Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2017.21664

99 (2017) 112–116
December

Inhibition of biofilm formation on FO membrane surface by plant-oriented organic molecules

Seung-Ju Choi, Dong-Kyu Park, Yeo-Myeong Yun, Seoktae Kang*

Department of Civil and Environmental Engineering, Korea Advanced Institute of Science and Technology, Daejeon 34141, Korea, Tel. +82-42-350-3635; Fax: +82-42-350-3610; email: stkang@kaist.ac.kr (S. Kang)

Received 27 August 2017; Accepted 28 October 2017

ABSTRACT

Quorum sensing inhibition (QSI) has been suggested as a potential solution to suppress the growth of biofilm on solid surfaces using pure enzymes or enzyme producing. In this study, three plant-oriented organic molecules (cinnamaldehyde, CIN; vanillin, VAN; zingerone, ZIN) were applied as QSIs in forward osmosis (FO) membrane system using *Pseudomonas aeruginosa* PAO1 as a model biofoulant. After 36 h of FO operation, all tested experiments with QSIs exhibited the retarded flux decline, and resulted in the increase in accumulated permeate volume by 5% (CIN), 21% (VAN), and 15% (ZIN) compared with that of control. It was due to the difference in the characteristics of biofilm formed on the membrane surface, that the biomass on the unit area of membrane surface with QSIs was decreased by 68%, 41%, and 15% in the presence of CIN, VAN, and ZIN, respectively. In the absence of QSIs, membrane surface turned more hydrophobic, which hindered the transport of permeate water due to the formation of hydrophobic biofilm, while those in the presence of QSIs possessed similar contact angle compared with that of the virgin membrane. Furthermore, the amount of extracellular polymeric substances per unit area of membrane was reduced significantly in the presence of QSIs. In conclusion, the addition of QSIs can be the economically feasible strategy to mitigate biofouling not only reducing the amount of biofilm on the membrane surface but also modifying properties of biofilm.

Keywords: Quorum sensing; Forward osmosis; Plant-oriented organic molecules; Permeate flux; Biofilm

1. Introduction

Water reuse and desalination processes have emerged as effective solutions to solve the problem of water scarcity around the world [1]. In particular, membrane processes including nanofiltration (NF), forward osmosis (FO), and reverse osmosis (RO) have been widely applied in both desalination and water reuse processes and core technologies including desalting and ultrapure water production [2]. Among them, FO is a technology where solvent passes through a semipermeable membrane by the osmotic pressure difference between the feed and draw side. In an FO–RO hybrid system, where effluent of wastewater and seawater enters the feed and draw side of FO, could be operated with lower energy consumption compared with an RO only process, as the fouling in RO is significantly reduced due to the rejection of potential foulants in FO [3]. Although fouling in FO is more reversible than RO [4], FO membrane is still prone to fouling because effluent of wastewater contains various foulants such as organics, inorganic salts, colloids, and microorganisms. Consequently, for the application of FO–RO hybrid membrane processes, extensive pretreatment would be required especially to minimize the formation of biofilm, or biofouling [5].

As a method to control biofilm, the application of physical cleaning by increasing the cross-flow velocity of the feed side has been shown to be ineffective in preventing or removing irreversible biofouling [6]. On the other hand, chemical cleaning has been utilized as an effective strategy,

* Corresponding author.

but long-term usage of cleaning agents was shown to hamper the structure of the membrane [7]. Thus, the novel strategy which can effectively hinder the build-up of biofouling on the membrane surface rather than the precursor removal of fouling is strongly required [8].

Bacteria are known to communicate with each other, a phenomenon known as quorum sensing (QS), using molecules named signal molecules during the formation of biofilm [9]. The quorum sensing inhibition (QSI), a concept of bacterial QSI, has been studied in membrane-based water treatment systems to mitigate biofouling as the form of pure enzymes such as acylase and lactonase, or carrying the enzyme producing bacteria [10–13]. However, the application of enzymes or enzyme producing bacteria is only limited to membrane bioreactor, and its economical feasibility and difficulties in maintaining enzyme producing bacteria are still a hurdle for wide application. Recently, plant-oriented organic molecules such as cinnamaldehyde (CIN), vanillin (VAN), and zingerone (ZIN) have been studied as potential alternative of enzymatic QSIs [14]. These plant-oriented organic molecules are known to have antibacterial activity at high concentration as well as effective in mitigating the formation of biofilm at the concentration even lower than growth inhibitory concentration [15–17]. Especially, CIN and ZIN have structural similarity with signal molecules such as acyl-homoserine lactone (AHL), and thus interfere signal molecules binding to the receptors [18,19]. In case of VAN, it was shown to degrade AHL without reducing the colony forming units of the bacteria used in the study [20]. When plant-oriented organic molecule, especially VAN, was applied in RO system, it effectively mitigated biofouling and also showed reduction in the amount of extracellular polymeric substances [21]. However, the detailed mechanism of these molecules has not been elucidated.

