Desalination and Water Treatment
www.deswater.com

1944-3994 / 1944-3986 © 2010 Desalination Publications. All rights reserved.
doi: 10.5004/dwt.2010.1772

18 (2010) 206–211
June

Nonwoven membrane modification by 4-vinyl pyridine grafted polyvinyl alcohol for resistance the adhension of bacteria

Chanchan Wang, Fenglin Yang*, Jinli Duan, Hanmin Zhang

School of Environmental and Biological Science and Technology, Dalian University of Technology, Dalian 116023, People's Republic of China
Tel.: +86-411-84706328; Fax: +86-411-84706328; email: yangfl@dlut.edu.cn, wangcc.dlut@yahoo.com.cn, zhhanmin@126.com

Received 2 June 2009; Accepted 16 December 2009

ABSTRACT

Membranes suffer from operational problems due to fouling in water filtration. Fouling is caused by the build-up of chemicals, bioorganic materials, and biofilms at the membrane surface. The focus of this study was the fabrication and characterization of 4-vinyl pyridine grafted polyvinyl alcohol (PVA-g-4VP) and the modification of nonwoven membrane (NWF) for the prevention of bacterial attachment. The graft copolymerization using Ce(IV) as an initiator was carried out in an aqueous solution. With keeping other conditions constant, the optimum conditions were shown as following: [Ce(IV)]=4 mmol/l, [4VP]=0.15 mol/l, reaction temperature=60°C, reaction time=240 min. NWF membranes were modified by coating of PVA-g-4VP with different graft concentrations, and quaternization of the pyridine groups with benzyl bromide. A significant surface enrichment of vinyl pyridine polymer side chains was observed by Fourier transform infrared-attenuated total reflectance. The permeability of the membrane was reduced after modification. The antimicrobial activity of the modified membrane was measured by 2,3,5-Triphenyl tetrazolium chloride-dehydrogenase. The PVA-g-4VP modified NWF membrane exhibited a higher antimicrobial activity at a higher graft concentration than PVA modified NWF membrane.

Keywords: 4-vinyl pyridine grafted polyvinyl alcohol (PVA-g-4VP); Microfiltration membrane; Antimicrobial activity

1. Introduction

Membrane systems are widely used to purify water of different qualities including microorganism solutions, seawater, wastewater and membrane bioreactor effluent [1–4]. They are very promising in water treatment because of their potential to remove particles, including microorganisms, organic pollutants, inorganic compounds and to achieve biologically-stable water to limit microbial regrowth in the distribution system. However, membrane separations suffer from operational problems due to fouling. Several types of fouling can occur in membrane systems, e.g., inorganic fouling or scaling, particulate and colloidal fouling, organic fouling and finally biofouling [5]. It is recognized that membrane fouling is affected by chemical and physical properties such as surface **hydrophilic-hydrophobic property**, pore size and porosity of membrane. Biofouling of microfiltration and ultrafiltration membranes was significantly, especially the rapid formation of a conditioning layer of largely organic molecules and the colonisation of bacteria and larger organisms [6]. As such, various approaches have been applied to attenuate the accumulation of particles on/in the membrane during filtration. The most

---

*Corresponding author.

common approach involves application of a shear force typically brought about by cross flower aeration at the membrane surface [6,7]. Even so, operational data from both full scale applications and laboratory studies [8–10] reveal that membrane fouling is inevitable unless managed appropriately.

More effective methods have been reported to prevent or control the fouling on/in the membrane. These include a modification of polymer membranes with low temperature plasma [11], photochemical technique [12], blend [13], coating [14] and adsorption [15]. Polyvinyl alcohol (PVA) is a possible candidate for the membrane separation of aqueous mixtures because of its good chemical stability, film forming ability and high hydrophilicity. It is an attractive material to be developed as a new type membrane modified material with good anti-fouling characteristics because it has been shown that PVA spontaneously adsorbs onto hydrophobic substrates [16]. Considerable interest has been focused on chemical modification by grafting synthetic polymers onto PVA. 4-vinyl pyridine (4VP) has been selected for improving physico-chemical properties of PVA and based polymers have inherent antimicrobial properties [17–19].

