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Protein fouling in carbon nanotubes enhanced ultrafiltration membrane: fouling mechanism as a function of pH and ionic strength

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Abstract

The protein fouling behavior was investigated in the filtration of the multiwall carbon nanotube (MWCNT) composite membrane and commercial polyethersulfone ultrafiltration (PES-UF) membrane. The effect of solution chemistry such as pH and ionic strength on the protein fouling mechanism was systematically examined using filtration model such as complete pore blocking, intermediate pore blocking and cake layer formation. The results showed that the initial permeate flux pattern and fouling behavior of the MWCNT composite membrane were significantly influenced by pH and ionic strength while the effect of PES-UF membrane on flux was minimal. In a lysozyme (Lys) filtration, the severe pore blocking in the MWCNT membrane was made by the combined

effect of intra-foulant interaction (Lys-Lys) and electrostatic repulsion between the membrane surface and the foulant at pH 4.7 and 10.4, and increasing ionic strength where the foulant-foulant interaction and membrane-fouling interaction were weak. In a bovine serum albumin (BSA) filtration, severe pore blocking was reduced by less deposition *via* the electrostatic interaction between the membrane and foulant at pH 4.7 and 10.4 and increasing ionic strength, at which the interaction between the membrane and BSA became weak. For binary mixture filtration, the protein fouling mechanism was more dominantly affected by foulant-foulant interaction (Lys-BSA, Lys-Lys, and BSA-BSA) at pH 7.0 and increase in ionic strength. This research demonstrates that MWCNT membrane fouling can be alleviated by changing pH condition and ionic strength based on the foulant-foulant interaction and the electrostatic interaction between the membrane and foulant.

Keywords: Bovine serum albumin; Carbon nanotube membrane; Isoelectric point; Lysozyme; Protein fouling; Solution chemistry

Abbreviation

BSA: Bovine serum albumin; CNTs: Carbon nanotubes; DI: Deionized; EfOMs: Effluent organic matters; IEP: Isoelectric point; Lys: Lysozyme; MWCO: Molecular weight cut-off; NF: Nanofiltration; NOM: Natural organic matter; PES: Polyethersulfone; UF: Ultrafiltration; UPW: Ultrapure water

1. Introduction

Ultrafiltration (UF)/nanofiltration (NF) membrane technologies have been increasingly used in the secondary effluent from wastewater treatment plant as well as been examined for whey protein fractionation on a laboratory scale [1-3], bioseparation [4-6] and cation macromolecule recovery in bioprocess [7] due to its superior separation performance. However, such applications appear to have several drawbacks in treating the feed waters containing effluent organic matters (EfOMs) such as

protein, polysaccharides, humic and fulvic acids [8]. Protein fouling in UF membrane is attributed to proteins adsorption/deposition on the membrane surface or inside the pores *via* pore blocking and/or cake layer formation, causing significant flux decline, increased chemical cleaning, and membrane replacement [9].

The separation performance of UF/NF membrane can be significantly influenced by the solution chemistry such as ionic strength, concentration and solution pH [10-12]. This is mainly due to the fact that ionic strength and solution pH strongly influence membrane surface and protein interaction, protein and protein interaction by shifting isoelectric point of protein and charge of the protein [13]. Thus, ionic compounds contained in wastewater lead to accelerating membrane fouling when the foulants are oppositely charged with the membrane and intensive chemical cleaning [14]. There have been numerous studies examining the effects of solution chemistry (ionic strength and solution pH) and model foulant in separation performance of UF/NF membrane [15-17]. Conventional polymeric membranes experience severe fouling by negatively charged protein at near to its isoelectric point (IEP) due to the decreased electrostatic repulsion between foulant-foulant, and foulant-membrane surface [18, 19]. Further, high ionic strength can induce membrane fouling due to electrical double layer compression effect on the foulants [20, 21]. Such effects of solution chemistry on protein adsorption on the membrane surface and pores are governed by electrostatic interaction/repulsion [22], indicating that the protein fouling in the UF membrane could be reduced by controlling the electrostatic interaction [23, 24]. Like negatively charged polymeric membranes, positively charged conventional membrane showed reduced fouling to the same charged proteins (Lys) mostly due to the less adsorption on the membrane surface *via* electrostatic repulsion [25].

Recent progress on carbon nanotubes (CNTs) membrane fouling reveals protein-fouling resistance due to its modified surface properties [26-28]. Celik et al. [29] also studied the protein fouling behavior in CNTs composite membrane at different solution pHs and membrane properties (such as hydrophilicity). The challenge in protein fouling stimulated further development on positively

charged nanohybrid UF membranes having antifouling properties due to electrostatic interaction with foulants. For example, poly (arylene ether sulfone) (PAES) block copolymers with carboxyl (COOH) functionalized CNTs incorporated UF hybrid membrane exhibited relatively low protein adsorption and irreversible protein fouling by increasing hydration of membrane surface and reducing the net charge density [30]. Further, currently fabricated positively charged graphene oxide (GO) nanosheet UF membrane presented enhanced antifouling properties due to the combined effect of hydrophilicity, surface charge and morphology of the membrane at different pH [31]. Recently fabricated CNTs/PANI composite membrane shows superior performance in natural organic matter (NOM) removal [32]. This membrane has unique physicochemical characteristics by incorporating the MWCNTs/PANI complex to UF membrane. It is positively charged below pH 9.2 (IEP), and removes NOM *via* electrostatic interaction with negatively charged NOM.

As such there have been numerous studies on protein fouling behavior in the positively charged membranes [33-35]. However, in-depth study on the influence of solution chemistry on protein fouling mechanism in a positively charged nanocomposite membrane has not reported in the literature. Therefore, this study is focused on the fouling behavior caused by positively/negatively charged protein on the positively charged membrane (CNT/PANI membrane) under different ionic strengths and solution pHs. It would give useful information to the wide application of CNT engineered membrane for wastewater reclamation and bioseparation.

