



Bio-entrapped membrane reactor for organic matter removal and membrane fouling reduction

Kok-Kwang Ng^a, Chien-Ju Wu^a, Li-Yuan You^a, Chin-Sheng Kuo^a, Cheng-Fang Lin^{a,*}, Andy Pui-Kwan Hong^b, Ping-Yi Yang^c

^aGraduate Institute of Environmental Engineering, National Taiwan University, 71 Chou-Shan Rd., Taipei 106, Taiwan

Tel. +886 2 3366 7427; Fax: +886 2 2392-8830; email: cflin@ntu.edu.tw

^bDepartment of Civil and Environmental Engineering, University of Utah, 110 South Central Campus Drive, 2068 MCE, Salt Lake City, UT 84112, USA

^cDepartment of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu HI 96822, USA

Received 14 December 2011; Accepted 8 May 2012

ABSTRACT

This study investigated the performance of a novel bio-entrapped membrane reactor (BEMR) in removal of organic matter and its membrane fouling condition in treating food processing wastewater. Three different hydraulic retention times (HRTs) with 6, 12 and 18 h, continuous aeration and intermittent aerations (1 h aeration and 1 h non-aeration; 1 h aeration and 2 h non-aeration) were studied. The results show that the HRT and aeration have significant impacts on the BEMR performance. The chemical oxygen demand and ammonia (NH₄⁺-N) removal efficiencies increased as the HRT increased with the continuous aeration condition. Results of this study indicated that the membrane fouling was severe at lower HRTs and intermittent aeration modes in the BEMR with a constant flux of 20 L/m² h. Conventional membrane bioreactor (CMBR) took 57 min to reach 55 kPa of trans-membrane pressure at HRT of 12 h while the BEMR could operate 215 min before reaching 55 kPa, which was 3.8 times longer than the CMBR. This may be attributed to the higher yield of biomass and production of soluble microbial products in the CMBR, because the CMBR produced 17% more of carbohydrate and 33% more of protein than the BEMR. Better performance of the BEMR was found at the longer HRTs (18 h > 12 h > 6 h) and continuous aeration was better than intermittent aeration.

Keywords: Bio-entrapped membrane reactor; Hydraulic retention time; Soluble microbial products; Intermittent aeration; Membrane fouling; Conventional membrane bioreactor

1. Introduction

Membrane technology especially membrane bioreactor (MBR) plays an important role as a biological treatment process in treating wastewater to achieve high quality effluent for water reclamation and reuse. MBR technology has been widely employed in recent

years and more than 2,200 MBRs have been installed and operated by 2004 [1]. MBR offers many advantages over the conventional activated sludge process (ASP) that includes tolerating high concentrations of mixed liquor suspended solids (MLSS), reducing waste sludge production while at the same time achieving high removals biological oxygen demand. However, a major concern in application of MBR is

*Corresponding author.

the permeate flux decline due to membrane fouling [2,3]. The primary membrane clogging mechanisms are deposition of soluble and particulate materials onto the membrane surface and/or penetrate into the membrane pore channels, which gradually decrease membrane permeability and the effectiveness of filtration.

To mitigate these disadvantages of conventional membrane bioreactor (CMBR), a new type of MBR was developed by employing bio-entrapped cells with the membrane system. This is termed bio-entrapped membrane reactor (BEMR), designed with an intention to handle high loads of dissolved organics, facilitate operation, and minimize the start-up period. In the recent years, Yang and his co-workers [4–6] investigated the effectiveness of bio-entrapped reactor (BER) in treating various types of wastewaters such as dilute swine wastewater, domestic sewage, and synthetic wastewater. Satisfactory results have been reported in eliminating pollutants such as phenol [7], odor producing substance [6], trimethylamine [8], and soluble organic matter [9]. In addition, BER produced less soluble microbial products (SMP) (71%) than that of the conventional ASP. When coupled with ultrafiltration (UF) membrane unit, BER had approximately 25–30% higher permeate flux than ASP [10]. Furthermore, various operating conditions of BER system were also investigated to optimize the efficiency of BER process. Song et al. [11] compared the process performance of multiple types of BER which includes fixed-bed BER, moving-bed BER and activated sludge process. It was discovered that both types of BER processes had better performance than the conventional ASP.

