Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.981928

56 (2015) 3196–3202
December

Taylor & Francis
Taylor & Francis Group

Antibacterial properties of membranes modified by acrylic acid with silver nanoparticles

Marta Bojarska[a,b,*], Wojciech Piątkiewicz[b]

[a]*Chemical and Process Engineering, Warsaw University of Technology, Waryńskiego 1, 00-045 Warsaw, Poland, Tel. +48 507 099 335; email: m.bojarska@ichip.pw.edu.pl*
[b]*PolymemTech Sp. z o.o. al., Niepodległości 118, 90 02–577 Warsaw, Poland, Tel. +48 508 236 694; email: w.piatkiewicz@ichip.pw.edu.pl (W. Piątkiewicz)*

Received 28 July 2014; Accepted 7 October 2014

ABSTRACT

Organic fouling is a very important problem in case of membrane filtration processes. Partially, impact of fouling might be overcome by: back puls, membrane cleaning, cross-flow filtration, or surface modification. In this paper, first, surface modification of membrane by acrylic acid and then silver nanoparticles will be presented. In the first step of modification, acrylic acid is grafted to the surface of the polypropylene capillary membrane, thanks to Fenton-type reaction. Then, membranes are further modified by the synthesis of silver nanoparticles directly on the membrane surface. It is well known that silver nanoparticles exhibit antibacterial properties for micro-organisms such as *Escherichia coli* and *Bacillus subtilis*. Also the presence of acrylic acid on the surface of the membrane should reduce adhesion of particles to the membrane surface. In the present work, results obtained for acrylic acid grafting will be presented including FT-IR analysis and the amount of grafted carboxylic groups. Results for membranes further modified by silver nanoparticles, as well as tests for antibacterial properties of such membranes, will be presented. Antibacterial properties of modified membranes were tested on two model gram positive and gram negative bacteria: *E. coli* and *B. subtilis*. Tests were performed in liquid and solid Lysogeny Broth. The presented membranes exhibit very good antibacterial/bacteriostatic properties.

Keywords: Membrane modification; Fenton-type reaction; Acrylic acid; Silver; Antibacterial

1. Introduction

Antibacterial properties of different forms of silver have been known for centuries. Also silver nanoparticles have many interesting properties such as electrical, optical, catalytic oxidation, and antimicrobial [1–3]. It is proved that silver nanoparticles in aqueous solutions release silver ions, which are biologically active [3,4]. Silver ions can destroy bacterial cell by interacting with proteins (for example, thiol groups form cysteine, forming Ag–S bond), which can result in inactivation of respiratory enzymes. They can also prevent the DNA replication and may have an impact on the membrane cell structure and permeability [2–10]. It is believed

*Corresponding author.

Presented at the MELPRO 2014 Conference Membrane and Electromembrane Processes, 18–21 May 2014, Prague, Czech Republic

that silver nanoparticles can increase permeability of the cell membrane due to their incorporation into the membrane structure, which ultimately leads to the release of cell contents and causes cell death [3,8,9]. In conclusion, it is suggested that the antibacterial effect of silver nanoparticles can be described by three main mechanisms:

(1) release of biocidal silver ions,
(2) direct impact on the cell membrane by silver nanoparticles,
(3) silver nanoparticles can generate reactive oxygen species [3].

It is postulated that immobilized silver nanoparticles may result in an easy-to-use disinfecting systems, with minimized danger of releasing silver nanoparticles to the environment [9]. Silver nanoparticles exhibit antibacterial properties against broad spectrum of bacteria, such as *Escherichia coli* [2,4,11], *Bacillus subtilis* [4], *Staphylococcus aureus* [4,11], *Streptococcus sp* [8], *Psuedomonas sp* [8], *Pseudomonas aeruginosa* [4], *Listeria monocytogenes* [11], *Salmonella typhi* [4,11], and *Streptococcus mutans* [12].