Therefore, the objective of this study is to apply plant-oriented organic molecules, specifically, CIN, VAN, and ZIN in FO membrane process to assess whether these molecules are effective in alleviating biofilm formation with lab-scale FO membrane system. Furthermore, membrane surface properties and foulants remaining on the membrane will be analyzed to identify antifouling mechanisms induced by QSIs.

2. Materials and methods

2.1. Types of QSIs

Three QSIs (CIN, VAN, ZIN) were purchased as analytical grade (Sigma-Aldrich, USA). Stock solutions of QSIs were made by dissolving each chemical in a DI solution with the final concentration of 10 mM.

2.2. FO experimental setup

2.2.1. Membrane and FO cell

FO membrane was purchased (Toray, Japan), and stored at 4°C in DI water. FO membrane was cut as a size of 9.5 cm × 4.5 cm, then placed inside of lab-scale FO cell with inner dimensions of 2.60 cm × 7.75 cm × 0.30 cm (W × L × H). The FO cell was then located in a lab-scale FO testing setup (Fig. 1). The temperature of both feed and draw solution was kept 30°C, and the cross-flow velocity of the feed and draw solution was maintained at 10 and 7 cm/s, respectively.

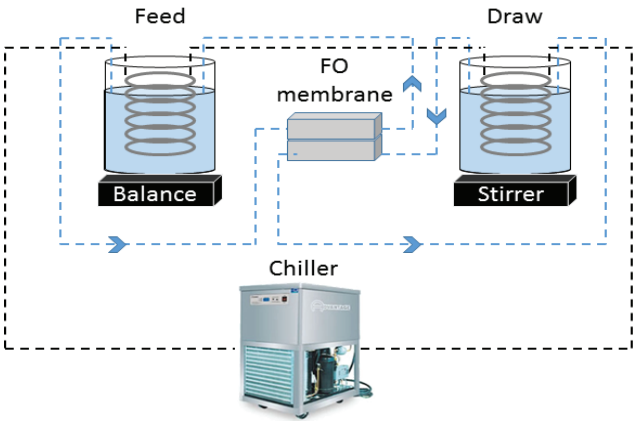

Fig. 1. Schematic diagram of continuous forward osmosis experiment.

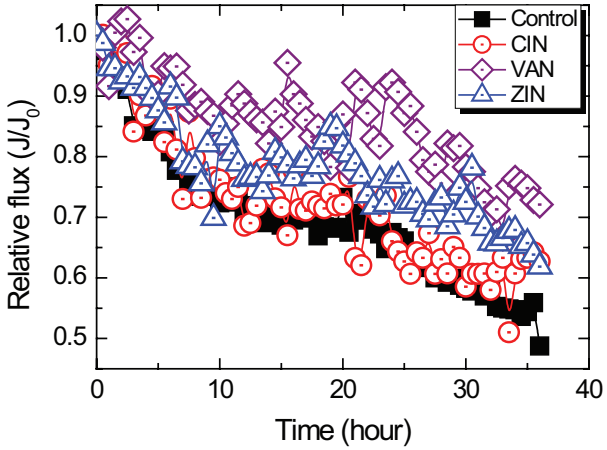

Fig. 2. Changes of permeate flux in the absence and presence of QSIs. Note that initial flux (J_0) was 31.2 ± 2.4 LMH.

2.2.2. Operating condition of FO system

As a feed solution, the synthetic wastewater effluent was prepared with the mixture of salts, phosphate buffer, and 0.2 mM sodium citrate as a carbon source as reported [22,23]. Three QSIs at the concentration of 1 mM and *Pseudomonas aeruginosa* PAO1 2 × 10^7 cell/mL were then added in the feed stream. Osmotic pressure was generated by draw solution of 0.5 M sodium chloride to simulate seawater. The permeate flux was calculated by measuring the difference in the weight of the feed solution reservoir using a digital scale (GF-4000, AND Co., Japan) placed under the reservoir as shown in Fig. 1.

