Bioresource Technology 171 (2014) 265–273

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Bio-entrapped membrane reactor and salt marsh sediment membrane bioreactor for the treatment of pharmaceutical wastewater: Treatment performance and microbial communities

Kok Kwang Ng, Xueqing Shi, Yinuo Yao, How Yong Ng *

Centre for Water Research, Department of Civil and Environmental Engineering, National University of Singapore, 1 Engineering Drive 2, Singapore 117576, Singapore

HIGHLIGHTS

- A novel salt marsh sediment membrane bioreactor (SMSMBR) was conducted.
- Pharmaceutical wastewater was treated by SMSMBR and bio-entrapped membrane reactor.
- SMSMBR thrived in high salinity environment and achieved higher TCOD removal.
- Marine microorganisms to degrade recalcitrant compounds were found in SMSMBR.
- Inhibition of nitrification was occurred due to the saline effect.

ARTICLE INFO

Article history:
Received 16 June 2014
Received in revised form 14 August 2014
Accepted 17 August 2014
Available online 23 August 2014

Keywords:
Pharmaceutical wastewater
High salinity
Membrane bioreactor
Entrapped biomass
Marine microorganisms

ABSTRACT

In this study, a bio-entrapped membrane reactor (BEMR) and a salt marsh sediment membrane bioreactor (SMSMBR) were evaluated to study the organic treatment performance of pharmaceutical wastewater. The influences of hydraulic retention time (HRT) and salinity were also studied. The conventional biomass in the BEMR cannot tolerate well of the hypersaline conditions, resulting in total chemical oxygen demand (TCOD) removal efficiency of 54.2–68.0%. On the other hand, microorganisms in the SMSMBR, which was seeded from coastal shore, strived and was able to degrade the complex organic in the presence of salt effectively, achieving 74.7–90.9% of TCOD removal efficiencies. Marine microorganisms able to degrade recalcitrant compounds and utilize hydrocarbon compounds were found in the SMSMBR, which resulted in higher organic removal efficiency than the BEMR. However, specific nitrifying activity decreased and inhibited due to the saline effect that led to poor ammonia nitrogen removal.

1. Introduction

Pharmaceutical wastewaters are mainly generated by chemical-synthetic industries and usually contain high chemical oxygen demand (COD), salinity and toxicity. Large volumes of complex and obstinate composition wastewaters, along with biological substances, cleaning agents and disinfectants are simultaneously produced and might constitute potential threats, such as endocrine disruption and have severe side effects on the aquatic environment (Lefebvre and Moletta, 2006; Kasprzyjk-Horden et al., 2008). Therefore, pharmaceutical wastewater is listed as one of the major industries targeted for pollutants reduction, and received more attention recently. Generally, pharmaceutical wastewaters are frequently treated by anaerobic process but the COD and recalcitrant organic compounds remain in the anaerobic process's effluent are still significantly high, which cannot meet requirements of discharge standards. Furthermore, the salinity in the pharmaceutical wastewater could inhibit the methanogenesis and disrupted the ordinary metabolic function of anaerobes (Lefebvre and Moletta, 2006; Chelliapan et al., 2011; Shi et al., 2014). For example, Chelliapan et al. (2011) reported that the COD removal efficiency was found as low as 45% in an anaerobic bioreactor when the organic loading rate (OLR) was approximately 3.7 kgCOD/m^3 d and the hydraulic retention time (HRT) was about 2 d. Energy recovery of the anaerobic process may not be economically feasible if the generation of methane is insignificant by the anaerobes.

For these reasons, aerobic treatment process such as aerobic membrane bioreactor (MBR) for the treatment of pharmaceutical wastewater can be recommended since the MBR technique has now become an attractive option for the treatment and reuse of industrial and municipal wastewater with several advantages.

* Corresponding author. Tel.: +65 6516 4777; fax: +65 6774 4202.
E-mail address: howyongng@nus.edu.sg (H.Y. Ng).

http://dx.doi.org/10.1016/j.biortech.2014.08.078
0960-8524/

Nomenclature

AOB	ammonia-oxidizing bacteria
BEMR	bio-entrapped membrane reactor
BOD	biological oxygen demand
COD	chemical oxygen demand (mg/L)
FISH	fluorescence in situ hybridization
HRT	hydraulic retention time (h)
MBBR	moving bed biofilm reactor
MBR	membrane bioreactor
MLSS	mixed liquor suspended solids (mg/L)
MLVSS	mixed liquor volatile suspended solids (mg/L)
NH_3-N	ammonia nitrogen (mg/L)
OLR	organic loading rate (kgCOD/m^3 d)
PCR	polymerase chain reaction
SMP	soluble microbial products
SMSMBR	salt marsh sediment membrane bioreactor
SRT	sludge retention time (day)
TCOD	total chemical oxygen demand (mg/L)
TDS	total dissolved solids (mg/L)
TEA	triethylamine
TMP	trans-membrane pressure (kPa)
TN	total nitrogen (mg/L)
TOC	total organic carbon (mg/L)
TP	total phosphorus (mg/L)
TSS	total suspended solids (mg/L)
UASB	up-flow anaerobic sludge blanket
VSS	volatile suspended solids (mg/L)

The advantages include reduced foot-print and reactor requirements, high-improved effluent quality, higher biomass concentration, good volumetric loading and less sludge production (Anderson et al., 1986; Ng et al., 2011). However, membrane fouling is one of the main challenges of MBR system, which has become a major obstacle for the wide-scale application of MBRs. Few studies were undertaken to investigate moving bed biofilm reactor (MBBR) (Artiga et al., 2005; Leiknes and Ødegaard, 2007) and entrapped biomass technique (Ng et al., 2011; Wang et al., 2012) coupled with membrane as an alternative to the conventional MBR. The MBBR was found to produce more suspended biomass in the effluent, leading to increased membrane fouling than the conventional MBR, and soluble microbial product (SMP) in the MBBR were found to be more abundant than in the conventional MBR (Yang et al., 2009). However, Ng et al. (2011) applied entrapped biomass coupled with membrane process, named as bio-entrapped membrane reactor (BEMR), to treat food and beverage wastewater, and achieved COD removal efficiencies of 93–98%, with longer membrane operating time and lesser SMP production than the conventional MBR. Wang et al. (2012) also developed bio-plates containing entrapped biomass that achieved 83–96% of ammonia removal for food processing wastewater. Yet, very little information is available on the application of entrapped biomass MBR for the treatment of pharmaceutical wastewater containing high salinity and high COD concentration.