Colloidal silver has been approved by the US Environmental Protection Agency as a disinfectant agent in hospitals and medical centers [9]. Currently, there is no clear evidence that silver nanoparticles are toxic for humans. The only known negative impact of high concentrations of silver ions in case of long-term exposure on humans is skin darkening [3,6]. Also long-term exposure to high concentrations of silver ions can cause a disease called *Argyria* (in case of some genetic determinants), which manifests the blue–gray staining of skin [2].

Precipitation of silver nanoparticles by reducing agent is one of the most frequently used method for producing silver nanoparticles [4]. In case of this method, silver ions derived from silver nitrate can be reduced by sodium borohydride. Also, if modified surface have carboxyl groups, it is possible to form bond between silver ions and carboxyl groups. Depending on the concentration of used precipitant, different sizes of nanoparticles may be produced [13]. In case of this modification, nanoparticles will be mostly placed on membrane surface. Another method of producing polymer membranes with nanoparticles is by adding the prepared nanoparticles to the spinning solution. Then, mix matrix membranes are prepared by phase inversion. In case of this modification, silver nanoparticles are trapped in the membrane structure. During continuous filtration performed on mix matrix membranes, problem of biofouling was not reduced significantly. Most likely this was due to continuous leaching of silver ions from the membrane structure. In this case, most of the silver nanoparticles were trapped in the membrane structure and did not have a direct contact with bacteria. Recent studies postulate that silver nanoparticles should be placed on membrane/contamination boundary. This arrangement of nanoparticles will allow direct contact of biocide with bacteria, which can result in better performance of the process [9,14].

2. Materials and methods

Polypropylene (PP) capillary membranes (PP ACUREL®V8/2HF) produced by MEMBRANA GmbH were used as a substrate during modifications. Basic properties of PP membrane are presented in Table 1.

Acrylic acid grafting to the membrane surface was performed by a Fenton-type reaction. This modification was performed to introduce carboxyl groups to the membrane surface. Grafting of acrylic acid by a Fenton-type reaction is a two-step process. In the first step, a PP membrane is placed in a solution of ethylene glycol dimethacrylate (EGDMA) and cumene hydroperoxide (CHP) in hexane. In the second step, membranes were placed for 15 min in a solution containing iron chloride(II), ascorbic acid, and an appropriate amounts of acrylic acid (0.1; 0.5; 1; 5; 7; 10; 13; 20%). EGDMA is used as a crosslinking agent which aims to improve the bond between PP and acrylic acid. Iron ions in the second step is oxidized during the Fenton reaction, and then regenerated by ascorbic acid (Fig. 1) [16].

Synthesis of silver nanoparticles on the membrane surface with carboxyl groups is also a few-step process. As a first step, premodified membranes were placed in sodium hydroxide (to convert –COOH groups into $–COO^-$). Then, membranes were placed in a 0.01 M solution of silver nitrate for 15 min. In this step, silver ions were most probably connected with $–COO^-$. Reduction of silver ions to metallic silver

Table 1
Basic properties of PP membranes (MEMBRANA GmbH) [15]

Properties	MEMBRANA GmbH
Average pore size, μm	0.2
Burst pressure, bar	>8
Implosion pressure, bar	>4
Outer diameter, mm	2.6
Inner diameter, mm	1.8
UFC, ml/barcm^2 min	2.0
Advancing contact angle,°	134.9

