A highly antibacterial achievement of hollow fiber polyethersulfone (PES) membrane loaded with silver nanoparticles

[1,*]Fachrul Razi, [2]Isao Sawada, [2]Hideto Matsuyama

[1]Department of Chemical Engineering, Syiah Kuala University, Banda Aceh 23111, Indonesia; [2]Department of Chemical Science and Engineering, Kobe University, 1-1 Rokkodai-cho, Kobe 657-8501, Japan.
*Corresponding Author: razybenseh@gmail.com

Abstract. A highly antibacterial of hollow fiber polyethersulfone (PES) membrane was prepared by loading silver nanoparticles within the PES graft acrylamide (AAm)-membrane. The grafted layers of AAm were provided the matrix for silver nanoparticles (AgNPs) entrapment. The characterization of the prepared hollow fiber (HF) PES membrane loaded with silver nanoparticles were examined by using transmission electron microscopy (TEM). To examine the antibacterial property of the prepared AgNPs-AAm-PES membrane, the halo zone and the shaking flask test were carried out. In these tests, both of unmodified PES membrane and AgNPs-AAm-PES membrane were exposed to pure culture suspension of *Escherichia coli* (*E. Coli*) bacteria with the concentration of 10^7 CFU/ml. The viable bacteria formed within the membrane surfaces and the membrane circumferences were observed by the halo zone formation, while the percentage of bacteria killing ratio was determined by shaking flask test method. The TEM results showed that the silver nanoparticles were formed within grafted layers of AAm-PES membrane and the size of silver nanoparticles were about 10 nm. The AgNPs-AAm-PES membrane were highly effective to prevent the membrane biofouling as shown by the clearly halo zone formation compared with the unmodified PES membrane. The shake flask test were also revealed that almost 99.9 percent of the *E. coli* bacteria were killed when they having exposed to the AgNPs-AAm-PES membrane. This was due to the silver ions are allowed to release from its membrane surface.
Key words: polyethersulfone (PES) membrane, silver nanoparticles (AgNPs), viable bacteria, silver ion, antibacterial.

Introduction

Biofouling phenomena on polymeric membrane have been attracted much more attention, particularly in the membrane application for water reclamation, waste water treatment, water desalination and drinking water production (Hilal et al. 2004, Tang et al. 2010). Generally, biofouling is generated by undesired attachment of microorganism communities such as bacteria to the membrane surfaces and produces a biopolymer matrix or complex structure called biofilm at the membrane surface (Malaysami et al. 2010, Bernstein et al. 2011). Biofilms begin with cell adhesion and progress to thick layers of extracellular polymeric substances (EPS), other organic chemicals, and a complex community of microbial cells which are difficult to remove (Ciston et al. 2009, Razi et al. 2012). Therefore, it is important to prevent the first step of cell adhesion to the membrane surface. Particularly for drinking water production and water treatment, biofilm formations can leads to a harmful bio-production because they can induce secondary pollution of purified water (Kochkodan et al. 2008). Moreover, biofouling caused the decrease in membranes performance which results in higher operation cost due to frequently cleaning and maintenance.

Silver is well known to have antibacterial activity. Silver ions demonstrate a broad spectrum of antibacterial activities at a low concentration and are used to aid wound healing (Feng et al. 2000). It was reported that cellulose acetate hollow fibers prepared by dissolving $AgNO_3$ in the polymer solution before casting had antibacterial activity against *E. coli* and *Staphylococcus aureus* (Chou et al. 2005). Compared with silver ions, silver nanoparticles are long lasting and are suitable for controlled release (Lv et al. 2009). Silver nanoparticles incorporated into polysulfone ultrafiltration membranes exhibited

antimicrobial properties towards a variety of bacteria, including *E. coli* K12 and *Pseudomonas mendocina* KR1 (Zoodrow et al. 2009).

In this work, we introduced hydrophilic polymers onto polyethersulfone (PES) membrane by graft polymerization of acrylamide (AAm) to improve membrane hidrophilicity and to provide the matrix for silver nanoparticles entrapment. Silver nanoparticles were subsequently loaded into the grafted layer to provide antibacterial activity. The aim of this work was to develop a new membrane possessing antibacterial properties. The achievement of antimicrobial of hollow fiber membrane, particularly in term of release killing property will be studied further.

Materials and Methods

Materials

Porous polyethersulfone (PES) hollow fiber membranes (molecular weight cutoff of 150 kDa) were purchased from Daicen membrane-systems, Ltd., Japan. Benzophenone, acrylamide, silver nitrate, sodium tetrahydroborate and Difco nutrient broth (NB) were purchased from Wako Pure Chemical Industries, Ltd., Japan. All the chemicals were used without further purification.

