Journal of Hazardous Materials 175 (2010) 551–557

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Simultaneous nitrogen and phosphorus removal by a novel sequencing batch moving bed membrane bioreactor for wastewater treatment

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ARTICLE INFO

Article history:
Received 22 May 2009
Received in revised form 11 October 2009
Accepted 12 October 2009
Available online 20 October 2009

Keywords:
Sequencing batch moving bed membrane bioreactor
Nitrogen removal
Phosphorus removal
Phosphorus accumulating organisms (PAOs)
Specific phosphorus uptake rate test

ABSTRACT

Biological nutrient removal (BNR) was investigated in a sequencing batch membrane bioreactor which used carriers instead of activated sludge named a sequencing batch moving bed membrane bioreactor (SBMBMBR). The SBMBMBR performed well on carbon and nitrogen removal at different COD/TN ratios. COD, TN and ammonium nitrogen removal efficiencies averaged at 93.5%, 82.6% and 95.6%, respectively. The TP removal was closely correlated with the length of anaerobic phase and aerobic phase. When anaerobic time and aerobic time were both 2 h, the average TP removal efficiency reached to 84.1% at influent TP concentration of 12.4 mg/L. DO in aerobic phase was an important factor affecting nutrient removal, and the optimal DO was about 3 mg/L. There was a small amount of denitrifying phosphorus accumulating organisms (DPAOs) in SBMBMBR which resulted from the anoxic microenvironment existed in the inner of the biofilm. Fluorescence in situ hybridization (FISH) results of microbes showed the composition and spatial structure of the microbial community in the reactor. Furthermore, sequencing batch mode operation was propitious to retard membrane fouling.

1. Introduction

Eutrophication of waters caused by nitrogen and phosphorus has become a focus of concern recently, and a number of biological nutrient removal processes had been developed. It is possible to achieve nitrification, denitrification and phosphorus removal in a single reactor when a sequencing batch reactor system (SBR) is used. Comparing with conventional activated sludge systems, the SBR systems have many advantages, e.g. reducing operational costs, improvements on nitrogen and phosphorus removal, and less bulking. In addition, its cycle format can be easily modified at any time to offset changes in process conditions, influent characteristics or effluent objectives [1–3].

Nitrogen can be removed as a final product of nitrogen gas by the combined of nitrification by autotrophs under aerobic conditions and denitrification by heterotrophs under anaerobic conditions. While phosphorus can only be removed by its uptake into biomass which can be discharged from the system as a surplus sludge [4]. According to current knowledge of biological phosphorus removal, under anaerobic conditions phosphorus accumulating organisms (PAOs) use the energy released from the hydrolysis of intracellular polyphosphate to transport volatile fatty acid (VFA) across their cell membranes and, hence produce polyhydroxybutyrate (PHB). The phosphate is released in connection with the storage of organic matter under anaerobic conditions. Under aerobic conditions PHB serves as an energy source for cell growth and storage of excess polyphosphate. The accumulation of phosphorus beyond that needed for normal cell growth for disposal with excess biomass is commonly known as enhanced biological phosphorus removal (EBPR) [5].

In conventional biological nutrient removal systems, both TN and TP removal require COD, which is often the limiting substrate in the incoming wastewater. The denitrification complicates the EBPR since the denitrifiers consume a portion of the substrate before the substrate can be utilized by the biological phosphorus removing organisms, in other words, the transfer of nitrate into the anaerobic phase inhibits phosphate release [6,7]. In order to resolve this conflict, a number of changes had been introduced in SBR systems, such as filling the SBR with carriers or aerobic granular instead of activated sludge [8]. Compared with the conventional SBR, the modified SBR adding zeolite powder could remove COD, NH_4^+-N, TN and TP significantly in a shorter cycle time. At the same operational time period, the modified SBR could treat wastewater as 1.22 times as that treated in conventional SBR [9]. Li et al. [10] reported that simultaneous removal of phosphorus and nitrogen was possible in the biofilm SBR as indicated by the respective removal ratio of around 90% and 57% at a COD loading of 1.00 kg COD/m^3 per day. Attached-growth biofilm can form aerobic zone, anoxic zone

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doi:10.1016/j.jhazmat.2009.10.040