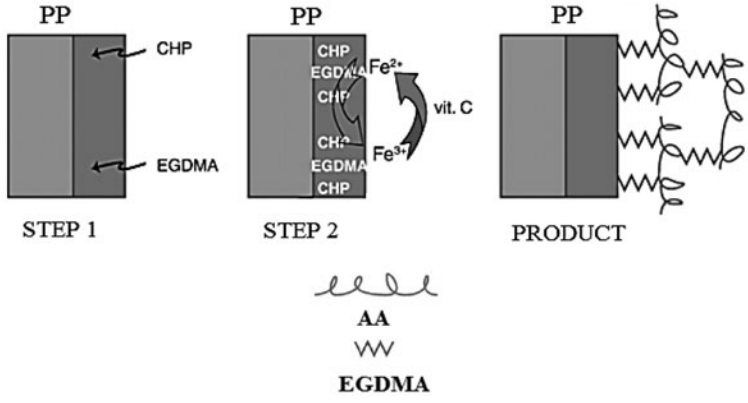

Fig. 1. Scheme of acrylic acid grafting by a Fenton-type reaction [16].

occurred thanks to $NaBH_4$. Concentration of sodium borohydride was few times greater than the concentration of silver salt. Modification method was developed on the basis of Mulfinger et al. [17]. Scheme of silver nanoparticles synthesis on premodified membrane surface is presented in Fig. 2.

FT-IR analysis was performed on a NICOLET 6700 (wave number range from 400 to 4,000 cm^{-1}). Thanks to this analysis, it was possible to confirm the presence of groups characteristic for acrylic acid on the membrane surface.

Plate test for antibacterial and bacteriostatic properties of modified membranes was performed. Membranes were placed vertically in LB (Lysogeny Broth) medium with 1% of agar, and culture of *E. coli* or *B. subtilis*. Then, plates were incubated in 37˚C for 24 h. Zone of growth inhibition present near the modified material proclaims its antibacterial properties.

Also test in liquid medium was performed. To assess the antibacterial properties of the modified membranes, measurement of optical density (OD_{550}) for LB after 24 h of incubation with bacteria (*E. coli* and *B. subtilis*) was performed. Adequate number of membranes were placed in 8 ml of inoculated LB. Cultures were then incubated in 37˚C for 24 h.

3. Results

In order to confirm the presence of functional groups characteristic for acrylic acid, FT-IR analysis was performed. In Fig. 3, spectra obtained for PP membranes and modified with different concentrations (0.1; 0.5; 1; 5; 7; 10; 13; 20%) of acrylic acid are presented. In case of all modified membranes, new peaks appeared. First peak appears in the 3,500–3,000 cm^{-1} region responsible for –O–H bond. Further in the range of 1,760–1,650 cm^{-1} appears peak responsible for carbonyl groups. In addition, peak 1,100 cm^{-1} is responsible for C–O– bond, which is probably responsible for ester groups.

As it is clearly presented, in case of all modifications, the presence of carboxyl groups, as well as the presence of acrylic acid on the membrane surface, is confirmed.

Membranes modified by acrylic acid with silver nanoparticles (Fig. 4) were then tested for antibacterial properties. Membranes modified by silver nanoparticles changed color from white to brownish and black.

First antibacterial/bacteriostatic test was performed in solid medium for two kinds of bacteria: *E. coli* and *B. subtilis*. Two plates inoculated with gram positive and gram negative bacteria and then incubated in

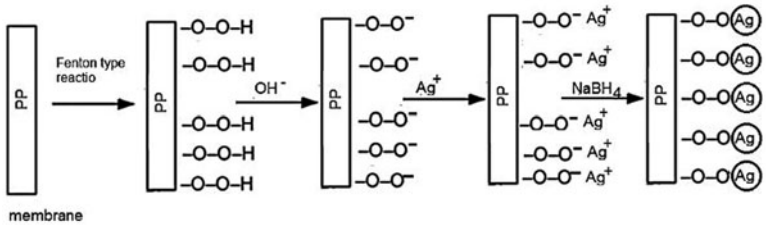

Fig. 2. Scheme of silver nanoparticles synthesis on membrane modified by acrylic acid [based on 18].