Researchers have shown sludge retention time (SRT) and hydraulic retention time (HRT) are the important parameters in MBR operations [12,13]. Different HRT operating conditions could affect the MBR's ability in treating wastewater and membrane fouling. Also, Cho et al. [14] stated that lower HRTs with high permeation flux operation could significantly hasten the membrane fouling. Several studies [10,12,15,16] were carried out for the effect of SRT on membrane fouling. They came to a consensus that extracellular polymeric substances (EPS) and SMP are noticeably higher when the SRT is shorter. Some bacteria undergo microbial aging and cell lysis and as a result, high volume of dead cells would accumulate on the membrane surface, clogging membrane pore channels. Fenu et al. [17] developed a model for SMPs in a dynamic environment, but the correlation between SMPs and fouling rates in full-scale MBR was still not well defined. Furthermore, Grady

et al. [18] also found out that high SRTs, result in a lower food to microorganism (F/M) ratio and create more interactions between substrate and microorganisms, thus increasing chemical oxygen demand (COD) removal efficiency. However, the prolonged SRT on the organic matter removal and membrane fouling behavior at various HRTs and aeration patterns are not yet well defined. Therefore, the aim of the present work was to investigate the performance of BEMR in removing organic matter and membrane fouling associated with various HRTs and aeration patterns using wastewater from a food processing plant. The results of this study could provide a promising solution to common problems encountered in MBR systems.

2. Methods

2.1. Membrane reactor set-up

Fig. 1 shows the schematic of the MBRs (BEMR and CMBR) used in this study. Both systems had a tank with a working volume of 50 L and a polyvinylidene fluoride (PVDF) hollow fiber UF membrane module air diffusers. The PVDF hollow fiber membrane module had an effective filtration area of 0.046 m² and nominal pore size of 0.036 μm (GE Zee-Weed-1, USA). The wastewater fed to both MBRs was taken from a food processing plant in Taoyuan, Taiwan. The wastewater was generated from manufacturing fresh milk, tea beverages, fruit juices, dairy products, and instant noodles. The influent was transported from the wastewater reservoir to each MBR by a peristaltic pump. Air flow-rate was controlled by a flow meter to maintain aerobic conditions of both MBRs. Dissolved oxygen (DO) concentrations were maintained at the levels of 7–8 and 2.5–3.5 mg/L for BEMR and CMBR, respectively. A timer was used to control the aeration and non-aeration schedule. Permeate was withdrawn through the UF hollow fiber membrane by a suction pump. An average constant flux of 20 L/m² h (LMH) was maintained and the rise of trans-membrane pressure (TMP) was monitored continuously. Operation parameters of the lab-scale BEMR and CMBR are presented in Table 1. SRT for the BEMR was determined following the calculations used in Qian et al. [19].

2.2. Membrane fouling analysis

The BEMR membrane fouling experiments were carried out with an average flux of 20 LMH at various HRTs (6, 12–18 h) and aeration patterns without backwash. The aeration modes in this study were

