Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2015.1024938

57 (2016) 7487–7494
April

Taylor & Francis
Taylor & Francis Group

Microbial behavior and characteristics of biomass during starvation and their influence on ultrafiltration of activated sludge

W. Lee[a], Y. Son[b], Y. Ahn[b], H. Kim[c], S. Kim[d], S. Kang[e,*], C. Lee[f,*]

[a]*Department of Environmental Engineering, Kumoh National Institute of Technology, Gumi, Gyeongbuk 730-701, Republic of Korea*
[b]*Department of Energy Engineering, Gyeongnam National University of Science and Technology, Jinju, Gyeongnam 660-768, Republic of Korea*
[c]*Department of Environmental Engineering, Chonbuk National University, Jeonju, Chonbuk 561-756, Republic of Korea*
[d]*Department of Environmental Engineering, Daegu University, Gyeongsan, Gyeongbuk 712-714, Republic of Korea*
[e]*Department of Civil Engineering, Kyung Hee University, Yongin, Gyeonggi 446-701, Republic of Korea, Tel. +82 31 201 3326; Fax: +82 31 202 8854; email: seoktae.kang@khu.ac.kr*
[f]*Department of Civil Engineering & Institute of River Environmental Technology, The University of Suwon, Hwaseong, Gyeonggi 445-743, Republic of Republic of Korea, Tel. +82 31 220 2470; Fax: +82 31 220 2522; email: chaeyoung@suwon.ac.kr*

Received 19 November 2014; Accepted 24 December 2014

ABSTRACT

Biological and physico-chemical properties of biomass under different growth phases are essential to characterize membrane fouling in membrane bioreactors (MBRs). This study assessed microbial behavior and characteristics under stationary and death phases as starvation progressed and their influences on membrane fouling during ultrafiltration of biomass in MBRs. As starvation progressed, samples were periodically drawn to be measured for biological and physico-chemical properties of the biomass, and for ultrafiltration (30,000 Daltons) tests. Ultrafiltration experiments exhibited fouling resistances caused by biomass and supernatant increased as starvation progressed, and the death phase of microbial growth exhibited more potential in membrane fouling than the stationary phase. Particulates were more responsible for ultrafiltration membrane fouling in the stationary phase of microbial growth, but soluble materials were more accountable in the death phase of microbial growth. Statistical analysis indicated biomass with lower activity could experience higher fouling potential during ultrafiltration of biomass.

Keywords: Membrane bioreactor; Membrane fouling; Microbial activity; Starvation; Biomass

1. Introduction

Membrane bioreactors (MBRs) have been given increased attention as one of the innovative and promising technologies for wastewater treatment and reclamation communities [1–4]. MBRs use ultrafiltration or microfiltration membranes for better separation of biomass. This leads to an increased microbial concentration in the reactors, a reduced sludge production, and a higher and more consistent quality effluent

*Corresponding authors.

Presented at IDW 2014 — The 7th International Desalination Workshop November 5–8, 2014, Jeju, Korea

compared with conventional activated sludge processes [1–3,5]. MBRs can effectively separate viruses as well as bacteria when ultrafiltration membranes are used. However, membrane fouling is one of the main obstacles to the wide range of applications of MBRs, which declines permeate flux and therefore increases operation costs [3,6].

In general, both particulate and soluble materials of activated sludge in MBRs deposit onto the membrane surface and are adsorbed onto the walls of the membrane pores, which causes membrane fouling in MBRs [7–9]. In addition to the physical classification of activated sludge, biological characteristics of biomass are also important to understand membrane fouling phenomena because MBR experiences adhesion of living and dead micro-organisms and their associated extracellular products on membrane surfaces and pores [1,9].

Each growth phase of micro-organisms in MBRs may demonstrate different membrane fouling behaviors. The biological and physico-chemical properties of biomass (or activated sludge) under different growth phases are essential to characterize membrane fouling in MBRs. Because MBR systems work generally under low food to micro-organism (F/M) conditions with an increased solid retention time (SRT), the activity of the microbial population is close to the one observed in the starvation phase [1,2]. Especially, biomass in small-scale MBRs often experience starvation conditions because the influents (raw wastewater) of the systems are not consistent in terms of water quantity as well as quality, thus limiting the availability of substrates. Under these low growth conditions, endogenous processes have a significant influence on the amount of active biomass and the overall system performance [10]. Overaged biomass and starving conditions can produce more cell debris and extracellular products [1], which may exacerbate membrane fouling. Mukai et al. [6] reported that growth phases of the sludge influenced the flux decline during ultrafiltration of centrifuged supernatants, and stationary culture solution showed the most time-dependent flux decline, and death phase culture solution showed the intermediate.