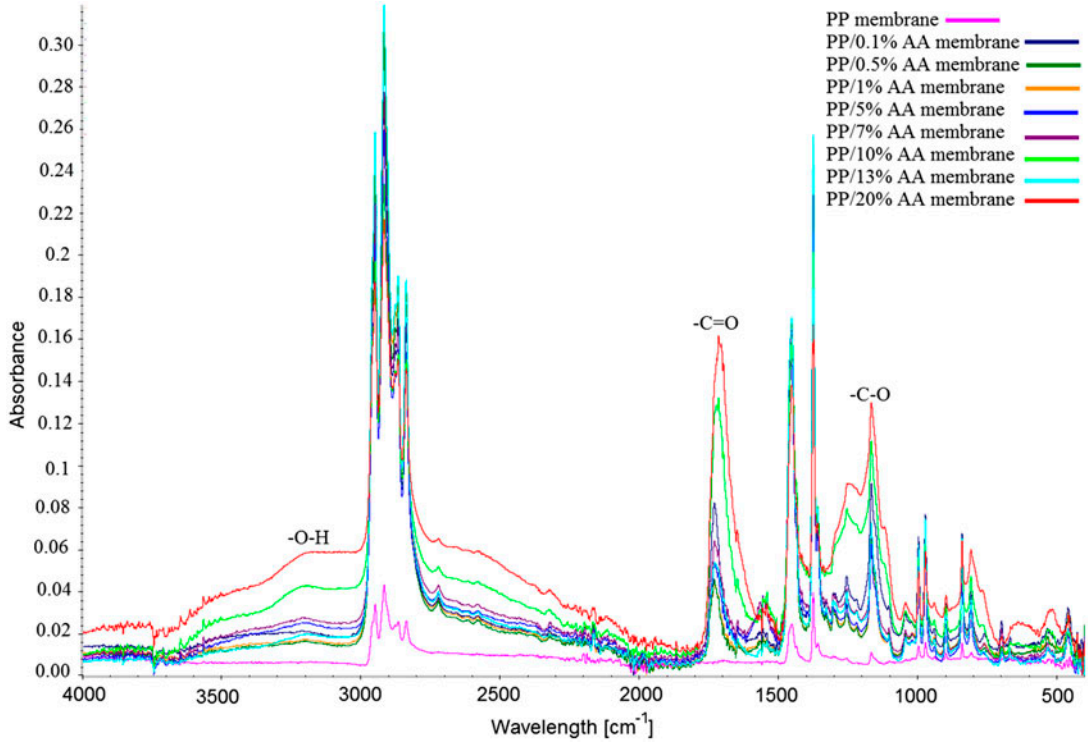

Fig. 3. FT-IR spectra obtained for PP membrane and membranes modified with 0.1, 0.5, 1, 5, 7, 10, 13, and 20% acrylic acid.

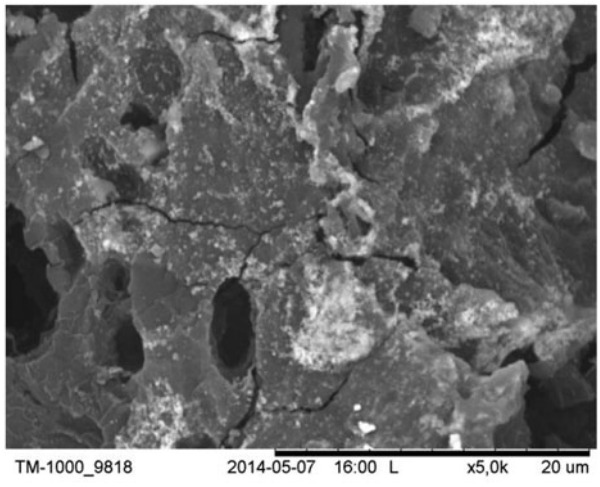

Fig. 4. SEM image of membrane outer surface modified by 10% of acrylic acid and silver nanoparticles.

