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EFFECT OF HYDROPHILIC AND HYDROPHOBIC ORGANIC MATTER ON AMOXICILLIN AND CEPHALEXIN RESIDUALS REJECTION FROM WATER BY NANOFILTRATION

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ABSTRACT

Antibiotics such as amoxicillin and cephalexin are a group of pharmaceutical compounds in human medicine practice that have been entered in water bodies. Presence of these compounds in the environment has raised concerns regarding the toxicity to aquatic organisms and the emergence of strains of antibiotic-resistant bacteria. Removal of these substances before entering the aquatic environment as well as water reuse plant is very important. The objective of this investigation was to evaluate the impact of hydrophilic and hydrophobic fractions of Natural Organic Matter (NOM) on the removal efficiency of cephalexin and amoxicillin, by using two different commercially available composite NF membranes (TFC-SR2 and TFC-SR3). In addition, the effect of NOM fractions on retention mechanism and permeates flux behavior was studied. Amoxicillin and cephalexin were used as models of antibiotics; alginate and humic acid were used as models of hydrophilic and hydrophobic fractions of NOM, respectively. It was observed that the rejection and permeate flux of amoxicillin and cephalexin were influenced by the membrane characteristics and properties of NOM. The results showed that as the alginate proportion was increased, the rejection improved. The permeate flux decreased with increasing alginate ratio. It was observed that the rejections of amoxicillin and cephalexin in TFC-SR2 were >97.3% and >95.8% in all experiments, respectively. In TFC-SR3, the rejection percentage were (95.9%-100%) and (86.1%-96.3%), respectively. Alginate and humic acid had synergistic effect on flux decline. In other words, increasing alginate concentration increased the rate and extent of flux reduction.

Key words: Natural Organic Matter fraction, Nanofiltration, Antibiotic removal, Fouling, Amoxicillin, Cephalexin

INTRODUCTION

In recent years, the occurrence of pharmaceutically active compounds (PhACs) or pharmaceutical personal care products (PPCPs) in the aquatic environment has been recognized as one of the emerging issues in environmental chemistry and a human health risk where water is subsequently used to supply drinking water (Heberer, 2002; Bendz *et al.*, 2005; Reif *et al.*, 2008). Antibiotics as an important group of PhACs, have been first produced in early 1940s and widely used in fighting against infectious bacteria and fungi(Li *et al.*, 2004).

The origin of antibiotics or their metabolites contamination in the aquatic environment (surface and groundwater) is considered to be mainly point and non-point source discharges mainly, such as human or animal excretions, municipal wastewater, landfill leachate and pharmaceutical industry wastewater (Yang and Carlson, 2003; Nghiem *et al.*, 2005). Researchers have shown that several classes of antibiotics and PPCPs are present in domestic effluents and aquatic environments (Kolpin *et al.*, 2002a; Weigel, 2003; Kolpin *et al.*, 2002b; Reverté *et al.*, 2003; Heberer, 2002; Yang and Carlson, 2003; Nghiem *et al.*, 2005), since they are often not

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fully assimilated by humans and animals during treatment (Nghiem *et al*., 2005). In addition, most of them show a recalcitrant behavior and are not easily removed from wastewaters in sewage treatment plants (STPs) *(*Reif *et al*., 2008).

Antibiotic residues in the wastewater seriously affect the biological treatment processes (Li *et al*., 2004). Xu *et al*., (2007) showed that antibiotics could not be eliminated at STPs and the highest elimination efficiency was 81%. Their elimination in the STPs was due to their sorption to the sludge, but not biodegradation (Xu *et al*., 2007). Another study showed that conventional wastewater treatment was observed to have great fluctuations and removal efficiency of PhACs was found to be much more influenced by changes in operating conditions compared with membrane filtration (Radjenovic *et al.*, 2007). Thus, these substances can be found in many secondary treated effluents and receiving freshwater bodies. Many studies conducted in different countries have indicated that their aquatic environments have been contaminated by more than 80 pharmaceutical substances and their metabolites (Nghiem *et al.*, 2005).

The presence of antibiotics in the aquatic environment has created two concerns. The immediate concern is the potential toxicity of these compounds to aquatic organisms and humans through drinking water. In addition, there is growing concern that release of antibiotics to the environment contributes to the emergence of strains of disease-causing bacteria that are resistant to high doses of these drugs (Yang and Carlson, 2003; Fent *et al.*, 2006). As a consequence, removal of pharmaceutical substance before entering the aquatic environment as well as for water reuse is very important.