Despite its importance to understand the relationship between biomass properties under different microbial growth phases and membrane fouling, there has been no control study to differentiate the effects of particulate and soluble materials of biomass on membrane fouling under different microbial growth phases. The objectives of this study were to evaluate various biological and physico-chemical properties of each particulate and soluble materials of biomass under stationary and death phases and assess their influence on ultrafiltration membrane fouling.

2. Materials and methods

2.1. Experimental methods

Sludge from a municipal wastewater treatment plant with a conventional activated sludge process was cultivated in a bioreactor for 90 d with synthetic wastewater. The influent chemical oxygen demand (COD) was 300 mg/L (COD:N:p = 100:10:2), and SRT was 10 d. Trace minerals in the synthetic wastewater included calcium (0.1 mg-Ca^{2+}/L), magnesium (0.5 mg-Mg^{2+}/L), manganese (0.1 mg-Mn^{2+}/L), zinc (0.1 mg-Zn^{2+}/L), iron (0.5 mg-Fe^{3+}/L), copper (0.1 mg-Cu^{2+}/L), cobalt (0.1 mg-Co^{2+}/L), and molybdenum (0.1 mg-Mo^{6+}/L). Alkalinity of the wastewater was 100 mg/L as $CaCO_3$. After 90 d of cultivation, the system reached pseudo-steady state [volatile suspended solids (VSS)–1,300 mg/L]. The sludge was delivered into two duplicated batch bioreactors, each with an effective volume of 8 L, and was aerated for 12 d without any additional feeding. The temperature of the reactors was maintained at 20˚C, and the dissolved oxygen concentration was kept about 4 mg-O_2/L. As starvation progressed, the sludge and supernatant samples were periodically taken at 0, 48, 96, and 288 h of the experiment from each bioreactor to determine the biological and physico-chemical properties and the relative contribution of each particulate and soluble materials of biomass to membrane fouling. The supernatant was separated from the sludge by centrifugation at 366 rad/s for 5 min.

2.2. Resistance analysis

Flux-decline tests and filtration resistance analysis were performed using a commercial dead-end stirred cell unit (Amicon 8200; Millipore Corp., USA) equipped with regenerated cellulose membranes (YM30, Millipore Corp., USA). The nominal molecular weight cut-off (MWCO) of the membrane was 30,000 Da. The membrane was symmetric and hydrophobic. Prior to ultrafiltration experiments, the VSS concentrations of the samples were adjusted to 1,000 ± 50 mg-VSS/L by diluting the sludge with supernatant (0 and 48 h samples) or condensing the sludge gravitationally (96 and 288 h samples) to avoid the effect of VSS concentrations on membrane fouling. All of the filtration experiments were performed at room temperature (22–25˚C) and the stirred cell unit was pressurized to 207 kPa with nitrogen gas. Stirring speed was 8 ± 0.5 rad/s.

The resistance-in-series model was used to analyze filtration resistance [1]. The total resistance (R_t) of ultrafiltration is the sum of intrinsic membrane resistance (R_m), plugging resistance (R_p) caused by cake

layer formation and pore blocking, and irreversible resistance (R_{ir}) by adsorption of organic and inorganic materials on the membrane surface and pores. R_m was obtained by filtering ultra-pure water; R_t by filtering the sludge and supernatant samples; R_p by filtering ultra-pure water after removing the cake layer physically from the membrane surface. R_{ir} was calculated from R_t, R_m, and R_p values (i.e. $R_{ir} = R_t - R_m - R_p$).

2.3. Analytical methods

Sizes of particulate materials were measured by PAMAS-2120 (PAMAS, Germany), and sizes of colloids were measured by a ZetaPlus (Brookhaven, UK) with a particle sizing software. Apparent molecular weight (MW) of the solute was determined using three different size ultrafiltration membranes (Amicon YM series with nominal MWCO of 3 k, 30 k, and 100 kDa; Millipore Corp., MA, USA) in parallel processing after prefiltering with 0.45 μm membrane filters (Whatman®, UK). A permeation coefficient model was used to correct apparent size distributions for membrane rejection [11].

