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Extracellular polymeric substance characteristics and fouling formation mechanisms in submerged membrane bioreactors

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ABSTRACT

In this study, the characteristics of extracellular polymeric substance (EPS) and the mechanism of fouling formation investigated using a submerged membrane bioreactor system consisting of a polytetrafluoroethylene (PTFE) membrane. During 85 days of operation, the bound EPS observed as the main component, which contributed major part of resistance to fouling. The protein to carbohydrate ratio was higher in the growth phase than in the stationary phase. The fouling resistances contributed by each individual resistances, i.e. loose cake layer, dense cake layer and internal fouling evaluated by batch filtration test after rinse, wash, and backwash operations respectively. The results showed that fouling of membrane and transmembrane pressure increased very quickly within 30 min of operation mainly due to resistance offered by immediate cake layer formation. The analysis of EPS contributed by different fouling layer revealed that most of the carbohydrate adsorbed quickly on the PTFE membrane first and then protein concentration increased with increasing filtration time. The protein content of EPS gradually increased when the foulant approach the membrane, whereas carbohydrate content showed opposite trend. Furthermore, the filtration of pure bacteria (*Ralstonia)* showed that most of proteins adsorbed on the membrane surface were hydrophobic and identified its contribution as the biological functions of translation.

Keywords: MBR; EPS; Fouling; PTFE membrane; Membrane cleaning; Membrane resistance; Protein nature; Size; Cellular function.

1. Introduction

In recent years, membrane bioreactor (MBR) technologies have been extensively applied as the advanced municipal and industrial wastewater treatment process because of its excellent separation ability and production of good effluent quality. However, membrane fouling remains a major drawback of this process because it deteriorates the membrane performance with regard to operational flux and transmembrane pressure (TMP). Therefore, frequent cleaning of the membrane by physical or chemical means is necessary to recover its initial performance [1].

The nature of foulant is predominantly organic and biological origin. Membrane biofouling initiates from bio-cake formation on the membrane surface through both the deposition of mixed liquor suspended solids (MLSS) and biofilm growth. The cake layer consist of

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rejected particulate materials (biomass) residing on the upstream face of a membrane and the biofilm is a viscous layer generally composed of water and slimy gum like substances e.g. extracellular microbial polymer matrix that envelops the attached cells [1–3]. The adsorption extracellular polymeric substances (EPS), which are excreted by bacteria and composed of a variety of organic substances mainly protein and carbohydrate on the membrane surface plays a key role in membrane fouling [4].

In the activated sludge process, EPS excreted by bacterial cells are release into the bulk solution and cover around the cell wall or entrap within the floc. A part of EPS released during cell lysis or diffuses through the cell membrane into the bulk solution is considered as soluble microbial products (SMP) or soluble EPS where as a part of EPS adhered to cell wall is considered as soluble EPS [1,5]. Many reports point out that EPS is the most significant factor affecting fouling in MBR [5–7]. The relationship between the EPS with different parameters such as operating conditions (e.g. solid retention time (SRT), hydraulic retention time (HRT), aeration, temperature, etc.), fluids characteristics (e.g. viscosity, mixed liquid suspended solid (MLSS) concentration, floc size, organic loading, etc.) and membrane properties (material, pore size, configuration, etc.) has been investigated [8].

Many researches have focused on investigating the factors that enhances fouling propensity such as characteristics of mixed liquor, includes soluble compounds [9], SMP [10–12], EPS [13], particle size distribution [14], and viscosity of mixed liquor [15]. These studies depicted that the concentration of carbohydrates and proteins in the reactor have a strong relationship with the propensity of membrane fouling in MBRs. In addition, few studies researchers have suggested that the bound EPS is mainly responsible for the formation of biological foulant and cake layer on the membrane surface and also responsible for increasing specific cake layer resistance [16–18]. However, previous studies not looked at distribution of different fraction of EPS content and the nature, characteristics and origin of foulant. Current understanding of membrane fouling in MBRs is still seems to be insufficient.

Membranes usually cleaned by different combination of physical, chemical and back flushing methods and depending on this, many studies divided total filtration resistance as sum of reversible and irreversible resistances [3], or sum of the cake layer, internal pore clogging and intrinsic membrane filtration resistance [19]. The primary objective of this research was to identify the resistance contributed from loose cake layer, dense cake layer, internal fouling, and intrinsic membrane filtration and to investigate the relationships among the EPS content with filtration period and finally to predict the origin, nature and characteristics of fouling substances.