This study aims to i) investigate the influence of feed solution chemistry on protein fouling behavior in the positively charged membrane: flux pattern under different pHs and ionic strengths, and ii) evaluate protein fouling mechanism using filtration models.

2. Materials and methods

2.1 Materials

2.1.1 Model protein foulants and chemicals

Bovine serum albumin (BSA) and Lysozyme (Lys) were used as model protein foulants. BSA (powder, Sigma-Aldrich) was used as the negatively charged model protein foulant. Its molecular weight (MW) is reported to be 67 kDa, and isoelectric point (IEP) is 4.7. Lys (powder, Sigma-Aldrich) was selected as the positively charged protein foulant. Its MW is reported to be 14.3 kDa, and IEP is 10.4. Each stock solution was prepared at a concentration of 1 g/L and stored in a glass bottle at 4 °C prior to use. The pH was adjusted using 1M-HCl and 1M-NaOH. The ionic strength of solution was adjusted by adding different concentrations of NaCl (VWR, ACS reagent). All stock solutions were prepared by ultrapure water (conductivity = 0.055 $\mu\text{S}/\text{cm}$).

2.1.2 Membranes

In this study, a multiwall CNT (MWCNT) enhanced membrane [32] was tested and compared with a commercial polyethersulfone (PES) membrane. Fabrication procedure of MWCNTs enhanced membrane can be found in a previous study [32]. Prior to use, all membranes were soaked in deionized (DI) water for 1-2 d to remove any impurities. Characteristics of membranes used in this study are summarized in **Table 1**.

2.2 Fouling experiments

The protein fouling experiments were carried out in a dead-end filtration mode under 2 bar for 12 h. The effective membrane area was 0.00146 m². Constant pressure was applied during the filtration test using a compressed nitrogen gas cylinder. The flux data were continuously acquired using an electronic balance connected to a computer. Each feed solution was prepared under the following conditions:

- Concentration: BSA = 10 mg/L, Lys = 10 mg/L, and BSA-Lys mixture = 20 mg/L (10mg/L BSA + 10mg/L Lys)
- pH: 4.7, 7.0, and 10.4
- Ionic strength: NaCl in the concentration of 0.001, 0.01, and 0.1 M

2.3 Membrane characterization

The zeta potential of membrane surface was measured by using the streaming potential technique (Anton Paar electrokinetic analyzer). The zeta potential measurements were carried out as a function of pH (4.0-11.0) and ionic strength (with 0.001, 0.005, and 0.010 M KCl at pH 7.0).

2.4 Membrane fouling model

Protein fouling mechanism of the MWCNT membrane by protein was examined. Three types of membrane fouling model were used to explain the flux decline with protein deposition during membrane filtration (complete and intermediate blocking, and cake layer formation) [36, 37]. The equations of each fouling models are summarized in **Table 2**.

In these equations, t is the filtration time, V is the cumulative permeate volume, A is the effective filtration area (0.00146 m^2), J_0 is the initial flux, k_b (1/s), k_i (1/m) and k_c (s/m^2) are the coefficients of complete blocking, standard blocking, intermediate blocking, and cake filtration models, respectively. From these equations, three data plots have been proposed where the linearity of the filtration data in the plot of $d(V/A)/dt$ vs V/A , $t/(V/A)$ vs t , $dt/d(V/A)$ vs t and $t/(V/A)$ vs V/A offers proof of the complete blocking, intermediate blocking, and cake filtration model, respectively. Two pore blocking models (complete and intermediate) were applied at the initial stage of filtration (~2 h) because those two blocking models are expected to be dominant in both the MWCNT and PES-UF membranes with bigger MWCO than the two model proteins. At the later stage (2 – 12 h), cake layer filtration was applied. The dominant filtration model was determined by comparing correlation coefficient (R^2). The fouling potential of both membranes at different solution chemistry was

examined by comparing the fouling coefficients (K_b , K_i and K_c), which were derived from those equations.

3. Results and discussion

3.1 Effects of solution chemistry on surface charge of the membranes

It is essential to study the surface charge of the membrane for an understanding of protein fouling mechanisms on membranes. Zeta potentials of both MWCNT and PES membranes were analyzed by streaming potential measurement in order to evaluate the effect of solution chemistry on the membrane surface charge. **Fig. 1(a)** shows the zeta potential of the MWCNT membrane, which varies from positive to negative value depending on the pH. The zeta potential of the MWCNTs membrane was shown to be highly positive (17.12 mV) under acidic condition (pH 4.7). Then, it slightly decreased to 10.9 mV when pH increased to 7.0. However, the membrane shifted to be a negative charge (-25 mV) at pH 10.4. Thus, it is noted that the isoelectric point (IEP) of the MWCNT membrane was 9.2 as shown in **Table 1**. On the other hand, the zeta potential of the PES-UF membrane remained in a negative value over the entire pH range, although its negative value slightly decreased (from -35 to -20 mV) as the pH decreased (pH 10.4 to 4.7). In addition to pH, ionic strength reduced the zeta potential of the MWCNT membrane as shown in **Fig. 1(b)**. The zeta potential of the MWCNT membrane dramatically dropped to the nearly zero (0) mV as the electrolyte (KCl) concentration increased to 0.01 M, indicating that the surface charge of the MWCNT membrane was strongly affected by ionic strength. Electrolyte ions could reduce the membrane surface charge density *via* charge neutralization. In contrast, there was no significant change in the negatively charged PES-UF membrane with the increase in electrolyte concentration. Meanwhile, the zeta potential at 0.1 M KCl could not be measured due to the limit of the equipment used. It is assumed that the zeta potential at 0.1 M would be almost zero (0) or near to negative.

Overall, solution chemistry, such as pH and ionic strength of feed solution, significantly influenced the surface charge of the MWCNT membrane while PES-UF membrane maintained being negatively charged regardless of solution chemistry.