2.3. Analysis of biofilm

2.3.1. Measurement of contact angle

After operation, the membrane was dried at the room temperature in the desiccator. The contact angle of the membranes was then measured using a contact angle analyzer (Phoenix 300 Plus, SEO Co., South Korea). Five measurements were conducted for each sample with sessile drop method, and the measured values were averaged.

2.3.2. SEM images

A biofouled membrane was fixed by following the protocol described elsewhere [24]. In brief, the membrane was washed three times with phosphate buffer saline (PBS), and then in 3 vol% glutaraldehyde in PBS for 5 h in 4°C. The membrane was then washed again with PBS and dehydrated stepwise by soaking in 25%, 50%, 75%, and 100% ethanol for 10 min respectively. Finally, the fixed membrane was observed through a field emission scanning electron microscopy (SEM; SU8230, Hitachi, Japan).

2.3.3. Mass and EPS content of biofilm

The biofilm was collected using a silicon knife, and then filtrated through 0.2 μm paper filter after vortexing. The membrane was then dried at 105°C for 2 h. The difference in weight before and after the filtration was defined as the biomass. Extracellular polymeric substances were extracted from the collected biofilm through a heat extraction method [25]. The carbohydrate and protein parts of EPS were quantified through Dubois and Bradford assays, respectively [26].

3. Results and discussions

3.1. Mitigation of biofouling by QSIs

Continuous FO experiments were conducted and relative flux (J/J_0) was monitored for 36 h in the absence (control) and presence (CIN, VAN, and ZIN) of QSIs. According to previous studies, major mechanism of flux decline in FO is due to the deposition of particulate matters such as colloids and microorganism, which induce colloid-enhanced osmotic pressure on the surface of feed side [23,27]. In Fig. 2, the decline of flux was retarded when QSIs were added in the feed solution. Especially, VAN showed significant retardation compared with CIN and ZIN. Thus, total permeate flux in the presence and absence of QSIs was compared. In Table 1, the total permeate flux normalized by that of the control was increased by 5%, 21%, and 15%, respectively, in the presence of CIN, VAN, and ZIN. Therefore, the effectiveness of QSIs in mitigating biofouling was shown to be in the order of VAN, ZIN, and CIN. This result implies that FO system could be operated more effectively in the presence of QSIs such as VAN.

To elucidate the structural difference of biofilm in the absence and presence of QSIs, biofilm on FO membrane surfaces were further analyzed by SEM and fluorescent microscopic images. As shown in Fig. 3, FO membranes without QSIs possessed more developed and dense structure of biofilm compared with those of QSIs (CIN). In contrast, the number of bacteria deposited on the membrane was significantly reduced in the existence of QSIs. As discussed in our previous study, deposition of microorganism and subsequent formation of biofilm layer on FO membrane surface hindered the back-diffusion of concentrated salts by cross-flow mixing [23]. Thus, we can conclude that the hindered deposition and inhibited growth of *P. aeruginosa* resulted in the modification of biofilm structure with QSIs in the solution. Moreover, the fully developed biofilm contributed to an increase in the hydrophobicity of membrane surface as shown in Table 1. These observations were also correlated with a decrease in