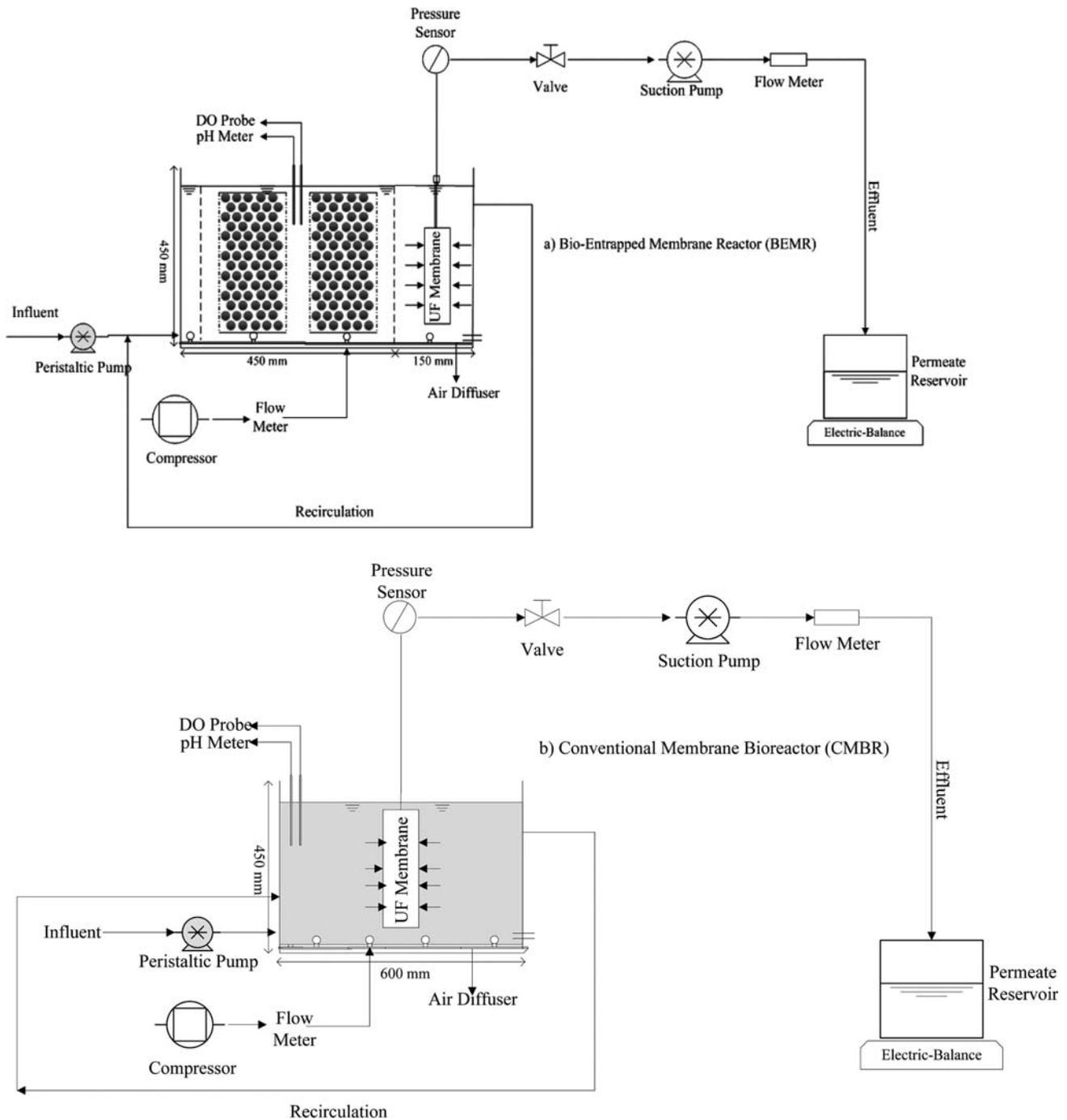


Fig. 1. Schematic diagrams of BEMR and CMBR.

operated under continuous aeration, intermittent aerations (1 h on/1 h off and 1 h on/2 h off) which followed the operating conditions by Yang et al. [5,6]. The aeration patterns with longer off periods (1 and 2 h) could be able to reduce the operating costs and energy costs of the system. Variations of HRTs at 6, 12, and 18 h were facilitated by operating the BEMR and the CMBR (12 h) with recirculation in the same

membrane area and same flux of 20 L/m^2 . For example, the BEMR in the first compartment (42 L) had a flow-rate of 3.5 L/h at the HRT of 12 h, while the membrane in the second compartment (8 L) was controlled by suction pump with a flow-rate of 0.92 L/h . Therefore, the excessive or overflow wastewater in the membrane tank would be recirculated to the first compartment. This was studied because changes in HRT

Table 1
Operation parameters of the lab-scale BEMR and CMBR

Parameter	BEMR	CMBR
Carrier diameter size (cm)	2.5	–
Reactor volume (L)	50	50
Void volume (L)	28	–
Packing ratio (%)	55.0	–
Dissolved oxygen (mg/L)	7.0–8.0	2.5–3.5
SRT (d)	500	15–20
MLSS (mg/L)	11,000 ^a	8,000
Temperature (°C)	25 ± 1.0	25 ± 1.0
pH	7.0–8.3	7.0–8.2
Average permeate flux (LMH)	20	20
HRT (h)	6, 12, 18	12

^aThe biomass in the bio-carrier.

could significantly impact the membrane fouling. The fouling experiment data were taken at the 9–10th day of each HRT in order to ensure the BEMR was operated in a steady-stage condition. For comparison, the CMBR was operated at a HRT of 12 h. The data acquisition was stopped when the TMP exceeded the maximum operating pressure (55 kPa) or the membrane fouling became significant. At that time, the membrane module would be taken out from the reactor and cleaned with sodium hypochlorite for 30 min. After cleaning operation with distilled water, the membrane was put back to the MBRs.

2.3. Analytical methods

The influent and effluent samples of the BEMR were taken daily and analyzed for COD and ammonia nitrogen ($\text{NH}_4^+\text{-N}$), using a closed reflux colorimetric method and the Nessler method with a spectrophotometer (HACH DR 2800), respectively [20]. DO and pH in the reactor were recorded daily with a portable DO/pH meter (HACH HQ20). The MLSS in the CMBR were measured with an MLSS meter (KPK SS-5Z, Japan). SMP was analyzed for total protein and total carbohydrate contents. Total protein contents were determined with the Lowry method with bovine serum albumin as the standard [10,21,22] and the modified Anthrone method was used for total carbohydrate contents with glucose as the carbohydrate standard [21–23].