Contact angle and specific ultraviolet absorbance at 254 nm (SUVA = UVA_{254}/DOC) were used to estimate the hydrophobicity of SS and supernatant, respectively. Contact angle was measured by a modified axisymmetric drop shape analysis using a contact angle meter (KRUSS G2, GmbH, Germany) [12]. The surface charges of microbial floc and supernatant were determined using a titration method [9,13] and a ZetaPlus (Brookhaven, UK), respectively.

Carbohydrate and protein concentrations in extracellular polymeric substances (EPS) were measured by the phenol-sulfuric acid method with glucose as standard [14] and the Lowry method with bovine serum albumin as standard [15], respectively, after heat extraction [13]. The sum of the amounts of total carbohydrates and proteins represented the total amount of EPS since those are the dominant components typically found in extracted EPS [16,17].

Oxygen uptake rate (OUR) was obtained by a respirometer (Challenge AER-200 system; Challenge Technology, Inc., AR, USA). The OUR value over the VSS concentration yields a value termed specific oxygen uptake rate (SOUR), and SOUR was adopted to measure the microbial activity [18]. COD, DOC, DO, pH, SS, and VSS were determined based on *Standard Methods* [19], and a statistical analysis of experimental data was performed using SPSS V.22 (SPSS Inc., IL, USA). Pearson's correlation coefficient (r) was used to measure the degree of linear dependence between two variables, giving a value between +1 and −1, where 1 is total positive correlation, 0 is no correlation, and −1 is total negative correlation. The significant value (p-value) is the probability that you would have found the current result if the correlation coefficient were in fact zero (null hypothesis). If the probability is lower than the conventional 5% ($p < 0.05$) the correlation coefficient is called statistically significant.

3. Results and discussion

3.1. Evolution of microbial population and activity

Variations of the population and activity of biomass were monitored for 288 h as starvation progressed (Fig. 1). VSS concentrations were considered to represent microbial population because synthetic wastewater was used. Initial VSS concentration was 1,300 mg/L. There was no noticeable variation of VSS concentration during the first 24 h of the experiment. Afterward, a continuous decrease in microbial population was observed throughout the 288 h of the experiment, leaving 62% of the initial VSS value. The lack of biodegradable organics in the reactor probably reduced microbial population and size.

Three distinct phases were observed in microbial activity as shown in Fig. 1. First, a gradual decrease in SOUR appeared between the initial stage and 60 h of the experiment, which were considered stationary phase. The initial SOUR value of the sludge was 8.3 mg-O_2/g-VSS/h. Second, SOUR steeply decreased between 60 and 100 h of the experiment, which may indicate a decrease in the active cell population and a strong potential of cell lysis (death phase). Finally, a further decrease occurred from 100 h but was not significant (death phase).

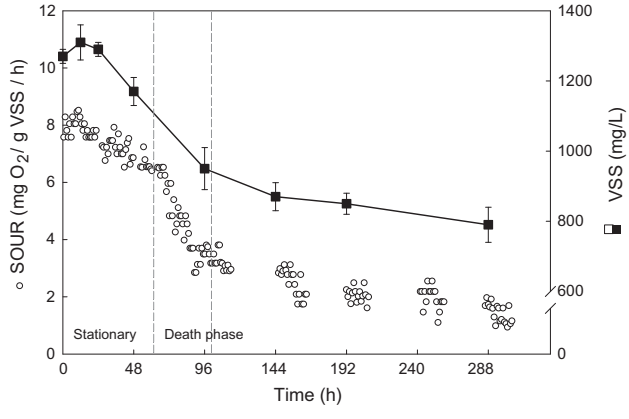

Fig. 1. Variations of VSS and SOUR values over time.

3.2. Ultrafiltration and resistance analysis

To compare ultrafiltration behavior of biomass and supernatant in different microbial growth phases, samples were harvested at 0, 48, 96, and 288 h of experiment. Fig. 2 presents the flux decline during ultrafiltration of the biomass and supernatant samples at different experiment times as starvation progressed. The initial rapid and subsequent gradual permeate flux declines were more severe at 96 and 288 h of the experiment in both biomass and supernatant samples. This result indicated there was a change in properties of potential foulants during the activity decline (60–100 h of the experiment). This could be confirmed by the biological and physico-chemical properties of biomass and supernatant samples, which will be discussed in the following sections.