In this paper, a novel 4-vinyl pyridine grafted polyvinyl alcohol (PVA-g-4VP) copolymer was fabricated by free radical graft copolymerzation. The resulting graft copolymers are shown to be promising modification materials for fabricating microfiltration membranes having the hydrophilic property, permeability to aqueous solutions and antimicrobial activity. The present investigation deals with the detailed study of some major factors which affect graft polymerization of 4VP onto PVA using ceric ammonium sulfate (CAS) initiator in aqueous medium. And the chemical characteristics, permeability and antimicrobial activity of the modified membrane were measured.

2. Materials and methods

2.1. Material

Nonwoven membrane (NWF) membranes were obtained from Tianjin TEDA Co., Ltd (China) with nominal pore diameters of 0.5 μm. PVA (Hunan Xiangwei Co. Ltd., China) with average molecular weight of 70000 and hydrolysis degree of 98% was used. 4VP (S.D. Lab-Chem Industry, China) and CAS (E. Merck, China) were purified before use. Glutaraldehyde (GA) was obtained as a 50% (w/w) aqueous solution. 2,3,5-Triphenyl tetrazolium chloride (TTC) was purchased from Sinopharm Chemical Reagent Co., Ltd and used as received. All other chemicals were of analytical grade and used without purification. The water was de-ionized water.

2.2. Graft copolymerization

The graft copolymerization of 4VP onto PVA was carried out in an aqueous solution. Firstly, PVA of 0.5 g was dissolved to 50 ml hot water (95°C) in a 100 ml three-necked round-bottom flask, which was fitted with a magnetic stirrer, a N_2 gas inlet and outlet, a reflux condenser, and a thermometer. The flask was kept in a water bath maintained at 4°C. After purged with nitrogen gas for about 30 min, a calculated amount of 4VP monomer was added into the flask, and mixed for 60 min before graft polymerization starting. The temperature was increased to a constant centigrade and a calculated amount of CAS was added to initiate the graft copolymerization. Nitrogen gas was supplied with a constant of flux during the graft copolymerization reaction. At the end of the reaction, the mixture was poured into a beaker containing an excess amount of ethanol, separated by filtration, and the products were extracted with acetone in a soxhlet apparatus for 6 h to dissolve all the 4VP monomers and homopolymers. The colorless products were dried under vacuum at 60°C for more than 12 h to a constant weight.

2.3. Preparation of 4-vinyl pyridine grafted polyvinyl alcohol adsorbed modification membranes

NWF membrane discs were shaken in ethanol for 24 h at 25°C, to wash the impurities on the membrane surface. After that, the membranes were incubated in 25 wt% GA for 6 h at 60°C to make GA adsorbed on the NWF surface. Afterwards, the membranes were washed three times with water. Certain amount of PVA-g-4VP was dissolved into water at 95°C to form a 2 wt% solution. GA (crosslinking agent) solution was added at a constant concentration ($\mathrm{mol}_{-\mathrm{CHO}}/\mathrm{mol}_{-\mathrm{OH}}$). The PVA-g-4VP modified NWF membranes were prepared by filtering 35 ml PVA-g-4VP solution through the NWF membranes with a diameter 19.6 cm^2 at 20 KPa using stirred cell assembly. The pressure applied was controlled by nitrogen gas. This was followed by curing the membranes in an oven at 60°C for 2 h. The quaternization was operated as follows: PVA-g-4VP modified membranes were placed in methanol solutions containing 20 vol% benzyl bromides and the reaction mixture was stirred for 48 h at 60°C. The products were then thoroughly rinsed with methanol and dried under vacuum.

2.4. Membrane characterization

To investigate the chemical changes of the PVA-g-4VP modified membranes and the original NWF surface, the surface analysis has been performed using Fourier transform infrared-attenuated total reflectance (FTIR-ATR). The FTIR-ATR spectra of membrane samples were measured by a Nicolet Magna IR 860 Instrument (Thermo Nicolet, Madison, WI, USA).