On the other hand, to ensure compliance with future discharge requirements, upgrading of existing water and wastewater treatment plants and implementation of new technologies are considered as the next steps in improvement of wastewater treatment (Radjenovic *et al*., 2007). During the last decade, nanofiltration (NF) membrane has been proposed as an attractive technology for removal of pharmaceutical substances from aquatic environment and for water reuse (Zularisam *et al*., 2006; Radjenovic *et al*., 2007). Previous studies have shown that nanofiltration has the potential to reject high rates of drugs (sulfamethoxazole, carbamazepine, and ibuprofen) from water and the retention of pharmaceutical substance is influenced by its physico-chemical property the solution chemistry, the membrane characteristics and the process conditions (Nghiem *et al*., 2005; Schafer and Elimelech, 2005).

Although nanofiltration has been increasingly used in water and wastewater treatment, but fouling remains as one of the major challenges in membrane applications which cause significant loss of productivity and increased operational costs (Zazouli *et al.*, 2008a). Natural Organic Matters (NOM), which are abundant in water resources, are considered as strong foulants during water treatment with membrane processes (Zazouli *et al.*, 2008a, and 2008b). The NOM can be broadly divided into two fractions of hydrophobic fraction such as humic acid and hydrophilic fraction such as alginic acid (Zazouli *et al.*, 2007).

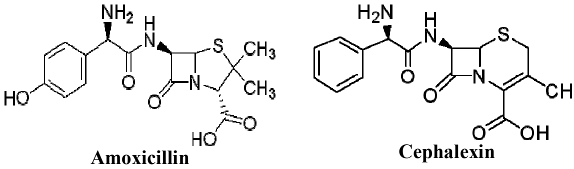
Membrane fouling caused by NOM is influenced by three major factors, i.e. characteristics of NOM and membranes, hydrodynamic conditions, and chemical environment in the feed. Hydrophilicity/phobicity, pore size, surface charge and surface roughness are the important membrane characteristics affecting the fouling behavior. Important feed conditions include the ionic strength, pH and the concentration of divalent ions. Various papers report the effect of NOM on retention of pollutants. In some cases the retention is raised in the presence of NOM (Devitt *et al.*, 1998); in other papers it is reduced (Nghiem *et al.*, 2002), while in a few researches almost no effect on total pollutant retention has been observed (Taylor *et al.*, 2002). Therefore, the presence of these substances may influence the removal efficiency of pharmaceutical substance by NF. Furthermore, NOM can promote exacerbating of the existing flux decline (Zhang *et al.*, 2006).

Natural Organic Matter has several fractions and each of them affect the performance of membrane in different manners. In addition, data on this subject is still very limited. Thus, the objective of this investigation was to evaluate the impact of hydrophilic and hydrophobic fractions of NOM on the removal efficiency of cephalexin and amoxicillin, by using two different NF membranes. In addition, the paper discusses the effect of NOM fraction on retention mechanism and permeate flux behavior.

MATERIALS AND METHODS

Reagents

Two high purity antibiotics (amoxicillin and cephalexin) were used. Molecular weight of amoxicillin and cephalexin are 365.4 and 347.39 g/mole, respectively (Fig.1). The antibiotics were purchased from Fluka Chemie AG (Buchs, Germany). These drugs are popular antibiotics in human medical care. Alginate acid sodium salt from brown algae, humic acid and glucose were purchased from Fluka Biochemical. Sucrose was purchased from Acros, Geel, Belgium. Magnesium sulphate ($MgSO_4$) and sodium chloride (NaCl) were purchased from Sigma Aldrich, Seelze, Germany GmbH and Carl Roth GmbH, Karlsruhe, Germany, respectively. Potassium chloride (KCl), potassium hydroxide (KOH) and hydrochloric acid (HCl) were purchased from Bernd Kraft GmbH, Duisburg, Germany. Water purified with a Milli-Q system from Millipore was used for all experiments.