37˚C for 24 h are presented in Fig. 5. Zone of growth inhibition (clear spot) around the membrane confirms the antibacterial or bacteriostatic properties of the modified material. In Fig. 5, results for PP membrane and 0.1, 0.5, 1, 5, 10 and 20% AA membranes modified by silver nanoparticles are presented. In case of plate inoculated by *E. coli*, clear zone of inhibited growth can be observed for membranes modified by nanoAg and 10% of acrylic acid. In case of other modifications, small clear zones were visible; however, it was impossible to show it in this picture.

In case of plate inoculated by *B. subtilis*, clear zone of inhibited growth appeared similarly as in the case of *E. coli* for the membrane with nanoAg and 10% of acrylic acid. Additionally, in case of membranes modified by 0.1, 1, 5, and 20% of acrylic acid and silver nanoparticles, zone with less frequent growth than in case of PP membrane appears.

Two plates inoculated with *E. coli* (on differentiating medium) and *B. subtilis* and incubated in 37˚C for 24 h are presented in Fig. 6. Presented results were obtained for PP membranes and 5, 7, 10, and 13% AA membranes modified by silver nanoparticles. In case of *E. coli*, growth-inhibited zones can be observed for membranes modified by 10 and 13% of acrylic acid and nanoAg. Also, minor clear zones are visible for membranes modified by 5 and 7% AA and silver nanoparticles.

In case of plates inoculated by *B. subtilis*, clear zones of inhibited growth are visible for membranes modified by 10 and 13% of acrylic acid and nanoAg.

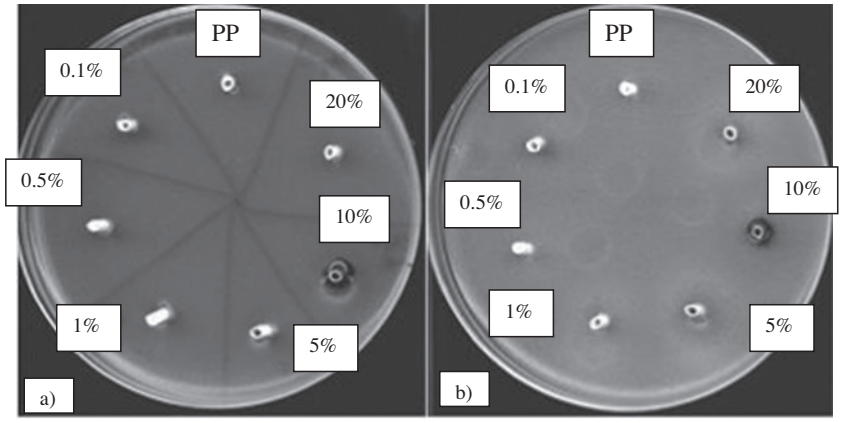

Fig. 5. Plates inoculated by (a) *E. coli* and (b) *B. subtilis* with PP and modified membranes and then incubated for 24 h at 37°C.

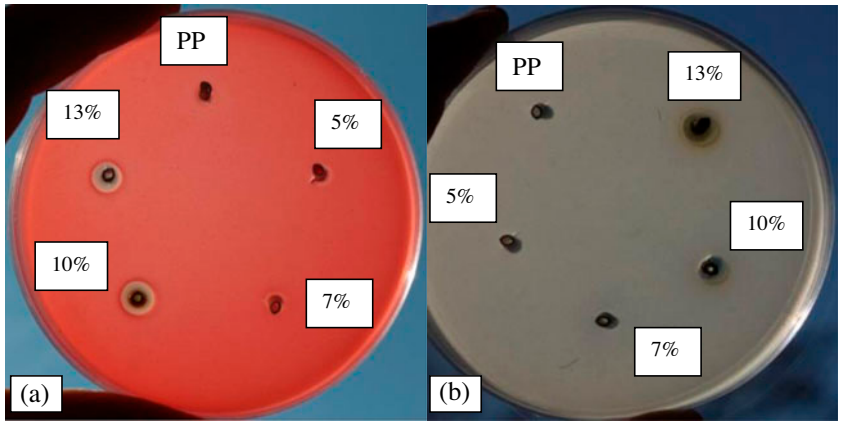

Fig. 6. Plates inoculated by (a) *E. coli* and (b) *B. subtilis* with PP and modified membranes, then incubated for 24 h at 37 °C.