It has been reported that high salinity in the pharmaceutical wastewater affect the performance of biological processes because the high salinity could cause unbalance osmotic stress across the cell wall that leads to plasmolysis as water is lost from microbial cells through osmosis (Lefebvre and Moletta, 2006; Ng et al., 2014; Shi et al., 2014). Biological treatment of saline wastewater usually results in low treatment efficiencies because of the disintegration and loss activity of cells in the present of high salt content (>2%) (Dinger and Kargi, 2001; Uygur and Kargi, 2004; Lefebvre and Moletta, 2006; Jang et al., 2013). Moreover, inorganic compounds such as salts in antibiotic wastewater cannot be removed effectively by biological treatment process (Kim et al., 2007). As a result, hypersaline wastewater was suggested to be diluted before feeding to the biological process, which possibly increases the consumption of water resources and additional operational costs (Zhang et al., 2012; Shi et al., 2014). Therefore, the coastal sediment microorganism coupled with membrane, named as salt marsh sediment membrane bioreactor (SMSMBR), was motivated that might not be easily affected by the high salinity environment and also could possibly improve the pharmaceutical wastewater treatment for meeting discharge standards and wastewater reuse. As reported by Wu et al. (2008), who extracted the microorganisms, *Aegiceras corniculatum,* from the constructed mangrove, found that the microorganisms were able to carry out the biodegradation process when treating the wastewater under high salinity environment.

In this study, it was the first time that two laboratory-scale aerobic MBRs (BEMR and SMSMBR) were studied for the treatment of pharmaceutical wastewater for over six months. The treatment performance and microbial communities were compared. The conventional MBR (suspended growth process) was not compared in this study as the conventional MBR has more severe fouling rate (Ng et al., 2011) and lower treatment efficiency (Ng et al., 2012) than the BEMR. The goal of this study was to develop an affordable and efficient MBR particularly for the treatment of high salinity pharmaceutical wastewater.

2. Methods

2.1. Wastewater characteristic

The wastewater was collected from the equalization tank of a pharmaceutical factory located in Singapore, which produce antibiotics of the penicillin family. It consisted of a mixed effluent combining the wastewater generated by the various productions lines in the plant (formulation effluent, cleaning of the equipment, etc.). It must be noted here that pH adjustment occurred on-site in the equalization tank using phosphoric acid, thus adding a source of phosphorus to the wastewater without taking the carbon content into account and nitrogen was supplemented in the form of urea. The collected wastewater was stored at 4 °C prior to use. The wastewater comprised 17,143 ± 1873 mg/L of TCOD, 26,664 ± 4594 mg/L of total dissolved solids (TDS), 216.4 ± 16.3 mg/L of total phosphorus (TP), 72.6 ± 17.6 mg/L of ammonia nitrogen (NH_3-N), 7238 ± 1623 mg/L of total organic carbon (TOC) and was fed to both the MBRs.

2.2. Sampling and reactor set up

Two aerobic MBR systems (i.e., a BEMR and a SMSMBR) were investigated for organic removal for the treatment of pharmaceutical wastewater. Each had a total effective working volume of 10-L, and a 300-nm pore sized ceramic microfiltration membrane (ITN, Germany) module with a surface area of 0.08 m^2 was installed in each MBRs. In the BEMR, the biomass was entrapped in a bio-carrier, each with a diameter of 2.5 cm. The bio-carriers occupied a void volume of 2-L, and thus having a packing ratio of 16.7%. The entrapped biomass technique was according to the procedure of Ng et al. (2011, 2014), and the entrapped biomass in the BEMR was activated sludge originated from a local wastewater treatment plant in Singapore. The salt marsh sediment was collected from the

coastal shore located at Lim Chu Kang, Singapore. Surface sediment (depth of 0 to 3 cm) was collected to ensure that the microorganisms in the sediments were aerobes. The surface sediment was oxidized, with a brownish color down to ~1 cm, and slightly uniformly gray color in the 1–3 cm depth. The coarse particles and sediments were sieved through a 1-mm screen and adjusted to approximately 10,000 mg/L of mixed liquor suspended solids (MLSS) concentration by the addition of seawater for reactor start-up.

2.3. Reactor configuration and operation

In this study, the BEMR and the SMSMBR were operated in parallel with different fluxes to investigate the effect of HRT on treatment efficiency. As shown in Fig. 1, peristaltic pumps were used to feed the pharmaceutical wastewater into the MBRs and withdraw permeates from the membrane modules. Level sensors were installed in the MBRs to control the feeding of influents and production of membrane permeates. The pH of the BEMR and the SMSMBR was maintained at approximately 7.02 using a pH controller (Etatron, DLX-PH-RX/MBB). Both the MBRs were installed with air diffusers to pump air into the reactors to provide sufficient oxygen for aerobic respiration and achieve membrane scouring. Both the MBR were operated at HRT of 120 and 60 h, mesophilic temperature, 27 ± 1 °C, a pro-long of sludge retention time (SRT) of 200 d, and an OLR ranging from 3.1 to 8.2 kgCOD/m^3 d. The configurations and operation parameters of the BEMR and the SMSMBR are listed in Table 1. The membrane cycle of the BEMR and the SMSMBR was 4-min suction and 1-min relaxation, and constant low-flux conditions (1.04 and 2.08 L/m^2 h) were maintained. The membrane was gently wiped with a sponge on the membrane surface on a weekly basis, and trans-membrane pressure (TMP) was observed to be stable with values of −2.1 to

Table 1
Operation parameters of the lab-scale BEMR and SMSMBR.

Parameter	BEMR	SMSMBR
Carrier diameter size (cm)	2.5	–
Reactor effective volume, (L)	10	10
Void volume (L)	2	–
Packing ratio (%)	16.7	–
Solids retention time, SRT (d)	~200	~200
Attached biomass (mg/L)	11,000	–
MLSS (mg/L)	3040–5380	16,880–21,080
Temperature (°C)	27 ± 1.0	27 ± 1.0
pH	~7.02	~7.02
Permeate flux (L/m^2 h)	1.04–2.08	1.04–2.08
Hydraulic retention time, HRT (h)	120, 60	120, 60
Organic loading rate, OLR (kg COD/m^3 d)	3.1–8.2	3.1–8.2