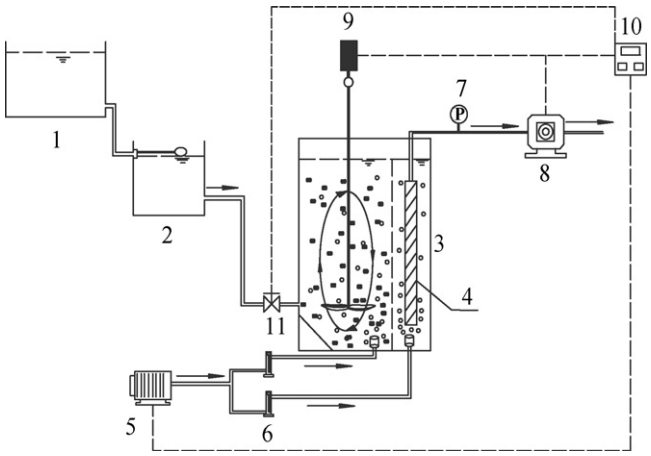

Fig. 1. Schematic diagram of experimental process. 1. Wasterwater reservoir; 2. Balance-box; 3. SBMBMBR reactor; 4. Membrane module; 5. Air pump; 6. Rotameter; 7. Vacuum gauge; 8. Peristaltic pump; 9. Agitator; 10. Timer; 11. Electromagnetic valve.

and anaerobic zone along the direction of mass transfer, providing favorable environment for simultaneous nitrification and denitrification (SND) [11–13]. It could be presumed that the biofilm can improve the TN removal in aerobic phase, and inhibit the transfer of nitrate into the anaerobic phase. As a result, the conflict on simultaneous nitrogen and phosphorus removal could be resolved.

Hence, the objective of this study was to enhance nitrogen and phosphorus removal efficiency by using carriers instead of activated sludge in the reactor. Compared with conventional SBR, another improvement of this study was introducing membrane filtration technology in SBR system. The combination of MBR enables good performance for removal of organic matter and biological sludge separation [14,15]. Based on the potential advantages of the SBMBMBR, it should be suitable for eutrophication water treatment (e.g. aquaculture wastewater).

The performance of the SBMBMBR on the nutrient removal was investigated at different influent COD/TN/TP ratios that ranged from 34.9/6.2/1 to 120.5/9.3/1. The suitable operational parameters under each condition were selected, the characteristics of nutrient removal were investigated, and membrane fouling behavior was monitored at the same time.

2. Materials and methods

2.1. Experimental set-up and operating conditions

Fig. 1 shows the schematic diagram of the experimental apparatus. The reactor was made of Plexiglas with a working volume of 30 L. Temperature was controlled at 25 °C using thermostats in it. A balance-box with a float-ball valve was used to control the water level of it. The membrane module used in the system was hollow-fiber membranes made of polypropylene (Hangzhou Kaihong, China) with a pore size of 0.1 μm and the filtration area was 0.4 m^2. A piece of clapboard with bores was fitted in the SBMBMBR, which divided the reactor into two parts with a volume ratio of 4:1. Certain proportional (30%, v/v) of carriers was filled in the bigger part, and the membrane module was fixed in the other part. The carriers used in the SBMBMBR were a new kind of non-woven carriers made in the laboratory. The carrier is hollow cylinder with outer diameter, inner diameter and height of 20 cm, 18 cm and 18 cm, respectively. The density of the carriers was 0.27 g/cm^3 and the effective specific surface area was 900 m^2/m^3. Adding the clapboard was to avoid the suspended carriers accumulating around the membrane module.

In the SBMBMBR system, the water was fed into the reactor in the anaerobic phase and the discharging of water occurred in the aerobic phase. The alternation between aerobic and anaerobic conditions was created by the on and off control of the air pump. To make it work this way a timer-controlled power supply system was used. For the anaerobic phase, the air pump and peristaltic pump were closed, while the electromagnetic valve placed in the influent pipeline was open and the agitator started to work to keep a complete mixing of sludge and water. The feed water flowed into the SBMBMBR by gravitational force and its level was controlled by the balance-box. During the aerobic phase, the electromagnetic valve and the agitator were closed, while the air pump and peristaltic pump worked simultaneously. This resulted in discharge of water and the descending of water level in the reactor. When this aerobic phase was over, the next cycle began with the anaerobic phase.

The SBMBMBR was operated uninterruptedly for about 5 months. Variations in operational parameters are listed in Table 1. The exchange ratio f_B is defined as the ratio between the volume discharged per cycle and the entire working volume of the reactor. During the experiment, solid retention time (SRT) was maintained at 15 days by wasting sludge mixed liquor at the end of aerobic phase. The aeration rate for the SBMBMBR was 0.45 m^3/h (0.30 m^3/h in the bigger compartment and 0.15 m^3/h in the lesser compartment).

The SBMBMBR were inoculated with activated sludge taken from the secondary settling tank of the municipal wastewater treatment plant in Dalian, China. Synthetic wastewater fed to the reactor consisted of sodium acetate, NH_4Cl, KH_2PO_4 and mineral solution containing $MgSO_4{\cdot}7H_2O$ (25 mg/L), $CaCl_2{\cdot}2H_2O$ (22 mg/L), $FeSO_4{\cdot}2H_2O$ (20 mg/L) and NaCl (25 mg/L). The initial influent contained 400 mg COD/L, 30 mg NH_4^+-N/L and 4 mg PO_4^{3-}-P/L. The pH was maintained at 7.6–8.5 in the reactor.