Amoxicillin **Cephalexin**

Fig. 1: Chemical structures of the antibiotics used in this study

Analytical methods

The concentration of antibiotics was determined from UV absorbance at 229 nm and 262nm for amoxicillin and cephalexin, respectively, and measured using the UV-Visible spectrophotometer CARY-50 Probe (Varian). In mixed cases, HPLC was also used by using a Dionex high-performance liquid chromatography (HPLC) system. The system comprised an autosampler (Gilson 235), a solvent delivery system, and a UV-Vis detector (Dionex UVD 340s or diode array). The solvent delivery system included two HPLC pumps (Dionex P 580), a degasser, and a gradient mixer. A reversed phase column Discovery C18 (PerfectSil Target ODS-3) with particle size of 5µm, and column length and diameter of 125 mm, and 2.1 mm, respectively, was used. Acetate ammonium (0.01 M) and acetonitrile (ACN) delivered at a constant flow rate of 0.3 mL/min were used as the mobile phase for gradient elution. Each analysis lasted 39 min and the injection volume was 20µL. pH and conductivity of the solution were measured by using a digital pH meter (WTW pH 525 model, Germany) and a conductivity meter (CG854 model, Germany), respectively.

Feed solutions

Ultrapure water (Millipore Milli-Q) was used for the preparation of all stock solutions and membrane performance experiments. The solution pH was adjusted with addition of either NaOH (0.1 N) or HCl (0.1 N) solution. Salt stock solutions were prepared using certified analytical grade sodium chloride (NaCl) dissolved in ultrapure water. In all filtration experiments, background electrolyte solution contained 10 mM NaCl. Also in all experiments, the feed solution contained 500 µg/L of each antibiotic. In addition, alginic acid (AA) and humic acid (HA) were used as models for hydrophilic and hydrophobic natural organic matter in water, respectively. Overall 25 mg/L organic matter was introduced into antibiotics solutions.

Nanofiltration experiments

Two commercial thin film composite NF membranes (TFC-SR2 and TFC-SR3) purchased from Koch, USA were used. The filtration experiments were conducted using a cross-flow laboratory-scale membrane test unit, which mainly consisted of feed tank (5 L), pump and membrane cell (Fig. 2). The membrane cell, the spacer inserted in the membrane cell, and all of the tubes were made of stainless steel. Full circulation mode was used during the experiments, i.e., the retentate and permeate were returned to the feed water reservoir in order to maintain constant concentration. For each experiment, a new membrane disk with the effective membrane area of 20 cm^2 was used. The experiments were conducted at room temperature (22±1°C), controlled by a water bath. All experiments were done at transmembrane pressure of 10 bar. Before use in experiments, the membrane was soaked overnight in water to remove impurities left over from the manufacturing process or additives used for stabilization. Pure water flux was firstly filtered for at least 1 h until an approximately constant flux was observed. During fouling experiments, the permeate flux was measured over time.

Experiments were conducted in the presence of six ratios of organic matter (without organic, only HA, 70% HA plus 30% AA, 50% HA plus 50% AA, 30% HA plus 70% AA and only AA). Overally, 25 mg/L organic matter was introduced into antibiotics solutions as alone or mixed. Amoxicillin and cephalexin were mixed in all experiments. Initially, filtration was performed without addition of any organic matter (AA and HA). All experiments were performed for 2 h. Drug concentrations in feed, permeate and retentate were analyzed. Rejection of pharmaceutical was calculated according to the following equation:

$$R\,(\%) = \left(1 - \frac{C_p}{C_f}\right) \times 100$$

Where R is the rejection (%); C_f and C_p are the solute concentrations in feed and permeate, respectively.

Measurements for membrane characterization

The membrane performance depends on the membrane properties such as zeta potential (ZP), contact angle (CA) and functional groups. ZP shows the membrane surface charge. The ZP experiments were carried out in a flat-sheet tangential flow module (Susanto and Ulbricht, 2005). The contact angle (CA) was measured by using the static captive bubble method that has been described in another study by the authors. The functional groups, molecular structures and the surface chemistry of the membrane were investigated by the Attenuated Total Reflection infrared spectroscopy (ATR-IR) technique.