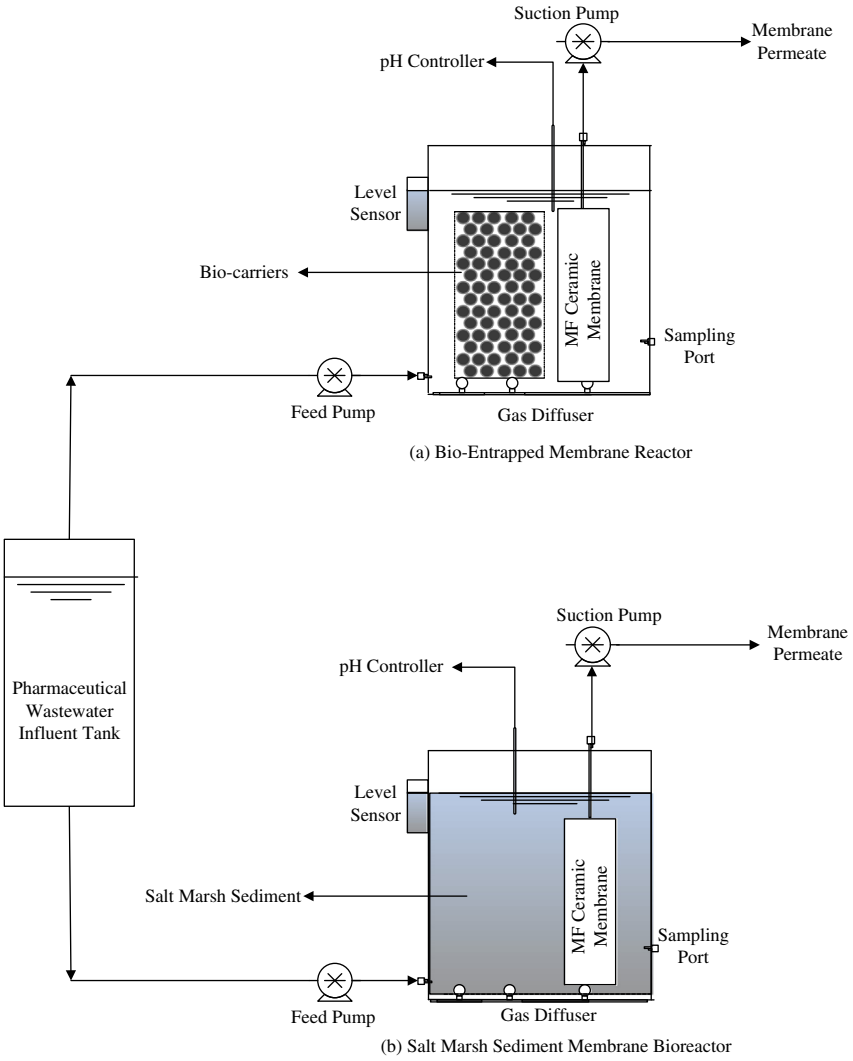


Fig. 1. Schematic of the membrane bioreactors. (a) BEMR and (b) SMSMBR.

−5.6 kPa at a HRT of 120 h, and −2.3 to −8.9 kPa at a HRT of 60 h. Both membranes did not require any chemical cleaning, as no severe membrane fouling was observed during the entire period of the study.

2.4. Analytical methods

Influents, effluents (i.e., membrane permeates) and mixed liquors were regularly taken from the outlets of feed pumps, permeate pumps and bioreactors, respectively, for analysis. The samples were centrifuged at 11,000 rpm for 20 min, and the supernatant was then analyzed for TCOD, TOC, TP and NH_3-N. The Standard Methods (APHA, 2005) were used for the measurement of COD concentration and TP was determined by ascorbic acid method (APHA, 2005). The TDS, total suspended solids (TSS) and volatile suspended solids (VSS) were determined by following the AFNOR recommendation (AFNOR, 2005). The TOC was analyzed by a TOC/TN analyzer (TNM-L/TOCL-CSH, Shimadzu, Japan). The NH_3-N was analyzed by the Hach salicylate method with a spectrophotometer (DR 5000, Hach) at 425 nm wavelength in the detection range of 0–50 mg/L. The ion elements were measured using ion chromatography (LC20 Chromatography Enclosure, DIONEX) for cation analysis and ion chromatography (ICS-1600, DIONEX) for anion analysis. Biomass samples taken at first day of inoculum process and day of 190th from both the BEMR and SMSMBR, were subjected to the Fluorescence In Situ Hybridization (FISH) analysis for detecting the presence of ammonia-oxidizing bacteria (AOB) with the oligonucleotide probe NSO1225 5′-CGCCATTGTATTA CGTGTGA-3′. The methodology was adopted from Tan et al. (2008). Hybridization was conducted in a humidified chamber at 46 °C for 3.5 h and subsequently subjected to DAPI counterstaining to visualize all bacteria.

2.5. Microbial community analysis

Bacterial community structures of each MBR were characterized using pyrosequencing method. Biomass samples at day of 190th from the BEMR and the SMSMBR were extracted for total DNA analysis using UltraClean DNA extraction kit (Mobio Laboratories, USA). Polymerase chain reaction (PCR) amplicon libraries were constructed for 454 pyrosequencing using bacterial primers 343F (5′-TACGGRAGGCAGCAG-3′) and 926R (5′-CCGTCAATTYYTTT RAGTTT-3′). The PCR reactions were performed according to the following procedure: 95 °C for 5 min, 35 cycles at 95 °C for 30 s, 55 °C for 1 min, 72 °C for 45 s, and finally 72 °C for 10 min. PCR products were sent to the Chinese National Human Genome Center at Shanghai for pyrosequencing using a 454/Roche GS-FLX Titanium Instrument (Roche, NJ, USA). After trimming of the barcodes and primers, sequences less than 200 bp or containing ambiguous bases were excluded from further analyses. The taxonomic identities of sequences were assigned using the Classifier program of the RDP-II at a confidence level of 80%.

3. Results and discussions

3.1. Start-up of the MBRs

The MBRs took about 1 month to complete the acclimation period and reached steady-state condition when the TCOD removal remained rather constant (Ng et al., 2011, 2014). During the start-up process, the proportion of the pharmaceutical wastewater (within the feed) was progressively increased: 10%, 30%, 50%, 80% and 100% of pharmaceutical wastewater, while the HRT was maintained at 120 h. Fig. 2 shows the MLSS concentrations and the MLVSS/MLSS ratio of both the MBRs. During the start-up period, the MLSS concentration of the BEMR increased 600–3040 mg/L and reached a steady value ranging from 3320 to 4120 mg/L and 3920 to 4860 mg/L at HRT of 120 and 60 h, respectively; whereas that of the SMSMBR increased from 11,000 to 15,840 mg/L during the start-up period, and reached a steady-state value of 15,160 to 17,770 mg/L regardless of the HRT. The MLVSS/MLSS ratio for both the MBRs was 0.8 ± 0.05, which indicates that the biomass in both the MBRs were healthy. Fig. 3a shows the BEMR and the SMSMBR in the new environment might not be favorable for the microorganisms, which led to a gradual decrease in TCOD removal efficiency when the proportion of the pharmaceutical wastewater was increased. However, at the end of the feeding period with 100% pharmaceutical wastewater from day 21 to 28, the TCOD removal efficiencies of the BEMR and the SMSMBR were maintained around 58.6–67.2% and 88.5–89.5%, respectively.

