

# Irreversible fouling of membrane bioreactors due to formation of a non-biofilm gel layer

E. Poorasgari, P. Larsen, X. Zheng, P. H. Nielsen, K. Keiding and M. L. Christensen

## ABSTRACT

Extra-cellular polymeric substances (EPS), known to contribute to fouling in membrane bio-reactors (MBRs), are generally divided into bound and free EPS. The free EPS are able to form a gel layer on the membrane active surface. The mechanisms involved in formation of such layer and its effects on performance of the MBR membranes were studied. The free EPS, extracted by centrifugation and microfiltration, contained a significant amount of humic-like substances. Under static contact to the membrane, adsorption of humic-like substances to the membrane occurred and could be explained by conventional adsorption kinetics. Due to static adsorption, surface roughness of the membrane declined significantly, indicating that adsorbed matters to the membrane filled the cavities of the membrane surface. Filtration of the free EPS caused 50% water flux decline. The fouling resistance linearly increased with the amount of the humic-like substances retained during filtration as predicted by gel growth theory. A low pressure backwash could re-establish the water flux only up to 70%.

**Key words** | adsorption, cake resistance, free EPS, gel formation, humic-like substances, irreversibility

E. Poorasgari  
P. Larsen  
P. H. Nielsen  
M. L. Christensen (corresponding author)  
Department of Biotechnology,  
Chemistry and Environmental Engineering,  
Aalborg University,  
Sohngaardsholmsvej 57,  
DK-9000, Aalborg,  
Denmark  
E-mail: [mhc@bio.aau.dk](mailto:mhc@bio.aau.dk)

X. Zheng  
K. Keiding  
Grundfos Holding, Poul Due Jensensvej 7,  
DK-8850 Bjerringbro,  
Denmark

## INTRODUCTION

A critical operational problem in membrane bio-reactors (MBRs) is fouling, which results in decreased permeability and necessitates periodical physical and chemical cleaning. It has been observed that during operation of MBRs a double-layer forms on the membrane active surface, e.g. an outer cake and an inner gel layer (Aryal *et al.* 2009). The outer cake builds up during filtration and falls off during relaxation, as observed by Bugge *et al.* (2012) where they modeled the permeate flux in a laboratory-scale MBR as a function of cake build-up and removal for short time operations. It has been observed that the inner gel layer, which is located between the loose layer and the membrane and which is not removed by relaxation (Ramesh *et al.* 2007), is composed of biopolymers (Aryal *et al.* 2009). Zhang *et al.* (2006) stated that accumulation of extra-cellular polymeric substances (EPS) is one of the stages of MBR fouling. Liu *et al.* (2012) have reported a correlation between the sludge EPS concentration and the membrane fouling. Thus, it has been hypothesized that

the EPS components are important in formation of the gel layer.

EPS components are generally divided into bound and free EPS (Nielsen & Jahn 1999). A recent study has shown that the free EPS components are in permanent contact with the MBR membrane and pass through the membrane during filtration (Poorasgari *et al.* 2014). It has been reported that the free EPS fraction contains a significant amount of humic-like substances (Meng *et al.* 2011). The humic-like substances are present in a gel layer formed on the MBR membranes (Aryal *et al.* 2009). It has been shown that adsorption of humic acid causes irreversible fouling of polymeric ultra-filtration membranes (Jermann *et al.* 2007).

Based on the mentioned findings, it can be expected that adsorption of EPS molecules and gel formation due to passive transport of free EPS towards the membrane surface are the mechanisms for irreversible fouling. Experiments have been conducted to determine whether adsorption or

gel layer formation or both are important for prediction of fouling in MBR systems by free EPS components.

## MATERIALS AND METHODS

### Sludge samples

The sludge was sampled from the MBR unit of a wastewater treatment plant which was designed for enhanced biological phosphorus removal of municipal wastewater. The municipal wastewater passed through an anaerobic (hydrolysis) and a de-nitrification unit before reaching the MBR unit. The MBR unit was of 6 m<sup>3</sup> volume and was operated at 30 mbar trans-membrane pressure in 10 min filtration and 2 min relaxation periods. pH, electrical conductivity and the concentration of volatile suspended solid of the MBR sludge were approximately 8, 1100  $\mu\text{S} \cdot \text{cm}^{-1}$ , and 6 mg  $\cdot \text{L}^{-1}$ , respectively. Flat sheet membranes of 40 m<sup>2</sup> were installed in the pilot plant. The active surface of the membrane was polyvinylidene fluoride (PVDF) hydrophilized with polyvinyl pyrrolidone (PVP). The nominal pore diameter was 0.2  $\mu\text{m}$ . The membrane was produced by Alfa Laval A/S.