3. Results and discussion

3.1. COD and ammonia removal efficiency in BEMR

The effectiveness of carbon and nitrogen removal with various HRTs (6, 12, and 18 h) and aeration

modes (continuous aeration, intermittent aeration [1 h on/1 h off and 1 h on/2 h off]) are shown in Fig. 2. Influent COD and ammonia nitrogen concentrations were measured to be in the range of 457–920 and 6.25–13.5 mg/L, respectively. During the operation of continuous aeration and HRT of 6 h, the effluent COD removal efficiency is 85–93%, while in the case of intermittent aerations (1 h on/1 h off and 1 h on/2 h off), COD removal efficiencies are 74.8–89.2 and 55.5–79%, respectively. On the other hand, at the HRT of 18 h, the removal efficiency of COD increased to 90.5–96.1% (continuous aeration), 87.1–93.3% (intermittent aeration with 1 h on/1 h off), and 75.7–86.6% (intermittent aeration with 1 h on/2 h off). In terms of ammonia removal rate under various aeration operation modes (Fig. 2 (b)), continuous aeration exhibits better performance than intermittent aerations. With continuous aeration at HRT of 6, 12, and 18 h, ammonia removal efficiencies are 79.1, 93.8, and 95.3%, respectively. Intermittent aeration (1 h on/2 h off) at HRT of 6, 12, and 18 h, the ammonia removal efficiencies are 55.3, 54.4, and 74.8%, respectively. Therefore, the results suggest that the increments in COD and ammonia removal in Fig. 2 are very modest in response to the increase of HRT.

In this study, a longer HRT generally leads to an enhanced removal of carbon and ammonia. Under lower HRT operations, there was not enough time for the microorganisms to degrade the excessive organic matter, which results in large quantities of substrate un-biodegraded in the BEMR effluent [12]. According to Dohanyos et al. [24], changes of operational parameters such as HRT might cause simultaneous increasing of volatile fatty acids that could result in a decrease in pH. Consequently, it would influence treatment efficiency negatively even though the COD removal had reached stable-state.

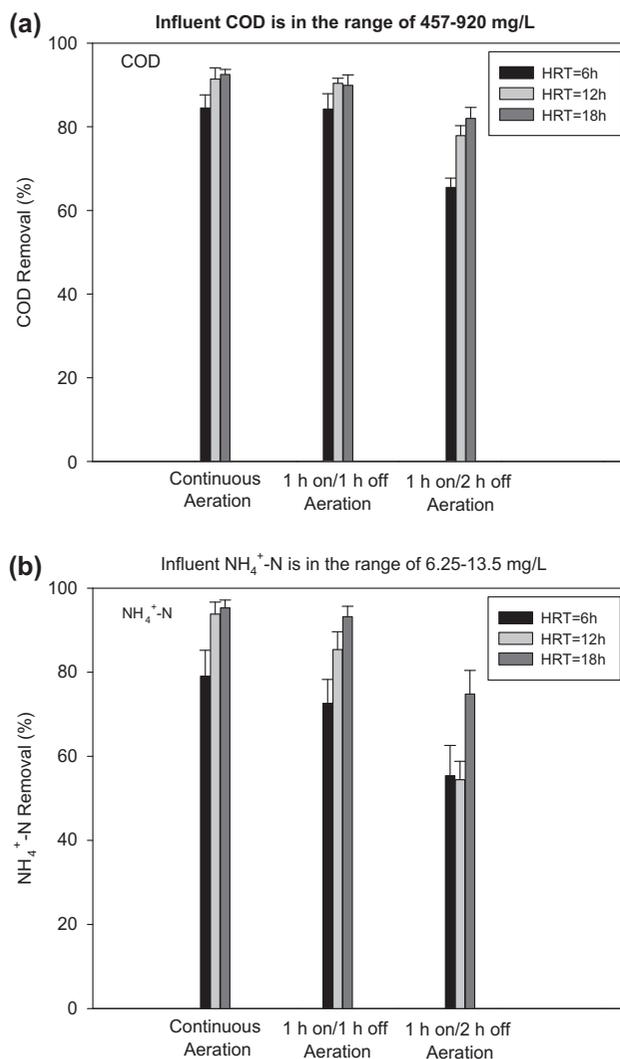


Fig. 2. Effect of HRTs and aerations on organic matter in BEMR (a) COD and (b) Ammonia.