3.2 Effects of pH on permeate flux with single proteins

3.2.1 MWCNT membrane

Fig. 2 shows the flux decline of MWCNT and PES-UF membrane filtration with Lys under different pH levels. Overall, the MWCNT membrane was found to be sensitive to the feed properties both in the beginning of filtration and the corresponding filtration process. A rapid permeate flux decline was observed at the initial stage of the MWCNT membrane filtration with Lys, which is associated with the membrane pore size. In addition, flux pattern of the MWCNT membrane was considerably affected by pH. It is noted that the IEP of Lys is pH 10.4, which indicates that the charge of Lys is neutralized at pH 10.4. As can be seen in **Fig. 2 (a)**, permeate flux was shown in the following order: pH 4.7 > pH 10.4 > pH 7.0. At pH 4.7, the initial permeate flux of MWCNT membrane was higher, and it was almost six times (from 50 to 340 LMH/bar) higher than that in pH 7.0. The high permeate flux at pH 4.7 in MWCNT membrane filtration may be due to less Lys adsorption on the membrane surface by the increased electrostatic repulsion between more positively charged membrane surface and positively charged Lys. Since MW of Lys (14.3kDa) was slightly larger than the pore size (10kDa) of the MWCNT membrane, feed solution with Lys had a possibility to block the pore of the membrane affecting the water transport through the membrane. In particular, at pH 4.7, the MWCNT membrane becomes more positively charged (17.12 mV) as shown in **Fig. 1(a)**, resulting in an increased electrostatic repulsion between membrane and Lys.

The higher flux at pH 10.4 than pH 7.0 can be explained by the foulant intermolecular interaction. As mentioned in the earlier section, the IEP is the pH at which a particular molecule carries no net electrical charge. The net charge on the molecule is affected by pH of its surrounding

environment as the solution and can become more positively or negatively charged due to the gain or loss of protons (H^+). The IEP value can affect the solubility of a molecule at a given pH. Such molecules have a minimum solubility in water or salt solutions at the pH that corresponds to their IEP and often precipitates out of solution. Hence, the weakened electrostatic repulsion between the membrane and Lys at pH 10.4 led to a higher flux of MWCNT membrane compared to at pH 7.0. This is probably due to the more aggregation between Lys molecules. It indicates that intermolecular interaction (Lys-Lys) could play a major role in permeate flux pattern in addition to the interaction between membrane and foulant.

In the case of negatively charged protein (BSA), the BSA adsorption on the surface of membranes with a much smaller pore size (MW of BSA = 67kDa vs. membrane pore size = 10 kDa) caused a rapid decline in permeate flux of the MWCNT membrane at the initial stage of filtration (**Fig. 2(c)**). Based on the flux decline, fouling by BSA on MWCNT membrane was less severe compared to that by Lys. BSA adsorption caused severe flux decline at around pH 7.0 where both the membrane and BSA are oppositely charged. However, higher fluxes were observed at pH 10.4 where the membrane is negatively charged and at the IEP of BSA (pH 4.7). As shown in **Fig. 2(c)**, the permeate flux in MWCNT membrane was different depending on pH, and it had the following order: pH 10.4 > pH 4.7 > pH 7.0. Such a result could be interpreted due to the shift of electrostatic interaction between BSA and membrane. At below pH 10.4, the surface charge of the MWCNTs membrane shifted to negative charge while BSA was still negatively charged. Thus, BSA was less adsorbed on the membrane surface due to the electrostatic repulsion between BSA and membrane, indicating that BSA in feed solution at pH 10.4 did not seriously hinder the water transport. The comparable flux was observed at pH 4.7. This may be due to the zero charged BSA at its IEP. Similar as Lys, weakened interactions between BSA molecules by neutralized charge resulted in less adsorption of BSA-BSA foulant on the membrane surface. BSA molecules were aggregated each other, rather than adsorption of BSA onto the membrane.

3.2.2 PES-UF membrane

Permeate flux in the PES-UF membrane filtration was almost similar even at different pH ranges. However, there was no flux difference by pH level from 4.7 to 10.4 in the PES-UF membrane (the initial flux was around 70 LMH/bar). There was no noticeable change by different pH. It may be interpreted that the electrostatic interaction between the membrane and Lys particle may not be the critical factor. Meanwhile, the relatively higher cake layer formation was observed at pH 7.0 while it was slightly reduced at IEP of Lys (pH 10.4). It is mostly due to the increase in the deposition of the neutrally charged Lys at the later stage of filtration. Consequently, the foulant-foulant interaction may have an important role in fouling in the PES-UF membrane filtration. Based on the flux decline and fouling potential, the PES-UF membrane fouling is more affected by electrostatic repulsion with negatively charged protein (BSA).

3.3 Effect of ionic strength on permeate flux with single protein

3.3.1 MWCNT membrane

With positively charged protein (Lys), the permeate flux in the MWCNT membrane was also significantly influenced by ionic strength (in terms of NaCl) at pH 7.0. It is noted that at pH 7.0, MWCNT membrane charged positively and the charge of membrane gradually decrease to nearly zero as ionic strength decreases to 0.01 M. As can be seen in **Fig. 3(a)**, the permeate flux of MWCNT membrane when 0.001 and 0.01 M of NaCl were added to the solution, was much higher than zero ionic strength (without NaCl addition), and it was rather slightly increased in the presence of 0.1M of NaCl. Slightly lower permeate flux at higher ion strength can be explained by the concentration polarization near to membrane. Overall, the low flux of the MWCNT membrane at pH 7.0 (**Fig. 2**) was overcome in the presence of ionic strength (0.001–0.1 M). Such a flux enhancement may appear due to the charge shielding effect of chloride ion (Cl^-) to the positive charged Lys (Lys^+). It probably prevented deposition of Lys on the pores and surface of MWCNT membrane.