In this study, the extents of fouling at different filtration period and corresponding variation in the EPS content in each fraction of loose, bound cake layer were investigated. In addition, the membrane fouling resistance offered by different fractions of the cake layer identified by applying the various cleaning methods. The bacteria that responsible for fouling identified and then type and characteristics of protein analyzed separately using batch filtration test with pure culture.

2. Materials and methods

2.1. The MBR experimental and conditions

A bench-scale MBR of 3 L volume consisting of two sets of flat polytetrafluoroethylene (PTFE) membranes (pore size of 0.22 μm, effective area of 0.0167 m^2) modules provided by the R&D Center for Membrane Technology (Chung Yuan Christian University, Taiwan) was setup for synthetic wastewater treatment. The composition of synthetic wastewater simulated was similar to the Nahu municipal wastewater treatment plant, Taipei (shown in Table 1). The clean PTFE membrane was hydrophobic in nature (contact angle – 127±4.5°). Permeate was produced by peristaltic pump (Cole-Parmer) and TMP was monitored using a pressure gauge (Pro-Instrument). The membranes fouling studied using long-term and short-term fouling experiments.

For long-term operation, permeate production was carried out using intermittent suction mode with a cycle of 5 min-pause and 10 min-run. The sludge retention time (SRT) and mixed liquid suspended solid (MLSS) was 30 days, and about 6500 mg/L respectively. Aerator was provided below membrane (flow rate –2 L/min) for mixing and maintaining dissolute oxygen concentration (over 2 mg/L) of the bulk solution. The fouling potential of filtration evaluated by the analysis of resistance based on Darcy's law as Eq.(1), where, *J* is the permeate

Table 1
Composition of simulated wastewater.

Component	Concentration in water (g/L)
Milk powder	72.855
$C_{12}H_{22}O_{11}$	7.25
CH_3COOH	4.465
KH_2PO_4	7.25
$(NH_4)_2SO_4$	5.125
Urea	16.07
$FeCl_3$	0.05
NaOH	neutralize pH to 6.8–7.2

flux (LMH, L/m²/h) and μ is the permeate viscosity (in mPa s). TMP is transmembrane pressure (kPa):

$$J = \frac{\text{TMP}}{\mu \times R_t}. \quad (1)$$

The short-term batch filtration experiments carried out in an aerobic MBR (vol- 3 L) under steady-state condition by keeping the EPS concentration in bulk sludge constant. Permeate was withdrawn continuously at different filtration time, 0.5, 3, 6, 12, 24 and 144 h. After filtration, the individual resistances offered by different fractions of the cake layer evaluated by measuring clean water flux after different cleaning. The resistance of fouling was divided into three parts: intrinsic membrane filtration resistance (R_m), cake layer resistance (R_c), and internal fouling (R_f) (Eq. (2)). R_c is subdivided into loose layer cake resistance (R_{lc}) and dense cake layer resistance (R_{dc}) which was evaluated after membrane rinse and brushing with sponge respectively (Eq. (3)). R_f is subdivided into reversible resistance (R_{bf}) and non-reversible resistance (R_{nf}) which was evaluated after using backwashing and chemical cleaning (5000 ppm sodium hypochlorite) respectively (Eq. (4)). The protein (p) and carbohydrate (c) concentration in extracted EPS samples of sludge in bulk and the different parts of the cake layer are measured:

$$R_t = R_m + R_c + R_f, \quad (2)$$

$$R_c = R_{lc} + R_{dc}, \quad (3)$$

$$R_f = R_{bf} + R_{nf}. \quad (4)$$

Apart from the monitoring of MBR performance and EPS, the relationship between PTFE membrane affinity to biofouling and protein excreted by microorganism investigated according to the procedure described in section 3.3.

2.2. EPS extraction method

The EPS of sludge samples were extracted according to procedure described by Fang and Jia (1996) [20]. The EPS of sludge samples collected from bulk solution, loose and bound cake layer and backwash foulant are extracted into two parts the soluble and bound EPS. Initially the sludge sample centrifuged at 9000 ×*g*, 4°C for 20 min (using an Avanti J-E type centrifuge with JA-14, JA-25.5 rotor). The supernatant obtained on centrifugation filtered through 0.22 µm cellulose acetate filters; this filtrate was labelled as soluble EPS. The pellets were washed by re-suspending in deionized water, and then centrifugation at 9000 ×*g*, 4°C for 20 min and remove the supernatant. After washing the sludge sample re-suspended in 0.06 mL 37% formaldehyde (kept at 4°C for 1 h) for fixing the cell, and then extracted with 4 mL 1N NaOH (kept at 4°C for 3 h). After this the reactive sample was centrifuged at 20,000 ×*g*, 4°C for 20 min, and supernatant obtained on centrifugation was filtered through 0.22 µm cellulose acetate filters; this filtrate was labelled as bound EPS. The soluble and bound EPS quantified by measuring the weight of solids after lyophilization.