### Extraction of free EPS from MBR sludge

Free EPS were extracted from the MBR sludge. Sludge sampling and EPS extractions were done on the same day. To extract the free EPS, the MBR sludge was centrifuged at 9,000 g for 1 h at 4 °C. The mentioned centrifugation intensity and time were chosen because rough calculations using Stock's equation had shown that single bacteria are removed with the applied conditions. The supernatant was firstly filtered through glass-fiber filters of 1.6  $\mu\text{m}$  in order to separate larger particles floating in the supernatant and secondly through a mixed cellulose ester of 0.45  $\mu\text{m}$  nominal pore diameter in order to remove and/or minimize the remaining microorganisms and their interference in the adsorption experiments. Similar procedures have been reported by Rosenberger *et al.* (2005) and Jiang *et al.* (2010).

### Characterization of free EPS

Measurement of concentration of EPS components was done less than 48 h after extraction. During this time the EPS samples were stored in ice-cold water at 4 °C. Concentrations of protein and humic-like substances were measured by the modified Lowry method using bovine serum albumin and humic acid as standards for protein

and humic-like substances, respectively (Frølund *et al.* 1995). Carbohydrate concentration was measured by the anthrone method using glucose as standard (Frølund *et al.* 1995). The measurements were triplicated.

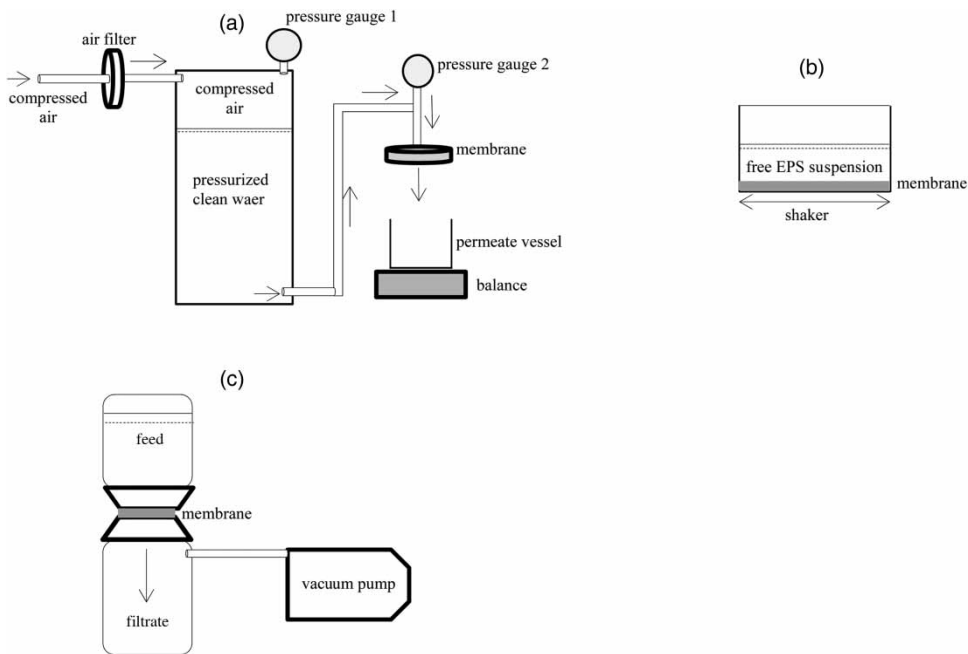
### Adsorption and filtration experiments

The membrane type was the same as the one used in the pilot plant MBR. The as-received membrane sheets were immersed and shaken in ethanol and subsequently in de-ionized water for 3 to 4 h to remove the glycerin protective coating from the membrane surface. Then, Milli-Q water was filtered through the membrane sheets at 2 bar pressure for 24 h to compact the membrane and get a fixed permeability. This procedure was done based on the information obtained from the manufacturer. The applied pressure and duration of compaction were based on the information obtained from the membrane manufacturer. After that, discs of 47 mm diameter were cut from the prepared membrane sheets for water flux and adsorption experiments.