After each ultrafiltration test, filtration resistances were analyzed to evaluate the contribution of each resistance to the total resistance (*R*t). Table 1 summarizes a series of resistances during ultrafiltration experiments of biomass and supernatant samples. Plugging resistance (R_p) appeared to dominate R_t in the ultrafiltration of both biomass and supernatant. R_p ranged 78 – 93% of R_t in biomass samples and 63 – 88% of R_t in supernatant samples. Physically irreversible resistance (R_{ir}) was not noticeable in either biomass or supernatant samples (<4%).

From the results of resistance analysis, the contributions of both particulate and soluble materials to flux decline were evaluated (Fig. 3). Fouling resistance ($R_p + R_{ir}$) by particulates SS was calculated from the difference between those of biomass and supernatant. Relative contribution of SS to the overall fouling resistance exhibited 56, 44, 44, and 47% at 0, 48, 96, and 288 h, respectively. At the beginning of the experiment, probably in the stationary phase of microbial growth, the contribution of SS to membrane fouling was slightly larger than that of supernatant. However, as starvation progressed leading to activity decrease, the supernatant was more responsible for membrane fouling, probably in the death phase of microbial growth. This result implied that the contribution of particulate and soluble materials to membrane fouling is closely related to cell lysis and surface properties of biomass as activity changed. To confirm the dominant parameters causing ultrafiltration fouling resistances in different growth phases, various biological and physico-chemical properties of biomass were investigated and are described in the following sections.

3.3. Biological properties of biomass and supernatant

SMP and EPS have been considered the major culprits in membrane fouling, which cause microbial adsorption and cake formation on membrane surfaces [1,9]. It is generally accepted that DOC concentrations in the supernatant liquor are attributed to either undecomposed organic substances contained in raw wastewater or the microbial metabolic products produced in biological reactions (i.e. SMP) [20]. Since the prepared synthetic wastewater was easily biodegradable, the DOC concentration can be an indicator of the

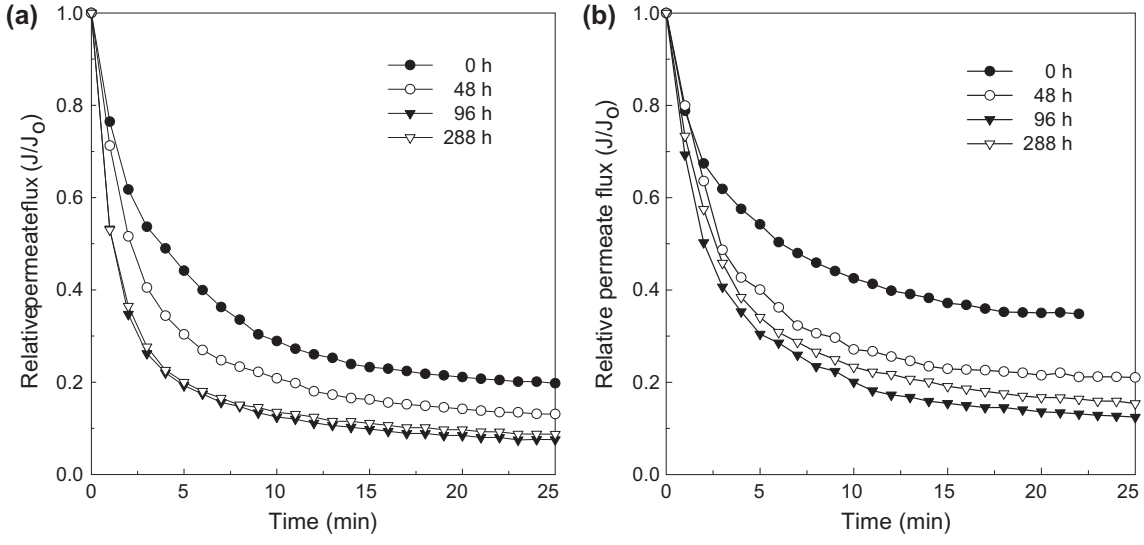

Fig. 2. Flux declines during ultrafiltration of (a) biomass and (b) supernatant at different experiment time as starvation progressed.