It may appear probably due to the effect of ionic strength on Lys particles. Due to the presence of ionic strength, Lys-Lys interaction decreased *via* increasing repulsion hydrating forces between Lys-Lys [10]. Further, Cl^- may interfere the interaction between the membrane and foulant by weakening surface charge of both membrane and foulant, leading to fouling mitigation. It demonstrates that ionic strength mainly lessened fouling potential *via* charge shielding effect on the model proteins. The increased fouling potential at the increasing ionic strength can be explained according to the interaction between both neutralized foulants and the membrane surface. Under increasing ionic strength, both the membrane and Lys particle became neutralized, leading to the increase in Lys deposition on the membrane surface.

Overall, ionic strength seemed to contribute considerably to permeate flux enhancement in the filtering of BSA with MWCNT membrane, but no significant difference was observed at the initial stage of filtration when the concentration of NaCl increased from 0.001 to 0.1 M. Then, there were minimal changes as increasing ionic strength at the final stage of filtration. As shown in **Fig. 3(c)**, permeate flux increased three times at the initial stage (2 h), continuing to have a stable flux for the rest filtration period (100-130 LMH/bar) when 0.001 – 0.1 M NaCl was added.

3.3.2 PES-UF membrane

In contrast to the MWCNT membrane, a different trend was observed in flux behavior of the PES-UF membrane by Lys fouling. The flux without ionic strength at pH 7.0 increased when 0.01 M of NaCl was added, but it showed a decreased flux with 0.001 and 0.1 M of NaCl. This indicates that there presented the critical point to neutralize the charge of Lys and PES-UF membrane. Ionic strength (NaCl) affected the charge of both Lys and membrane, especially negatively charged PES-UF membrane. However, at relatively high or low ionic strength, residual ions (Na^+ and Cl^-) after their reaction with Lys and membrane affected again to them. Thus, it is assumed that flux decline behavior in the PES-UF membrane is governed by complex mechanism due to the altered surface

charge of both membrane and protein at different ionic strengths, which affects the interaction between protein and the membrane.

Fig. 3(d) shows that the permeate flux in BSA filtration increased by nearly 30 % at the initial stage (2 h) as ionic strength increased, but it remained nearly same over the whole filtration period (12 h). It indicates that ionic strength did not significantly influence BSA fouling on the PES-UF membrane surface at the final stage of filtration. The result is in agreement with previous research presenting that increase in ionic strength can enhance fouling due to decrease in electrostatic repulsion via hydration repulsion forces between BSA-BSA and membrane-BSA [10].

3.4 Effect of solution chemistry on permeate flux with binary mixture

3.4.1 Permeate flux pattern as a function of pH

3.4.1.1 MWCNT membrane

Fig. 4 shows the permeability MWCNT membrane in filtering mixture of two positively/negatively charged proteins (Lys + BSA). Overall, extremely low flux pattern was observed in the filtration with MWCNT membrane compared to that with a single protein. Interestingly, an opposite trend - the lowest flux in the vicinity of nearly IEP of each model protein was observed in permeate flux under protein mixture filtration test, compared to those in the single protein filtration. As seen in **Fig 4 (a)**, the permeate flux exhibited a considerable decrease in flux pattern (10 LMH/bar) at pH 4.7 and 10.4 compared to that (80 LMH/bar) at pH 7.0.

Apart from contributing to membrane transport *via* electrostatic interaction (repulsion) between single proteins and membrane surface, mixing of oppositely charged proteins seemed to generate more complicated intermolecular interactions between macro solutes (Lys-BSA, Lys-Lys, BSA-BSA) and charged membrane surface. Based on the fouling and permeate flux behavior at different pH, protein mixture fouling mechanism tends to be mainly governed by the intermolecular interaction

(Lys-BSA). At pH 7.0, Lys-BSA mixture may agglomerate by intermolecular interaction in the feed solution. It decreased the deposition of the binary mixture on the membrane surface, leading to fouling alleviation, which is comparable to the BSA filtration. However, at pH 4.7 and 10.4 where each of proteins is neutrally charged, the proteins deposited on the membrane, then induced the deposition of the counter charged proteins by bridge effect.

3.4.1.2 PES-UF membrane

In contrast, negatively charged PES-UF membrane showed the lowest permeate flux (20 LMH/bar) at pH 7.0 while the permeate flux was improved at pH 10.4 and 4.7 (to 24 and 43 LMH/bar, respectively) (**Fig. 4(b)**). It indicates that oppositely charged foulants were easily adsorbed on the membrane surface, leading to the severe pore blocking at the initial stage. The fouling at the initial stage was significantly alleviated at pH 4.7 and 10.4, at which each protein is neutralized. It means that the interaction between the neutralized protein, and charge membrane and counter protein may reduce pore blocking at the initial stage, but it promoted cake layer formation at the later stage *via* bridge effect, particularly at pH 4.7 where the membrane and model protein are oppositely charged.

3.4.2 Permeate flux pattern as a function of ionic strength

3.4.2.1 MWCNT membrane

Similar to the effect of pH, ionic strength also caused extremely low flux pattern in the filtering of protein mixture with MWCNT membrane. As seen in **Fig 4 (c)**, permeate flux of the MWCNT membrane remained at 80 LMH/bar for 12 h in the absence of NaCl. However, at the low concentration (0.001-0.01 M), flux decreased significantly to 20-27 LMH/bar, and then was recovered to that without NaCl. Such a trend in the presence of binary mixture is opposite to that of a single protein. It may be due to the charge shielding effect of NaCl ion, which probably had a negative impact on flux decline behavior. The intermolecular interaction (Lys-BSA, Lys-Lys and

BSA-BSA) may contribute to protein aggregation on the membrane surface, leading to an extremely low permeate flux behavior in the MWCNT membrane.

The addition of 0.001 M NaCl led to severe pore blocking and cake layer formation by reducing Lys-BSA interaction. However, the membrane fouling was reduced gradually when ionic strength increased to 0.1 M. It is probably due to the charge shielding effect of NaCl ion on the two oppositely charged proteins and the membrane surface, and it reduced the membrane-foulant interaction. Such effects may induce protein adsorption at a low concentration of NaCl, and then tends to prevent deposition of binary protein particles on the membrane surface *via* charge shielding effect.