Three different setups were used; one for water flux measurement, one for static adsorption and, one for filtration of EPS and backwash after filtration of EPS (Figure 1). The water flux measurement of the prepared membrane discs was done in a dead-end filtration setup where Milli-Q water was filtered through the prepared membrane discs at 0.5 bar pressure and the permeate flow was measured by gravimetric method. The pressure was supplied from a compressed air source and the compressed air was filtered before reaching the water of the feed tank. The air filter was of the same nominal pore diameter as the PVDF MF to remove the particles that can foul the membranes of 0.2  $\mu\text{m}$ . The permeate flow was constant over the time of Milli-Q water filtration at constant pressure. The permeate flow measurements were done before and after adsorption, and after backwashing the membrane discs which were fouled by EPS filtration. The water flux was calculated by dividing permeate flow by the surface area of the membrane disc – 0.00119 m<sup>2</sup>. The water flux values were corrected for 20 °C by applying the viscosity correction function:

$$J_{20^\circ\text{C}} = \frac{\mu_T}{\mu_{20^\circ\text{C}}} \times J_T \quad (1)$$

where  $\mu_{20^\circ\text{C}}$  and  $\mu_T$  are the dynamic viscosity of water as Pa  $\cdot$  s at 20 °C and actual water temperature, respectively.  $J_{20^\circ\text{C}}$  and  $J_T$  are the water flux as m  $\cdot$  s<sup>-1</sup> at 20 °C and at actual temperature, respectively. Normalized water flux was obtained by dividing the corrected water flux values



**Figure 1** | Experimental setups; Milli-Q water flux measurement (a), static adsorption (b), dynamic adsorption and backwash (c). In setup (c), free EPS suspension and Milli-Q water were used as feed for dynamic adsorption and backwash, respectively.

measured after adsorption and backwash by those measured for the same prepared membrane discs before adsorption experiments. The fouling resistance was calculated by the Darcy equation:

$$R_C = \frac{\Delta P}{\mu_{20^\circ\text{C}} \times J_{20^\circ\text{C}, \text{foul}}} - R_m \quad (2)$$

where  $R_C$  is the cake resistance as  $\text{m}^{-1}$ ,  $\Delta P$  the trans-membrane pressure as  $\text{N} \cdot \text{m}^{-2}$ ,  $\mu$  the dynamic viscosity of water,  $J_{20^\circ\text{C}, \text{foul}}$  is the temperature-corrected water flux after adsorption experiments, with the same unit as mentioned in Equation (1).  $R_m$  is the membrane resistance as  $\text{m}^{-1}$ , which was calculated from the water flux of the clean prepared membrane discs.

The adsorption experiments were done in static and dynamic modes. In the current work, the static adsorption means the adsorption due to contact between the membrane and the free EPS at no applied pressure, and dynamic adsorption means adsorption due to filtration of free EPS through the membrane.

The static adsorption tests were done in the cells of 20 mL volume made of Teflon. A volume of 10 mL free EPS was used for the static adsorption experiments which were conducted at room temperature for different contact times up to 4 h. No adsorption of humic-like substances to the Teflon cells was detected. The experiments were done with and without

shear. The shear was provided by a moving shaker of 1.2 Hz frequency and 13 cm displacement length. For each experiment, a fresh prepared membrane disc was used. The static adsorption experiments were triplicated. Adsorption of humic-like substances to the membrane was monitored by measuring UV254 absorbance of the free EPS suspension before and after static contact. The amount of adsorbed humic-like substances was calculated by Equation (3):

$$\theta = \left( \frac{\text{UV254}_{\text{init}} - \text{UV254}_{\text{f}}}{\text{UV254}_{\text{init}}} \right) \times \frac{C_{\text{init}} \times V}{M} \quad (3)$$

where  $\theta$  is concentration of humic-like substances on the membrane as  $\mu\text{g} \cdot \text{g}^{-1}$ ,  $\text{UV254}_{\text{init}}$  the UV254 absorbance of the initial free EPS,  $\text{UV254}_{\text{f}}$  the UV254 absorbance of the free EPS after contact to the PVDF MF membrane,  $C_{\text{init}}$  the concentration of humic-like substances of the initial free EPS as  $\mu\text{g} \cdot \text{mL}^{-1}$ . Measuring UV254 absorbance of the dilutions of the free EPS showed that the UV254 absorbance linearly decreases with increasing dilution factor. Also, measurements showed the UV254 absorbance of the free EPS was linearly correlated to the concentration of the humic-like substances measured by the modified Lowry method (Frølund *et al.* 1995). Therefore, a positive linear relationship has been assumed between UV254 absorbance and concentration of humic-like substances.  $V$  is the volume of free EPS used for the static contact experiments as mL,