2.2. Analytical methods

Ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), total phosphorous (TP), COD, mixed liquor suspended solid (MLSS), mixed liquor volatile suspended solid (MLVSS), and sludge volume index (SVI) were analyzed according to the standard methods for the examination of water and wastewater [16]. The amounts of biomass fixed in carriers were determined as follows: A certain amount of carriers was taken out from the bioreactor and put in a beaker with 500 ml deion-

Table 1
Operation parameters in the experiment.[a]

Phase	Operational days (day)	COD_{in} (mg/L)	TN_{in} (mg/L)	TP (mg/L)	COD/TN/P	HRT (h)	f_B (%)	Biofilm (mg/L)	Suspended MLSS (mg/L)	MLVSS/MLSS	Cycle time of SBMBMBR (min)	
											Anaerobic	Aerobic
I	1–36	433.8(33.6)	33.6(12.0)	3.6(0.9)	120.5/9.3/1	15.3	12	886	3080	0.86	30	80
II	37–72		45.1(5.3)	7.4(1.0)	58.6/6.1/1	19.6	15.3	935	3174	0.90	60	120
III	73–105		43.3(6.9)	7.3(1.4)	59.4/5.9/1	26	15.3	1062	3236	0.90	120	120
IV	106–165		76.9(8.5)	12.4(2.1)	34.9/6.2/1	26	15.3	1125	3428	0.92	120	120

[a] Standard deviation is given between parentheses; COD_{in}, influent COD concentration; TN_{in}, influent TN concentration; TP_{in}, influent TP concentration.

ized water in it. Then, the carriers were stirred with a magnetic stirrer for 60 min, and the biomass fixed in carriers was washed out from carriers. The suspension was dried and weighed to calculate the concentration of the biofilm in SBMBMBR. Dissolved oxygen (DO) and pH in reactor were measured by a DO meter (YSI 55/12 FT, USA) and a pH meter (Sartorius PB–10, Germany), respectively (Aqualytic). The total nitrogen (TN) was based on the sum of NH_4^+-N, NO_2^--N and NO_3^--N rather than an independent TN test. The contents of PHB and glycogen were measured using the methods reported by Hahn et al. [17] and Wang et al. [18], respectively.

2.3. Fluorescence in situ hybridization

The composition and spatial structure of the microbial community in the reactor (including the biofilm and the suspended biomass) were analyzed by fluorescence in situ hybridizations (FISH) in this study. FISH were performed according to Hibiya et al. [19]. The microbes sample was dispersed into individual cells by ultrasonication, and placed in hybridization well on a gelatin-coated microscopic slide. NSO190 targeted halophilic and halotolerant β-proteobacterial AOB [20]. Ntspa662 and Nit3 are specifically used to target Nitrospira and Nitrobacter [21,22]. PAOMIX probes (comprising equal amounts of probes PAO462, PAO651 and PAO846) are used to target PAOs [23]. EUB338 is used to target all eubacteria [24]. After hybridization, the microbes samples on slides were examined using an epifluorescence microscope (OlympusBX51, Japan) together with the standard software package delivered with the instrument (version 4.0).

2.4. Experimental procedures

2.4.1. General experiments

These experiments were conducted for investigating the removal of nitrogen, phosphorus and COD. Effluent of one cycle was collected in a beaker and the mixture was used for analysis.

2.4.2. Cyclic studies

This test was carried out when the performance of SBMBMBR was stable. It was applied to the SBMBMBR for optimizing nutrient removal, which includes a series of sampling and analysis during one cycle. $t = 0$ min was set for the starting of the anaerobic phase. The sampling was done by manual adjustment peristaltic pump during the anaerobic phase.

2.4.3. Batch experiments

In order to investigate PAOs in more detail, batch experiments were carried out using the methods reported by Wachtmeister et al. [25]. The sludge was transferred from the reactor to a sealed vessel at the end of aerobic phase. Here, suspended and biofilm biomass were used for batch experiments together, the ratio of the two fractions was adopted according to the state at that time in the bioreactor. It was incubated anaerobically with excess sodium acetate for 90 min to increase the PHB stored by sludge. Then the sludge was divided into two parts. One part was exposed to aerobic condition to measure aerobic phosphorus uptake rate (APUR) and the other part was exposed to anoxic condition to measure anoxic phosphorus uptake rate (NPUR). Phosphate uptake rate was evaluated from the slop of the line describing the linear decrease in phosphate concentration. Based on the assumption of the denitrifying phosphorus bacteria (DPB) sludge having a nearly identical phosphorus uptake under anoxic and aerobic conditions, the ratio of NPUR to APUR was used to analyse the proportion of DPAOs in PAO [26,27].