Continuous aeration consistently exhibited higher carbon and ammonia at removal efficiencies than those of intermittent aeration (1 h on/1 h off and 1 h on/2 h off) regardless of HRT. The result was supported by Cho et al. [25] that the removal of ammonia nitrogen was more efficient in the continuous aeration mode than in the intermittent aeration mode. The higher ammonia removal efficiency in the continuous aeration operation is primarily due to cell synthesis and nitrification in the entrapped biomass of the BEMR. The longer SRT of the BEMR could remove a larger portion of influent carbon and ammonia the same time interval. The diffusion limitation of DO in the inner part of the entrapped biomass may trigger denitrification reaction in the oxic part of the BEMR [4].

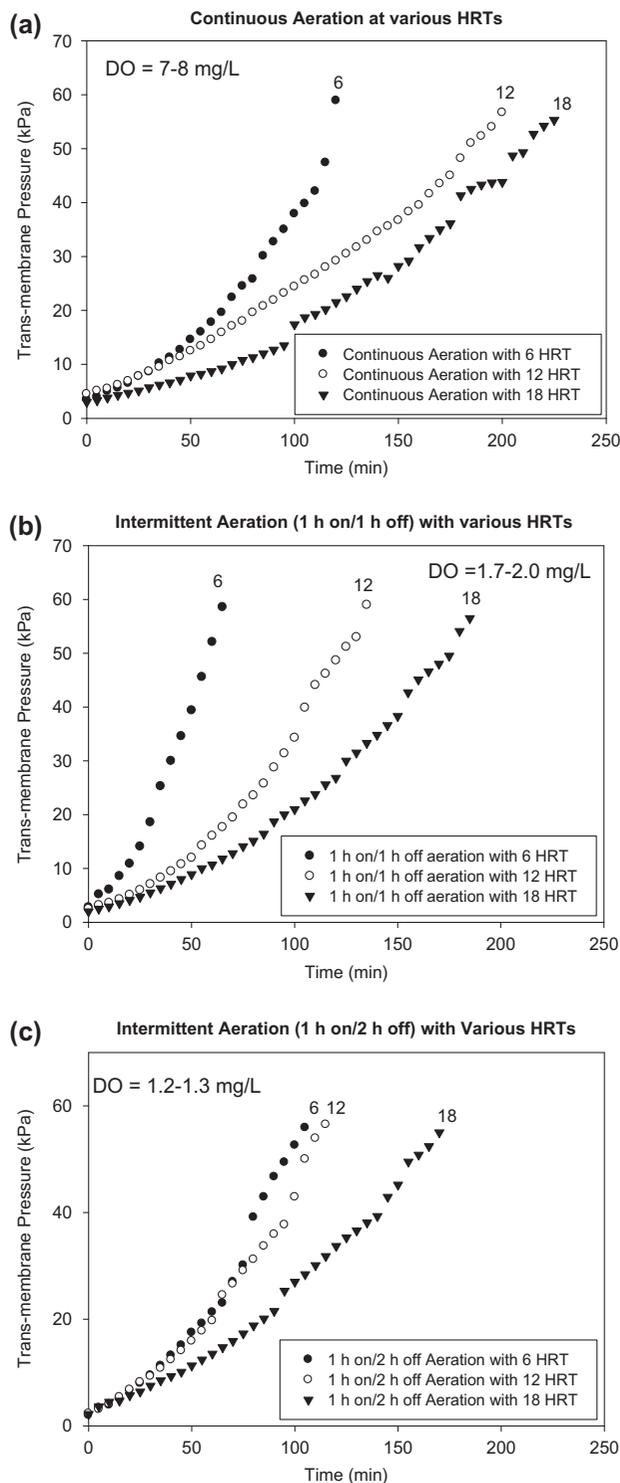


Fig. 3. Influence of HRTs and aerations on BEMR-membrane fouling.

The nitrogen removal efficiency significantly decreased in the mode of 1 h aeration and 2 h non-aeration (Fig. 2(b)). A significant increment of ammonia removal efficiency can be observed under the 1 h

aeration and 1 h non-aeration operation mode. The operation mode of 1 h aeration and 2 h non-aeration did not exhibit high carbon and ammonia removal efficiency in the BEMR as compared with the mode of 1 h aeration and 1 h non-aeration, although less aeration has the advantage of cost saving from less energy consumption. The 1 h aeration and 1 h non-aeration achieved approximately 10–20% and 17–30% higher COD and ammonia removal efficiencies respectively than 1 h aeration and 2 h non-aeration. This might be due to the highly aerated reactor from previous cycle of 1 h aeration. The 1 h aeration and 1 h non-aeration mode reactor has enough DO that sustains during the subsequent 1 h non-aeration. On the other hand, 1 h aeration and 2 h non-aeration was non-aerated for 2 h, which results in shortage of oxygen concentration for nitrification to occur [25].