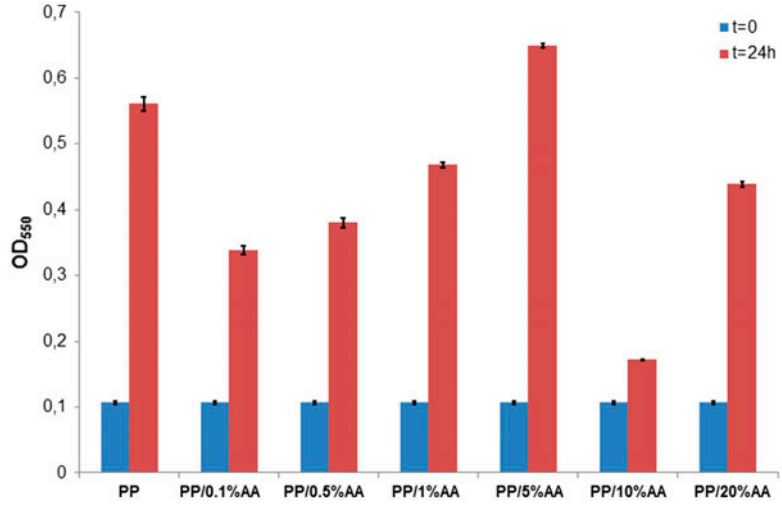

Fig. 7. Effect of acrylic acid concentration on antibacterial properties of membranes with immobilized silver nanoparticles on *E. coli*.

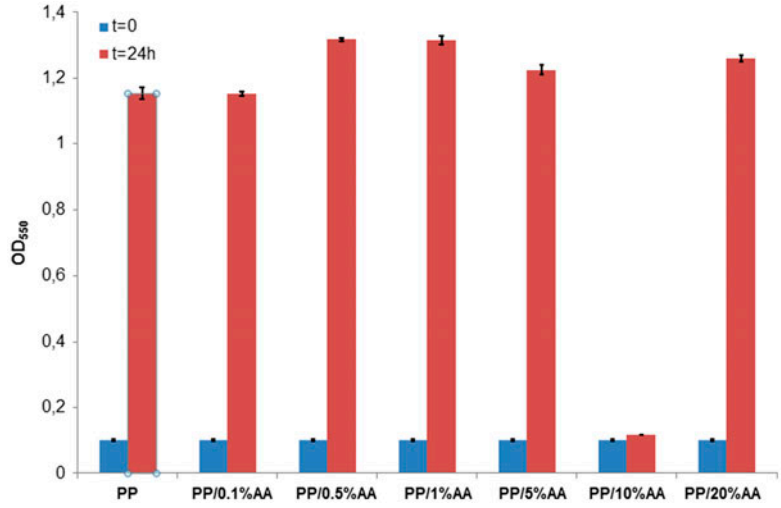

Fig. 8. Effect of acrylic acid concentration on antibacterial properties of membranes with immobilized silver nanoparticles on *B. subtilis*.

Also antibacterial test in liquid medium was performed. In case of this experiment, LB was inoculated with *Escherichia coli*. Initial OD_{550} of culture was 0.1, and then culture was incubated with membranes for 24 h at 37˚C. After 24 h of incubation, OD_{550} was measured once again. Increase in this parameter indicated micro-organism growth. It is important to stress out that this test does not differentiate live and dead bacteria. Only in case of cell lysis, the reduction of OD_{550} will be observed. Graph presenting antibacterial effect of modified membranes is presented in Fig. 7. The biggest bactericidal effect was obtained for membranes modified by 10% of acrylic acid with silver nanoparticles. Then, the order of antibacterial properties will be membranes modified by 0.1, 0.5, 20, 1, and 5% of acrylic acid with silver nanoparticles. In case of this method of modification, growth of AA concentration does not go in pair with the growth of a number of free carboxyl groups. Also, antibacterial effect is strictly connected with the amount of silver nanoparticles, which are connected with the number of free carboxyl groups present on the membrane surface.

