IOP Conf. Series: Materials Science and Engineering **434** (2018) 012191 doi:10.1088/1757-899X/434/1/012191

Enhanced antibiofouling properties of chitosan-based membranes by coating and blending of Moringa Oleifera L extracts

M K Pratiwi[1], L Masyrifah[1], L C Hawa[2], S R Dewi[1], N Izza[1], B D Argo[1], S Sucipto[3] and Y Wibisono[1,4,*]

[1] Bioprocess Engineering, Universitas Brawijaya, Jl. Veteran 1, Malang 65145, Indonesia
[2] Agriculture Engineering, Universitas of Brawijaya, Jl. Veteran 1, Malang 65145, Indonesia
[3] Agroindustrial Technology, Universitas of Brawijaya, Jl. Veteran 1, Malang 65145, Indonesia
[4] MILI Institute for Water Research, PO Box 301 ML, Malang 65101, Indonesia

Y_Wibisono@ub.ac.id

Abstract. During application of ultrafiltration membrane for fruit juice clarification, biofouling occurred as the process deals with food substances. Chitosan-based membrane has potential to reduce membrane biofouling, however the performance is still requiring improvement. Natural antibiotics such as Moringa oleifera leaves and nuts extracts might be used to improve the antibiofouling properties of ultrafiltration membrane. We investigated the method to adhere Moringa oleifera extracts into chitosan-based ultrafiltration membranes. Two methods were investigated, i.e. coating and blending. The modified membranes were then immersed in Escherichia coli solution to test the anti-biofouling effect, alongside clean water flux test. The results show that by increasing Moringa oleifera extract concentration from 0%, 2.5% and 5% by using coating method, the mean clean water fluxes are similar in ±5000-6000 L/m2h, while blending method provide less clean water fluxes, i.e. ±6.000 L/m2h, 2.150 L/m2h, 4.462 L/m2h, for blending Moringa oleifera extract concentration from 0%, 2,5% and 5%, respectively. The lower clean water flux for blending method is due to smaller pore sizes of chitosan membrane which was filled by Moringa oleifera extract. With regards to bacteria adhesion, by observing 200µm membrane area under microscope, and analyzing the images, the total area of Escherichia coli attachment onto membrane surface are 38.07%, 4.57%, 0.57% for coating Moringa oleifera extract concentration of 0%, 2,5% and 5%, respectively. This result is better than blending methods that promotes 38.7%, 11.22% and 32.87% total area of Escherichia coli attachment onto membrane surface, by increasing blending concentration of 0%, 2,5% and 5%, respectively.

1. Introduction

Membrane is a separation instrument that has more advantages compared with separation methods [1, 2]. Separation using membranes is a continuous, relatively low energy used, possible to combine with other separation processes, clean and relatively low waste produced [3]. Moreover, properties and

characteristics of the membranes can be adjusted [4] and operational conditions can be controlled [5, 6].

Having these advantages, membranes can be used in a wide range of applications, such as seawater and brackish water desalination, separation and concentration of industrial wastewater, purification and sterilization of drinking water, blood separation for kidney patients, gas separation, and membrane application in biotechnology and food production. In relation with food production, membrane might serve as high quality separator, meet food safety standard and support *halalan toyyiban* production system [7, 8].

Membranes made from chitosan are also relatively safe for food application. However, chitosan-based membrane which has positively charged, could bind negatively charged microorganisms. Microorganisms bound to membrane surface might promote fouling called biofouling [9, 10].

Membranes contains biofouling can cause performance degradation of membrane. However, membrane fouling needs to be prevented, one of which was providing antibiotic materials on membrane. One of natural antibiotic material is *Moringa oleifera* plant [11, 12]. Moringa leaves contain antibacterial compounds such as alkaloids, flavonoids, and phenols that have a working mechanism of damaging bacterial cells [13]. The addition of Moringa leaves extract on chitosan-made membranes is expected to prevent bacterial growth on membranes surfaces. In this study therefore, we investigated the addition of Moringa leaves extract to chitosan-based membrane by comparing two methods, coating and blending method, with different Moringa leaves extract concentration.

2. Experiments

2.1. Materials

Chitosan was derived from tiger shrimp waste from local fisherman. Fresh Moringa leaves were taken from local farmer. Some chemicals e.g. NaOH, acetic acid, ethanol and E. *coli* bought from local suppliers.