In general, the BEMR responded properly in various modes of operating conditions: continuous aeration, intermittent aeration (1 h on/1 h off only) and different HRTs to achieve high levels of organic and ammonia nitrogen removals.

3.2. Membrane fouling analysis

Fig. 3 illustrates the development of TMP under different HRTs (6, 12, and 18 h) and aerations patterns (continuous and intermittent aeration) for the BEMR. The BEMR was set up to have no backwash while maintaining a constant flux of 20 LMH. The membrane fouling tests with the development of TMP were monitored in 9th or 10th day of each run. From day 1–day 8 was the acclimation period for the entrapped biomass to adapt to the new feed water and to reach pseudo-steady state conditions in the BEMR until the COD removal efficiency reached a stable condition without much fluctuation. Fig. 3 shows that the development of TMP significantly increases as the HRT decreases regardless the aeration modes, which is in agreement with the previous studies [3,12,14,22]. Membrane TMP during the continuous aeration (Fig. 3(a)) reached 55 kPa after 135, 215, and 240 min at HRT of 6, 12, and 18 h, respectively. Membrane TMP under intermittent aeration (1 h on/1 h off) approached 55 kPa (Fig. 3(b)) after 65, 135, and 185 min at HRT of 6, 12, and 18 h, respectively. At the intermittent aeration (1 h on/2 h off) membrane TMP exceeded 55 kPa (Fig. 3(c)) after 105, 115, 170 min at HRT of 6, 12, and 18 h.

Fig. 3 also shows that the BEMR exhibits a better performance in terms of membrane fouling reduction under the operation mode of intermittent aeration (1 h

on/1 h off and 1 h on/2 h off) at various HRTs. The DO concentrations with continuous aeration was 8–9 mg/L, while DO concentration at 1 h aeration and 1 h non-aeration, and 1 h aeration and 2 h non-aeration were 1.7–2.0 and 1.2–1.3 mg/L, respectively. The concentrations of suspended solids (SS) under 1 h aeration and 2 h non-aeration at HRT of 6, 12, and 18 h were measured at 34–58, 29–73 and 18–56 mg/L, respectively. While the concentrations of SS in 1 h aeration and 1 h non-aeration at HRT of 6, 12 and 18 h were 75–156, 59.7–155 and 38–79 mg/L, respectively. This can be explained by the fact that longer non-aeration period (120 min vs. 60 min) allows for better settling efficiency of SS. However, even though 1 h aeration and 1 h non-aeration pattern operates under higher SS concentration, membrane filtration period was actually slightly longer (~10%) than 1 h aeration and 2 h non-aeration pattern, except for HRT of 6 h. This phenomenon could be explained by Ng et al. [10] in which he indicated that SS was in fact the minor cause of membrane fouling, instead, the presence of SMP as a result of substrate metabolism and biomass decay was the primary factor for membrane operation decline.

3.3. Comparison of BEMR and CMBR

In addition to correlating the impact of SMP to membrane fouling tendency, the operation of BEMR was compared with the CMBR at an HRT of 12 h with a constant flux of 20 LMH, Fig. 4 shows that BEMR takes a longer filtration period (215 min) to reach 55 kPa, while the CMBR took only 57 min to approach 55 kPa. In other words, the operation time before reaching the unacceptable membrane fouling for BEMR is 3.8 times longer than the CMBR. The longer

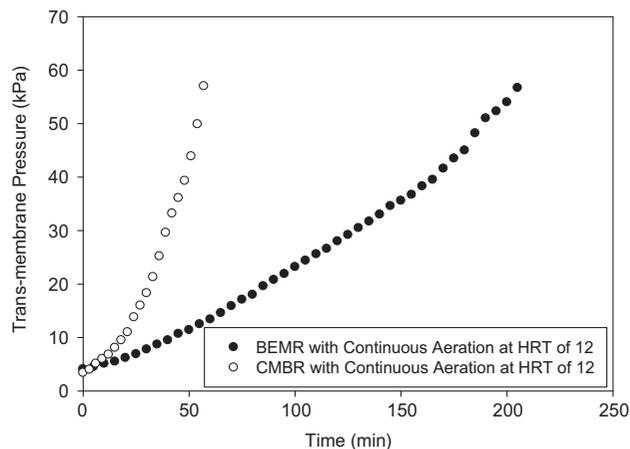


Fig. 4. Comparison of BEMR and CMBR on membrane fouling.