2.3. Analytical methods

COD, BOD, TN, TP of the influent and the mixed liquid suspended solid concentration (MLSS) were measured by the standard method announced by Environmental Analysis Lab, EPA Taiwan. The contents of protein and carbohydrate in EPS were measured by the modified Lowry's method [21] and phenol-sulfuric acid method [22] using bovine serum albumin and glucose as the respective standards. Contact angle was used to estimate the hydrophobicity of the membrane. Sludge particle size distribution was measured by Mastersizer/E (MALVERN, UK) and surface Contact Angle was measured by Meter CA-D type (Kyowa Kaimenagaku Co., Japan). The protein purification was carried by HPLC (Gilson, USA).

3. Results and discussion

3.1. Long-term performance of MBR

The average concentration of MLSS and MLVSS maintained at 6,599 ± 1,718 mg/L and 5,938 ± 1,722 mg/L, respectively. The floc size of sludge plays important role in sludge filterability. The changes in the particle size distribution (PSD) of sludge floc on different days of operation (2, 7, 17, 21, 35 and 45 days) during the course of the experiment for 85 days represented in Fig. 1(a). The sludge floc PSD showed unimodal distribution on all days but it shapes became narrow as operation days increases. As the operation period increases, the sludge floc PSD shifted towards higher particle size and the number of average particle size increases and after certain days of operation (~ 35 days), it remained almost constant. Fig. 1(b) shows the average particle size (APS) of sludge floc during 2–45 days. Results also showed that of the average particle size in bulk sludge increased rapidly from 80.8 µm to 118.9 µm during initial 35 days and then remained almost stable around ~120 µm after operating reactor equivalent to one SRT period. Therefore, from these results it can be concluded that as operation time proceeds, the aggregation of the microorganisms

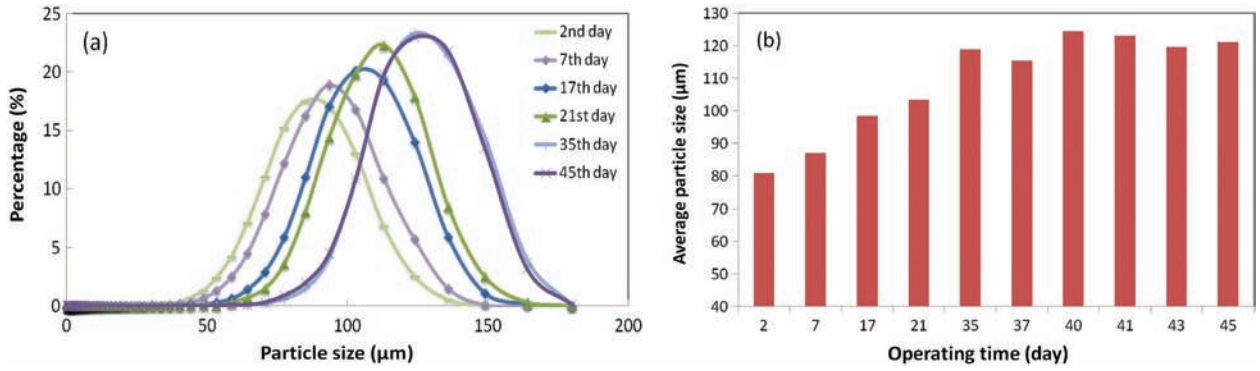

Fig. 1. (a) Particle size distribution and (b) average particle size of sludge floc on different days in long-term MBR operation.

results in development of larger floc size and the floc size remained almost stable after operating more than 1 SRT days.

Fig. 2 shows the concentration profile of soluble and bound fraction of protein and carbohydrate during operation. The soluble protein and carbohydrate of EPS in mixed liquid were 16.4 ± 3.2 mg/L and 4.1 ± 0.5 mg/L, respectively, where as the average concentration of bound protein and carbohydrate were 519.9 ± 54.6 mg/L and 158.9 ± 9.2 mg/L respectively. A slight increment with suddenly rising of bound protein and carbohydrate concentration observed during initial period only. Then the concentration of bound EPS became stable after operating MBR for 55 days, which is equivalent to ~2 SRTs period. The distribution of soluble and bound P/C ration were 0.37–23.6 and 2.59–4.35, respectively. The values of P/C ratio for bound EPS were close to that observed in other MBR system studies (which ranged from 2.44 to 4.5), but the values for the soluble EPS were smaller than that observed in other MBR studies form literature (which range from 1.25 to 3.91)[23–26].