3.2. Organic removal by the MBRs

3.2.1. TCOD removal

In this study, the MBRs were investigated under two different operating conditions (i.e., at HRT of 120 and 60 h), and the treatment performance (TCOD and ammonia nitrogen) of the BEMR and the SMSMBR is shown in Fig. 3. The SMSMBR achieved 88.9 ± 1.2% and 82.5 ± 2.3% at the HRT of 120 and 60 h, respectively; while TCOD removal efficiency of 65.0 ± 1.7% and 59.1 ± 2.4% was achieved by the BEMR. The results (Fig. 3a and Table 2) show that the SMSMBR was able to achieve approximately 23.6 ± 1.8% of higher TCOD and 26.5 ± 3.1% of higher TOC removal efficiency than the BEMR, and it could be attributed to the microorganisms in the SMSMBR being able to adapt and strive in the high salinity environment of the MBR as they were retrieved from the coastal environment. The conventional biomass obtained from domestic wastewater treatment plant that entrapped in the BEMR could have been their ordinary metabolic functions and degradation disrupted by the strong salinity mixture environments (Woolard and Irvine, 1994) and toxicity of the pharmaceutical wastewater (Shi et al., 2014), which led to lower organic removal rate than the SMSMBR. Ludzack and Noran (1965) investigated the effects of salt concentrations up to 20 g/L on the conventional biomass and high salt concentration resulted in low biological oxygen demand (BOD) removal, nitrification and sedimentation efficiencies. This statement was consistent with the results obtained by Dinger and Kargi (1999), who observed that the effluent COD removal efficiency fell from 85% to 59% when the salinity was increased from 0% to 5%. In our previous investigation by Shi et al. (2014), the up-flow anaerobic sludge blanket (UASB) could only achieve a COD removal efficiency of 43.3 ± 2.2% with an OLR of 8.1 kg/m^3 d. However, when the UASB was used as a pre-treatment process for conventional MBR, the UASB + MBR achieved excellent COD removal rate of 94.7%. Therefore, the SMSMBR in this study had showed its ability to withstand high salinity of the pharmaceutical wastewater alone without the aid of anaerobic process as pre-treatment process.

Decreasing the HRT did affect the TCOD removal efficiency of the BEMR and the SMSMBR. As shown in Fig. 3a, at a HRT of 120 h (OLR = 3.3 ± 0.2 kgCOD/m^3 d), the average TCOD removal efficiency of 65.0 ± 1.7% and 88.9 ± 1.2% were achieved by the BEMR and the SMSMBR, respectively. However, when the HRT was reduced to 60 h (OLR = 7.2 ± 1.0 kgCOD/m^3 d), the average TCOD removal efficiencies decreased to 59.0 ± 2.3% and 82.5 ± 2.3% for the BEMR and the SMSMBR, respectively. A lower HRT correspond to a higher OLR, resulting in the biological system being unable to utilize the nutrients and degraded the organics efficiency under overloading conditions. The above findings are consistent with the result reported by Nandy and Kaul

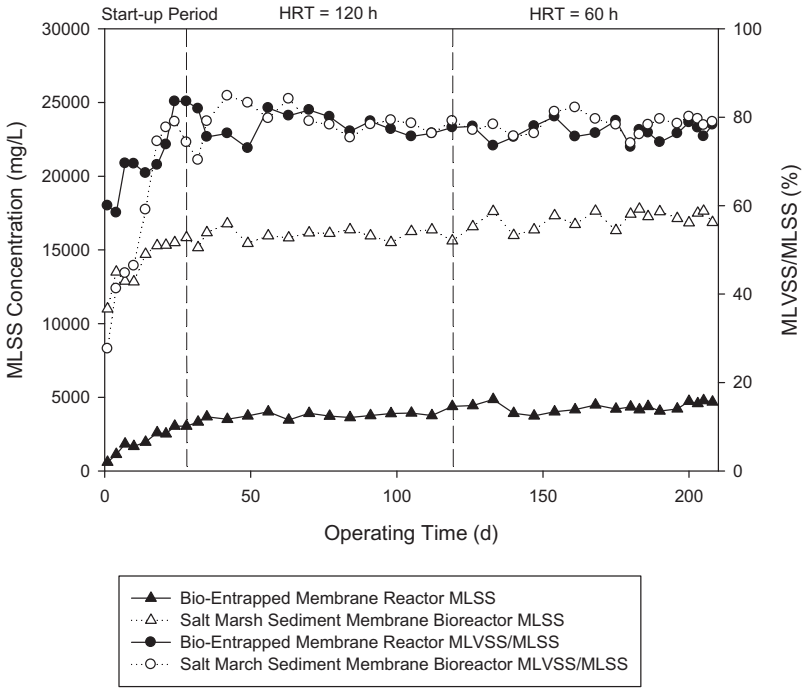


Fig. 2. MLSS concentration and MLVSS/MLSS ratio of the BEMR and the SMSMBR.

(2001), whereby a fixed film reactor treating herbal-based pharmaceutical wastewater could achieve 76–98% of COD removal efficiency at an OLR of 10 kgCOD/m^3 d, while the COD removal efficiency was reduced to about 50% when the OLR was increased to 48 kgCOD/m^3 d. Shi et al. (2014) reported that the UASB could achieve 41.1–45.5% of COD removal efficiency at a HRT of 96 h for the treatment of pharmaceutical wastewater, and that the COD removal efficiency was dropped remarkably to 26.2–34.1% when the HRT was reduced to 36 h. Moy et al. (2002) also suggested that the biological system should avoid for high OLR operation because it can disturb microbial activities, reduce the strength of anaerobic granules to lose their structural integrity and cause disintegration of anaerobic granules.