3.4.2.2 PES-UF membrane

An opposite trend was observed in the filtration of the PES-UF membrane with protein mixture solution compared with that of the MWCNT membrane. **Fig. 4(d)** shows that there seemed to have a slight flux decline in the presence of 0.001 M NaCl at the initial filtration period. However, as ionic strength increased up to 0.1 M, the permeate flux increased nearly 1.5-fold (from 17 to 27 LMH/bar) as a function of ionic strength. The permeate flux with protein mixture seemed to decrease in the presence of 0.001 M NaCl, but it gradually increased as the ionic strength increased to 0.1 M. Further, severe flux decline at the initial stage was alleviated by the increase in ionic strength. It indicates that combined effect of membrane-foulant and foulant-foulant interaction which resulting in a serious fouling at pH 7.0 was reduced by ionic strength *via* charge shielding effect on the foulant and the membranes. These results are in agreement with a previous study that increase in ionic strength can enhance the permeate flux by suppressing Lys-BSA interaction [11].

Overall, a linear trend in permeate flux was observed as a function of ionic strength, compared to the irregularly increased flux pattern under single protein filtration test (Lys and BSA are individually added) (**Figs. 3(b) and 3(d)**). With protein mixture, the charge-shielding effect seemed to contribute

to the flux enhancement of the PES-UF membrane in the binary mixture filtration presumably due to the different mechanisms of negatively charged membrane.

3.5 Fouling mechanisms under solution chemistry

Filtration models were used to explaining MWCNT filtration with two proteins (Lys and BSA) both at initial (~2h) and final (2-24h) stages of filtration. The fouling potential as K_b , K_i and K_c was compared in each membrane filtration under different solution chemistry condition. Detailed are presented in supporting information (**Table S1** and **S2**). Overall, pore blocking (complete and intermediate) at the initial stage and cake layer formation at the final stage were dominant during MWCNTs filtration both with individual Lys and BSA solution, and protein mixture under different pHs and ionic strengths.

Based on the fouling potential estimated by these two fouling models and change of zeta potential at different pHs and ionic strengths in the MWCNT membrane and protein, fouling mechanisms can be explained by the electrostatic interaction between the proteins and the membrane surface and intermolecular interaction (Lys-Lys, BSA-BSA and Lys-BSA). The dominant organic matter removal mechanism was found to be increased by the electrostatic interaction between charged model compound and positively charged membrane surface [32]. Current research also reports that membrane fouling can be determined by the surface chemistry such as foulant-membrane interactions [38]. Thus, protein fouling can be explained by the pH and ionic strength influence on the electrostatic interaction between positively charged MWCNT membrane and both charged proteins (BSA and Lys).

The effects of charged proteins and solution chemistry such as pH and ionic strength on fouling mechanisms were examined by comparing coefficients from filtration model and flux decline behavior.

3.5.1 MWCNT membrane (Fig. 5)

a. For a same charged protein (Lys) filtration in the MWCNT membrane, the membrane fouling was found to be more influenced by intrafoulant interaction (Lys-Lys) based on the effect of increasing ionic strength on the flux decline and fouling mechanisms. For the effect of pH, at pH 4.7 the fouling alleviation was achieved due to the increased electrostatic repulsion between the oppositely charged membrane surface and the foulants. At pH 10.4, Lys aggregation due to the neutralized particle reduced membrane fouling compared to at pH 7.0 thus higher flux was achieved at IEP of Lys (pH 10.4). Even if electrostatic repulsion still exists at pH 7.0, highly hydrated Lys may decline permeate flux, resulting in severe membrane fouling.

b. For an oppositely charged protein (BSA) filtration in the MWCNT membrane, the membrane fouling was more governed by the electrostatic interaction between the membrane surface and the foulants according to the effects of pH and ionic strength. The membrane fouling was significantly alleviated at pH 4.7 and 10.4 at which the electrostatic interaction between the membrane and BSA weakened. Increasing ionic strength also reduced membrane fouling *via* charge shielding effect, but still higher than at different pH, demonstrating that the foulant-foulant attraction may have a less significant contribution to the membrane fouling than the membrane-fouling attraction.

c. For binary mixture filtration test in the MWCNT membrane, the foulant-foulant interaction was found to affect its fouling mechanisms dominantly. At pH 7.0 at which oppositely charged proteins are neutralized by agglomeration, fouling was alleviated. However, at pH 4.7 and 10.4, the neutralized protein deposited on the membrane surface, inducing deposition of counter charged proteins *via* bridging effect. Thus, severe fouling was observed. It demonstrates that foulant-foulant interaction played a major role under different pH condition. Meanwhile, NaCl addition seemed to promote binary proteins deposition on the membrane surface by reducing intermolecular reaction (Lys-BSA). However, as the ionic strength increased, the protein deposition was reduced by charge shielding effect on the foulant-foulant and membrane-foulant interactions.

3.5.2 PES-UF membrane (Fig. 6)

a. For an oppositely charged protein (Lys) filtration in the PES-UF membrane, combined effect of membrane-foulant and foulant-foulant interactions was found to control the fouling mechanism at different pH. Particularly, at different ionic strength, membrane-foulant interaction played a major role in fouling *via* charge shielding effect on the protein and membrane surface. Different solution chemistry mainly affected building up the cake layer at the later stage of filtration.

b. For an oppositely charged protein (BSA) filtration in the PES-UF membrane, fouling was mainly governed by the membrane-fouling interaction at different solution chemistry. Severe pore blocking at the initial stage and cake layer formation at the later stage at pH 4.7 was alleviated at pH 7.0 and 10.4 due to the increased electrostatic repulsion. However, increase in ionic strength induced fouling due to the decreased electrostatic repulsion *via* charge shielding effect.

c. For binary mixture filtration test in the PES-UF membrane, combined effect of membrane-foulant and foulant-foulant interaction played an important role in fouling. Different pH affected oppositely charged foulants and membrane. It led to severe pore blocking at pH 7.0 via electrostatic interaction between the membrane and foulants. At pH 4.7, at which neutralized protein and oppositely charged protein promoted building up the cake layer at the later stage. Severe fouling was alleviated by an increase in ionic strength due to charge shielding effect.