Table 1
Summary of filtration resistances over time from ultrafiltration experiments of biomass (1,000 mg-VSS/L) and supernatant

Time (h)	R_m (10^{12} m^{-1} (%))	R_p (10^{12} m^{-1} (%))	R_{ir} (10^{12} m^{-1} (%))	R_t (10^{12} m^{-1} (%))
Biomass				
0	2.1[a] (20.0)[b]	8.2 (78.1)	0.2 (1.9)	10.5 (100)
48	2.0 (12.7)	13.5 (85.4)	0.3 (1.9)	15.8 (100)
96	1.9 (06.5)	27.1 (92.8)	0.2 (0.7)	29.2 (100)
288	2.0 (08.1)	22.0 (89.4)	0.6 (2.5)	24.6 (100)
Supernatant				
0	2.0 (35.1)	3.6 (63.2)	0.1 (1.7)	5.7 (100)
48	2.0 (20.6)	7.5 (77.3)	0.2 (2.1)	9.7 (100)
96	1.9 (11.0)	15.1 (87.8)	0.2 (1.2)	17.2 (100)
288	2.0 (14.9)	10.9 (81.4)	0.5 (3.7)	13.4 (100)

[a]The values are average of two runs
[b]The percentage to total resistance (R_t)

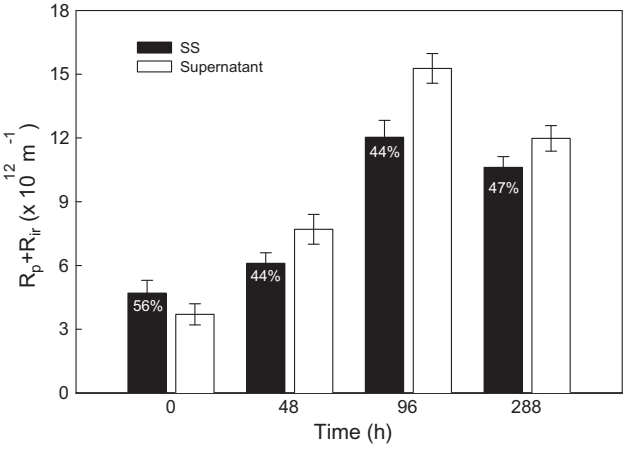

Fig. 3. Fouling resistances ($R_p + R_{ir}$) of particulates (SS) and soluble materials (supernatant) over times as starvation progressed.

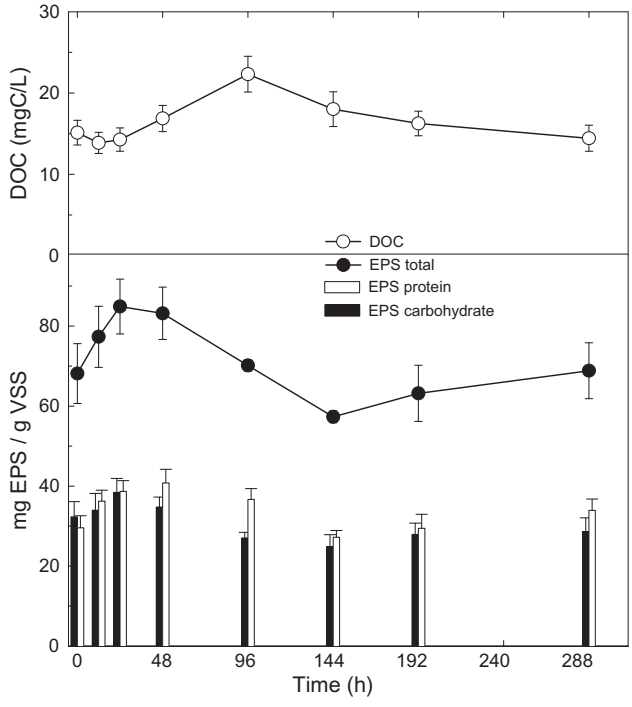

Fig. 4. Changes in DOC of supernatant and EPS.

latter. The DOC of the supernatant recorded the maximum value around 96 h of the experiment (Fig. 4). This coincided with the release of organic materials from the cell partially due to the death of micro-organisms that has been explained by the decrease in microbial activity as starvation progressed (Fig. 1). Micro-organisms excrete organic materials under starvation conditions to obtain energy for maintenance by endogenous respiration or metabolism of intracellular components [21,22].