The best results were obtained for membranes modified by 10% of acrylic acid and silver nanoparticles. However, that test does not distinguish dead cells from alive. Only in case of membranes modified by 10% AA/nanoAg colony-forming units, test was performed. For the modified membranes, there was ~10^{-2} cfu/ml, but for PP membrane ~3×10^{-8} cfu/ml after 24 h of incubation in 37˚C.

In case of next experiment in liquid media, LB was inoculated by *B. subtilis*. Initial OD_{550} of culture was 0.1, then culture was incubated with membranes for 24 h at 37˚C. After 24 h of incubation, OD_{550} was measured once again. However, in case of this experiment, bacterial cultures were infected, which was most likely due to fungal infection. Nevertheless, it was decided to run this experiment and see how modified membranes cope with multicultural culture. Graph presenting antibacterial effect of modified membranes on infected *B. subtilis* culture is presented in Fig. 8. Antimicrobial properties were observed only in case of membrane modified with 10% of acrylic acid with nanosilver. In case of other modifications, no antimicrobial effect was observed.

Based on the obtained data, it can be concluded that membranes modified with 10% of acrylic acid and silver nanoparticles have strong antibacterial properties. Also membranes modified by 10% acrylic acid with silver nanoparticles exhibited antibacterial properties against gram positive and gram negative bacteria both in solid medium and in liquid medium.

4. Conclusions

It is possible to modify PP membranes by acrylic acid by a Fenton-type reaction. Thanks to this modification, free carboxyl groups were introduced to the membrane surface. Also the presence of free carboxyl groups most probably allowed to form bond between silver nanoparticle and membrane surface.

Modified with silver nanoparticles, membranes were then tested for antibacterial properties. The best results were obtained for the membranes modified by 10% of acrylic acid nanoAg. This membrane had a strong bacteriostatic/antibacterial effect against both

E. coli and *B. subtilis* in solid medium. Similarly, in liquid medium, 10% AA/nanoAg membranes exhibited significant decrease in the content of micro-organisms after 24 h of incubation in case of both types of bacteria. It is also worth noting that in the case of infected *B. subtilis* cultures, membranes modified with 10% of acrylic acid/nanoAg were able to reduce the number of micro-organisms, including those derived from infections, most likely fungi. Thanks to those modifications, membranes got additional reactive properties. Membranes have become not only a physical barrier but also a reactive one. Based on the obtained results, it can be concluded that examined issues require further study.

Acknowledgments

Authors would like to thank MSc. Jarosław Skowroński form the Institute for Sustainable Technologies—National Research Institute form Radom, Poland. This work was partially supported by the NCBiR project EraNet/MNT/NFSM/1/2011.

References

[1] S. Sridhar, T.M. Aminabhavi, S.J. Mayor, M. Ramakrishna, Permeation of carbon dioxide and methane gases through novel silver-incorporated thin film composite pebax membranes, Ind. Eng. Chem. Res. 46 (2007) 8144–8151.

[2] N.L. Yong, A.W. Mohammad, C.P. Leo, N. Hilal, Polymeric membranes incorporated with metal/metal oxide nanoparticles: A comprehensive review, Desalination 308 (2013) 15–33.

[3] P. Dallas, V.K. Sharma, R. Zboril, Silver polymeric nanocomposites as advanced antimicrobial agents: Classification, synthetic paths, applications, and perspectives, Adv. Colloid Interface Sci. 166(1–2) (2011) 119–135.

[4] K. Chaloupka, Y. Malam, A.M. Seifalian, Nanosilver as a new generation of nanoproduct in biomedical applications, Trends Biotechnol. 28(11) (2010) 580–588.