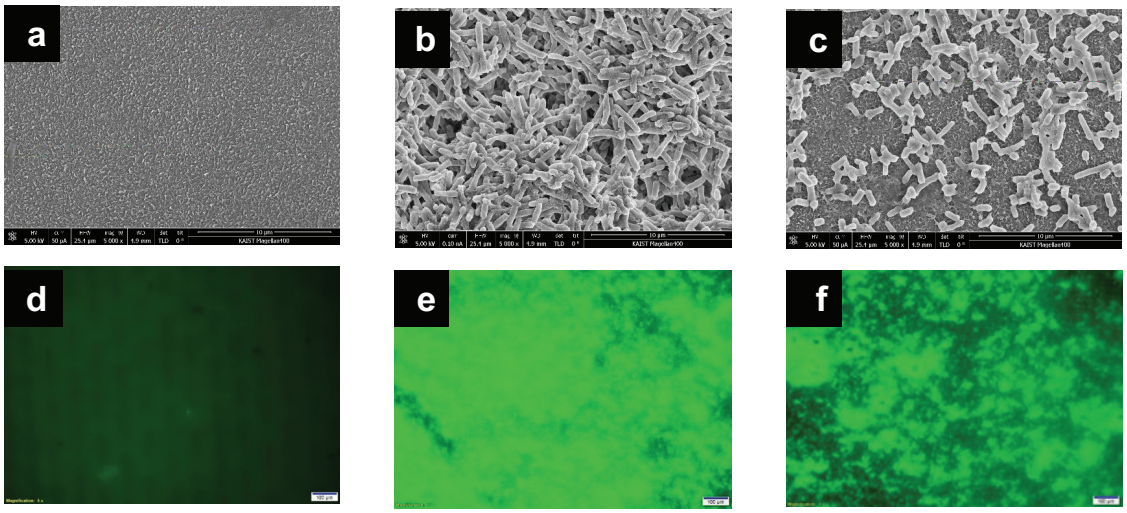

Fig. 3. SEM (top) and fluorescence (bottom) images of FO membrane surface. (a) and (d) Virgin membrane, (b) and (e) control, (c) and (f) with QSI (cinnamaldehyde). Note that fluorescence images were taken after 21 h of operation.

Table 1
Properties of virgin and fouled membranes with and without QSIs

	Virgin	Control	CIN	VAN	ZIN
Relative flux after 36 h (J/J_0)	1	0.49	0.63	0.72	0.62
Contact angle (°)	30.0 ± 5.0	61.0 ± 1.0	52.9 ± 2.5	27.4 ± 3.5	49.9 ± 5.0
Mass of biofilm (g/m^2)	–	4.23	1.35	2.52	3.59

the amount of biocake in the presence of QSIs compared with the absence of QSIs, which discussed in the next section.

3.2. Characterization of biofilm

3.2.1. Contact angle and amount of biofilm

To identify the changes in the membrane surface properties, the contact angle of virgin and biofouled membranes were measured. As shown in Table 1, there were clear differences in contact angles between the virgin and fouled membranes, where fouled membranes turned hydrophobic. However, in the presence of QSIs, the surface of FO membrane was more hydrophilic, and interestingly, the decrease of flux was strongly correlated with the increase of contact angle. In particular, the contact angle of VAN specimen was almost as same as that of virgin membrane. This is in accordance with the previous result that the total permeate flux was the highest in the presence of VAN.

The amount of biocake remaining on the membrane without QSIs was 4.23 g/m^2, and it was decreased by 68%, 41%, and 15% in the presence of CIN, VAN, and ZIN, respectively. It has been reported that the biofilm formed on FO membrane process is much weaker than that of RO membrane process due to the lack of hydraulic pressure across the membrane [28]. Although the amount and thickness of the biofilm cannot induce a significant hydraulic resistance, thicker biofilm layer on FO membrane surface can hinder the back-diffusion of concentrated salts accumulated in the biofilm layer [29]. Thus, the mechanism of biofouling mitigation in the presence of QSIs was shown to be not only to alter the biofilm hydrophobicity, but also to be effective in mitigating the formation of biofilm on the membrane.

3.2.2. Properties of EPS

EPS, a typical biopolymer that is known to enhance structural stability of biofilms and further lead to irreversible fouling. The amounts of both the carbohydrate and protein parts of EPS per unit area were quantified after the biofouling experiments. As shown in Fig. 4, the protein part of EPS decreased by 5%, 30%, and 42%, and the carbohydrate part was reduced by 38%, 41%, and increased 5% in the presence of CIN, VAN, and ZIN, respectively. In the previous study, protein part of EPS was shown to play crucial roles in embedding cells and forming clusters and further resulted in the quick growth of biofouling layer, and carbohydrate fractions played stabilizing the biofilm structure by forming a scaffold within the fruiting-body structure [30]. Consequently, these results corresponded to the results from permeate flux data, that the amount of biofilm was significantly weaker in the presence of QSIs than control due to the deficit of structural building block of biofilm such as EPS. In our results, specifically, the amount of protein part of EPS was reduced significantly in the presence of QSIs. Therefore, QSIs were proven to be effective in mitigating biofilm formation.

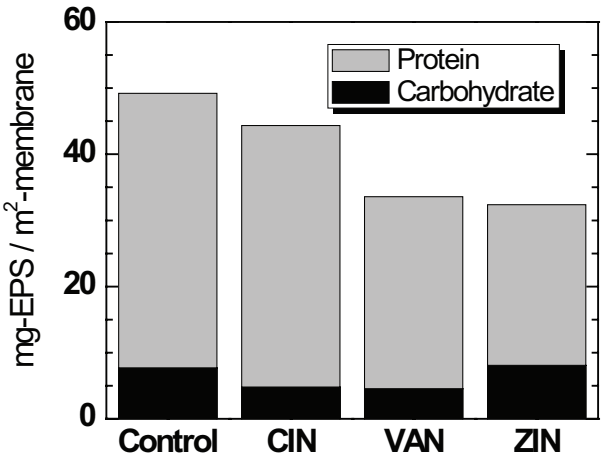

Fig. 4. The amount of protein (gray bar) and carbohydrate (black bar) parts of EPS per unit area for the presence and absence of QSIs.