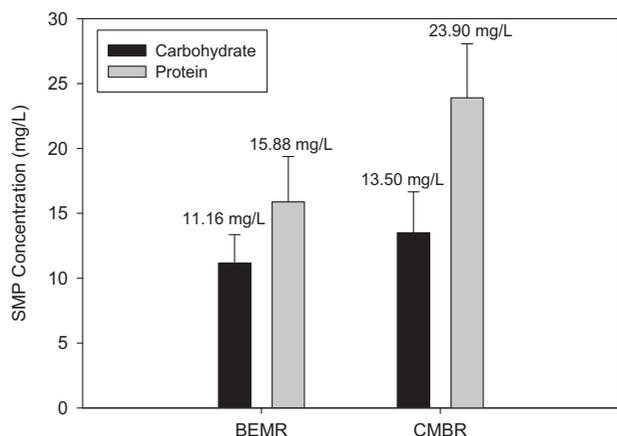


Fig. 5. The concentration of SMP in BEMR and CMBR.

membrane filtration run of the BEMR can be attributed to significantly lower SS concentration in the reactor as well as higher organic removal efficiency. This is because the BEMR has longer SRT than the CMBR, which allows slow-growing microorganisms to adapt the environment and increase biological activities that help to reduce membrane fouling [26]. Fig. 5 shows that the total SMP consists of SMP_c (total carbohydrate) and SMP_p (total protein) contents in the BEMR and the CMBR. The CMBR produced greater amount of SMP_c and SMP_p concentrations than BEMR by 17 and 33%, respectively. In the BEMR, F/M ratios was lower at higher SRTs. As reported by several researchers [12,15,16,22], lower SMP concentrations resulted from longer SRT would enhance membrane filterability. Furthermore, Ng et al. [10] reported that the entrapped biomass with longer SRT in the BEMR provided sufficient contact with the nutrients and thus, reduced the number of dead cells that contributed to less production of EPS and SMP. Therefore, it is reasonable to conclude that longer SRT and low SS production in the BEMR contributes a significantly better MBR operation in terms of membrane fouling than CMBR.

4. Conclusion

The performance of the novel BEMR in terms of membrane fouling tendency at various HRTs and types of aeration modes were studied and compared with a CMBR.

- (1) In the BEMR, the increment of HRTs and continuous aeration has a better performance in removing COD and ammonia.
- (2) The membrane fouling tendency is dramatically decreased as the HRT increased under the continuous aeration mode.

- (3) The BEMR is less susceptible to fouling. It has longer filtration period than the CMBR (215 min vs. 57 min) due to the slow-growing microorganisms with long SRTs in the BEMR.
- (4) The BEMR produced less SMP_c (17%) and SMP_p (33%) than the CMBR.

Acknowledgements

The funding of this work was partially supported by the National Science Council of the Republic of china (NSC98-2221-E-002-029-MY3).