3.2.2. Ammonia nitrogen removal

Ammonia is one of the hydrolysis products formed during the degradation of proteinaceous organic materials. NH_3-N was monitored regularly after start-up period, on the 28th day. The influent NH_3-N, effluent NH_3-N, and NH_3-N removal efficiencies of the BEMR and the SMSMBR are shown in Fig. 3b. The influent NH_3-N was in the range of 52–83 mg/L, while the NH_3-N concentrations in the permeate of the BEMR and the SMSBR were gradually found to be higher than those in the influent. The BEMR achieved NH_3-N removal efficiencies of −287.0% to −33.3% and −294.4% to −103.6% at HRT of 120 and 60 h, respectively, while the SMSMBR had NH_3-N removal efficiencies of −373.6% to −48.5% and −353.0% to −170.1% at HRT of 120 and 6 h, respectively. NH_3-N was originated from triethylamine (TEA), which was found in the pharmaceutical wastewater as it was used as a solvent or a raw material in organic synthesis. The TEA biodegradation pathway under aerobic condition was proposed by Cai et al. (2011). From the chemical molecular structure of TEA, $N(CH_2CH_3)_3$, it was found that TEA can be completely oxidized into ammonia and carbon dioxide under the aerobic condition, according to Eq. (1):

$$N(CH_2CH_3)_{3(aq)} + 9\ O_{2(aq)} \rightarrow NH_{3(aq)} + 6\ CO_{2(aq)} + 6\ H_2O \quad (1)$$

The negative results of the NH_3-N removal efficiency observed could also be attributed to the high salinity wastewater, which inhibited the nitrification process (Dinger and Kargi, 1999; Dinger and Kargi, 2001; Lefebvre and Moletta, 2006; Bassin et al., 2011; Jang et al., 2013) and the complex organic composition in the pharmaceutical wastewater (Bassin et al., 2011). For example, Dinger and Kargi (1999) reported that the nitrification could be reduced when the salt concentration was higher than 2%, and Jang et al. (2013) reported that the removal efficiency of ammonia decreased from 87% to 46% when the salt concentration was increased from 0 to 20 g of NaCl/L. These results were similar to those of the moving-bed reactor, which showed that the removal of ammonia was very low (17%) and indicated pronounced inhibition of the nitrification process (Bassin et al., 2011). In addition, the high salinity and biorefractory compounds, even at low concentration, might not favor the growth of nitrifying bacteria or accelerate the elimination of nitrifying bacteria. According to Moussa et al. (2006), the ammonia oxidizers are more sensitive to salt stress than heterotrophs removing organic matter, and Bassin et al. (2011) concluded that the nitrification process is difficult to be occurred effluents from chemical industry due to the complex organic composition in the wastewater.

3.3. Microbial community analysis

3.3.1. Pyrosequencing analysis

Microorganisms in the conventional biomass and salt marsh sediment are the main contributors to degradation and removal of pollutants. Therefore, the microbial community in the MBRs was investigated in this study at a HRT of 60 h. A total of 620 and 576 bacterial 16S rRNA gene sequences were recovered from the BEMR and the SMSMBR, respectively; and the total identified phyla for the BEMR and the SMSMBR were 7 of each as shown in Fig 4. Samples in the SMSMBR were mainly represented by *Bacteroidetes* (51.3%), *Proteobacteria* (36.5%), *Firmicutes* (4.5%) and *Actinobacteria* (3.6%); while the major bacteria groups in the BEMR were

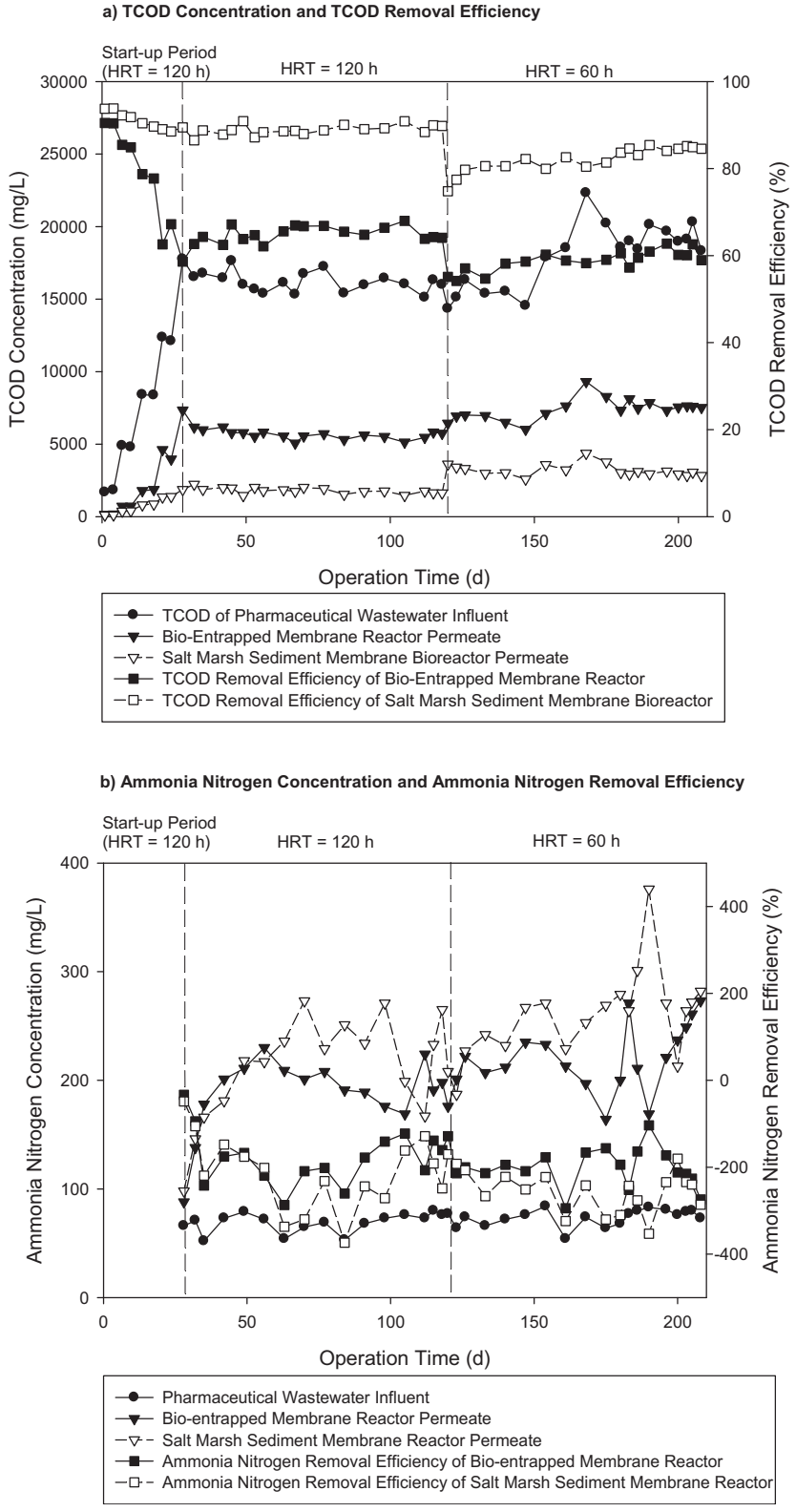


Fig. 3. Experimental results of the BEMR and the SMSMBR. (a) TCOD concentration and TCOD removal efficiency. (b) Ammonia nitrogen concentration and ammonia nitrogen removal efficiency.