Combining the results in Figs. 3 and 4, the contribution of supernatant to membrane fouling was relatively distinct at 96 h of the experiment when the SMP appeared the highest. After 96 h, DOC levels in the system slightly decreased possibly because a relatively readily degradable portion of SMP was eliminated. However, DOC remained at the end of the experiment due to undecomposed SMP, which could be considered refractory under this experimental condition.

Fig. 4 also presents the concentrations of carbohydrates and proteins in EPS as starvation progressed. At the beginning of the experiment, EPS proteins (EPS_p) were slightly less than EPS carbohydrates (EPS_c) ($EPS_p/EPS_c = 0.9$), but the ratios of proteins to carbohydrates of EPS increased after 48 h ($EPS_p/EPS_c = 1.2–1.5$). This was probably due to the excretion

of intracellular polymers or cell lysis, which could be explained by increased DOC concentrations (Fig. 4) and decreased microbial activities (Fig. 1). Carbohydrates are extracellularly synthesized for a specific function; proteins exist in the extracellular polymer networks due to the excretion of intracellular polymers or cell lysis [17]. Since there was no additional feeding, the portion of carbohydrates in microbial floc, which reflected the available carbon, declined. However, the portion of proteins on the cell surface increased as starvation progressed, while the total EPS concentration decreased.

Statistical analysis of the experimental data was performed to find the key contributors to membrane fouling caused by SS and supernatant as starvation progressed. The protein/carbohydrate ratio in EPS ($r = 0.84$) appeared more significant than the quantity of total EPS ($r = -0.36$) to predict the fouling resistance caused by SS during starvation. Supernatant DOC, which represents SMP in this study, correlated ($r = 0.84$) with the fouling resistance caused by supernatant. Microbial activity had strong negative correlations with both the fouling resistance caused by SS ($r = -0.94$) and supernatant ($r = -0.83$), implying that a lower activity has more fouling potential by particulate and soluble fractions of biomass.

3.4. Physico-chemical properties of biomass and supernatant

Sizes of microbial floc and colloids, which could mainly affect the plugging resistance, decreased as starvation progressed. The floc had average sizes of 5.9, 5.6, 5.1, and 4.9 μm at 0, 48, 96, and 288 h of the experiment, respectively. The floc sizes were inversely proportional to the experiment time, partially explaining higher R_p values at 96 and 288 h than at 0 and 48 h in both biomass and supernatant ultrafiltration tests (Table 1). As the sizes of particulate materials decreased, the cake layer became denser. The difference in sizes was probably due to a continuous hydraulic stress and different physiological states of biomass as starvation progressed.

Apparent MW distributions of soluble organic fractions were different from each other as starvation progressed (Fig. 5). All the solutions contained a wide distribution of MW ranging from <3 k to >100 k Da. Lower MW fraction (<3 k Da) dominated at the beginning of the experiment but decreased over time as starvation progressed. Portions of higher MW fraction (>30 k Da) were larger after the breakthrough cell lysis (96 and 288 h) than before (0 and 48 h). The portions of higher MW over 30 kDa were 75% at 96 h and 70% at 288 h. As cell lysis increased, high MW compounds

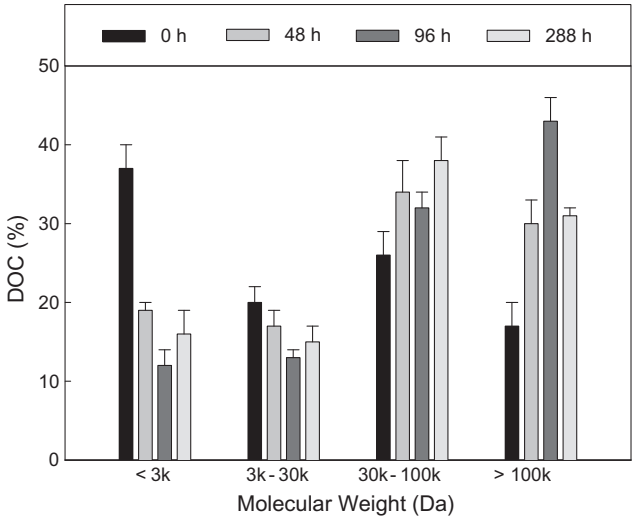

Fig. 5. MW distribution of DOC in supernatant.

such as RNA, enzymes, and proteins probably leaked out [10,23], and these molecules larger than 30 kDa probably adsorbed onto the membrane pores and led to the higher relative contribution of supernatant to overall membrane fouling (Fig. 3).