2.2. Methods

Chitosan was extracted from 500-gram tiger shrimp shell that previously washed, dried and powdered. The powder then deproteinated by using 5% NaOH, decolorized by using acetone, deacetilized two times by using 50% NaOH. Moringa leaves extraction was prepared by maceration method. Dried leaves crushed and sieved on 60 mesh, then soaked into 96% ethanol (1:5 b/v) for 4 days. Afterwards, the solution was filtered, while the residue was soaked with 96% ethanol for one day at a ratio of (1:2.5 b/v) and then filtered again and obtained filtrate 1 and 2. Both filtrates then mixed. The mixed filtrate was evaporated on a rotary evaporator at 65 rpm, temperature 45^{o}C for 40 min. Viscous extracts obtained from evaporation then diluted to make concentration variation of 0%, 2.5% and 5%. The total phenol content of Moringa leave extract was determined using sulfanilic acid reagent and analyzed by using UV-VIS spectrophotometry. The results then compared with the standard total phenol curve. FTIR characterization were done to check the components of the extracts.

The next step was preparation of chitosan-based membrane with the addition of Moringa extract by comparing two methods *i.e.:* coating and blending. In order to make coated chitosan-based membrane, at first chitosan powder was dissolved on 1% acetic acid with ratio 1:50 (w/v) and stirred until homogen solution obtained. The solution then kept in room temperature for about 2 days to let the air bubbles disappear. After the solution ready, it was the poured-on membrane mold and casted. The membrane then dried in oven with temperature of 40^{o}C for 24 hours then left at room temperature for 24 hours. The dried membrane then immersed in 2% NaOH solution to facilitate ease membrane release from the casting mold [14]. The membrane then immersed in 150 ml of the extract solution (with concentration of 0%, 2.5%, 5%, respectively) for 2.5 hours. The coated membranes, then dried at room temperature. The Moringa impregnated chitosan-based membrane (blending method) was prepared by dissolving the powdered chitosan on 1% acetic acid with ratio 1:50 (w/v). The Moringa leaves extract with different concentration of 0%, 2,5%, and 5% was added into the solution, respectively, then stirred until homogen. The solution then kept in room temperature for about 2 days.

The solution was then poured in membrane casting, dried in the oven at temperature of 40°C for 24 hours and left for 24 hours at room temperature. The membrane released from the casting by immersing in 2% NaOH solution.

3. Results and discussion

3.1. FTIR characterization

Figure 1 shows the IR spectra of functional groups of chitins, chitosan and Moringa leaves extract.

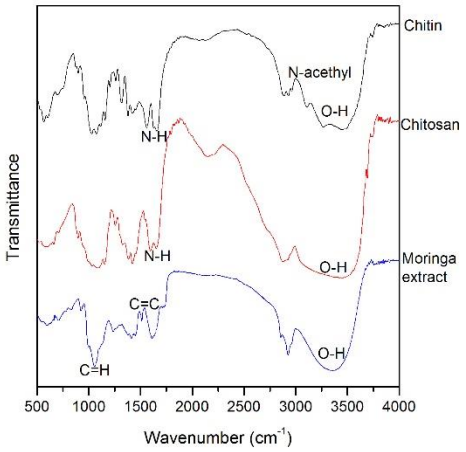

Figure 1. IR spectra of chitin, chitosan, and moringa leaves extract.

It is shown in IR spectra of chitin where an N-acetyl group observed in absorption band of 3267.95 cm^{-1}, but N-acetyl is not detected in chitosan IR spectra. NH_2 group present in absorption band of 1597.71 cm^{-1}. The loss of the N-acetyl group indicated that the deacetylation process has been successful and the results obtained were chitosan [14]. From IR spectra of Moringa leaves extract at wavelength of 3333.53 cm^{-1} was O–H group. There were OH groups of carboxylic acids and phenol compounds such as –OH, C=C aromatics and –CH aromatics at 3116 cm^{-1}, where the OH stretching group will appear at wavelength intensities 3116.11 cm^{-1} to 3577.11 cm^{-1}. At the wavelength of 1237.05 cm^{-1} there was a stretch of C–O from phenol or alcohol [15].

Figure 2 IR spectra of pristine, blended and coated membranes. It showed that peak wavelength intensity increased from 1651.72 cm^{-1} to 1666.5 cm^{-1} which was the wavelength of the C=O group due to the bonding with acetic acid group. There was no significant change in the results of IR spectra, but it can be observed that there was a widening of –OH bands initially at 3333.53 cm-^{1} to 3358.07 cm^{-1} on the chitosan membrane with coating of Moringa leaves extract of 5%. It is due to the addition of –OH from water at chitosan dissolution process, as well as the addition of slightly –OH from phenol. Slightly –OH group from phenols can be due to the concentration of Moringa leaves extract was 5% only. Furthermore, it was also detected C=C aromatics content, –OH bend at wavelength of 1380 cm^{-1}, and aromatic C–H at 908.47 cm^{-1}, also found in IR spectrum results of Moringa leaves extract, it can be concluded that there was an interaction between chitosan membrane with Moringa leaves extract during coating process. Membrane with blending of Moringa leaves extract experienced constriction of hydrogen bonding stretch on chitosan due to phenolic group of extracts which appear at wavelength from 3116.11 cm^{-1} to 3577.11 cm^{-1} [15]. There were increase of wavelength intensity at 1672 cm^{-1} and 1614.42 cm^{-1} which was C=O group area due to mixing with Moringa leaves extract. At wavelength range of 1750 cm^{-1} and 1625 cm^{-1} there was C=O group which was an aliphatic ketone compound. There was no significant changed because low addition of Moringa leaves extract.