4. Conclusions

In this study, the applicability of economic QS inhibitors such as CIN, VAN, and ZIN in mitigating biofilm formation on an FO membrane surface was verified. The propensity for declining flux was delayed and the total permeate volume was increased significantly in the presence of QSIs. The mitigation of biofouling was induced by the change in the membrane surface properties, where the contact angle of the fouled membrane in the presence of QSIs became more hydrophilic compared with that in the absence of QSIs. Moreover, the amount of biocake and EPS was significantly reduced in the presence of QSIs. In conclusion, the addition of QSIs to the feed water which is prone to membrane biofouling is effective to mitigate biofilm formation, thus an effective strategy in FO–RO hybrid system.

Acknowledgments

First and second authors are supported by U-City Master and Doctor Course Grant Program, and this research was supported by grant (code 17IFIP-B088091-04) from Industrial Facilities & Infrastructure Research Program and funded by Ministry of Land, Infrastructure and Transport of Korean government.

References

[1] M.A. Shannon, P.W. Bohn, M. Elimelech, J.G. Georgiadis, B.J. Marinas, A.M. Mayes, Science and technology for water purification in the coming decades, Nature, 452 (2008) 301–310.
[2] A.J. Ansari, F.I. Hai, W.E. Price, J.E. Drewes, L.D. Nghiem, Forward osmosis as a platform for resource recovery from municipal wastewater – a critical assessment of the literature, J. Membr. Sci., 529 (2017) 195–206.
[3] T.Y. Cath, N.T. Hancock, C.D. Lundin, C. Hoppe-Jones, J.E. Drewes, A multi-barrier osmotic dilution process for simultaneous desalination and purification of impaired water, J. Membr. Sci., 362 (2010) 417–426.
[4] B. Mi, M. Elimelech, Organic fouling of forward osmosis membranes: fouling reversibility and cleaning without chemical reagents, J. Membr. Sci., 348 (2010) 337–345.
[5] M. Kumar, S.S. Adham, W.R. Pearce, Investigation of seawater reverse osmosis fouling and its relationship to pretreatment type, Environ. Sci. Technol., 40 (2006) 2037–2044.
[6] H. Yoon, Y. Baek, J. Yu, J. Yoon, Biofouling occurrence process and its control in the forward osmosis, Desalination, 325 (2013) 30–36.