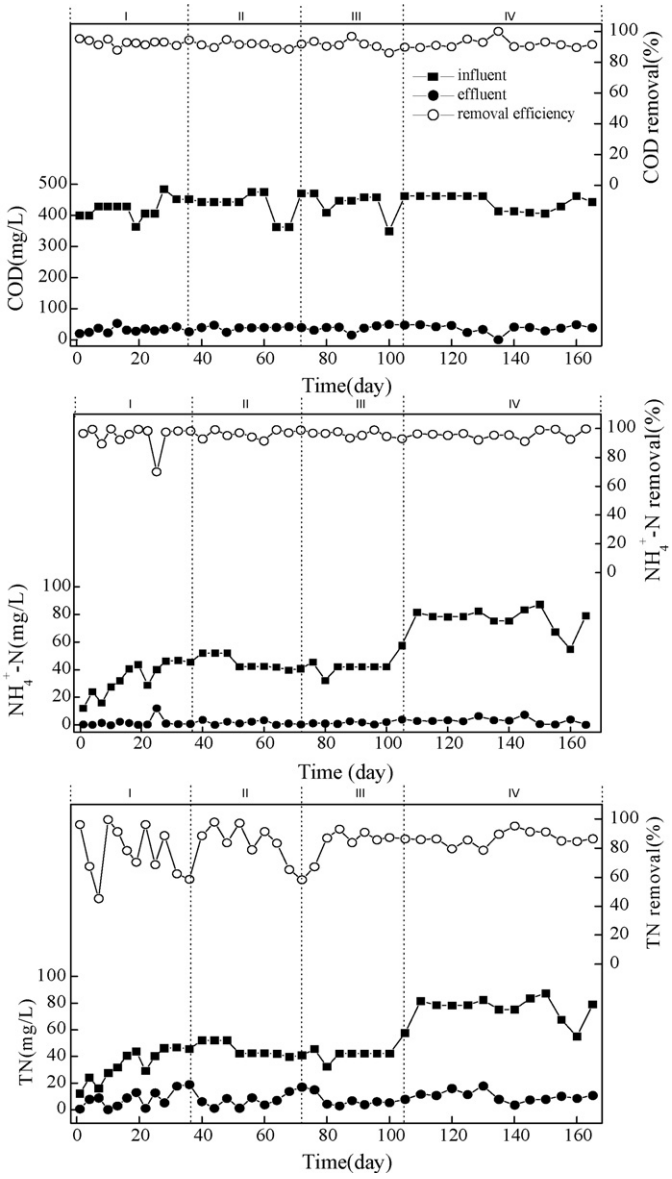

Fig. 2. COD, NH_4^+-N, TN concentrations and removal efficiencies in SBMBMBR.

3. Results and discussions

3.1. Performance of the SBMBMBR

3.1.1. Organic substance and nitrogen removal

Fig. 2 illustrates variations in COD, NH_4^+-N and TN concentrations as well as their removal efficiencies throughout whole experimental period. The SBMBMBR showed good performance on organic carbon removal. Major part of COD was consumed during anaerobic phase and the removal efficiency was 84.0%. It was similar to other nitrogen and phosphorus removal researches [28]. COD was mainly removed during the anaerobic phase, and microorganisms stored COD as PHB under anaerobic condition for later use during the aerobic phase. At the end of aerobic phase, COD concentration stabilized at 0–50 mg/L and the corresponding removal efficiency achieved 93.5% on average. It can be seen that changes of f_B or COD/TN/TP ratio in influent almost did not show any influence on the COD removal.

The SBMBMBR performed well on NH_4^+-N removal (Fig. 2). The NH_4^+-N removal efficiency averaged at 95.4%. Results indicated that increase nitrogen loading rate (NLR) or change of COD/TN ratio

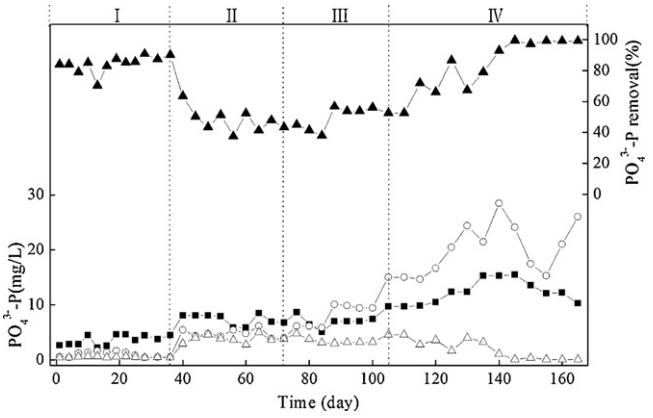

Fig. 3. TP concentrations and removal efficiencies in SBMBMBR.

did not affect NH_4^+-N removal efficiency significantly. Most of the organic substances had been removed in anaerobic phase, hence, autotrophic bacteria could grow easily in aerobic phase, resulting in a good performance on NH_4^+-N removal.