To know the effect of modification on membrane hydraulic permeability, the water flux was measured before and after modification at a constant pressure of 40 KPa. All water fluxes were measured (by the volume method) until the consecutively recorded values were considered to be constant (i.e., they differed by less than 4%).

2.5. Antimicrobial activity of 4-vinyl pyridine grafted polyvinyl alcohol modified membrane

The bacteria in the biofilm were separated from the hydrophobic polypropylene membrane surface, which was used in membrane bioreactor. They were chosen and analyzed as the bacterial model because those bacteria were commonly present throughout the water filtration and readily colonize membrane surface as biofilms. The bacteria were grown in a synthetic nutritious water solution containing sucrose, NH_4Cl, KH_2PO_4 at 37°C, rotating at 100 rpm until it reached optical density at 600 nm (OD_{600}) of about 0.8 (measured by spectrophotometer, Spectrum722E, China). The solution was diluted in sterile phosphate buffered saline (PBS) to bring the solution cell concentration to approximately 10^6 CFU/ml (OD_{600}=0.6). Disc samples (diameter 25 mm) were taken from each type of modified membrane plus an original membrane as control. These samples were placed in 50 ml centrifuge tubes and sterilized with ultraviolet irradiation for 30 min, and 20 ml of the diluted cells culture was added. These samples were rotated at 100 rpm on an orbital shaker for 3 h at 37°C, and poured the solution from the centrifuge tubes. Then, samples were gently washed twice with PBS to get rid of any non-adherent cells. TTC solution of 10 ml at concentration of 0.001% was added to each centrifuge tubes and incubated for 20 min at 37°C. After that, the products were transferred to fresh tube and 50 ml ethanol was added to each tubes to dissolve the dye inside cells. The absorbance of liquid that dissolved triphenyl formazan (TF) was measured at the wavelength of 490 nm with 722E spectrophotometer (SPECTRUM).

3. Results and discussion

3.1. Grafting parameters

Grafting efficiency (GE) and grafting percentage (GP) were used to evaluate the graft copolymerization according to Eqs. (1) and (2), respectively:

$$GP(\%) = \frac{w_1 - w_0}{w_0} \times 100\% \tag{1}$$

$$GE(\%) = \frac{w_1 - w_0}{w_2} \times 100\% \tag{2}$$

where w_0, w_1, and w_2 denoted the weight of PVA, graft copolymer, and monomer charged, respectively. The percentage of grafting was included so as to give a more clear idea about the level of grafting in graft copolymer.

3.2. Effect of concentration of 4-vinyl pyridine

Fig. 1 showed the effect of 4VP concentration on graft copolymerization. With the increase in 4VP concentration, GP increased continuously, reached the maximum value when the concentration of 4VP was 0.16 mol/L, and then decreased. This behavior could be explained by the fact that an increase of monomer concentration led to the accumulation of monomer molecules in close proximity to the PVA backbone. The decrease of GP after saturation could be associated with depletion in the available 4VP concentration as well as a reduction in the active sites on the PVA backbone as the graft copolymerization proceeded. The decrease of GE with the increase in 4VP concentration may be due to the amount of 4VP which was more helpful to copolymerization than grafting in the concentrations determined.

3.3. Effect of initiator concentration

To study the effect of the initiator concentration, graft copolymerization was studied at various CAS concentrations by keeping other reaction conditions constant. As shown in Fig. 2, with the increase of the initiator concentration, GP and GE had a rapidly increase at first, then a slow increase to a maximum value, and finally a decrease. The increase of GP may be ascribed to the increase of macroradicals. With the increase of initiator concentration, more CAS attacked the unit of