4. Conclusion

The fouling behavior of positively (Lys) and negatively charged (BSA) proteins was observed in the positively charged MWCNT membrane with high sensitivity to the pH and ionic strength of the feed solution. Overall, the MWCNT membrane with high sensitivity to the feed properties considerably affected the initial flux and fouling behaviour.

- The permeate flux of the MWCNT membrane was found to be strongly influenced by pH and ionic strength under single protein filtration test. In comparison, solution chemistry did not affect considerably the permeate flux in PES-UF membrane. Although severe flux decline behavior appeared on the MWCNT membrane at the initial stage of filtration, overall permeate flux outperformed the PES-UF membrane due to the extremely high flux of the MWCNT membrane. Further, ionic strength resulted in flux enhancement in single protein filtration with the MWCNT membrane. Meanwhile, protein mixture filtration had different permeate flux behavior in both membranes.
- For the MWCNT membrane filtration, fouling by Lys was controlled by combined effect of membrane-foulant and foulant-foulant interaction at different solution chemistry. Severe pore blocking was alleviated at pH 4.7 and increase in ionic strength, at which electrostatic interaction increased and Lys became aggregated due to charge shielding effect. Severe pore blocking by BSA was alleviated at pH 4.7 and 10.4 where electrostatic interaction decreased, and an increase in ionic strength due to charge shielding effect. Intermolecular interaction (BSA-Lys) dominantly affected the MWCNT membrane fouling mechanism at different pHs, Meanwhile, in the presence of ionic strength, intermolecular interaction mainly controlled the membrane fouling, but as ionic strength increased, both the foulant-foulant and membrane-foulant interaction by charge shielding effect became a dominant factor in the fouling mechanism.
- For the PES-UF membrane filtration, severe pore blocking by single proteins was dominantly controlled by the interaction with foulants. However, in a protein mixture filtration, severe cake layer formation was developed at pH, and it was alleviated by combined effect of membrane-foulant and foulant-foulant interaction.
- Severe flux decline and fouling, despite of very high initial flux in the MWCNT membrane could be significantly alleviated by controlling solution pH and chemistry. It is suggested that

the MWCNT membrane sensitive to pH and ionic strength can be widely applied in the wastewater reclamation.

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References

1. S. Bhattacharjee, et al., Studies on the fractionation of β -lactoglobulin from casein whey using ultrafiltration and ion-exchange membrane chromatography. *J. Membr. Sci.* 275(2006) 141-150.
2. G. Konrad and T. Kleinschmidt, A new method for isolation of native α -lactalbumin from sweet whey. *International Dairy Journal*. 18(2008) 47-54.
3. B. Cheang and A.L. Zydney, A two-stage ultrafiltration process for fractionation of whey protein isolate. *J. Membr. Sci.* 231(2004) 159-167.
4. D. Yu, et al., Purification of monoclonal antibody from tobacco extract using membrane-based bioseparation techniques. *J. Membr. Sci.* 323(2008) 159-166.
5. A.L. Zydney and R. van Reis, 5.40 - *Bioseparations: Membrane Processes*, in *Comprehensive Biotechnology (Second Edition)*, M.-Y. Editor-in-Chief: Murray, Editor. 2011, Academic Press: Burlington. 499-520.
6. L. Wang, et al., Purification of humanized monoclonal antibodies by membrane-based hybrid bioseparation technique. *Journal of Immunological Methods*. 314(2006) 1-8.
7. S.Y. Cheng, et al., Positively charged nanofiltration membranes: Review of current fabrication methods and introduction of a novel approach. *Advances in Colloid and Interface Science*. 164(2011) 12-20.
8. M. Rebhun and J. Manka, Classification of organics in secondary effluents. *Environ. Sci. Technol.* 5(1971) 606-&.
9. A.D. Marshall, et al., The Effect of Protein Fouling in Microfiltration and Ultrafiltration on Permeate Flux, Protein Retention and Selectivity - A Literature-Review. *Desalination*. 91(1993) 65-108.

10. R. Miao, et al., Enhancement and Mitigation Mechanisms of Protein Fouling of Ultrafiltration Membranes under Different Ionic Strengths. *Environ. Sci. Technol.*(2015).
11. Y.-N. Wang and C.Y. Tang, Fouling of Nanofiltration, Reverse Osmosis, and Ultrafiltration Membranes by Protein Mixtures: The Role of Inter-Foulant-Species Interaction. *Environ. Sci. Technol.* 45(2011) 6373-6379.
12. S.P. Palecek and A.L. Zydney, Intermolecular electrostatic interactions and their effect on flux and protein deposition during protein filtration. *Biotechnology Progress.* 10(1994) 207-213.
13. G. Singh and L.F. Song, Quantifying the effect of ionic strength on colloidal fouling potential in membrane filtration. *J. Colloid Interface Sci.* 284(2005) 630-638.
14. Y. Yu, et al., Effect of solution chemistry on organic fouling of reverse osmosis membranes in seawater desalination. *J. Membr. Sci.* 351(2010) 205-213.
15. R. Chan, et al., Quantitative analysis of membrane fouling by protein mixtures using MALDI-MS. *Biotechnology and Bioengineering.* 85(2004) 190-201.
16. M. Nilsson, et al., The influence of pH, salt and temperature on nanofiltration performance. *J. Membr. Sci.* 312(2008) 97-106.
17. L. Palacio, et al., Fouling with protein mixtures in microfiltration: BSA-lysozyme and BSA-pepsin. *J. Membr. Sci.* 222(2003) 41-51.
18. Y.N. Wang and C.Y.Y. Tang, Protein fouling of nanofiltration, reverse osmosis, and ultrafiltration membranes-The role of hydrodynamic conditions, solution chemistry, and membrane properties. *J. Membr. Sci.* 376(2011) 275-282.
19. W.S. Ang and M. Elimelech, Protein (BSA) fouling of reverse osmosis membranes: Implications for wastewater reclamation. *J. Membr. Sci.* 296(2007) 83-92.
20. Q. She, et al., The role of hydrodynamic conditions and solution chemistry on protein fouling during ultrafiltration. *Desalination.* 249(2009) 1079-1087.
21. H. Mo, et al., Fouling of reverse osmosis membrane by protein (BSA): Effects of pH, calcium, magnesium, ionic strength and temperature. *J. Membr. Sci.* 315(2008) 28-35.
22. K.L. Jones and C.R. O'Melia, Protein and humic acid adsorption onto hydrophilic membrane surfaces: effects of pH and ionic strength. *J. Membr. Sci.* 165(2000) 31-46.
23. M.M. Rohani and A.L. Zydney, Role of electrostatic interactions during protein ultrafiltration. *Advances in Colloid and Interface Science.* 160(2010) 40-48.
24. V. Chen, et al., The Use of Anionic Surfactants for Reducing of Ultrafiltration Membranes - Their Effects and Optimization. *J. Membr. Sci.* 67(1992) 249-261.
25. J. Xu, et al., Positively charged aromatic polyamide reverse osmosis membrane with high anti-fouling property prepared by polyethylenimine grafting. *Desalination.* 365(2015) 398-406.
26. S. Qiu, et al., Preparation and properties of functionalized carbon nanotube/PSF blend ultrafiltration membranes. *J. Membr. Sci.* 342(2009) 165-172.