Hydrophobicity and surface charge of both SS and supernatant samples were summarized in Table 2. Contact angles and SUVA values represent hydrophobicity of SS and supernatant, respectively. High SUVA values indicate a relatively high degree of hydrophobicity due to its high aromaticity. Hydrophobicity of SS and supernatant appeared to increase by 96 h as starvation progressed but decreased at the end of the experiment (288 h). SUVA of the supernatant was the largest at 96 h (Table 2). SUVA values are relatively low compared to typical wastewater samples because the synthetic wastewater used glucose as carbon source (influent $UVA_{254} = 0.0\ cm^{-1}$).

Surface charges of SS and zeta potential of supernatant similarly varied as starvation progressed (Table 2). Net negative charge decreased as starvation progressed. EPS_p/EPS_c had a strong positive influence on the surface charge of SS ($r = 0.95$), but the total EPS had no notable influence ($r = -0.07$). In addition, EPS protein had a weak positive correlation ($r = 0.46$) with surface charge, implying that the charge properties of protein could affect the surface charge of SS. Positively charged and non-polar (hydrophobic) amino acids in protein might be dominant in biomass samples [24]. As starvation progressed, microbial activity decreased (Fig. 1), and proteins that were excreted from the cell onto the cell surface increased. These could neutralize the negative charges and therefore decreased the net

Table 2
Hydrophobicity and surface charge of SS and supernatant

	SS		Supernatant	
Time (h)	Contact angle (degrees)	Surface charge (meq g^{-1}-VSS)	SUVA (L mg m^{-1})	Zeta potential (mV)
0	36 ± 2	−0.63 ± 0.05	0.35 ± 0.05	−12.5 ± 0.9
48	43 ± 2	−0.54 ± 0.03	0.66 ± 0.04	−10.2 ± 0.7
96	52 ± 3	−0.44 ± 0.04	0.72 ± 0.05	−8.9 ± 0.7
288	41 ± 4	−0.47 ± 0.03	0.22 ± 0.03	−8.0 ± 0.8

negative surface charges of SS and supernatant. Hydrophobicity ($r = 0.71$) and surface charge ($r = 0.95$) of SS demonstrated a relatively strong positive correlation with the fouling resistance caused by SS, and zeta potential ($r = 0.85$) exhibited a good correlation with the fouling resistance caused by supernatant during starvation.

4. Conclusions

The biological and physico-chemical characteristics of microbial floc and supernatant were investigated as starvation progressed. The death phase of microbial growth exhibited potential in membrane fouling higher than that of the stationary phase. Changes in biological and physico-chemical properties of biomass and supernatant correlated with changes in fouling resistances during ultrafiltration of biomass and supernatant. The contributions of particulates and soluble materials in biomass to membrane fouling depended on microbial growth phases. Particulates were more responsible for membrane fouling in the stationary phase, while soluble materials were more accountable in the death phase. EPS compositions were dependent on the excretion of intracellular polymers or cell lysis as starvation progressed. The compositions and properties of EPS appeared more related to membrane fouling than the amount of EPS. A decrease in microbial activity and a potential of cell lysis induced a distinct permeate flux decline after 96 h of starvation condition in both biomass and supernatant filtration. Strong correlations between microbial activity and fouling resistances indicated that biomass with a lower activity can experience higher membrane fouling potentials.

Acknowledgments

This study was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2012R1A1A1039250). This study was also supported by Korea Small and Medium Business Administration (Grant No. S2092789) and by Industrial Facilities & Infrastructure Research Program (Grant No. 14IFIP-B087385-01) funded by the Ministry of Land, Infrastructure and Transport of Korean government.