Photografting polymerization and Ag nanoparticle formation

For the graft polymerization, a PES membrane (150 kDa) was immersed in 0.4 wt% benzophenone (photoinitiator) dissolved in methanol. After drying, the membrane was immersed in the monomer solution (acrylamide). The acrylamide concentrations were varied from 0.07 to 0.21 mol/dm^3 to control the grafting amount. The hollow fiber PES membranes were UV irradiated with UV curing unit (HB100A-1, SEN LIGHT Corp., Japan) equipped by a high pressure Hg-UV Lamp (UM-102, Ushio Inc., Japan) with intensity of 170 mW/cm^2 and at wavelength of 350 nm.

After the reaction was completed, the grafted PES membrane was washed with deionized (DI) water and stored for 2 nights. The grafting amount (GA) was calculated as the dried weight of grafted polymer per outer surface area. The graft polymerized membrane was then immersed in a silver nitrate solution, with the silver nitrate concentrations varied from 1.0×10^{-3} to 1.0×10^{-1} mol/dm^3. Finally, silver nanoparticles were formed in the graft layer by reducing the silver ions with 1.0×10^{-2} mol/dm^3 sodium tetrahydroborate aqueous solution for 20 min.

Membrane characterization

To observe the chemical structure changes of modified and unmodified PES membranes, The X-ray photoelectron spectroscopy (XPS) measurement was carried out to analyze the change of the chemical composition of the membrane surfaces (XPS, ESCA-3400, Shimadzu Co., Ltd., Japan). The X-ray gun was operated at 10 kV and 20 mA.

The morphologies of PES hollow fiber membranes and the formation of silver nanoparticles were observed with a transmission electron microscope (TEM, Tecnai G2, FEI Co., Japan) with an accelerating voltage of 120 kV. Samples were embedded in epoxy resin and sliced to 50 nm sections by an ultramicrotome (Reichert Ultracut S, Leica Co., Germany).

Halo zone and shake flask tests

The antibacterial activity of the polymer membrane containing silver nanoparticles was tested using *Escherichia coli* NovaBlue as model Gram-negative bacteria. *E. coli* was purchased from Merck KGaA, Germany. The test bacteria were cultured at 37 °C by shaking in a nutrient broth (NB) overnight. The bacterial suspension was diluted in NB or phosphate buffered saline (PBS) to 1-5$\times10^7$ cells/ml of test bacterial suspension. The halo zone test was performed as follows. The diluted bacteria solution was placed on NB agar culture medium and the original PES membrane and the silver-loaded membrane then placed on the NB agar. After 24 hr of cultivation, the width of inhibitory halo around the membranes was observed. For the shake flask test, membranes loaded with 0.2-2.2 mg silver

nanoparticles were immersed in a 40 ml PBS solution containing 400 µl of 1-5×10^7 cells/ml bacterial suspension. After the samples were incubated for 8 hr at 37 °C, the number of viable bacteria was counted.

Results and Discussion

Characteristics of grafted PES membrane

The grafted PES membrane with silver nanoparticles (abbreviated "AgNPs-AAm-PES") was characterized using XPS. As shown in Figure 1(a), the AgNPs-AAm-PES membrane exhibited intense peaks at around 374.5 and 368.5 eV, attributed to Ag $3d_{3/2}$ and $3d_{5/2}$ cores, respectively. This indicates that the AgNPs-AAm-PES contained silver nanoparticles. The nitrogen peak was also detected, as shown in Figure 1(b). This peak is attributed to the polyacrylamide grafted on the PES membrane and provided further evidence of acrylamide grafting on the membrane surface.

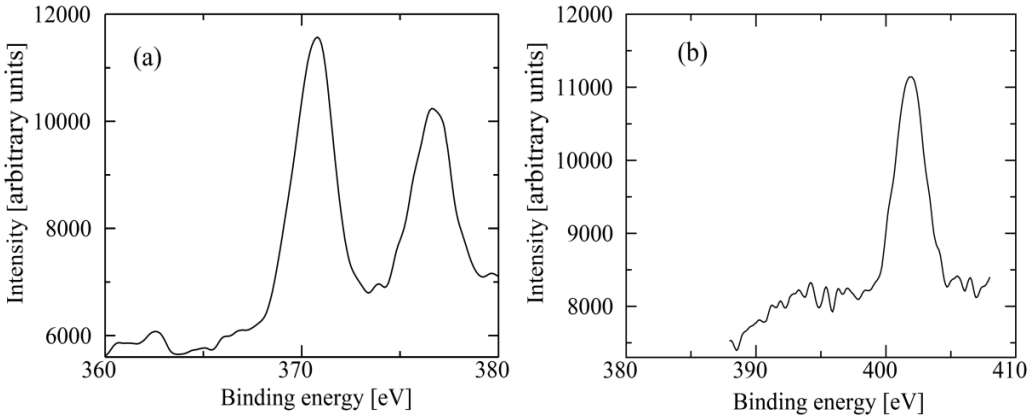

Figure 1. XPS results for the AgNPs-AAm-PES membrane (GA: 3.44 mg/cm^2, $AgNO_3$ concentration during nanoparticle formation: 1.0×10^{-1} mol/dm^3). (a) Ag peak and (b) N peak.