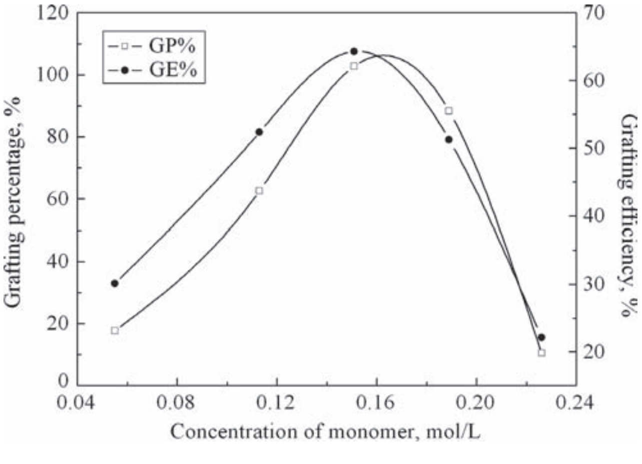

Fig. 1. Effect of concentration of monomer on GP and GE under reaction conditions: PVA = 8 g/L; CAS=4 mmol/L; Time=120 min; Temperature=60°C.

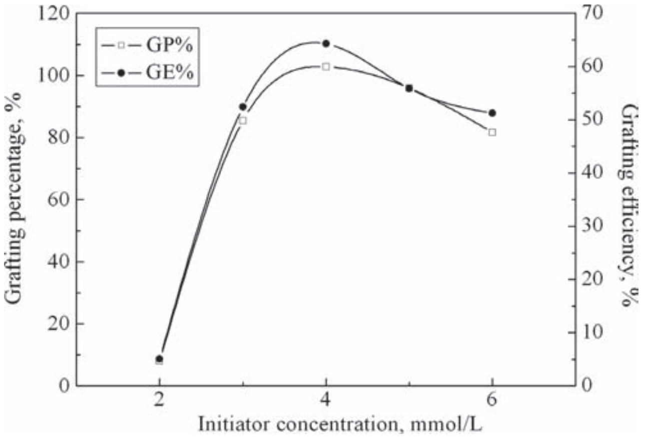

Fig. 2. Effect of initiator concentration on GP and GE under reaction conditions: PVA=8 g/L; 4VP=0.16 mol/L; Time=120 min; Temperature=60°C.

PVA, which made more PVA macroradicals generate, thus more active sites of PVA could react with CAS, which initiated the propagation reaction of 4VP. With the further increase of amount of CAS (>4 mmol/L), the concentration of Ce(IV) radicals increased, and thus initiated the copolymerization of 4VP. This resulted in the decrease of GP and GE.

3.4. *Effect of temperature*

The effect of temperature was studied by changing the reaction temperature from 20 to 80°C and keeping other reaction conditions constant, as shown in Table 1. GP and GE reached a maximum at 60°C and then decreased. At low temperature, diffusion of 4VP and CAS molecules onto the PVA structure and increased rate of decomposition of the complex formed between Ce(IV) and PVA was slow, the amount of the radicals was small and thus GP was low. With the increase in temperature, the collision chance of PVA and CAS increased and resulted in the increase of PVA macroradicals, and thus enhanced the graft polymerization. GP and GE showed a decrease above this optimum temperature, which should be related to the following facts: at a higher reaction temperature, the thermal decomposition rate of CAS increased. The small radicals obtained will be helpful to the copolymerization of 4VP. Besides, the mobility of macroradicals would be enhanced and may lead to termination at a higher temperature.

Table 1
Effect of temperature on grafting parameters; PVA=1.0 g/L, 4VP=0.16 mol/L, CAS=4 mmol/L, time=240 min.

Temperature (°C)	GE%	GP%
20	23.71	37.87
40	39.48	66.5
60	64.3	102.82
80	52.4	85.43

Table 2
Effect of time on grafting parameters; PVA=1.0 g/L, 4VP= 0.16 mol/L, CAS=4 mmol/L, temperature=60°C.