[7] Z. Wang, J. Tang, C. Zhu, Y. Dong, Q. Wang, Z. Wu, Chemical cleaning protocols for thin film composite (TFC) polyamide forward osmosis membranes used for municipal wastewater treatment, J. Membr. Sci., 475 (2015) 184–192.
[8] S. Zhao, L. Zou, C.Y. Tang, D. Mulcahy, Recent developments in forward osmosis: opportunities and challenges, J. Membr. Sci., 396 (2012) 1–21.
[9] D.G. Davies, M.R. Parsek, J.P. Pearson, B.H. Iglewski, J.W. Costerton, E.P. Greenberg, The involvement of cell-to-cell signals in the development of a bacterial biofilm, Science, 280 (1998) 295–298.
[10] K.-M. Yeon, W.-S. Cheong, H.-S. Oh, W.-N. Lee, B.-K. Hwang, C.-H. Lee, H. Beyenal, Z. Lewandowski, Quorum sensing: a new biofouling control paradigm in a membrane bioreactor for advanced wastewater treatment, Environ. Sci. Technol., 43 (2009) 380–385.
[11] J.-H. Kim, D.-C. Choi, K.-M. Yeon, S.-R. Kim, C.-H. Lee, Enzyme-immobilized nanofiltration membrane to mitigate biofouling based on quorum quenching, Environ. Sci. Technol., 45 (2011) 1601–1607.
[12] H.-S. Oh, K.-M. Yeon, C.-S. Yang, S.-R. Kim, C.-H. Lee, S.Y. Park, J.Y. Han, J.-K. Lee, Control of membrane biofouling in MBR for wastewater treatment by quorum quenching bacteria encapsulated in microporous membrane, Environ. Sci. Technol., 46 (2012) 4877–4884.
[13] S.-R. Kim, H.-S. Oh, S.-J. Jo, K.-M. Yeon, C.-H. Lee, D.-J. Lim, C.-H. Lee, J.-K. Lee, Biofouling control with bead-entrapped quorum quenching bacteria in membrane bioreactors: physical and biological effects, Environ. Sci. Technol., 47 (2013) 836–842.
[14] V. Plyuta, J. Zaitseva, E. Lobakova, N. Zagoskina, A. Kuznetsov, I. Khmel, Effect of plant phenolic compounds on biofilm formation by *Pseudomonas aeruginosa*, APMIS, 121 (2013) 1073–1081.
[15] P. Jia, Y.J. Xue, X.J. Duan, S.H. Shao, Effect of cinnamaldehyde on biofilm formation and sarA expression by methicillin-resistant *Staphylococcus aureus*, Lett. Appl. Microbiol., 53 (2011) 409–416.
[16] L. Kumar, S. Chhibber, K. Harjai, Zingerone inhibit biofilm formation and improve antibiofilm efficacy of ciprofloxacin against *Pseudomonas aeruginosa* PAO1, Fitoterapia, 90 (2013) 73–78.
[17] K. Ponnusamy, D. Paul, J.H. Kweon, Inhibition of quorum sensing mechanism and aeromonas hydrophila biofilm formation by vanillin, Environ. Eng. Sci., 26 (2009) 1359–1363.
[18] G. Brackman, T. Defoirdt, C. Miyamoto, P. Bossier, S. Van Calenbergh, H. Nelis, T. Coenye, Cinnamaldehyde and cinnamaldehyde derivatives reduce virulence in *Vibrio* spp. by decreasing the DNA-binding activity of the quorum sensing response regulator LuxR, BMC Microbiol., 8 (2008) 149.
[19] L. Kumar, S. Chhibber, R. Kumar, M. Kumar, K. Harjai, Zingerone silences quorum sensing and attenuates virulence of *Pseudomonas aeruginosa*, Fitoterapia, 102 (2015) 84–95.
[20] J.H. Choo, Y. Rukayadi, J.K. Hwang, Inhibition of bacterial quorum sensing by vanilla extract, Lett. Appl. Microbiol., 42 (2006) 637–641.
[21] S. Kappachery, D. Paul, J. Yoon, J.H. Kweon, Vanillin, a potential agent to prevent biofouling of reverse osmosis membrane, Biofouling, 26 (2010) 667–672.
[22] C. Boo, M. Elimelech, S. Hong, Fouling control in a forward osmosis process integrating seawater desalination and wastewater reclamation, J. Membr. Sci., 444 (2013) 148–156.
[23] K. Ruengruehan, P. Fagkeaw, D. Jang, S. Ko, S. Kang, Impact of feed ionic concentration on colloidal and organic fouling of osmotically driven membrane process, Desal. Wat. Treat., 57 (2016) 24551–24556.
[24] S. Kang, M. Herzberg, D.F. Rodrigues, M. Elimelech, Antibacterial effects of carbon nanotubes: size does matter! Langmuir, 24 (2008) 6409–6413.
[25] M. Herzberg, S. Kang, M. Elimelech, Role of extracellular polymeric substances (EPS) in biofouling of reverse osmosis membranes, Environ. Sci. Technol., 43 (2009) 4393–4398.
[26] P. Gerhardt, Methods for General and Molecular Bacteriology, American Society for Microbiology, Washington D.C., 1994.
[27] C. Boo, S. Lee, M. Elimelech, Z. Meng, S. Hong, Colloidal fouling in forward osmosis: role of reverse salt diffusion, J. Membr. Sci., 390 (2012) 277–284.
[28] S.E. Kwan, E. Bar-Zeev, M. Elimelech, Biofouling in forward osmosis and reverse osmosis: measurements and mechanisms, J. Membr. Sci., 493 (2015) 703–708.
[29] A. Bogler, S. Lin, E. Bar-Zeev, Biofouling of membrane distillation, forward osmosis and pressure retarded osmosis: principles, impacts and future directions, J. Membr. Sci., 542 (2017) 378–398.
[30] B. Yuan, X. Wang, C. Tang, X. Li, G. Yu, In situ observation of the growth of biofouling layer in osmotic membrane bioreactors by multiple fluorescence labeling and confocal laser scanning microscopy, Water Res., 75 (2015) 188–200.