As far as the TN removal efficiency was concerned, during the reactor start-up phase (phase I), the effluent TN concentration had some fluctuation, the TN removal efficiency averaged at 76.9% and average effluent TN concentration was 7.76 mg/L. In order to enhance denitrification and increase the organic carbon supply, a higher f_B (15.3%) was adopted on day 37. As the increase of influent TN concentration and the adjustment of the cycle time during phase II and phase III, the TN removal efficiency increased to 82.7% and 85.1%, and the average effluent TN concentrations were 7.80 mg/L and 6.45 mg/L, respectively. In the SBMBMBR, denitrification can occur during the whole cycle time. In the aerobic phase, the TN removal mainly depends on SND performance, which resulted from DO concentration gradients arising from diffusional limitations. Nitrification took place at the carrier interface, which was an aerobic layer, and denitrification occured in the deeper layer of the biofilm, where anaerobic conditions were present [13]. In the anaerobic phase, the residual nitrate and nitrite could be removed by using the influent COD as substrate. Whereas, the transfer of residual nitrate and nitrite into the anaerobic phase could consume a portion of the substrate before the substrate being utilized by PAOs, which might inhibit phosphate release [5–7]. Therefore, in order to ensure simultaneous nitrogen and phosphorus removal, the SND performance must be enhanced during aerobic phase. In SBMBMBR, along with the attached-growth biofilm became thicker, the SND performance improved and the effluent TN concentration maintained at a low level. In phase IV, the TN removal efficiency averaged at 86.6% and average effluent TN concentration was 10.30 mg/L.

3.1.2. Phosphorus removal

Fig. 3 shows variations in the TP concentrations as well as its removal efficiency in the SBMBMBR during the whole experiment. When COD/TN/TP was at about 120.5/9.3/1 (phase I), TP removal efficiency averaged at 84.9% and average effluent TP concentration was 0.54 mg/L. It can be seen that the TP concentration at the end of anaerobic phase was close to that in the effluent. The phenomena of released phosphate in the anaerobic phase and uptake phosphate in the aerobic phase cannot be observed. In the starting period, the influent TP concentration was comparatively low and PAOs had not been accumulated in the reactor. It could be concluded that the TP removal mainly depended on biological assimilation in this period. With increasing the influent TP concentration and lengthening the anaerobic time and aerobic time (phase II), a higher f_B (15.3%) was adopted on day 37. The cycle time was adjusted to 60 min anaerobic and 120 min aerobic from 30 min anaerobic and 80 min aerobic. The TP removal efficiency decreased rapidly to about 47.8% and average effluent TP concentration was 3.86 mg/L. From Fig. 3, it can be seen that in this period TP concentration at the end of anaerobic phase was not obviously higher than that in the effluent. It meant PAOs did not release phosphate adequately in anaerobic phase. In biological phosphorus removal, phosphorus release in anaerobic conditions is important to the uptake of phosphorus in the subsequent aerobic phase, eventually affecting the phosphorus removal efficiency. Thus, a condition favourable for the anaerobic phosphorus release is necessary to effectively remove phosphorus. Hence, a longer anaerobic time (120 min) was adopted on day 73 (i.e. phase III), the phenomenon of phosphate release was evidently improved and TP removal efficiency increased to about 49.6% and average effluent TP concentration was 3.68 mg/L. In order to accumulate PAOs further, the influent TP concentration was increased to 12.4 mg/L in phase IV. In this period, as PAOs accumulated gradually in the reactor, the TP removal increased sharply and averaged at 84.1% and average effluent TP concentration was 1.97 mg/L. The phenomena of released phosphate in the anaerobic phase and uptake phosphate in the aerobic phase can be observed obviously. Compared with phase IV, Zhang et al. [14] reported that in the SBMBR without adding carriers, TN and TP removal efficiencies averaged at 31.9% and 49.9%, respectively, with the similar C/N ratio (6.3) and f_B (12%). It indicated that adding carriers could enhance simultaneous nitrogen and phosphorous removal.