and  $M$  the membrane mass as g. By using MATLAB, Equation (4) was applied as a kinetic model for fitting the amount of the adsorbed humic-like substances versus time in the static adsorption experiments:

$$\theta = \theta_{\text{eq}} \left( 1 - e^{(-K'' \cdot t)} \right) \quad (4)$$

$$K'' = K \frac{a}{v}$$

where  $\theta_{\text{eq}}$  is the equilibrium concentration of humic-like substances on the membrane as  $\mu\text{g} \cdot \text{g}^{-1}$ ,  $K$  the mass transfer coefficient as  $\text{h}^{-1}$ ,  $t$  the contact time as  $\text{h}$ ,  $v$  the volume of the adsorbent as  $\text{m}^3$ , and  $a$  the surface of the adsorbent as  $\text{m}^2$ . The  $\theta$  was calculated as  $\mu\text{g} \cdot \text{g}^{-1}$ .

The dynamic adsorption was done in a dead filtration setup at room temperature where the flow was driven by a vacuum pump of 0.07 bar pressure. No adsorption of humic-like substances to the setup was detected. EPS filtration experiments were done in triplicate by filtering 100 mL free EPS suspension divided in five equal volumes of 20 mL at room temperature. The retention of humic-like substances was calculated by Equation (5):

$$R = \frac{\text{UV254}_{\text{feed}} - \text{UV254}_{\text{filtrate}}}{\text{UV254}_{\text{feed}}} \quad (5)$$

where  $R$  is the retention of the humic-like substances,  $\text{UV254}_{\text{feed}}$  the UV254 absorbance of the free EPS before filtration and  $\text{UV254}_{\text{filtrate}}$  the UV254 absorbance of filtrate of the free EPS. It has been assumed that the amount of total retained humic-like substances is equal to summation of the amount of EPS retained at each filtration stage. The amount of the humic-like substances retained by the membrane was calculated by Equation (6):

$$\omega = \sum_{i=0}^n \left( \frac{R \times C_{\text{filtrate}} \times V_{\text{filtered}}}{M} \right)_n \quad (6)$$

where  $V_{\text{filtered}}$  is the volume of free EPS filtered through the PVDF MF membrane in each step and  $n$  is the number of the steps.  $C_{\text{filtrate}}$  is the concentration of the humic-like substances in permeate. The  $\omega$  was calculated as  $\text{mg} \cdot \text{m}^{-2}$ .

### Membrane surface analyses

A NTEGRA NT-MDT atomic force microscope was used in tapping mode for roughness analysis which was conducted on the active surface of the clean membrane and the active

surface of the membrane after static contact with the free EPS. The static contact was done for 12 h at 4 °C. A surface area of  $15 \mu\text{m} \times 15 \mu\text{m}$  was scanned on each sample.

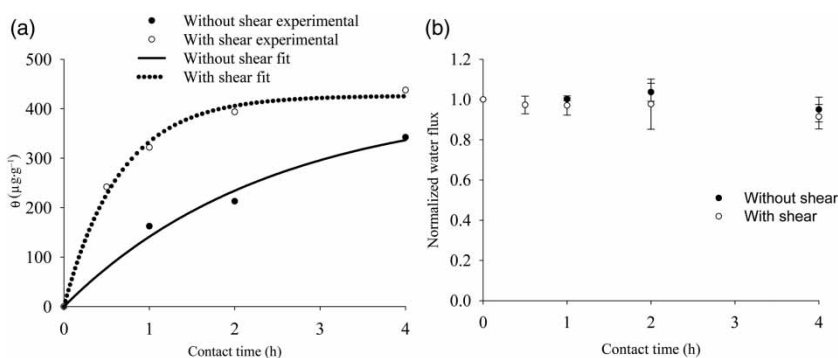
## RESULTS AND DISCUSSION

### Free EPS components of MBR sludge

The free EPS extracted from the MBR sludge was analysed. The measurements showed that the extract contained  $20 \pm 2 \text{ mg} \cdot \text{L}^{-1}$  humic like substances and no protein. The carbohydrate concentration was  $4 \pm 1 \text{ mg} \cdot \text{L}^{-1}$ . The mentioned concentration of humic-like substances of the free EPS is comparable to the one reported by Liu *et al.* (2012) but they had also observed presence of protein and a much higher concentration of carbohydrate. The reason for the absence of protein and very low carbohydrate concentration in our study could be the decomposition of these compounds in the hydrolysis and de-nitrification units. However, the humic-like substances are expected to be more resistant against biological degradation because of their poly-aromaticity.