References

[1] J. Mallevialle, P.E. Odendaal, M.R. Wiesner (Eds.), Water Treatment Membrane Processes, McGraw-Hill, New York, NY, 1996.
[2] S. Adham, P. Gagliardo, L. Boulos, J. Oppenheimer, R. Trussell, Feasibility of the membrane bioreactor process for water reclamation, Water Sci. Technol. 43 (2001) 211–218.
[3] W. Yang, N. Cicek, J. Ilg, State-of-the-art of membrane bioreactors: Worldwide research and commercial applications in North America, J. Membr. Sci. 270 (2006) 201–211.
[4] X. Li, F.I. Hai, L.D. Nghiem, Simultaneous activated carbon adsorption within a membrane bioreactor for an enhanced micropollutant removal, Bioresour. Technol. 102 (2011) 5319–5324.
[5] K. Brindle, T. Stephenson, The application of membrane bioreactor for the treatment of wastewaters, Biotechnol. Bioeng. 49 (1996) 601–610.
[6] T. Mukai, K. Takimoto, T. Kohno, M. Okada, Ultrafiltration behaviour of extracellular and metabolic products in activated sludge system with UF separation process, Water Res. 34 (2000) 902–908.
[7] L. Defrance, M. Jaffrin, B. Gupta, P. Paullier, V. Geaugey, Contribution of various constituents of activated sludge to membrane bioreactor fouling, Bioresour. Technol. 73 (2000) 105–112.
[8] E. Bouhabila, R.B. Aim, H. Buisson, Fouling characterisation in membrane bioreactors, Sep. Purif. Technol. 22–23 (2001) 123–132.
[9] W. Lee, S. Kang, H. Shin, Sludge characteristics and their contribution to microfiltration in submerged membrane bioreactors, J. Membr. Sci. 216 (2003) 217–227.
[10] C. Lopez, M.N. Pons, E. Morgenroth, Endogenous processes during long-term starvation in activated sludge performing enhanced biological phosphorus removal, Water Res. 40 (2006) 1519–1530.
[11] B.E. Logan, Q. Jiang, Molecular size distributions of dissolved organic matter, J. Environ. Eng. ASCE 116 (1990) 1046–1062.

[12] W.C. Duncan-Hewitt, Z. Policova, P. Cheng, E.I. Vargha-Butler, A.W. Neumann, Semiautomatic measurement of contact angles on cell layers by a modified axisymmetric drop shape analysis, Colloids Surf. 42 (1989) 391–403.
[13] J.W. Morgan, C.F. Forster, L. Evison, A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges, Water Res. 24 (1990) 743–750.
[14] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–356.
[15] O.H. Lowry, N.H. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[16] B. Frølund, R. Palmgren, K. Keiding, P.H. Nielsen, Extraction of extracellular polymers from activated sludge using a cation exchange resin, Water Res. 30 (1996) 1749–1758.
[17] R. Bura, M. Cheung, B. Liao, J. Finlayson, B.C. Lee, I.G. Droppo, G.G. Leppard, S.N. Liss, Composition of extracellular polymeric substances in the activated sludge floc matrix, Water Sci. Technol. 37 (1998) 325–333.
[18] C.N. Hass, Oxygen uptake rate as an activated sludge control parameter, J. Water Pollut. Control Fed. 51 (1979) 938–943.
[19] APHA, AWWA and WEF, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, DC, 2012.
[20] D.J. Barker, D.C. Stuckey, A review of soluble microbial products (SMP) in wastewater treatment systems, Water Res. 33 (1999) 3063–3082.
[21] E.A. Dawes, D.W. Ribbins, Some aspects of the endogenous metabolism of bacteria, Bact. Rev. 28 (1964) 126–149.
[22] C.W. Boylen, J.C. Ensign, Intracellular substrates for endogenous metabolism during long-term starvation of rod and spherical cells of *Arthrobacter crustallopoietes*, J. Bacteriol. 103 (1970) 578–587.
[23] V. Urbain, B. Mobarry, V. Desilva, D.A. Stahl, B.E. Rittmann, J. Manem, Integration of performance, molecular biology and modeling to describe the activated sludge process, Water Sci. Technol. 37 (1998) 223–229.
[24] M.F. Dignac, P. Ginestet, D. Rybacki, A. Bruchet, V. Urbain, P. Scribe, Fate of wastewater organic pollution during activated sludge treatment: Nature of residual organic matter, Water Res. 34 (2000) 4185–4194.