Firmicutes (47.9%), *Actinobacteria* (21.9%), *Bacteroidetes* (13.5%), and *Proteobacteria* (14.9%). These four predominant phylawere consistent with results shown in Xia et al. (2010), who found *Proteobacteria, Firmicutes, Actinobacteria*, and *Bacteroidetes* are the predominant and subdominant groups in five MBRs in China and USA. Further looking at the class level of bacteria, *Bacteroidia* and *Betaproteobacteria* were abundant in the aerobic systems as *Betaproteobacteria* and *Bacteroidetes* could capture and express diverse resistance genes and are central players in the worldwide problem of antibiotic resistance (Deng et al., 2012). Moreover, *Clostridia*,

Table 2
Experimental results of the BEMR and the SMSMBR.

Water quality parameter	Pharmaceutical wastewater influent	BEMR		SMSMBR	
		HRT = 120 h	HRT = 60 h	HRT = 120 h	HRT = 60 h
TCOD (mg/L)	17,143 ± 1873	5743 ± 488	7399 ± 733	1803 ± 203	3193 ± 412
OLR (kgCOD/m^3 d)	–	3.3 ± 0.1	7.2 ± 1.0	3.3 ± 0.1	7.2 ± 1.0
TOC (mg/L)	7238 ± 1623	3243 ± 450	3574 ± 919	923 ± 218	1277 ± 421
TDS (mg/L)	26,664 ± 4594	24,367 ± 3253	24,919 ± 4419	27,225 ± 1983	26,325 ± 3413
SS (mg/L)	398.6 ± 149.1	–	–	–	–
NH_3-N (mg/L)	68.3 ± 8.7	187.6 ± 34.6	218.5 ± 31.5	213.4 ± 51.1	253.0 ± 47.4
Flux (L/m^2 h)	–				
Chlorides, Cl^- (mg/L)	19,960 ± 3391	15,345 ± 2141	14,359 ± 3744	18,899 ± 4032	17,068 ± 1578
Fluoride, F^- (mg/L)	104 ± 37	28 ± 11	20 ± 17	21 ± 5	26 ± 23
Sulfates, SO_4^{2-} (mg/L)	126 ± 33	87 ± 21	183 ± 91	140 ± 67	128 ± 50
Phosphate, PO_4^{3-} (mg/L)	138 ± 29	123 ± 38	174 ± 69	138 ± 15	105 ± 34
Sodium, Na^+ (mg/L)	9825 ± 1749	7277 ± 883	7583 ± 1639	7928 ± 1717	8480 ± 823
Potassium, K^+ (mg/L)	3837 ± 780	2551 ± 447	2921 ± 821	2820 ± 484	3081 ± 421
Calcium, Ca^{2+} (mg/L)	56 ± 26	30 ± 16	55 ± 13	24 ± 7	51 ± 5

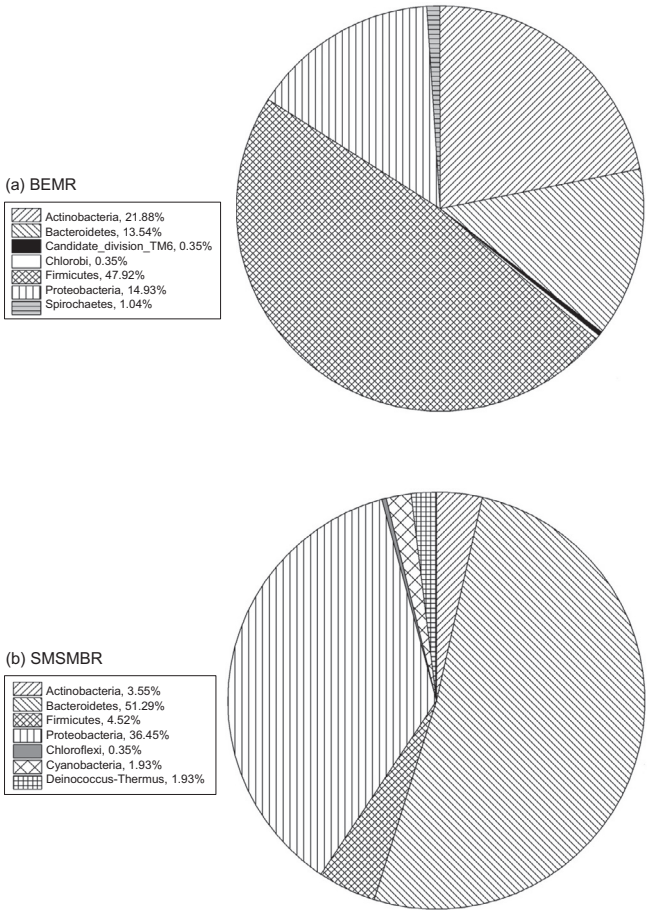


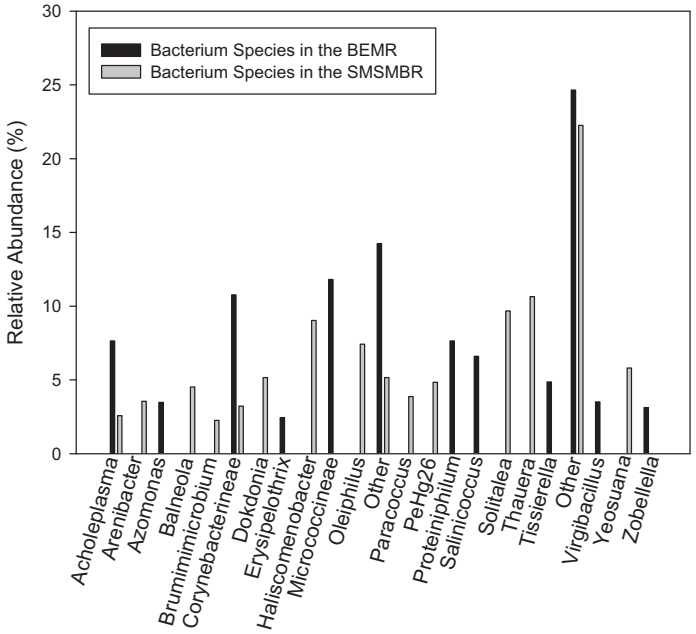
Fig. 4. Comparison of the quantitative contribution of the sequences affiliated with different phyla to the total number sequences from the BEMR and SMSMBR. (a) BEMR and (b) SMSMBR.