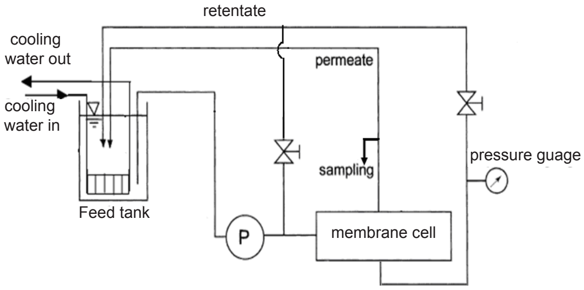


Fig.2: Flat-sheet cross-flow membrane cell used in this study

The ATR-IR spectroscopy measurement were done by using of a Bruker Equinox 55 instrument (Bruker Optics Inc., Billerica, MA, USA) equipped with a liquid nitrogen detector. All spectra were collected between 4000 and 400 1/cm, averaging 32 scans at a nominal instrument resolution of 4 1/cm using a diamond crystal; the temperature was 21 ± 1°C. A program written for the Opus software from Bruker was used to calculate difference spectra versus the corresponding background spectra. NaCl and MgSO4 salt solution were used for salt rejection tests. Sucrose (MW = 342 g/mole) and glucose (MW = 180 g/mole) were used for organic rejection tests.

RESULTS

Membrane characterization

Membrane characteristics have been discussed in another study by authors (Zazouli *et al.,* 2009); however, some results are shortly presented. Contact angle measurement showed that CA was 40.1±1.8 and 44.6±4.9 in the membranes TFC-SR2 and TFC-SR3, respectively. CA data indicated that both membranes had CA within the range of a hydrophilic material; however the membrane TFC-SR3 was slightly more hydrophobic than the membrane TFC-SR2. The measured permeability measurements showed that permeability were 7.5 and 2.1(L/m^2.h.bar) for membranes TFC-SR2 and TFC-SR3, respectively. Hence, the TFC-SR2 membrane was more permeable than TFC-SR3 membrane, but as shown in Table 1, it had lower solute rejection for all substances (the rejections of sodium chloride, magnesium sulfate, sucrose and glucose).

The ATR-IR spectra of two fresh membranes showed that the IR spectra of two membranes were basically similar; however, peak bands at 3200-3500 1/cm due to N-H stretch, 1640-1650 1/cm due to NH bend and 1720-1750 1/cm due to C=O stretch were significantly different.

The absorbance of TFC-SR2 membrane was higher than TFC-SR3. This may indicate that the content of polyamide on the surface of composite membrane was higher for TFC- SR2 than for TFC-SR3. Furthermore, the peak of secondary amide (amide II) at wavelength~1540 1/cm was not observed indicating that the membranes had been prepared from polyamide, which does not have secondary amine. Also, survey of the membranes Zeta potential ZP as function of pH showed that the surface of two NF membranes had negative charge with the absolute zeta potential value decreasing towards acidic pH values. The membrane TFC-SR3 was more negative charged than TFC-SR2.

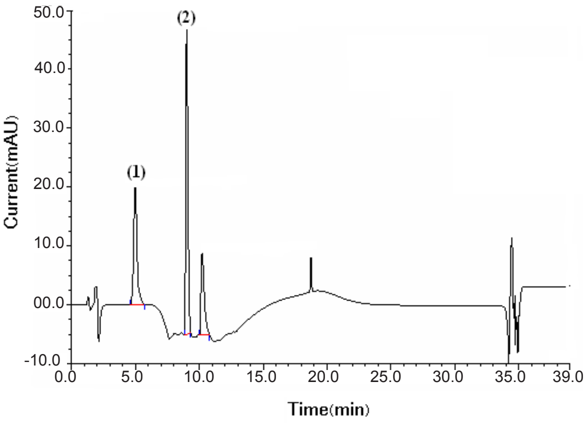
Table 1: Resuls of solutes rejection(R) by membranes (%)

Membrane	NaCl [a]	$MgSO_4$ [b]	Sucrose [c]	Glucose [c]
TFC-SR2	24	92	98	73
TFC-SR3	38	93	99	98

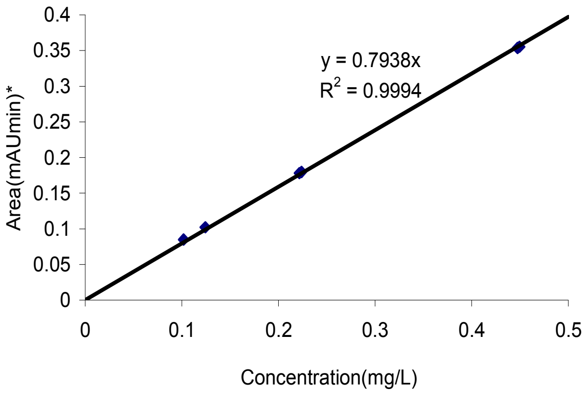
[a] Experimental condition: 10mM NaCl at 10 bar and pH = 7.5.
[b] $MgSO_4$ rejection as conductivity rejection: 5mM $MgSO_4$, 5 bar and pH = 7.5.
[c] Sucrose and glucose rejection as TOC rejection percentage: 1000 ppm, 5 bar and pH = 7.5.