Figure 2. shows TEM images of the AgNPs-AAm-PES membrane (GA: 3.44 mg/cm^2) at three different magnifications. The region covered by a red circle in Figure 2(a) is enlarged in Figure 2(b) and the region covered by the circle in Figure 2(b) is enlarged in Figure 2(c). According to Figure 2(a), the grafted layer thickness was about 10 µm for the membrane with 3.44 mg/ cm^2 grafted acrylamide while the size of silver nanoparticles of about 10 nm in diameter were clearly observed in the grafted layer region within 100 nm from the outer surface as shown in Figure 2(b) and 2(c) as well.

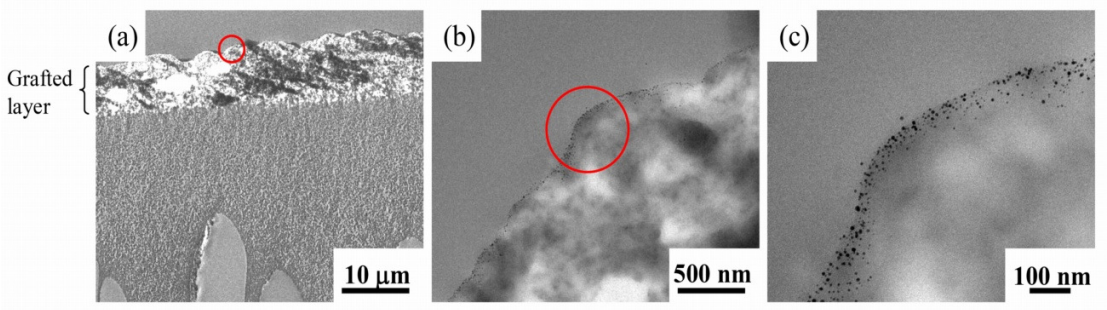

Figure 2. TEM images of an AgNPs-AAm-PES membrane (GA: 3.44 mg/cm^2, $AgNO_3$ concentration during nanoparticle formation: 1.0×10^{-1} mol/dm^3).

Evaluation of antibacterial activity

Figure 3 shows the results of the halo zone test. The original PES membrane had no antibacterial activity, as evidenced by bacteria growing near the PES membrane surface. In contrast, clear halo zones were observed around the AgNPs-AAm-PES membranes. For the silver-loaded membranes, the membrane prepared with a higher silver nitrate solution concentration showed a clearer and wider halo zone. These results show the high antibacterial activity of the membranes with silver nanoparticle.

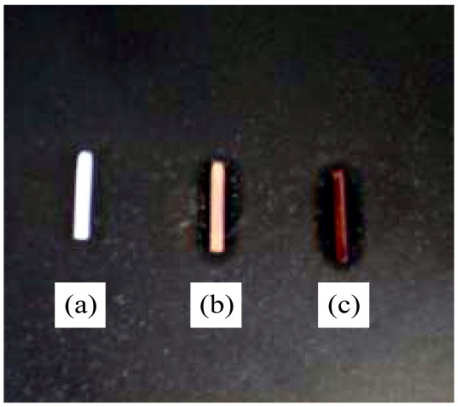

Figure 3. Measurement of antibacterial property by the halo zone test. (a) PES membrane (150 kDa), (b) AgNPs-AAm-PES membrane (GA: 3.44 mg/cm^2, $AgNO_3$ concentration during nanoparticle formation: 1.0×10^{-2} mol/dm^3) and (c) AgNPs-AAm-PES membrane (GA: 3.44 mg/cm^2, $AgNO_3$ concentration during nanoparticle formation: 1.0×10^{-1} mol/dm^3).