Gammaproteobacteria, *Mollicutes* and *Erysipelothrix* are excised in the BEMR, while *Flavobacteria*, *Sphingobacteria* and *Crymorphaceae* are found in the SMSMBR, which showed the microbial community in both systems was complex and could maintain system stability.

Fig. 5 shows the quantitative contribution of bacterial species in the BEMR and the SMSMBR. Bacteria species affiliated with *Corynebacterineae* sp., *Acholeplasma* sp., *Micrococcineae* sp., *Proteiniphilum* sp., and *Salinicoccus* sp. were abundant in the BEMR (Fig. 5a); while *Thauera* sp., *Haliscomenobacter* sp., *Solitalea* sp., *Oleiphilus* sp., and *Yeosuana* sp. were most abundant in the SMSMBR system (Fig. 5b). The predominant abundance of major bacterial species groups was totally different between these two MBRs since their biomass origin and characteristics are different. As discussed earlier, the better organic treatment performance found in the SMSMBR, rather than BEMR, could be attributed to the marine and ocean bacterial species detected in the SMSMBR. For example, bacterium species of *Dokdonia* sp. (5.2%), *Oliephilaceac* sp. (7.4%), *Yeosuana* sp. (5.8%), *Balneola* sp. (4.5%), and *Arenibacter* sp. (3.5%), could increase the TCOD removal efficiency of pharmaceutical wastewater. The *Dokdonia* sp. is a representative of phylum *Bacteroidetes* and the cells are gram-negative, non-spore-forming rods with strictly aerobic environment (Yoon et al., 2005), and is found able to degrade complex and recalcitrant organic matter (Gonzalez et al., 2011). Furthermore, *Oliephilaceac* sp. has been reported by Golyshin et al. (2002), who identified the *Oliephilaceac* sp. is a novel strictly aerobic marine bacterium that only aliphatic hydrocarbons are used as carbon sources for growth. Kwon et al. (2006) also studied the *Yeosuana* sp. from estuarine sediment of South Sea, Korea, and reported that the *Yeosuana* sp. cells formed on marine agar 2216 are strictly aerobic, yellowish-brown colonies are able to degrade benzopyrene and recalcitrant organic compounds. Therefore, it can suggest that the application of the SMSMBR with the marine bacterium could achieve better TCOD and TOC removal efficiencies for the treatment of pharmaceutical wastewater containing high TDS, toxicity and poorly biodegradable with the biorefractory characteristics of the active ingredients for antibiotics production.

3.3.2. Fluorescence in situ hybridization (FISH) analysis

Through the pyrosequencing analysis of microbial community and population for the BEMR and the SMSMBR, the presence of *Nitrosospira* sp., *Nitrosomonas* sp., *Nitrosococcus* sp., and *Nitrosolobus* sp. and composition of nitrifying population were not found in both MBRs samples (Fig. 4). Therefore, FISH technique was performed to identify and analyze the nitrifying microbial communities in the BEMR and the SMSMBR, for the purpose of explaining the unsatisfactory ammonia nitrogen removal efficiencies observed. The AOB of the BEMR and the SMSMBR were verified using the FISH technique (Fig. S1). With the same magnification and sample dilution, the BEMR and the SMSMBR had AOB present in them during the start-up period (Fig. S1a, b, A and B). However, when the hypersaline and recalcitrant compounds of pharmaceutical wastewater were fed to both the MBRs, the AOB in both the MBRs were hardly detected (Fig. S1c, d, C and D). This observation suggested that the pharmaceutical wastewater containing a significant amount of refractory contaminants and salinity, should require a chemically or physically pre-treatment process prior to



NOTE: Other in the BEMR (<2 %): Alkalibacter, Bacillus, Devosia, Incertae_sedis, Jeotgalicoccus, Myroides, Spirochaeta, Sporanaerobacter, Thalassobacillus, vadinBC27

NOTE: Other in the SMSMBR (< 2%): Catelibacterium, Defluvibacter, Devosia, Incertae_sedis, Lishizhenia

Fig. 5. Comparison of the quantitative contribution of the sequences affiliated with different bacterial species to the total number sequences from the BEMR and SMSMBR. Sequences not classified to any known species are included as unclassified bacteria.

the biological treatment in order to reduce the concentration of inhibitory or remove the inhibitory substances that disturb the nitrifying microbes.

4. Conclusions

BEMR with conventional biomass and SMSMBR seeded from marine sediment were investigated for the treatment of pharmaceutical wastewater. The SMSMBR demonstrated good treatment performance, achieving approximately 23.6 ± 1.8% of higher TCOD and 26.5 ± 3.1% higher TOC removal efficiency than the BEMR at OLR of 3.1–8.2 kgCOD/m^3 d. Several marine bacterial species found in the SMSMBR were able to degrade complex and recalcitrant organic matter, resulting in the higher organic removal efficiencies observed. Decreasing HRT or increasing OLR resulted in slightly lower organic removal efficiencies. High salinity and recalcitrant compounds in the pharmaceutical wastewater could affect the organic removal performance and inhibit the nitrification.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.08.078.