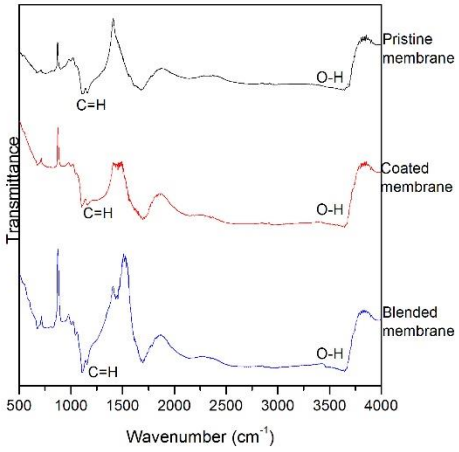

Figure 2. IR spectra of pristine, blended and coated membranes.

3.2. Total phenol content of moringa extract

Total phenol test of Moringa leaves extract using sulfanilic acid reagent with UV-spectrophotometric method performed by Duplo. The total phenol content of Moringa extract was 7.01 ± 0.03%. The results obtained were relatively quite high compared with reported in the literature. Phenol can inhibit bacterial growth.

3.3. Membrane thickness

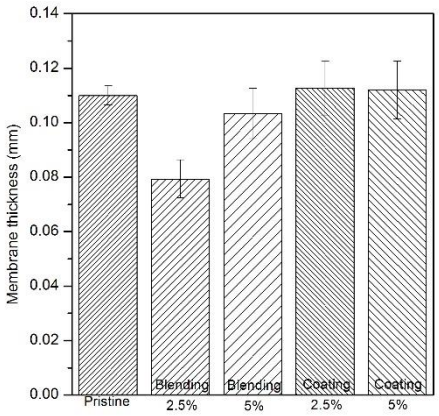

Figure 3. Membrane thickness of pristine, blended and coated membranes.

As shown in **Figure 3**, the thickness of chitosan-based membranes have a non-uniform thicknesses. It is due to differences in volume of solution, total soluble particles in solution, or molding condition. The blended membrane have relatively lower thickness due to interaction change between chitosan polymer and Moringa leaves extract. The average membrane thickness is 0.1 mm, which could be included in microfiltration membrane thickness range (0.01 mm to 0.15 mm).

3.4. Uniaxial compressive stress

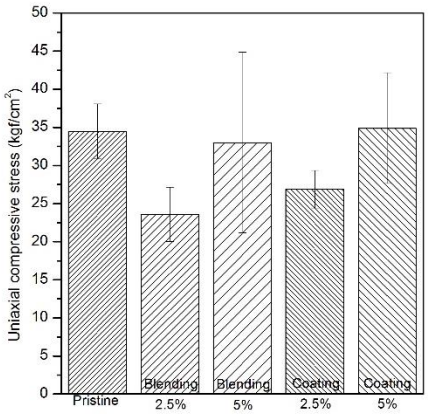

Figure 4. Uniaxial compressive stress of pristine, blended and coated membranes.

The uniaxial compressive stress of the chitosan-based membrane was ranged from 30,34 kg/cm^2 to 32,07 kg/cm^2 (as shown in **Figure 4**). The uniaxial compressive stress were tested under dry membrane condition.

3.5. Clean water flux

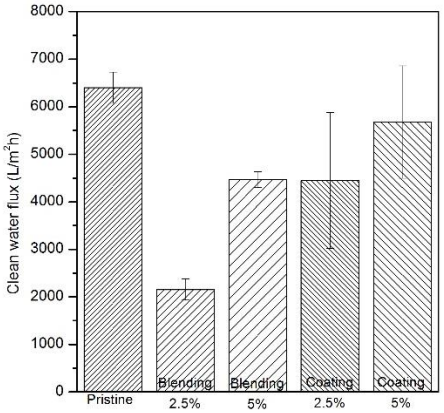

Figure 5. Clean water flux of pristine, blended and coated membranes.

Figure 5 shows clean water flux of pristine, blended and coated membranes. The clean water flux was tested under constant pressure 0.75 bar. As shown, the blended membranes have lower flux, due to Moringa leaves extract narrowed the pores and also might adsorb water.

The chitosan-based membranes flux testing also showed that the membranes is categorized in microfiltration membrane, which is normally produced flux value >50 L/m^2 hours under pressure range of 0.1 bar to 0.2 bar.