[5] H.J. Mohammad, K.M. Fromm, A.A. Ashkarran, D. Jimenez de Aberasturi, I. Ruiz De Larramendi, T. Rojo, V. Serpooshan, W.J. Parak, M. Mahmoudi, Antibacterial properties of nanoparticles, Trends Biotechnol. 30 (10) (2012) 499–511.

[6] L. Qilin, S. Mahendra, D.Y. Lyon, L. Brunet, M.V. Liga, D. Li, P.J.J. Alvarez, Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications, Water Res. 42(18) (2008) 4591–4602.

[7] H. Jafar, R.J. Crawford, E.P. Ivanova, Antibacterial surfaces: The quest for a new generation of biomaterials, Trends Biotechnol. 31(5) (2013) 295–304.

[8] M. Ndeke, M. Thwala, M. Nota, The antibacterial effects of engineered nanomaterials: Implications for wastewater treatment plants, J. Environ. Monit. 13(5) (2011) 1164–1183.

[9] P. Gunawan, C. Guan, X. Song, Q. Zhang, S.S.J. Leong, C. Tang, Y. Chen, M. Chan-Park, M.W. Chang, K. Wang, R. Xu, Hollow fiber membrane decorated with Ag/MWNTs: Toward effective water disinfection and biofouling control, ACS Nano 5 (2011) 10033–10040.

[10] E.-S. Kim, G. Hwang, M. Gamal El-Din, Development of nanosilver and multi-walled carbon nanotubes thin-film nanocomposite membrane for enhanced water treatment, J. Membr. Sci. 394–395 (2012) 37–48.

[11] I. Azócar, E. Vargas, N. Duran, A. Arrieta, E. González, Preparation and antibacterial properties of hybrid-zirconia films with silver nanoparticles, Mater. Chem. Phys. 137(1) (2012) 396–403.

[12] J.F. Hernández-Sierra, F. Ruiz, D.C.C. Cruz Pena, F. Martínez-Gutiérrez, A.E. Martínez, A.D.J.P. de Jesús Pozos Guillén, H. Tapia-Pérez, G.M. Martínez Castañón, The antimicrobial sensitivity of *Streptococcus mutans* to nanoparticles of silver, zinc oxide, and gold, Nanomed. Nanotechnol. Biol. Med. 4(3) (2008) 237–240.

[13] J.-H. Li, The double effects of silver nanoparticles on the PVDF membrane: Surface hydrophilicity and antifouling performance, Appl. Surf. Sci. 265 (2013) 663–670.

[14] M.S. Mauter, Y. Wang, K.C. Okemgbo, C.O. Osuji, E.P. Giannelis, M. Elimelech, Antifouling ultrafiltration membranes via post-fabrication grafting of biocidal nanomaterials, ACS Appl. Mater. Interface. 3 (8) (2011) 2861–2868.

[15] Information from MEMBRANA GmbH data sheet, Data provided by membrane manufacturer with acquired membranes. Available from: http://www.membrana.com/index.php/filtration-membranes-241/accurel-membrane.

[16] B. Butruk, M. Trzaskowski, T. Ciach, Fabrication of biocompatible hydrogel coatings for implantable medical devices using Fenton-type reaction, Mater. Sci. Eng. C 32(6) (2012) 1601–1609.

[17] L. Mulfinger, S.D. Solomon, M. Bahadory, A.V. Jeyarajasingam, S.A. Rutkowsky, C. Boritz, Synthesis and study of silver nanoparticles, J. Chem. Educ. 84(2) (2007) 322.

[18] M. Bryjak, T. Janecki, I. Gancarz, K. Smolińska, Plazmowa modyfikacja membran polimerowych Membrany i procesy membranowe zeszyt III, Wykłady monograficzne i specjalistyczne, Toruń, 2009, pp. 64–79.