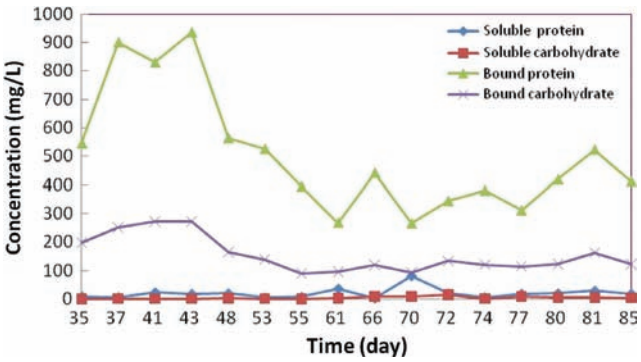

Fig. 2. The variation of the soluble and bound EPS in long-term MBR operation.

Results from the analysis of floc particle size and EPS concentration revealed that the concentrations of bound protein and bound carbohydrate increases during growth phase while the quantity of protein occupies most part of the components of EPS. Furthermore, the bound EPS concentration decreased to a constant state when the bacteria growth situation trended to the stationary. The soluble EPS concentration has not changed significantly during entire operation period.

3.2. *Study the effect of filtration period on fouling using short-term MBR batch filtration test*

The protein and carbohydrate concentration contributed by different parts of fouling varies depending on the time of filtration. As filtration time proceeds longer, the loose cake layer may starts compacting and will partially converts it in to the dense cake layer. Therefore, in this study the extent of fouling on different periods of filtration studied. After operating for two SRTs period MBR biomass characteristics was stabilized, therefore the short-term batch filtration experiments were carried out at different continuous filtration periods, 0.5, 3, 6, 12, 24 and 144 h. Table 2 shows the difference components of the fouling resistance observed during different filtration period. Membrane fouling develops rapidly and TMP rise quickly due to generation of the membrane resistance within short operation (30 min) of this MBR system. It was also observed that the change of resistances while rising the filtration time up to 24 h were not significant.

In order to evaluate the rate of resistance rate ($R/\Delta t$), the resistance obtained form each test was divided by its filtration period (0.5, 3, 6, 12, 24 and 144 h). The Δt was used in calculation of the resistance per unit time and it is referred as filtration period. The resistance rates of each portion of foulant layer contributed during different filtration time normalized and then plotted in Fig. 3.

Table 2
The variations of the resistance at different filtration periods.

	The different components of the fouling resistance ($10^{12}m^{-1}$)							
Time (hr)	R_t	R_k	R_{dc}	R_{nf}	R_{bf}	R_{ic}/R_t	R_{dc}/R_t	R_{ic}/R_{dc}
0.5	34.4	31.8	0.7	0.9	0.4	92.2%	1.9%	48.2
3	34.7	32.1	1	0.4	0.3	91.6%	3.0%	31.1
6	35.7	32.4	2.1	0.5	0.4	89.7%	5.9%	15.4
12	33.6	29.1	2.1	0.1	0.4	85.5%	5.9%	13.5
24	35.3	29.8	3.2	1.2	1.1	84.5%	8.5%	13.1
144	29.3	18.1	6.0	4.0	0.1	62.9%	19.2%	3.0

From these results, the loose cake layer identified as the main source of the fouling resistance, which contributed nearly 88.7 ± 3.5% of total resistance within 24 h. Moreover, as filtration time increases the loose cake layer resistance decreases while dense cake layer resistance increases (Fig. 3). This indicates that strength of loose cake layer decreases with increase in filtration time and it contributes to an increase in dense layer cake resistance. In other words, a part of loose cake layer nearby the membrane site might have compressed and then became more compact by suction force. Finally, this part transformed to a portion of dense cake layer. Although the driving force supplied by pump was constant, the increasing resistance of dense cake layer weakened the attraction between the loose cake and the membrane. Consequently, the resistance and thickness of loose cake layer decreased with time. The resistance of the loose cake layer reduced to three times than the dense cake layer after operating of 144 h. This result was close to that observed by other studies in which the reversible fouling contributed the most of part of resistance [27–29].