27. V. Vatanpour, et al., Fabrication and characterization of novel antifouling nanofiltration membrane prepared from oxidized multiwalled carbon nanotube/polyethersulfone nanocomposite. *J. Membr. Sci.* 375(2011) 284-294.
28. A. Rahimpour, et al., Novel functionalized carbon nanotubes for improving the surface properties and performance of polyethersulfone (PES) membrane. *Desalination*. 286(2012) 99-107.
29. E. Celik, et al., Protein fouling behavior of carbon nanotube/polyethersulfone composite membranes during water filtration. *Water Res.* 45(2011) 5287-5294.
30. M. Kumar and M. Ulbricht, Novel antifouling positively charged hybrid ultrafiltration membranes for protein separation based on blends of carboxylated carbon nanotubes and aminated poly(arylene ether sulfone). *J. Membr. Sci.* 448(2013) 62-73.
31. M. Kumar, et al., Quaternized polysulfone and graphene oxide nanosheet derived low fouling novel positively charged hybrid ultrafiltration membranes for protein separation. *Rsc Advances*. 5(2015) 51208-51219.
32. J. Lee, et al., High flux and high selectivity carbon nanotube composite membranes for natural organic matter removal. *Sep. Purif. Technol.* 163(2016) 109-119.
33. S. Nakao, et al., Separation of Proteins by Charged Ultrafiltration Membranes. *Desalination*. 70(1988) 191-205.
34. M. Hadidi and A.L. Zydney, Fouling behavior of zwitterionic membranes: Impact of electrostatic and hydrophobic interactions. *J. Membr. Sci.* 452(2014) 97-103.
35. M. Ouammou, et al., Flux decay in protein microfiltration through charged membranes as a function of pH. *Colloids and Surfaces a-Physicochemical and Engineering Aspects*. 298(2007) 267-273.
36. L.J. Zeman and A.L. Zydney, *Microfiltration and ultrafiltration : principles and applications*. 1996: Marcel Dekker, INC.
37. S. Jeong, et al., Submerged membrane hybrid systems as pretreatment in seawater reverse osmosis (SWRO): Optimisation and fouling mechanism determination. *J. Membr. Sci.* 411(2012) 173-181.
38. G. Mustafa, et al., New insights into the fouling mechanism of dissolved organic matter applying nanofiltration membranes with a variety of surface chemistries. *Water Res.* 93(2016) 195-204.

List of tables

Table 1 Characteristics of the membranes used in this study [32].

Table 2 Fouling mechanism equations.

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Table 1 Characteristics of the membranes used in this study [32].

	UPW [*] permeability (LMH ^{**} /bar)	Pore size by BET (nm)	MWCO ^{***} (kDa)	Contact angle (°)	Isoelectric pH
MWCNT	490.8 ± 46.3	4.4-4.6	10	45.4 ± 0.1	9.2
PES	85.5 ± 8.5	-	10	57.6 ± 0.4	2.9

^{*}UPW: Ultrapure water, ^{**}LMH: L/m²h; and ^{***}MWCO: Molecular weight cut-off.

Table 2 Fouling mechanism equations.

Fouling mechanisms	Equations
<i>Complete blocking</i>	$\frac{d(\frac{V}{A})}{dt} = J_0 - k_b(\frac{V}{A})$
<i>Intermediate blocking</i>	$\frac{dt}{d(\frac{V}{A})} = \frac{1}{J_0} + k_i t$
<i>Cake layer formation</i>	$\frac{t}{V/A} = \frac{1}{J_0} + \frac{k_c}{2}(\frac{V}{A})$

List of Figures

Fig.1 Zeta potential of MWCNT membrane and PES membrane (a) at different pHs (4.7, 7.0 and 10.4) and (b) different ionic strengths by adding different concentrations of KCl (background electrolyte) (0.001, 0.005 and 0.010 M) at pH 7.0.

Fig.2 Comparison of permeate flux pattern of MWCNT and PES-UF membrane filtration with positively charged protein (Lys) and negatively charged protein (BSA) under different pHs (4.7, 7.0 and 10.4).

Fig.3 Comparison of permeability pattern with positively charged protein (Lys) and negatively charged protein (BSA) fouling under different ionic strength (concentration, monovalent/divalent ionic strength) (0.001, 0.01, 0.1 M NaCl).