References

AFNOR, 2005. Qualité de l'eau, recueil des normes françaises. Agence Française de Normalisation, Paris.
Anderson, G.K., Saw, C.B., Fernandes, M.I.A.P., 1986. Application of porous membranes for biomass retention in biological wastewater treatment processes. Process Biochem. 12, 174–182.
APHA, 2005. Standard Methods for Water and Wastewater Examination. American Public Health Association, Washington.
Artiga, P., Oyanedel, V., Garrido, J.M., Mendez, R., 2005. An innovative biofilm-suspended biomass hybrid membrane bioreactor for wastewater treatment. Desalination 179, 171–179.
Bassin, J.P., Dezotti, M., Sant'Anna Jr., G.L., 2011. Notification of industrial and domestic saline wastewaters in moving bed biofilm reactor and sequencing batch rector. J. Hazard. Mater. 185, 242–248.
Cai, T., Chen, L., Ren, Q., Cai, S., Zhang, J., 2011. The biodegradation pathway of triethylamine and its biodegradation by immobilized Arthrobacter protophormiae cells. J. Hazard. Mater. 186 (1), 59–66.
Chelliapan, S., Wilby, T., Yuzir, A., Sallis, P.J., 2011. Influence of organic loading on the performance and microbial community structure of an anaerobic stage reactor treating pharmaceutical wastewater. Desalination 271, 257–264.
Deng, Y.Q., Zhang, Y., Gao, Y.X., Li, D., Liu, R.Y., Liu, M.M., Zhang, H.F., Hu, B., Yu, T., Yang, M., 2012. Microbial community compositional analysis for series reactors treating high level antibiotic wastewater. Environ. Sci. Technol. 46, 795–801.
Dinger, A.R., Kargi, F., 1999. Salt inhibition of nitrification and denitrification in saline wastewater. Environ. Technol. 20, 1147–1153.
Dinger, A.R., Kargi, F., 2001. Performance of rotating biological disc system treating saline water. Process Biochem. 36, 901–906.
Golyshin, P.N., Chernikova, T.N., Abraham, W.R., Lunsdorf, H., Timmis, K.N., Yakimov, M.M., 2002. *Oleiphilaceae* fam. nov., to include *Oleiphilus messinensis* gen. nov., sp. nov., a novel marine bacterium that obligately utilizes hydrocarbons. Int. J. Syst. Evol. Microbiol. 52, 901–911.
Gonzalez, J.M., Pinhassi, J., Fernandez-Gomez, B., Coll-Llado, M., Gonzalez-Valazquez, M., Puigbo, P., Jaenicke, S., Gomez-Consarnau, L., Fernandez-Guerra, A., Goesmann, Al., Pedros-Alio, C., 2011. Genomic of the proteorhodopsin-containing marine *Flavobacterium dokdonia* sp. strain MED134. Appl. Environ. Microbiol. 77, 8676–8686.
Jang, D., Hwang, Y., Shin, H., Lee, W., 2013. Effects of salinity on the characteristics of biomass and membrane fouling in membrane bioreactors. Bioresour. Technol. 141, 50–56.
Kasprzyjk-Horden, B., Dinsdale, R.M., Guwy, A.J., 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res. 42, 3498–3518.
Kim, S.D., Cho, J., Kim, I.S., Vanderford, B.J., Snyder, S.A., 2007. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking and waste waters. Water Res. 41, 1013–1021.
Kwon, K.K., Lee, H.S., Jung, H.B., Kang, J.H., Kim, S.J., 2006. *Yeosuana aromativorans* gen. nov., sp. nov., a mesophilic marine bacterium belonging to the family *Flavobacteriaceae*, isolated from estuarine sediment of the South Sea Korea. Int. J. Syst. Evol. Microbiol. 56, 727–732.
Lefebvre, O., Moletta, R., 2006. Treatment of organic pollution in industrial saline wastewater: a literature review. Water Res. 40 (20), 3761-3682.
Leiknes, T., Ødegaard, H., 2007. The development of biofilm membrane bioreactor. Desalination 202, 135–143.

Ludzack, F.J., Noran, D.K., 1965. Tolerance of high salinities by conventional wastewater treatment processes. J. Water Pollut. Control Fed. 37, 1404–1416.
Moussa, M.S., Sumanasekera, D.U., Ibrahim, S.H., Lubberding, H.J., Hooijmans, C.M., Gijzen, H.J., van Loosdrecht, M.C., 2006. Long term effects of salt on activity, population structure and floc characteristics in enriched bacterial cultures of nitrifiers. Water Res. 40 (7), 1377–1388.
Moy, B.Y.P., Tay, J.H., Toh, S.K., Liu, Y., Tay, S.T.L., 2002. High organic loading influences the physical characteristics of aerobic sludge granules. Appl. Microbiol. 34 (6), 407–412.
Nandy, T., Kaul, S.N., 2001. Anaerobic pre-treatment of herbal-based wastewater using fixed-film reactor with recourse to energy recovery. Water Res. 35, 351–362.
Ng, K.K., Lin, C.F., Panchangam, S.C., Hong, A.P.K., Yang, P.Y., 2011. Reduced membrane fouling in a novel bio-entrapped membrane reactor for treatment of food and beverage processing wastewater. Water Res. 45, 4269–4278.
Ng, K.K., Shi, X., Tang, M.K.Y., Ng, H.Y., 2014. A novel application of anaerobic bio-entrapped membrane reactor for the treatment of chemical synthesis-based pharmaceutical wastewater. Sep. Purif. Technol. 132, 636–643.
Ng, K.K., Wu, C.J., You, L.Y., Kuo, C.S., Lin, C.F., Hong, A.P.K., Yang, P.Y., 2012. Bio-entrapped membrane reactor for organic matter removal and membrane fouling reduction. Desalin. Water Treat. 50, 59–66.
Shi, X., Lefebvre, O., Ng, K.K., Ng, H.Y., 2014. Sequential anaerobic–aerobic treatment of pharmaceutical wastewater with high salinity. Bioresour. Technol. 153, 79–86.
Tan, N.C., Kampschreur, M.J., Wanders, W., van der Pol, W.L., van de Vossenberg, J., Kleerebezem, R., van Loosdrecht, M.C., Jetten, M.S., 2008. Physiological and phylogenetic study of an ammonium-oxidizing culture at high nitrate concentrations. Syst. Appl. Microbiol. 31, 114–125.
Uygur, A., Kargi, F., 2004. Salt inhibition on biological nutrient removal from saline wastewater in a sequencing batch reactor. Enzyme Microb. Technol. 34, 313–318.
Wang, C.H., Liu, J.C.W., Ng, K.K., Lin, C.F., Hong, P.K.A., Yang, P.Y., 2012. Immobilized bioprocess for organic carbon and nitrogen removal. Desalin. Water Treat. 37, 296–301.
Woolard, C.R., Irvine, R.L., 1994. Treatment of hypersaline wastewater in the sequencing batch reactor. Water Res. 29 (4), 1159–1168.
Wu, Y., Tam, N., Wong, M., 2008. Effects of salinity on treatment of municipal wastewater by constructed mangrove wetland microcosms. Mar. Pollut. Bull. 57, 727–734.
Xia, S., Duan, L., Song, Y., Li, J., Piceno, Y.M., Anderson, G.L., Alvarez-Cohen, L., Moreno-Andrade, I., Huang, C.L., Hermanowicz, S.W., 2010. Bacterial community structure in geographically distributed biological wastewater treatment reactors. Environ. Sci. Technol. 44, 7391–7396.
Yang, S., Yang, F., Fu, Z., Lei, R., 2009. Comparison between a moving bed membrane bioreactor and a conventional membrane bioreactor on membrane fouling. Bioresour. Technol. 100, 6655–6657.
Yoon, J.H., Kang, S.J., Lee, C.H., Oh, T.K., 2005. *Dokdonia donghaensis* gen. nov., sp. nov., isolated from sea water. Int. J. Syst. Evol. Microbiol. 55, 2323–2328.
Zhang, J., Zhang, Y., Quan, X., 2012. Electricity assisted anaerobic treatment of salinity wastewater and its effect on microbial communities. Water Res. 46, 3535–3543.