3.2. Effect of DO on nitrogen and phosphate removal

To examine the effect of DO concentration on nitrogen and phosphorus removal, three different DO concentrations (1, 3 and 6 mg/L) were adopted during aerobic phase for 15 days, respectively. The average removal efficiencies of NH_4^+-N, TN and TP are shown in Fig. 4. It was observed that the NH_4^+-N removal efficiency was only 70.1% with DO of 1 mg/L. Low DO was in favor of denitrification, but could make against to nitrification. Hence, nitrification became the limiting step for TN removal and the TN removal efficiency was only 68.9%. Also due to the low DO, the rate of phosphate uptake was slowed down and the TP removal efficiency was 78.8%. With DO of 3 mg/L, the NH_4^+-N, TN and TP removal efficiencies were 92.8%, 88.4% and 89.5%, respectively. Both the SND and the biological phosphorus removal performed well in the reactor. As DO concentration increased to 6 mg/L, the NH_4^+-N removal efficiency raised to 97.2%, while the TN removal efficiency

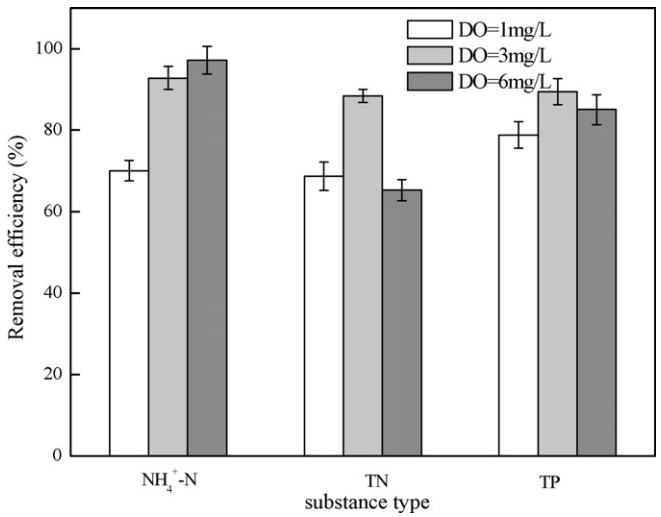

Fig. 4. Comparison of the NH_4^+-N, TN and TP removal efficiencies under different DO conditions.

reduced to 65.3% since high DO went against with denitrification. Simultaneously, a considerable amount of remaining nitrate transferred into the anaerobic phase. It affected phosphate release and finally reduced phosphorus removal. Consequently, the TP removal efficiency was 85.1%, lower than that with DO of 3 mg/L. It is concluded that DO during the aerobic phase was an important factor affecting simultaneous nitrogen and phosphorus removal, and the optimal DO concentration was about 3 mg/L in the SBMBMBR system.

3.3. Cyclic studies of SBMBMBR

Cyclic studies could clarify the transformation of various pollutions during the cycle time and provide information for adjusting the operation parameters. During phase IV, a higher removal efficiency of NH_4^+-N, TN and TP persisted in the SBMBMBR. On day 135, the cyclic test was carried out and variations in NH_4^+-N, NO_3^--N, NO_2^--N and other typical substances concentrations during one cycle were present in Fig. 5. It was clear that COD was mainly removed during the anaerobic stage, with removal efficiency of 60.7%. (The COD removal rate was calculated as the removed COD during the anaerobic time/the removed COD during the entire cycle.) NH_4^+-N concentration achieved to the maximum after 60 min feeding and then almost not changed in the anaerobic phase. In the aerobic phase, NH_4^+-N concentration decreased rapidly in the initial 30 min and decreased to 1.77 mg/L in the end. As to NO_3^--N, in the anaerobic phase, residual NO_3^--N of the last cycle was removed by utilizing the feeding COD as carbon substrate. In aerobic phase, the maximum of NO_3^--N concentration was detected at the 30th min and then decreased gradually. At the end of the aerobic phase, NO_3^--N concentration was only 2.08 mg/L, which showed that the biofilm presented good SND performance in SBMBMBR. NO_2^--N nearly could not be detected during the whole cycle. At the end of the cycle, NH_4^+-N and TN removal efficiencies reached 87.7% and 73.5%, respectively. The SND performance in the aerobic phase played a dominant role for the TN removal. At the end of the aerobic phase, the sum of residual NO_3^--N and NO_2^--N was low, which ensured the denitrifying bacteria could not intensely scramble for limited carbon substrate with PAOs in the next anaerobic phase.