The EPS content in each foulant layer separated by different cleaning methods analyzed at different filtration time and results represented in Table 3. The amount of carbohydrate adsorbed on the PTFE membrane was almost constant, where as the amount of protein adsorption increases with the increase in the sucking time (Table 3). It implied that most of carbohydrate adsorbed on the PTFE membrane first and the protein concentration increases with increasing the operation time. Moreover, the P/C ratios were getting small due to the significant increment of protein after operating of 144 h. From the comparisons between the protein and carbohydrate of each part of foulant classified by rinse, wash, and backwash showed the compositions of EPS of the bulk sludge, loose cake layer, dense cake and internal fouling were different. With the foulant located far away the membrane, it revealed that Protein content of EPS gradually increase when the foulant approach the membrane. Whereas the carbohydrate content of EPS displayed the opposite trend. Metzger et al. (2007) divided the cake layer into upper layer, intermediate layer, and lower layer by rinsing, back washing and chemical cleaning, respectively. The *Results* also revealed that a larger concentration of biopolymers observed in the upper layer than the other part of layer. As for SMP, higher concentration observed in the lower layer [24].

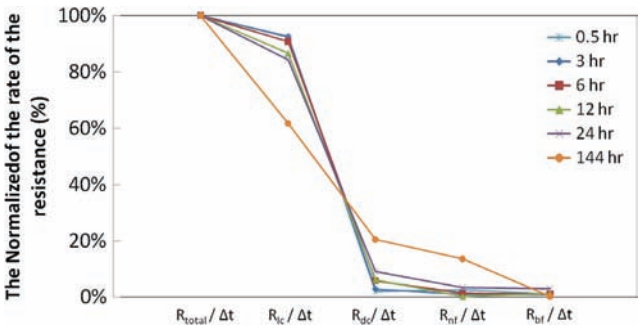

Fig. 3. The proportion of resistance transition in the operating duration depict.

The comparison of P/C ratio of EPS from bulk sludge, loose cake layer, dense cake layer and internal fouling showed decreasing trend at all filtration period but the quantity of bound protein in the dense cake layer increased with operation time. As a whole, the P/C ratio of EPS from bulk sludge, loose cake layer, dense cake layer and internal fouling are 3.28, 2.21, 0.66 and 0.45 respectively. The EPS concentration of the loose cake layer was higher than the dense cake layer and internal foulant. Additionally, the correlation coefficients of P/C ratio are relatively low to the flux, TMP, and MLVSS in the bench-scale MBR test (data not shown here). The P/C ratios of bulk sludge and loose cake layer was almost closer and higher than other layer. Since the loose cake layer is in direct

Table 3
The variations of content of bound protein, carbohydrate and P/C ratios in different foulant layers at different filtration durations.

Cleanings	Filtration duration (h)					
	0.5	3	6	12	24	144
Bound protein (mg/m^2)						
Rinse	244.7 ± 107.4	266.3 ± 91.4	312.8 ± 100.1	228.3 ± 60.1	289.9 ± 32.1	510.5 ± 50.3
Wash	17.6 ± 8.5	18.7 ± 11.7	23.0 ± 11.0	9.6 ± 5.8	12.7 ± 3.0	35.0 ± 10.4
Backwash	13.2 ± 6.3	15.8 ± 16.7	15.1 ± 15.3	5.1 ± 2.9	9.5 ± 3.8	11.7 ± 5.1
Bound carbohydrate (mg/m^2)						
Rinse	119.0 ± 21.8	95.5 ± 25.7	160.6 ± 37.4	120.6 ± 36.6	134.1 ± 30.6	299.7 ± 74.0
Wash	28.1 ± 10.5	31.9 ± 7.5	34.5 ± 11.1	32.5 ± 6.6	31.2 ± 3.5	35.9 ± 1.4
Backwash	31.9 ± 14.0	28.1 ± 10.9	36.0 ± 10.9	27.5 ± 5.4	28.1 ± 7.2	23.6 ± 15.1
P/C ratio (mg/mg)						
Rinse	2.52	2.78	1.93	1.92	2.39	1.70
Wash	0.80	0.66	0.85	0.29	0.41	0.97
Backwash	0.65	0.61	0.36	0.21	0.34	0.50

contact with bulk liquid, the concentration of EPS was higher in loose cake layer. Some studies described that the carbohydrate/protein in the reactors were found to have a strong relationship with the evolution of membrane fouling in MBRs but there was no a clear relationship between carbohydrate/protein in the reactors and membrane fouling in MBRs [30,31].