### Static adsorption and its effects

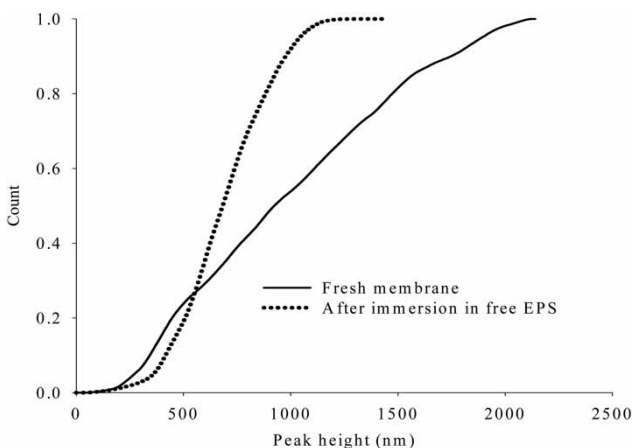
Static adsorption experiments were conducted by exposing the membrane active surface to the microfiltered free EPS extracted from MBR sludge. Figure 2(a) shows adsorption rate of the humic-like substances is higher at the beginning of the static contact and it decreases over the contact time. The higher initial adsorption rate is more pronounced for the experiments with shear than for the experiments without shear. For the experiments without shear, the adsorption seemed to be continuing after 4 h contact time, while the adsorption almost levelled off at 4 h contact time for the with-shear experiments. All of the mentioned observations can be explained by film theory. At the beginning, the adsorption sites are free and the concentration difference between the adsorptive suspension and the membrane is at a maximum, but it decreases over the contact time resulting in a decrease of adsorption rate. The shear reduced the thickness of the film, increased the mass transfer coefficient and shortened equilibration time to 4 h. The equilibration time and the adsorbate mass observed in the present study are comparable to those observed in the static adsorption of polyphenols of the flavan-3-ol family to a polyethersulfone–PVP MF membranes (Cartalade & Vernhet 2006).



**Figure 2** | Static adsorption of free EPS (a) and its effect on water flux (b).

There is a good agreement between the experimental results and the values predicted by Equation (4) which is based on the film theory. From the static adsorption experiments with shear, the equilibrium concentration has been calculated as  $426 \mu\text{g}\cdot\text{g}^{-1}$  and equilibrium concentration of static adsorption without shear has been adjusted to the same value. The  $K''$ , directly proportional to mass transfer coefficient, has been calculated as  $0.3961 \text{ h}^{-1}$  for the experiments without shear and  $1.521 \text{ h}^{-1}$  for the experiments with shear, respectively. Figure 2(b) shows that the static adsorption did not cause a significant change in water flux.

The surface roughness of the membrane surface was analysed before and after contact to free EPS. Figure 3 shows that the peak height and its distribution become less after contact to free EPS suspension. The free EPS components attach to the membrane surface and they fill the cavities and reduce the height of the peaks. This observation confirms adsorption of free EPS components to the membrane surface under static contact condition.



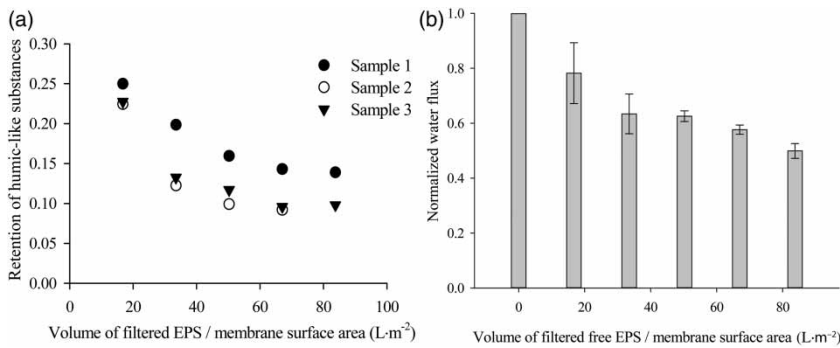
**Figure 3** | Effect of static adsorption of free EPS suspension on membrane surface roughness.