References

- [1] W.B. Yang, N. Cicek, J. Ilg, State-of-the-art of membrane bioreactors: Worldwide research and commercial applications in North America, *J. Membr. Sci.* 270(1–2) (2006) 201–211.
- [2] F. Meng, H. Zhang, Y. Li, X. Zhang, F. Yang, J. Xiao, Cake layer morphology in microfiltration of activated sludge wastewater based on fractal analysis, *Sep. Purif. Technol.* 44 (3) (2005) 250–257.
- [3] S.R. Chae, Y.T. Ahn, S.T. Kang, H.S. Shin, Mitigated membrane fouling in a vertical submerged membrane bioreactor (VSMBR), *J. Membr. Sci.* 280(1–2) (2006) 572–581.
- [4] P.Y. Yang, Z.Q. Zhang, B.G. Jeong, Simultaneous removal of carbon and nitrogen using an entrapped-mixed-microbial-cell process, *Water Res.* 31(10) (1997) 617–2625.
- [5] P.Y. Yang, M. Shimabukuro, S.J. Kim, A pilot scale bioreactor using EMMC for carbon and nitrogen removal, *Clean Technol. Environ. Policy* 3 (2002) 407–412.
- [6] P.Y. Yang, H.J. Chen, S.J. Kim, Integrating entrapped mixed microbial cell (EMMC) process for biological removal of carbon and nitrogen from dilute swine wastewater, *Bioresour. Technol.* 86 (2003) 245–252.
- [7] P.Y. Yang, T.S. See, Packed entrapped mixed microbial cell process for removal of phenol and its compounds, *J. Environ. Sci. Health., Part A* 26(8) (1999) 1491–1512.
- [8] C.T. Chang, B.Y. Chen, I. Shiu, F.T. Jeng, Biofiltration of trimethylamine-containing waste gas by entrapped mixed microbial cells, *Chemosphere* 55 (2004) 751–756.
- [9] C.H. Wang, J.C.W. Liu, K.K. Ng, C.F. Lin, P.K.A. Hong, P.Y. Yang, Immobilized bioprocess for organic and nitrogen removal, *Desalin. Water Treat.* 37 (2012) 1–6.
- [10] K.K. Ng, C.F. Lin, S.K. Lateef, S.C. Panchangam, A.P.K. Hong, P.Y. Yang, The effect of soluble microbial products on membrane fouling in a fixed carrier biological system, *Sep. Purif. Technol.* 72 (2010) 98–104.
- [11] C.Y. Song, E. Cho, Z. Wang, P.Y. Yang, Removal of organic and nitrogen and molecular weight distribution of residual soluble organic from entrapped mixed microbial cells and activated sludge process, *Water Environ. Res.* 78 (2006) 2501–2507.
- [12] F. Meng, S.R. Chae, A. Drews, M. Kraume, H.S. Shin, F. Yang, Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material, *Water Res.* 43(6) (2009) 1489–1512.
- [13] N. Fallah, B. Bonakdarpour, B. Nasernejad, M.R. AlaviMoghadam, Long-term operation of submerged membrane bioreactor (MBR) for the treatment of synthetic wastewater containing styrene as volatile organic compound (VOC): Effect of hydraulic retention time (HRT), *J. Hazard. Mater.* 178(1–3) (2010) 718–724.
- [14] J. Cho, K.G. Song, S.H. Lee, K.H. Anh, Sequencing anoxic/anaerobic membrane bioreactor (SAM) pilot plant for advanced wastewater treatment, *Desalination* 178(1–3) (2005) 219–225.

- [15] S. Liang, C. Lui, L. Song, Soluble microbial products in membrane bioreactor operation: Behaviors, characteristics, and fouling potential, *Water Res.* 41(1) (2007) 95–101.
- [16] S. Malamis, A. Andreadakis, Fractionation of proteins and carbohydrates of extracellular polymeric substances in a membrane bioreactor system, *Bioresour. Technol.* 100(13) (2009) 3350–3357.
- [17] A. Fenu, T. Wambecq, C. Thoeye, G. De Gueldre, B. Van de Steene, Modeling soluble microbial products (SMPs) in a dynamic environment, *Desalin. Water Treat.* 29 (2011) 210–217.
- [18] C.P.L. Grady, G.T. Daigger, Jr., H.C. Lim, *Biological Wastewater Treatment*, Marcel Dekker Inc., New York, NY, 1999.
- [19] X. Qian, P.Y. Yang, T. Maekawa, Evaluation of direct removal of nitrate with entrapped mixed microbial cell technology using ethanol as the carbon source, *Water Environ. Res.* 73(5) (2011) 584–589.
- [20] Hach, DR 2800 Spectrophotometer. Procedures Manual Edition 2, Hach Company Publication, Halle (2007).
- [21] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* 193 (1951) 265–275.
- [22] K.K. Ng, C.F. Lin, S.C. Panchangam, P.K. Huang, P.Y. Yang, Reduced membrane fouling in a novel bio-entrapped membrane reactor for treatment of food and beverage processing wastewater, *Water Res.* 45 (2011) 4269–4278.
- [23] K. Raunkjaer, T.H. Jacobsen, P.H. Nielsen, Measurement of pools of protein, carbohydrate and lipid in domestic wastewater, *Water Res.* 28 (1994) 251–262.
- [24] M. Dohanyos, B. Kosova, J. Zabranksa, P. Grau, Production and utilization of volatile fatty acids in various types of anaerobic reactor, *Water Sci. Technol.* 17 (1985) 191–205.
- [25] E.S. Cho, J. Zhu, P.Y. Yang, Intermittently aerated EMMC-Biobarrel (entrapped mixed microbial cell with Bio-barrel) process for concurrent organic and nitrogen removal, *J. Environ. Manage.* 84 (2007) 257–265.
- [26] S.S. Han, T.H. Bae, G.G. Jang, T.M. Mak, Influence of sludge retention time on membrane fouling and bioactivities in membrane bioreactor system, *Process Biochem.* 40 (2005) 2393–2400.