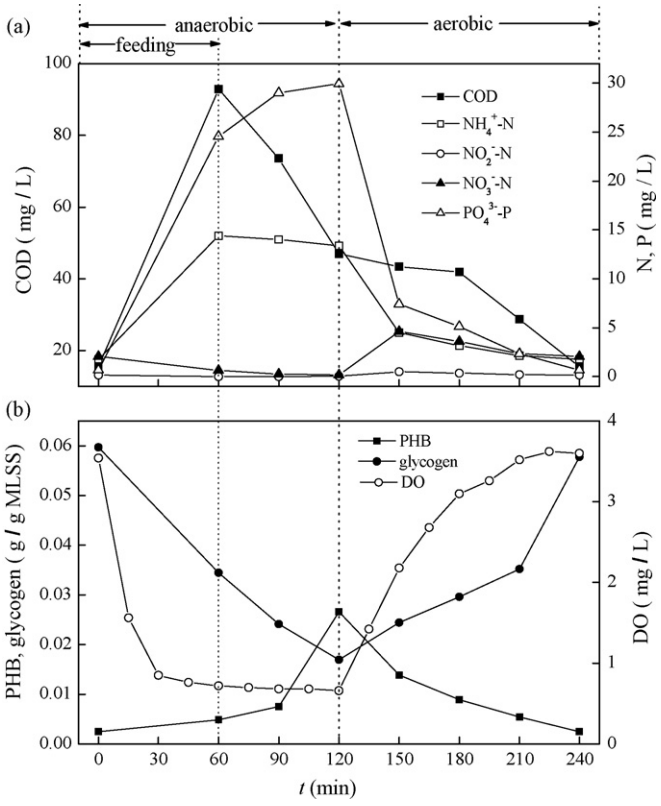

Fig. 5. Nutrient profiles during typical cyclic test on day 135. For (a), COD, NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P profiles during the cyclic test. For (b), PHB, glycogen and DO profiles during the cyclic test.

The phosphate concentration increased to 29.93 mg/L at the end of the anaerobic phase. In the aerobic phase, the phosphate uptake rapidly in the initial 30 min and phosphate concentration achieved 0.67 mg/L in the end. At the same time, the content of PHB and glycogen changed in accordance with phosphate release and uptake. At the end of the anaerobic phase, PHB accumulated to 0.027 g/gMLSS and glycogen decreased to 0.017 g/gMLSS, and at the end of aerobic phase, PHB was consumed to 0.002 g/gMLSS and glycogen accumulated to 0.058 g/gMLSS. It has been concluded that there are three criteria for a compound to function as an energy-storage compound: (1) Accumulation under conditions when the supply of energy from exogenous sources is in excess of that required by the cell for growth. (2) Utilization when the supply of exogenous sources is no longer sufficient for maintenance of the cell. (3) Degradation to produce energy and use as an electron donors for nutrient removal [29–31]. In our experiment, variations in the PHB and glycogen concentrations in microbial cells showed that in the feast period (feeding and anaerobic phase) PAOs utilized glycogen glycolysis to uptake external carbon source in wastewater and accumulate stored substances – PHB. While in the famine period (aerobic phase) PAOs consumed PHB and stored glycogen in microbial cells. The cycle test showed that the phosphate removal in the SBMBMBR was in accordance with the current knowledge of biological phosphorus removal. Meanwhile, variations in DO concentration during the whole cycle showed that after the aerobic phase the massive high volume influent can decrease the DO concentration quickly.

3.4. Specific phosphorus uptake rate test

On day 150, TP removal efficiency achieved 96.7% and the SBMBMBR exhibited good phosphate uptake phenomenon. In order to study the sludge characteristics and explore whether there was denitrifying phosphorus accumulating organisms (DPAOs) in phosphorus removing organisms, batch tests were carried out to calculate specific phosphorus uptake.

After anaerobic phosphate release, the sludge exhibited good aerobic phosphate uptake performance with the APUR of 14.9 mg/(gMLVSS h). The NPUR was 0.5 mg/(gMLVSS h) and the corresponding proportion of DPAOs in the total PAOs was 3.3%. Results indicated that there exists a small amount of DPAOs in the anaerobic/aerobic mode of SBMBMBR. DPAOs could utilize nitrite or nitrate instead of oxygen as electron acceptors to accumulate phosphate under anoxic conditions [32,33]. It can be speculated that the special microenvironment existed in the biofilm supplied anoxic condition for DPAOs in aerobic phase. Since in the aerobic phase the anoxic microenvironment existed in the inner of the biofilm was narrow. PAOs which utilize oxygen as electron acceptors were the main contributor for phosphorus removal, while DPAOs only took a small proportion.