HPLC chromatographic analysis

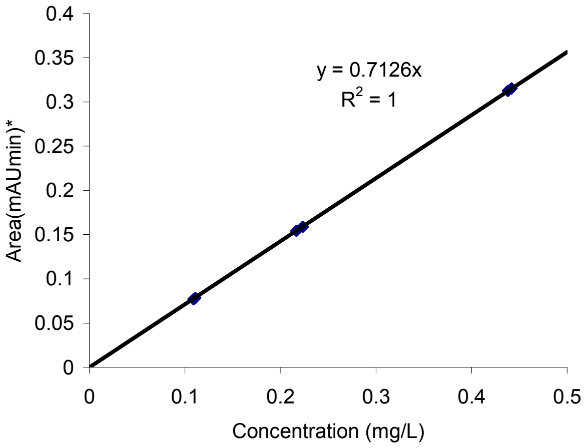
An alternative HPLC-UV method has been used for amoxicillin and cephalexin quantification .Results as typical chromatograms of the amoxicillin and cephalexin forms are shown in Fig. 3. As shown in Fig. 3, under described chromatographic conditions, the retention times for amoxicillin and cephalexin were about 4.8 min and 9 min, respectively. The calibration curve was linear over the range 0.1 mg/L to 0.5 mg/L. Regression coefficients were 0.999 and 1 for amoxicillin and cephalexin, respectively (Fig. 4).



ig. 3: Chromatogram obtained from a standard solution containing 500ppb cephalexin and amoxicillin. Peaks: 1= Amoxicillin; 2= Cephalexin.



A) Amoxicillin



B) Cephalexin

Fig. 4: Calibration curve (HPLC Correlation Coefficient) of cephalexin and amoxicillin

Rejection and permeate flux behavior in nanofiltration

Table 2 shows the influence of organic ratio on permeate concentration and rejection of amoxicillin and cephalexin for TFC-SR2 and TFC-SR3 NF membranes. It was observed that the rejection of amoxicillin and cephalexin in TFC-SR2 at pH=7.5 and 10 bar pressure was more than 97.3% and 95.8% in all experiments; respectively.

The rejection of amoxicillin and cephalexin in TFC-SR3 membrane was 95.9-100, 86.1-96.3 as percentage, respectively. The rejection efficiency of TFC-SR3 membrane was higher than TFC-SR2 membrane. It was also observed that the presence of organic matter in solution (except for amoxicillin solution with TFC-SR2 nanofiltration membrane) increased the antibiotic rejection for both NF membranes and two antibiotics. This effect was more pronounced with increasing

Table 2: Effect of organic matter ratio on amoxicillin and cephalexin rejection: 10 bar pressure; Initial concentration =500 ppb; Ion strength=10 mM NaCl

Membrane	**Antibiotics**	**No organics**	**HA**[a]	**70% HA+ 30% AA**	**50% HA+ 50% AA**	**30% HA+ 70% AA**	**AA**[b]
TFC-SR3	Amoxicillin	97.3	ND[c]	ND	ND	ND	ND
	Cephalexin	95.8	ND	ND	ND	ND	96.9
TFC-SR2	Amoxicillin	97.4	96.5	96.6	95.9	96	ND
	Cephalexin	86.1	89.1	93.6	94.7	95.2	96.3

