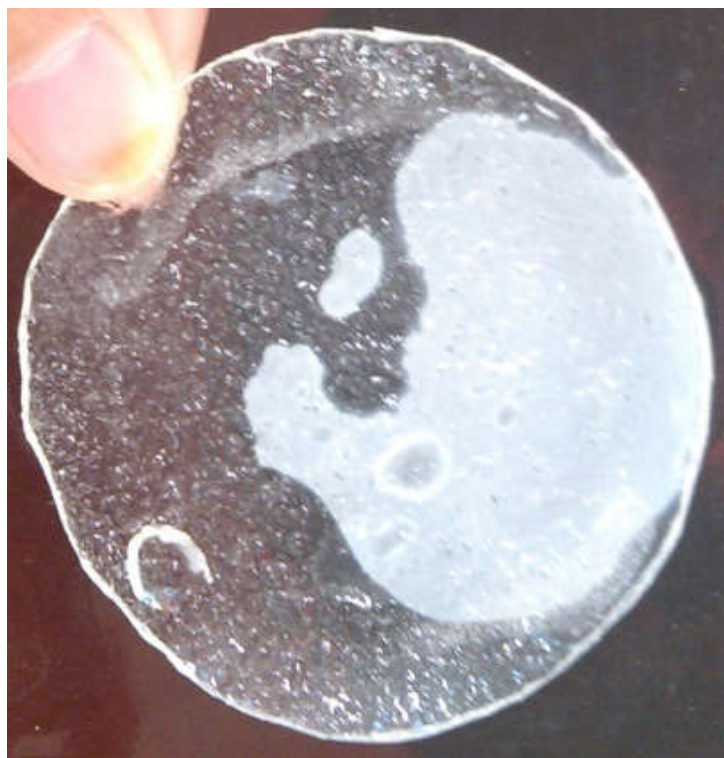


Figure 11. Result of metal Pb filtration using biomembrane

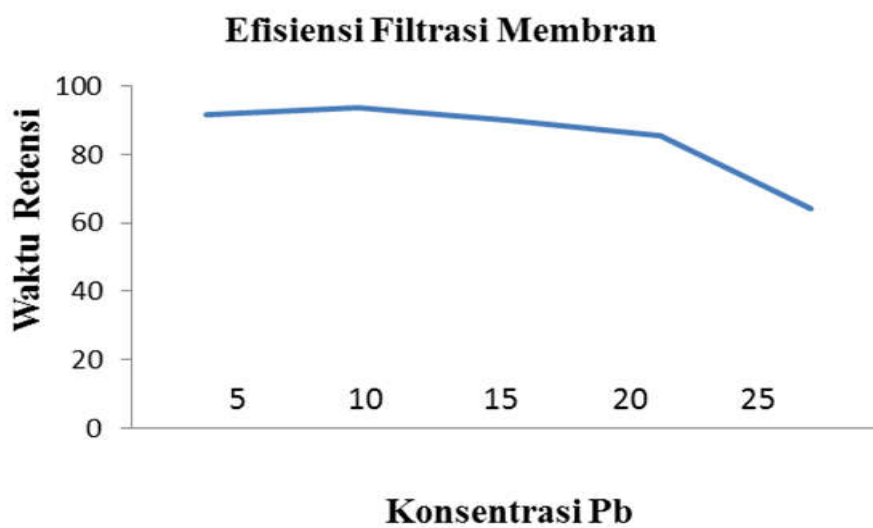


Figure 12. Curve of efficiency of Pb metal filtration biomembrane

Tablet 1. Result of AAS analysis of Pb metal filtrate using biomembrane

Feed concentration (ppm)	Permeate concentration (ppm)	Coefficient of Rejection (%)
5	0.42	91.64
10	0.63	93.70
15	1.52	89.87
20	2.88	85.60
25	8.95	64.20

4. CONCLUSION

From this research, we have successfully convert the waste banana stems with high cellulose content into membrane biofilter. This banana stem was used as a source for making an excellent microfiltration membrane for Pb removal process. The pore sizes of the prepared membrane were 5 micrometers with filtration efficiency of 93.70% for 10 ppm of Pb metal solution. Based on this study,

5. ACKNOWLEDGEMENTS

We thank to Deviyanthi Nur Afifah of Chemistry Department at Universitas Islam Negeri Sunan Gunung Djati Bandung for supporting and assisting this research.

6. AUTHOR'S NOTES

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article. Authors confirmed that the data and the paper are free of plagiarism.

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