Fig.4 Comparison of permeability pattern with binary mixture fouling under different pH (4.7, 7.0, 10.4) and ionic strength (NaCl 0.001, 0.01 and 0.1 M)

Fig.5 Fouling mechanisms in the MWCNT membrane under different solution chemistry (a) Lys protein filtration, (b) BSA filtration and (c) Mixed protein filtration.

Fig.6 Fouling mechanisms in the PES membrane under different solution chemistry (a) Lys protein filtration, (b) BSA filtration and (c) Mixed protein filtration.

(a)

(b)

Fig. 1 Zeta potential of MWCNT membrane and PES membrane (a) at different pHs (4.7, 7.0 and 10.4) and (b) different ionic strengths by adding different concentrations of KCl (background electrolyte) (0.001, 0.005 and 0.010 M) at pH 7.0.

(a) Lys filtration with MWCNT membrane

(b) Lys filtration with PES-UF membrane

(c) BSA filtration with MWCNT membrane

(d) BSA filtration with PES-UF membrane

Fig. 2 Comparison of permeate flux pattern of MWCNT and PES-UF membrane filtration with positively charged protein (Lys) and negatively charged protein (BSA) under different pHs (4.7, 7.0 and 10.4).

(a) Lys filtration with MWCNT membrane

(b) Lys filtration with PES-UF membrane

(c) BSA filtration with MWCNT membrane

(d) BSA filtration with PES-UF membrane

Fig. 3 Comparison of permeability pattern with positively charged protein (Lys) and negatively charged protein (BSA) fouling under different ionic strength (concentration, monovalent/divalent ionic strength) (0.001, 0.01, 0.1 M NaCl).

(a) MWCNT filtration at different pH

(b) PES-UF filtration at different pH

(c) MWCNT filtration at different ionic strength

(d) PES-UF filtration at different ionic strength

Fig. 4 Comparison of permeability pattern with binary mixture fouling under different pH (4.7, 7.0, 10.4) and ionic strength (NaCl 0.001, 0.01 and 0.1 M).

Foulant	Schematics	Fouling mechanisms
(a) Positively charge protein	<p>Pressure</p> <p>Lys</p> <p>pH 4.7</p> <p>Less deposited proteins</p> <p>Fouling alleviation</p> <p>pH 7</p> <p>Severe fouling</p> <p>pH 10.4</p> <p>Less deposited proteins</p> <p>Fouling alleviation</p>	-Membrane-foulant interaction -Foulant-foulant interaction (Lys-Lys)
		Increased electrostatic repulsion at away from IEP
	<p>Pressure</p> <p>Lys</p> <p>Cl⁻ ion</p> <p>0 M</p> <p>Protein adsorption</p> <p>Fouling</p> <p>0.001 M</p> <p>0.01 M</p> <p>0.1 M</p> <p>Less deposited proteins</p> <p>Fouling alleviation</p>	- Foulant-foulant interaction Lys aggregation by charge shielding effect
(b) Negatively charged protein	<p>Pressure</p> <p>BSA</p> <p>pH 4.7</p> <p>Less deposited proteins</p> <p>Fouling alleviation</p> <p>pH 7</p> <p>Protein adsorption</p> <p>Fouling</p> <p>pH 10.4</p> <p>Less deposited proteins</p> <p>Fouling alleviation</p>	-Membrane -foulant interaction Decreased electrostatic interaction at near IEP of both membrane and foulant
	<p>Pressure</p> <p>BSA</p> <p>Na⁺ ion</p> <p>0 M</p> <p>Protein adsorption</p> <p>Fouling</p> <p>0.001 M</p> <p>0.01 M</p> <p>0.1 M</p> <p>Less deposited proteins</p> <p>Fouling alleviation</p> <p>Ion shielding effect</p>	-Membrane -foulant interaction Decreased electrostatic interaction by charge shielding effect

Fig. 5 Fouling mechanisms in the MWCNT membrane under different solution chemistry (a) Lys protein filtration, (b) BSA filtration and (c) Mixed protein filtration.

Foulant	Schematics	Fouling mechanisms
(a) Positively charged protein	<p>Pressure</p> <p>Lys</p> <p>pH 4.7 pH 7 pH 10.4</p> <p>Less deposited proteins Protein adsorption Less deposited proteins</p> <p>Low Fouling Fouling Low Fouling</p>	<p>Membrane-foulant interaction and Foulant-foulant interaction</p> <p>Deposition of neutralized Lys on the PES membrane</p>
	<p>Pressure</p> <p>Lys Cl⁻ ion</p> <p>0 M 0.001 M 0.01 M 0.1 M</p> <p>Protein adsorption</p> <p>Fouling Charge shielding effect Fouling alleviation</p>	<p>Membrane-foulant interaction</p> <p>Decreased protein adsorption by charge shielding effect</p>
(b) Negatively charged protein	<p>Pressure</p> <p>BSA</p> <p>pH 4.7 pH 7 pH 10.4</p> <p>Protein adsorption</p> <p>Fouling Electrostatic repulsion Less deposited proteins</p> <p>Fouling alleviation</p>	<p>Membrane-foulant interaction</p> <p>Increased electrostatic repulsion between BSA(-) and PES membrane (-)</p>
	<p>Pressure</p> <p>BSA Na⁺ ion</p> <p>0 M 0.001 M 0.01 M 0.1 M</p> <p>Protein adsorption</p> <p>Fouling Charge shielding effect More deposited proteins</p> <p>Fouling promotion</p>	<p>Membrane-foulant interaction</p> <p>Decreased electrostatic repulsion by double layer compression and charge shielding effect</p>

Fig. 6 Fouling mechanisms in the PES membrane under different solution chemistry (a) Lys protein filtration, (b) BSA filtration and (c) Mixed protein filtration.

Highlights

- The MWCNT membrane was found to have very high sensitivity to the feed properties.
- The MWCNT membrane considerably affected the initial flux and fouling behaviour.
- Fouling in the MWCNT membrane could be alleviated by controlling solution chemistry.
- Fouling was controlled by the membrane-foulant and foulant-foulant interaction.