Time (min)	GE%	GP%
30	11.5	18.37
60	20.7	32.95
120	49.1	78.2
240	64.3	102.82

3.5. *Effect of time*

Table 2 depicted the GE of 4VP onto PVA at different reaction periods. The GE exhibits progressive improvement with the increase of reaction period up to 240 min and then it remains constant. This effect of time on grafting can be explained as follows: it is obvious that the higher contact time of monomer molecules with the PVA macroradicals, the higher will be grafting, but the level of GE after a certain time can be attributed to the depletion of initiator and monomer with the progress of reaction. GP follows the same trend as GE. These may be indicated the complete depletion of CAS initiator in the systems at 240 min. With the increase in reaction time, the concentration of monomer and free radicals in the system deduced, and would result in the leveling off of GP and GE.

3.6. *Characteristics of the 4-vinyl pyridine grafted polyvinyl alcohol modified membrane*

It is clear in their FTIR spectras that the PVA-g-4VP modified membrane had both characteristic peaks of PVA (O–H stretching modes in 3350–3400 cm^{-1}) and the pyridine ring stretching modes (1653 cm^{-1}) of the grafted 4VP polymer, which proved the effective modification of PVA-g-4VP on NWF. Furthermore, the peak intensity of the band at 1653 cm^{-1} increased with the bulk graft concentration of PVA-g-4VP. Since the concentration of a functional group is directly proportional to its absorption band area in the FTIR spectrum, the ratio of the absorption band area at 1653 cm^{-1} to that at 2940 cm^{-1} (the absorption band associated with the CH_2 groups of PVA) was directly related to the bulk graft concentration of the PVA-g-4VP copolymer (Fig. 3, curve b and c). The FTIR spectroscopic results suggested that the modification has indeed occurred.

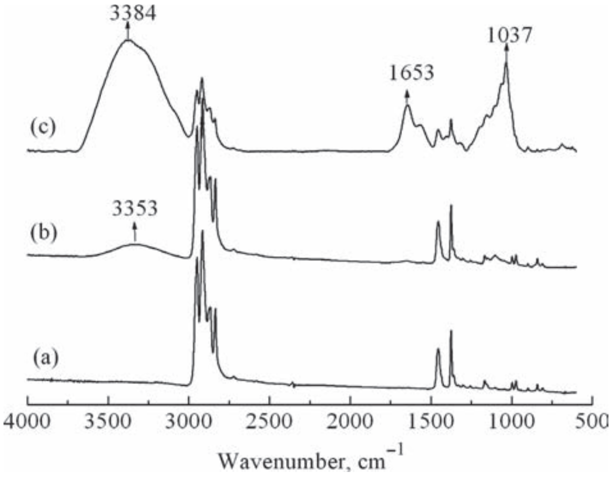

Fig. 3. FTIR spectra of (a) the original NWF membrane, the PVA -g-4VP copolymers modified membrane of graft percentage, (b) 68%, (c) 108%.

Hydraulic permeability of the membranes in water filtration was measured and used to evaluate the permeability properties of modified membranes. Fig. 4 presented the results of the pure water flux of a series of membranes. The water flux of the membrane decreased from 15860 L/m^2. h to 1292 L/m^2. h after coating PVA-g-4VP hydrogel layer. It was due to the pore size was decreased after modification.

3.8. Determination of antibacterial activity

TTC is a small substance, which can be incepted by live bacteria and pass through the cell wall and membrane. Under the action of dehydrogenase in live cells, TTC will be reduced into the product TF, which is red. If immerse the membrane with bacteria in the TTC solution, the dehydrogenase in live bacteria will transform TTC to TF, the membrane will change to red. So the adsorption of live bacteria on the membrane surface can be estimated by measuring the produced amount of TF.