[a] HA: Humic acid

[b] AA: Alginic acid

[c] ND: Not detected

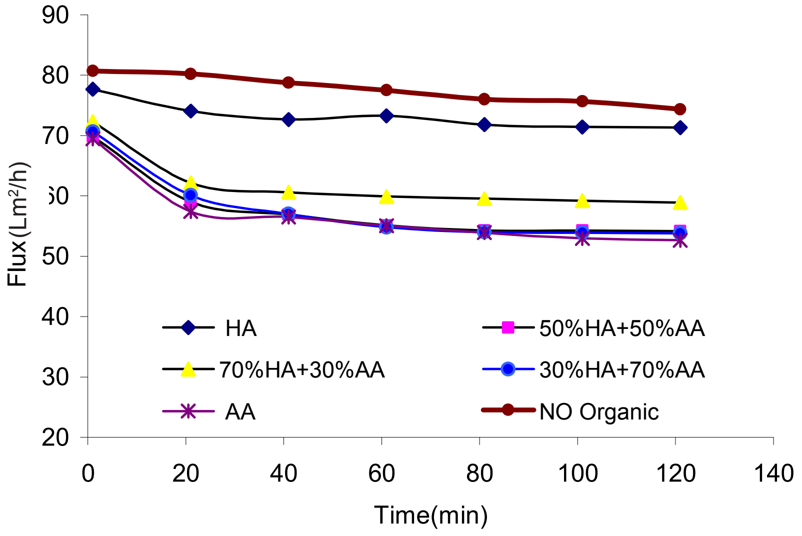


Fig. 5: Permeate flux behavior of TFC-SR2 membrane in presence of alginic acid and humic acid with various ratios (total 25 ppm); 500 ppb amoxicillin; 500 ppb cephalexin (pH = 7.5, 10 mM NaCl).

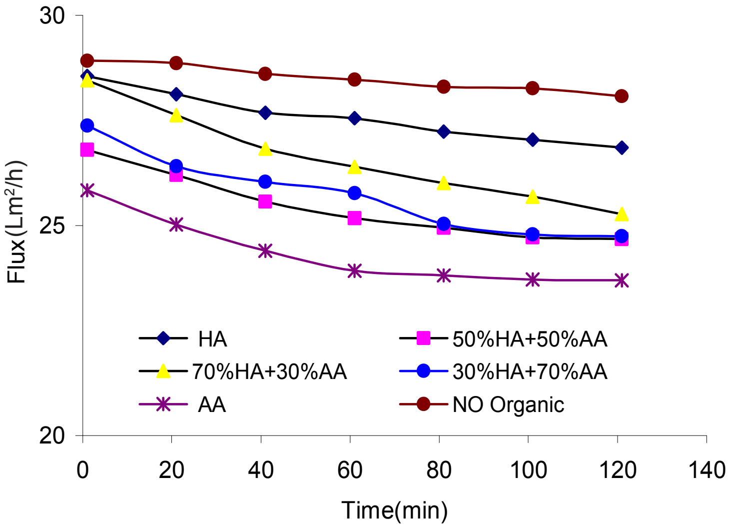


Fig. 6: Permeate flux behavior of TFC-SR3 membrane in presence of alginic acid and humic acid with various ratio (total 25 ppm); 500 ppb amoxicillin; 500 ppb cephalexin (pH = 7.5, 10 mM NaCl).

alginate ratio.

Figs. 5 and 6 show the influence of alginate and humic acid containing different mass ratios on permeate flux behavior vs. time for TFC-SR2 and TFC-SR3, respectively. It clearly shows that the flux decline was much more substantial at the higher mass ratio of alginate to humic acid. It was observed that the addition of alginate ratio decreased the permeate flux for both NF membranes. However, the decrease in flux was more pronounced for TFC-SR2 membranes.

DISCUSSION

Membrane characterization

The membrane characteristics as well as solution characteristics have the important effects on membrane performance. As stated and shown in Table1, the TFC-SR2 membrane was more permeable than TFC-SR3, but it had lower solute rejection for all substances used.

It seemed that there was trade-off between flux and rejection; however, for charge solute having molar mass higher than sodium chloride, $MgSO_4$, the difference in rejection was very small. Similar phenomenon was observed for no charge solute (glucose and sucrose): significant different rejection was observed for solute having smaller molar mass (glucose). Comparision of rejection data for $MgSO_4$ and glucose for TFC-SR2 membrane indicates that the rejection of solute was influenced not only by molar size, but also by solute charge. If the solute rejection was influenced only by solute size, the rejection of glucose should be higher than the rejection for $MgSO_4$. Such observation was not clearly found for TFC-SR3 membrane. Therefore, the effect of charge interaction on separation was significant for TFC-SR2 membrane.

In order to discuss the hyrophilicity of membrane, CA was measured. Results showed that both membranes had CA within the range of hydrophilic materials. Thus, these NF membranes should be hydrophilic with TFC-SR3 slightly more hydrophobic than TFC- SR2. The reason would be that the porosity of TFC-SR2 was higher than TFC-SR3, as noticed by its higher hydraulic permeability. But ATR-IR spectra confirmed that both membranes were made from the same material. The possible reason for this difference is the degree of swelling of the active layer of membrane that for TFC-SR2 was higher than the membrane TFC-SR3.