3.3. Identification of nature, characteristics and origin of foulant from the dense cake layer

This study also isolated the microorganisms form the dense cake layer during the long-term operation. The microorganisms were isolated from the colonies by TGC media. According to the sequencing results, it observed that the bacterium with fastest growth rate among all strains identified as *Rlastonia*. In order to investigate the specific protein that excreted by microorganism, the PTFE membranes set in the respective tanks with pure culture of *Rlastonia*. The membrane filtration of the pure bacterial liquid carryout until a certain thickness of cake layer formed on membrane surface.

Afterwards, the EPS attached on the membrane purified by HPLC and then identified for its functions. From the measurement of protein hydrophilicity, it observed that the most of proteins adsorbed on the PTFE membrane surface were hydrophobic for *Ralstonia* (Fig. 4(a)). Furthermore, Fig. 4(b) showed the distribution of molecular weight and 75% of proteins were in the MW range larger than 40 kDa, indicating that majority of proteins attached on the PTFE membrane surface have high MW. Fig. 5 showed the characteristics of 11 proteins isolated from the related with cellular functions. Most proteins attached on the PTFE membrane surface were in connected with biological function of translation, and the next were functions of carbohydrate metabolism, protein refolding.

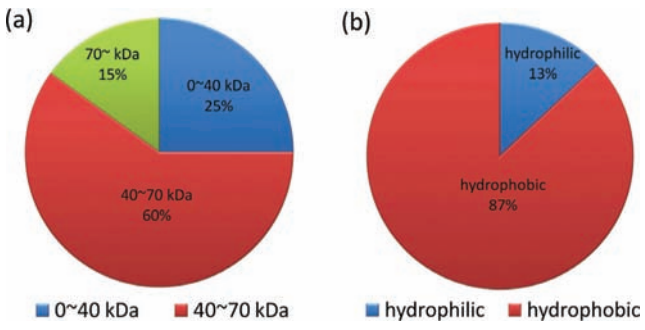

Fig. 4. The properties of proteins in the dense cake layer. (a) Hydrophilicity distribution and (b) molecular weight distribution.

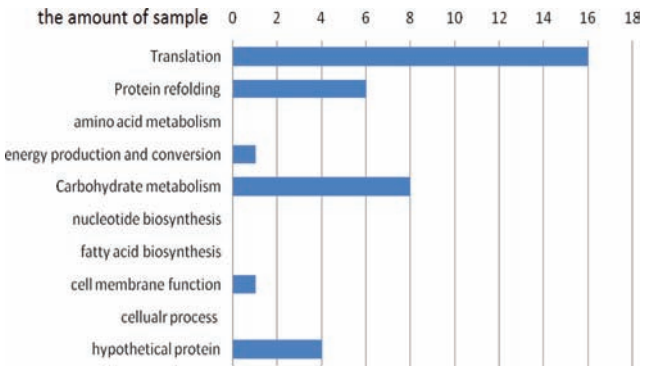

Fig. 5. The cellular function for the proteins in the dense cake layer.

4. Conclusion

In this study, a submerged MBR bench-scale system with PTFE membranes operated to treat the simulated municipal wastewater. The average particle size in bulk sludge increased from 80.8 μm to 118.9 μm and then remained almost constant after operating more than one SRT period. The bound EPS increased in the growth phase while the quantity of protein occupied most components of the EPS. Furthermore, the bound EPS concentration decreased to a constant state when the bacteria growth situation trended to the stationary. The soluble EPS does not change during the entire operation period. The concentration of bound EPS became stable after operating two SRTs period.

The fouling resistance rapidly generated within 30 min of filtration operation; thereafter, no significant change on resistance while rising the filtration time up to 24 h. A part of the loose cake layer nearby the membrane site compressed and then became more compact due to continuous suction force during the longer filtration period. The main resistance of fouling contributed by the loose cake layer was around 80–95% in the short-term operation.

The most of carbohydrate adsorbed on the PTFE membrane first and the protein concentration increase with increasing the operation time. The P/C ratio seems mostly related with the bulk sludge and loose cake layer on membrane fouling.

The filtration of pure bacteria liquid containing *Ralstonia* using the PTFE membrane showed that most of proteins adsorbed on membrane surface were found to be hydrophobic, and it was identified as about the biological functions of translation, and the next were functions of carbohydrate metabolism, protein refolding.

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