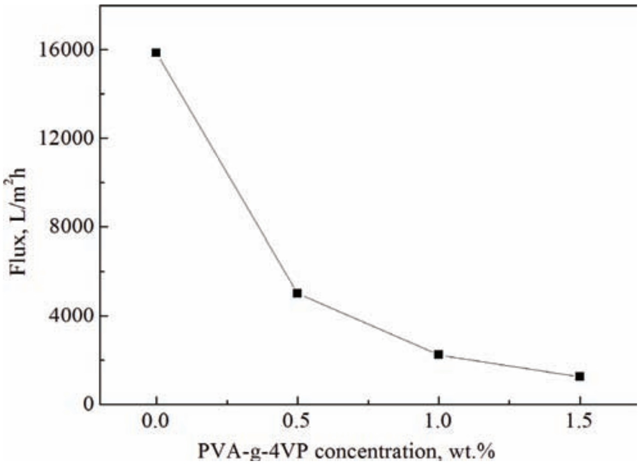

Fig. 4. Flux profile as a function of PVA-g-4VP concentration for various modified membranes during the filtration of water at a transmembrane pressure of 40 KPa.

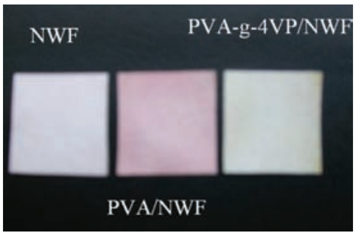

Fig. 5. The photo show the TTC incepted by live bacteria on the PVA-g-4VP/NWF composite membrane surface.

The adhesion and accumulation of live cells on the membrane surface via hydrophobic interaction would result in the formation of bacterial colonies and membrane fouling [21,22]. Fig. 5 showed that the original NWF membrane surface and the PVA modified membrane both have red color, while the PVA-g-4VP modified membrane was colorless. Fig. 6 showed the calculated absorbance data by TF. These absorbance data were calculated by subtracting the absorbance of control samples that contain no cell from the raw absorbance data of each sample. Thus, the absorbance data of the samples that were incepted with cells represent only the amount of adhered cells on the membrane surfaces. The results indicated that cells adhere strongly on the original NWF membrane surface and the bacteria may be entrapped

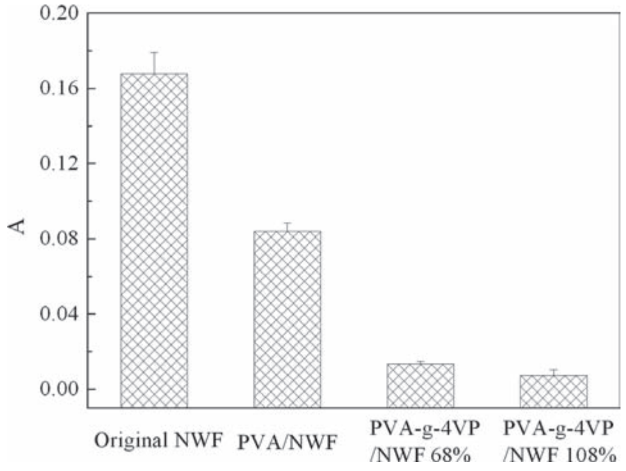

Fig. 6. The image shows absorption of TF.

deep in the membrane. PVA adsorption modified membrane showed a low degree of bacterial attachment with strong cell viability (Fig. 6), this may be due to the hydrophilic and smooth surface has lower interaction with bacterial. In contrast, the disc modified by PVA-g-4VP showed very sparse cell attachment and a high degree of cell inactivation (Fig. 6), which would prevent new cell or biofilm growth. The anti-fouling properties of the quaternized PVA-g-4VP modified membrane can be accounted for follows: (a) the good antibacterial properties of quaternary ammoniation PVA-g-4VP inhibit the formation of bacterial colonies and (b) membrane fouling is reduced by hydrophilic surface.