### Dynamic adsorption and its effects

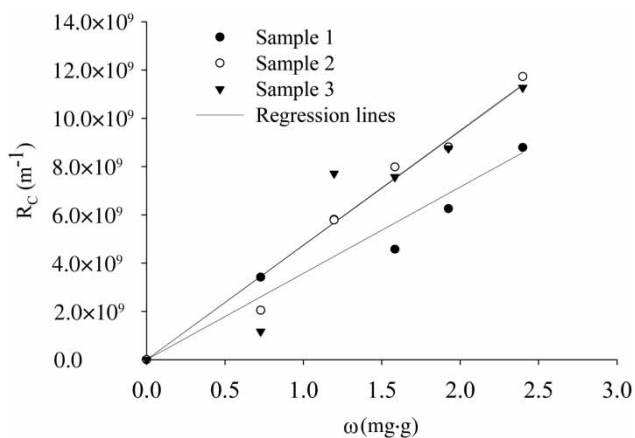
The free EPS suspension was filtered through PVDF MF membrane to study the effects of the dynamic adsorption on the PVDF MF membrane. Figure 4(a) shows that retention of humic-like substances is higher at the beginning of filtration and it decreases as filtration continues. The retention almost levelled off when the ratio between filtered EPS suspension and membrane surface area reached  $50 \text{ L}\cdot\text{m}^{-2}$ . This indicates that the part of the initial retention is controlled by the adsorption capacity of the membrane. Part of the decrease of the retention may be due to concentration polarization. As more EPS suspension is filtered, less adsorption sites remain free and the retention becomes less. The retention does not become zero; i.e. the humic-like substances continue to accumulate on the membrane surface. This documents the build-up of a gel layer by the retained humic-like substances since polymers are able to produce a gel network above a certain concentration (Stevens 1999).

Figure 4(b) shows that water flux decreases after filtration of free EPS. The normalized water flux decreased with increase of filtered EPS and it declined to 50% when the ratio between filtered EPS suspension and membrane surface area reached  $84 \text{ L}\cdot\text{m}^{-2}$ .

There is a similarity between the trend of normalized water flux and retention versus the volume of filtered free EPS. Therefore, a correlation was expected between the fouling resistance which is calculated from the flux data and the amount of the retained humic-like substances which is calculated from the retention data. Figure 5 shows the fouling resistance is in an acceptable positive linear correlation with the amount of humic-like substances retained during filtration of free EPS. The calculated coefficients of determination ( $r^2$ ) are 0.8948, 0.9048 and 0.9747 for samples 1, 2 and 3, respectively. The regression lines of sample 2 and sample 3 lie on each other. The linearity



**Figure 4** | Retention of humic-like substances (a) and flux decline due to filtration of free EPS suspension (b).



**Figure 5** | Correlation between cake resistance and the amount of retained humic-like substances in dynamic adsorption.

indicates the applicability of the gel growth theories for prediction of flux decline (Christensen *et al.* 2006) during filtration of free EPS. The amount of the humic-like substances retained during filtration of free EPS was almost eight times higher than that adsorbed during static adsorption. This may explain the significant effect of dynamic adsorption on water flux compared to the static adsorption, which did not affect water flux significantly. The high amount of adsorbed humic-like substances can be explained by the fact that during filtration of free EPS the humic-like substances are transported to deeper adsorption sites of the membrane, which might not be accessible by static adsorption. Further, some of the humic-like substances might be larger than the membrane pores or some of the pores and therefore rejected by the membrane.

### Irreversibility of fouling

Irreversibility of fouling was studied by backwash. The flux decline caused by filtration of free EPS is only up to 70%

recovered by backwash (data not shown). This observation is in line with the study done by Ramesh *et al.* (2007) where they found humic-like substances as one of the components of the irreversible fouling layer formed on the membrane active surface.

### CONCLUSION

Multi-layer fouling is observed in membrane bioreactors where an inner gel layer forms between the loose cake and the membrane surface. It has been shown that formation of the inner layer was due to adsorption and accumulation of the EPS components which are present in the supernatant of the MBR sludge and extracted as free EPS. The free EPS contained a significant amount of humic-like substances. It has also been shown that the humic-like substances adsorbed to the membrane surface during static contact but this adsorption caused no significant change in water flux, whereas filtration of the free EPS caused formation of a gel layer resulting in a significant decline in water flux. Backwash could re-establish the water flux only up to 70% of the initial. There was a linear correlation between the amount of humic-like substances retained by the membrane and the resistance produced during free EPS filtration. This correlation may enable us to predict irreversible flux decline from the amount of retained humic-like substances.

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