3.6. Anti-biofouling properties

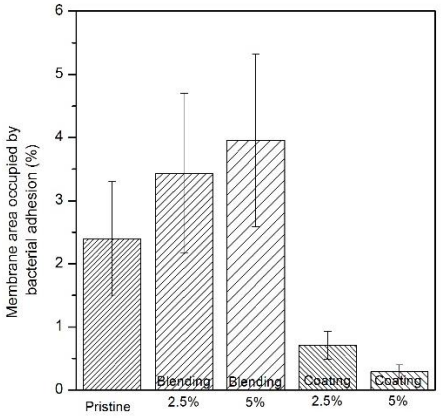

Figure 6. Membrane area occupied by *E coli* adhesion of pristine, blended and coated membranes.

As shown in **Figure 6**, there were barely any effect of blending Moringa leaves extract into the membrane as the *E coli* occupied larger area of membrane surfaces. The interaction of phenol substance with the bacteria cells more effectively occurred in coating method. Moringa leaves contained minerals such as calcium and magnesium, which could make strong and complex bonds when mixed with chitosan, thereby decreasing binding ability of bacterial cell into membranes surface. The higher Moringa leaves extract, the more anti-bacterial effect on the coated membrane (as shown in **Figure 7**).

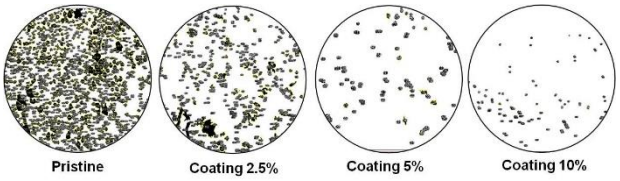

Figure 7. Microscopic images of coated membranes occupied by *E coli* adhesion with different concentration of Moringa leaves extract coating.

Anti-biofouling properties of Moringa leaves extract coating is comparable with anionic hydrogel coating reported [4]. However, the mechanism is not similar. Moringa extract coating tends to prevent bacterial adhesion by phenolic biocidal effect, while anionic hydrogel coating was employed hydrophilic properties of hydration shells which prevent negatively charge *E coli* bacterial attach onto membrane surface.

In practical application however, coating method is merely preventing early attachment of bacterial cells onto membrane surface. Whether the coating could prevent bacterial attachment in longer time is still need further investigation. Furthermore, the stability of coating in different environments condition (such as pH, salinity, solvent etc.) is unknown, this is also need further research.

4. Conclusion

The addition of Moringa leaves extract with coating method was more effective used to reduce the biofouling occurrence on the surface of chitosan-based membranes compared with blending method. It was also found that Moringa leaves extract, might prevent the membrane surface from bacterial adhesion, in which higher extract concentration could provide more antibacterial effect.

References

[1] Wibisono Y 2014 *Dissertation* Universiteit Twente
[2] Wibisono Y, Nugroho W A and Chung T W 2014 *Procedia Chemistry* **9** 210
[3] Devianto L A 2018 paper presented at the I*OP Conference Series: Earth and Environmental Science*
[4] Wibisono Y 2015 *Water Research* **71** 171
[5] Wibisono Y, Ahmad F, Cornelissen E R, Kemperman A J B, Nijmeijer K 2016 *Desalination and Water Treatment* **57** 17625
[6] Wibisono Y, Cornelissen E R, Kemperman A J B, Nijmeijer D C, Van Der Meer W G J 2012 paper presented at the *Procedia Engineering*
[7] Wibisono Y, Sucipto S, Perdani C G, Astuti R and Dahlan M 2018 paper presented at the *Proceedings of the 3rd International Halal Conference (INHAC 2016)*
[8] Nugroho W A, Nugraha R and Wibisono Y 2013 paper presented at the *Proceeding of Sharia Economics Conference-Hannover*
[9] Wibisono Y, El Obied K E, Cornelissen E R, Kemperman A J B and Nijmeijer K 2014 *Journal of Membrane Science* **475** 131
[10] Wibisono Y and Widodo S 2015 *Procedia Environmental Sciences* **28** 224
[11] Viera G H F, Mourao J A, Angelo Ã n M, Costa R A and Vieira R H S d F 2010 *Rev. Inst. Med. trop. S. Paulo* **52** 129
[12] Zaffer M 2014 *Pak. J. Pharm. Sci* **27** 1857
[13] Pal S K, Mukherjee P K, Saha K, Pal M and Saha B P 1995 *Ancient Science of Life* **14** 197
[14] Blair Hal S, Guthrie J, Law T K and Turkington P 1987 *J. Appl. Polym. Sci* **33** 641
[15] Poljansek I and Krajnc M 2005 *Acta Chim. Slov.* **52** 238