4. Conclusion

4VP can be easily graft copolymerized onto PVA using Ce(IV) as an initiator in acidified aqueous medium. The reaction variables such as monomer concentration, initiator concentration, polymerization time and temperature of the system affect considerably the GE of 4VP onto PVA. With keeping other conditions constant, the optimum conditions were shown as following: [Ce(IV)] = 4 mmol/L, [4VP] = 0.15 mol/L, reaction temperature = 60°C, reaction time = 240 min. A significant surface enrichment of the more hydrophilic and antimicrobial activity vinylpyridine polymer side chains was observed. The quaternary ammoniation PVA-g-4VP modified membrane exhibited a higher antimicrobial activity at a higher graft concentration than both original NWF membrane and PVA modified membrane. We believe that this hydrophilization approach has great potential in industrial production and can also be easily applied to other common commercial hydrophobic membrane materials, such as polyethylene, polyvinylidene fluoride and poly(tetrafluoroethylene), to confer new properties on them.

Nomenclature

GP: Grafting percentage
GE: Grafting efficiency
w_0: the weight of PVA
w_1: the weight of graft copolymer
w_2: the weight of monomer charged

Acknowledgements

Financial supports from the China state water pollution control and management of major special science and technology (No. 2008ZX0 7208).

References

[1] M.H. Liu, S.C. Yu, J. Tao and C.J. Gao, J. Memb. Sci., 325 (2008) 947–956.

[2] Y.Y. Wang, F. Hammes, M. Düggelin and T. Egli, Environ. Sci. Technol., 42 (2008) 6749–6754.

[3] M. Hesampour, A. Krzyzaniak and M. Nyström, J. Memb. Sci., 325 (2008) 199–208.

[4] Y. Miura, Y. Watanabe and S. Okabe, Environ. Sci. Technol., 41 (2007) 632–638.

[5] F.G. Meng, H.M. Zhang, F.L. Yang and L.F. Liu, Environ. Sci. Technol., 41 (2007) 4065–4070.

[6] N. Hilal, O.O. Ogunbiyi, N.J. Miles and R. Nigmatullin, Sep. Sci. Technol., 40 (2005) 1957–2005.

[7] S. Judd, Water Sci. Technol., 51 (2005) 27–34.

[8] B.Q. Liao, D.M. Bagley, H.E. Kraemer, G.G. Leppard and S.N. Liss, Water Environ. Res., 76 (2004) 425–436.

[9] S. Rosenberger, H. Evenblij, S.T. Poele, T. Wintgens and C. Laabs, J. Memb. Sci., 263 (2005) 113–126.

[10] W. B. Yang, N. Cicek and J. Ilg, J. Memb. Sci., 270 (2006) 201–211.

[11] H.Y. Yu, Z.Q. Tang, L. Huang, G. Cheng, W. Li, J. Zhou, M.G. Yan, J.S. Gu and X.W. Wei, Water Res., 42 (2008) 4341–4347.

[12] H. Susanto and M. Ulbricht, Langmuir, 23 (2007) 7818–7830.

[13] Y.Q. Wang, Y.L. Su, X.L. Ma, Q. Sun and Z.Y. Jiang, J. Memb. Sci., 283 (2006) 440–447.

[14] C.H. Zhang, F.L. Yang, W.J. Wang and B. Chen, Sep. Puri. Technol., 61 (2008) 276–286.

[15] G.G. Shubhangi and V.B. Manohar, J. Phys. Chem. B, 109 (2005) 13941–13947.

[16] M. Kozlov, M. Quarmyne, W. Chen and T.J. McCarthy, Macromolecules, 36 (2003) 6054–6059.

[17] B.J. Gao, S.X. He, J.F. Guo and R.X. Wang, Mater. Lett., 61 (2007) 877–883.

[18] C. Yao, X.S. Li, K.G. Neoh, Z.L. Shi and E.T. Kang, J. Memb. Sci., 320 (2008) 259–267.

[19] F.X. Hu, K.G. Neoh, L. Cen and E.T. Kang, Biotechnol. Bioeng., 89 (2005) 474–484.

[20] M.F.A. Goosen, S.S. Sablani, H.A. Hinai, S.A. Obeidani, R.A. Belushi and D. Jackson, Sep. Sci. Technol., 39 (2004) 2261–2297.

[21] B.K. Li and B.E. Logan, Colloids. Surf. B, 36 (2004) 81–90.