As stated in the results, zeta potential (ZP) of the membranes had negative charge with the absolute zeta potential value decreasing towards acidic pH values. This charge variation as function of pH is due to the dissociation of membrane functional groups, such as carboxylic and amide, and adsorption of hydroxide ion. All these effects may be influenced by solution pH. It should be noted that ZP data represent the effects of (macro)ion adsorption from solution as well as of dissociation of acidic or basic groups of (macro) ions on the surface. Charge interaction between such groups can modify the membrane pore size to some extent (Childress and Elimelech, 2000).

Rejection and permeate flux behavior

As shown in Table 2, the rejection efficiency of TFC-SR3 membrane was higher than TFC-SR2 membrane, because membrane SR3 has negative surface as zeta potential had shown. In addition, SR3 was less permeable than SR2. The presence of organic matter in solution (except for amoxicillin solution with TFC-SR2 nanofilter) increased the antibiotic rejection for both membranes and two antibiotics. This effect was more pronounced with increasing alginate ratio.

Figs. 5 and 6 clearly show that the flux decline was much more substantial at the higher mass ratio of alginate to humic acid and the addition of alginate ratio decreased the permeate flux for both NF membranes. However, the decrease in flux was more pronounced for TFC-SR2 with greater pore size. The alginate and humic acid might form solid layer (fouling layer) on the membrane surface and thus increased the permeate flow resistance; therefore, it might hinder the antibiotic transport through the membrane. The adsorption of organic foulants on the membrane surface may also increase the negative charge of the membrane surface, and thus increase the repulsion forces (Yoon *et al.,* 1998). Same trends was observed for humic acid fouling, however alginate fouling was significant than humic acid fouling. This result was in accordance with other studies (Lee *et al.,* 2006 and Tang *et al.,* 2007).

Figs. 5 and 6 also clearly show that individual alginate fouling was more detrimental than individual humic acid fouling, and alginate exhibited greater flux decline than humic acid alone at similar conditions. Moreover, amoxicillin and cephalexin rejection increased with increasing proportions of alginate (Table 2). As it was shown earlier, the much greater observed adhesion force with alginate than humic acid was due to the gel-forming nature of alginate, where intermolecular adhesion needs to be strong enough to sustain the structural integrity of the gel network. It has been reported that gelation of charged macromolecules by intermolecular bridging is predominant for hydrophilic organic macromolecules compared to hydrophobic organic macromolecules such as humic acids(Lee and Elimelech, 2006). In addition to the greater intermolecular adhesion of the alginate gel network, the larger size of alginate compared to humic acid may also be responsible for the formation of a thicker fouling layer on the membrane surface, and thus, more severe flux decline during fouling.

Similar results have been reported for algogenic organic matter (AOM) and Suwannee River humic acid(SRHA) fouling by Her *et al*. It was shown that a higher flux decline was observed with increasing proportions of AOM. The hydrophilic fraction was more AOM than SRHA (Her *et al.,* 2004). However, Li *et al* (2007) have reported that considerable synergistic effect may be observed when bovine serum albumin (BSA) and alginate were both present in solution. The larger foulant particle sizes measured in the co-existence of BSA and alginate, indicate formation of BSA-alginate aggregates, which resulted in greater fouling rates. Unlike BSA fouling, rapid membrane flux decline occurred immediately after alginate was introduced into the feed solution (Li *et al.,* 2007).

Lee and Elimelich have studied the fouling of reverse osmosis membrane by alginate and SRNOM (Suwannee River natural organic matter) and have reported that alginate (hydrophilic) fouling was more severe than SRNOM (hydrophobic) because the gelation of charged macromolecules by intermolecular bridging is predominant for alginate compared to SRNOM. Another reason for the formation of a thicker fouling layer on the membrane surface may be the larger size of alginate compared to SRNOM and, thus, more severe flux decline during fouling (Lee and Elimelech, 2006).

This study showed that alginate and humic acid have synergistic effect on flux decline. In other words, increasing alginate concentration increased the rate and extent of flux reduction. Also amoxicillin and cephalexin rejection almost increase with increasing proportions of alginate or hydrophilic fraction.

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