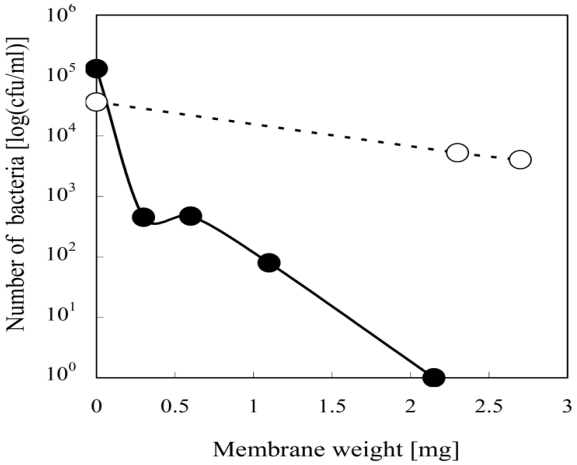

Figure 4. Measurement of antibacterial property by the shake flask test. ○: PES membrane (150 kDa), ●: AgNPs-AAm-PES membrane (150 kDa) (GA: 3.44 mg/cm^2, $AgNO_3$ concentration during nanoparticle formation: 1.0×10^{-1} mol/dm^3).

The antibacterial activity of the AgNPs-AAm-PES membrane against *E. coli* was also examined by the shake flask method. The numbers of bacteria after incubation with membranes are plotted against the membrane weight in the PBS solution in Figure 4. The presence of the original PES membrane resulted in no clear decrease in bacteria numbers. On the other hand, the AgNPs-AAm-PES membrane was quite effective in reducing the

viable bacteria numbers. For example, when the silver-loading membrane of 2.2 mg was added to the solution, the bacteria count was almost zero, meaning the antibacterial efficiency was about 99.99%.

Conclusions

A higly antimicrobial hollow fiber PES membrane was succesfully prepared by loaded silver nanoparticles (AgNPs) within grafted layer of Am-PES membrane. The size of AgNPs formed within the grafted layer was about 10 nm. This AgNPs was effective to prevent the microbial attachment on the modified PES membrane, as it was confirmed by both the halo zone test and a shake flast test methods. The possed of antimicrobial property of PES membrane loaded with AgNPs was due to reléasing of silver ions of its PES membrane surface. Silver ions are thought to disrupth the bacteria cell-membrane and killed the bacteria upon contact. Shaking flask test revealed that almost of 99,99% bacteria were reduced. These results sugessting that AgNPs-AAm-PES membrane exhibited high antibacterial properties.

References

Hilal N., Kochkodan V., Al-Khatib L., Levadna T. 2004. Surface modified polymeric membranes to reduce (bio) fouling: a microbiological study using *E. coli*. Desalination, 167 : 293-300.

Tang X., Flint S. H., Bennett R. J., Brooks J. D. 2010. The efficacy of different cleaners and sanitizers in cleaning biofilms on UF membranes used in the dairy industry, J. Membr. Sci, 352: 71-75.

Malaisamy R., Berry D., Holder D., Raskin D., Lepak L., Jones. K.L. 2010. Development of reactive thin film polymer brush membranes to prevent biofouling. J. Membr. Sci, 350: 361-370.

Bernstein R., Belfer S., and Freger V. 2011. Bacterial attachment to RO membranes surfaced-modified by concentration-polarization-enhanced graft polymerization. Environ. Sci. Technol, 45: 5793-5980.

Ciston S., Lueptow R. M., Gray K. A. 2009. Controlling biofilm growth using reactive ceramic ultrafiltration membranes. J. Membr. Sci, 342: 263–268.

Razi F., Sawada. I, Ohmukai. Y, Maruyama. T, Matsuyama. H. 2012. The improvement of antibiofouling efficiency of polyethersulfone membrane by functionalization with zwitterionic monomers. J. Membr. Sci, 401-402: 292-299.

Kochkodan V., Tsarenko S., Potapchenko N., Kosinova V., Goncharuk V. 2008. Adhesion of microorganisms to polymer membranes a photobactericidal effect of surface treatment with TiO_2. Desalination, 220: 380–385.

Feng Q. L., Wu J., Chen G. Q., Cui F. Z., Kim T. N., Kim J. O. 2000. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*, J. Biomed. Mater. Res, 52: 662–668.

Chou W. L., Yu D. G., Yang M. C. 2005. The preparation and characterization of silver-loading cellulose acetate hollow fiber membrane for water treatment. Polym. Adv. Technol, 16: 600–607.

Lv Y., Liu H., Wang Z., Liu S., Hao L., Sang Y., Liu D., Wang J., Boughton R. I. 2009. Silver nanoparticle-decorated porous ceramic composite for water treatment. J. Membr. Sci, 331: 50–56.

Zodrow K., Brunet L., Mahendra S., Li D., Zhang A., Li Q., Alvarez P. J. J. 2009. Polysulfone ultrafiltration membranes impregnated with silver nanoparticles show improved biofouling resistance and virus removal. Water Res, 43: 715–723.