3.5. The microbiological community and distribution in SBMBMBR

Biofilm and suspended biomass samples were taken from the reactor on day 125. To assess the composition of the biofilm cultured on the non-woven materials in steady state, FISH was performed with the 16S rRNA targeting oligonucleotide probes

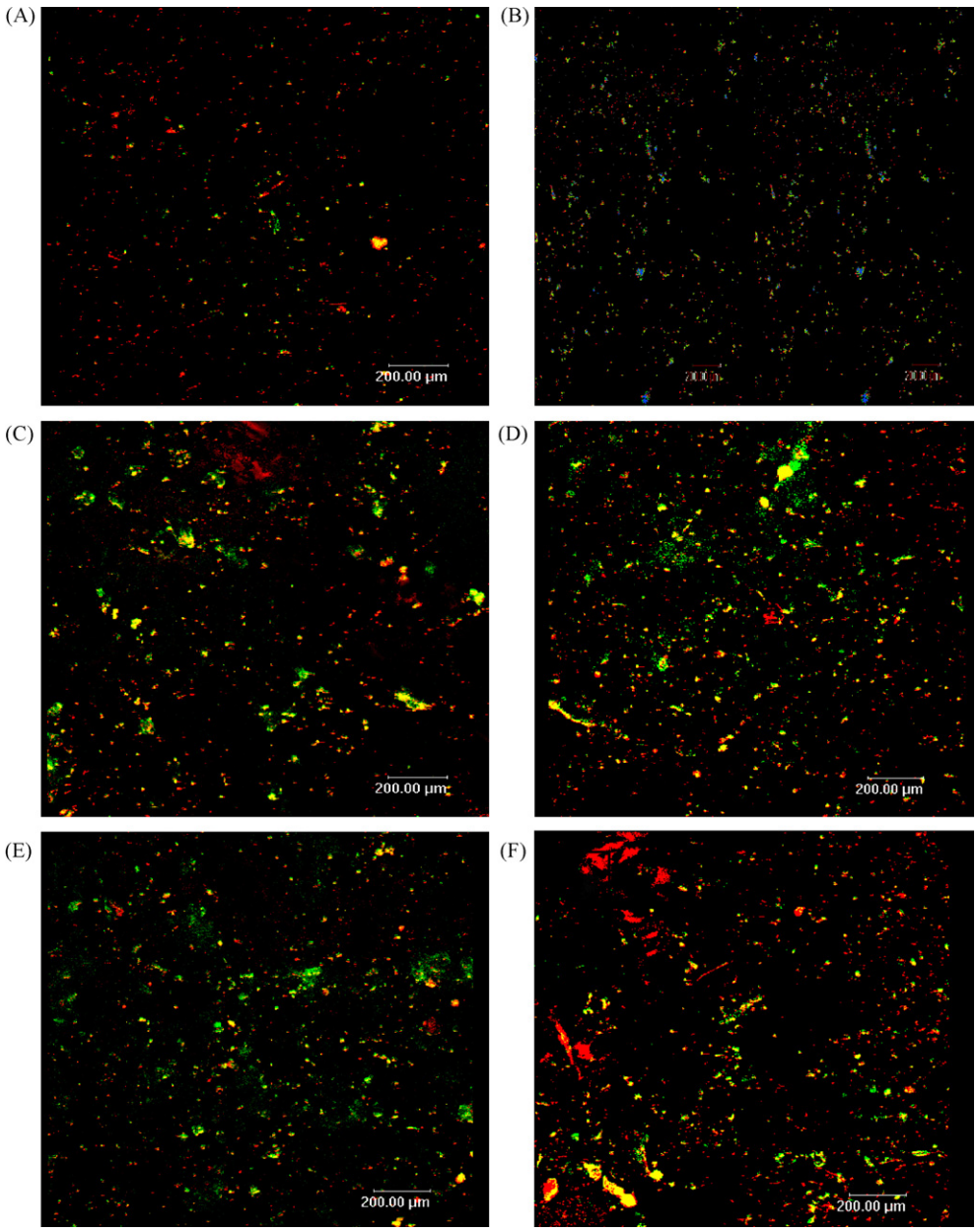

Fig. 6. FISH micrographs of microbes samples. For (A), (C) and (E), FISH with a CY3-labeled NSO190 (red) probe, a FITC-labeled Ntspa662 (green) probe and a FITC-labeled Nit3 (green) probe (A, for the inner layer of the biofilm; C, for the outer layer of the biofilm; E, for the suspended biomass). For (B), (D) and (F), FISH with a CY3-labeled EUB338 (red) probe and a FITC-labeled PAOMIX (green) probe (B, for the inner layer of the biofilm; D, for the outer layer of the biofilm; F, for the suspended biomass). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

NSO190, Ntspa665, Nit3, PAOMIX and EUB338. The inner layer of the biofilm consisted of a large quantity of AOB reacting with NSO190, and small amounts of NOB reacting with Ntspa665 and Nit3. About 78 ± 5% of the total biomass aera was detected by probe NSO190, which indicating that the low DO concentration existed in this layer (Fig. 6A). Simultaneously, a small amount of PAOs also was observed, about accounted for 16 ± 5% of the total biomass (Fig. 6B). The situation was quite different for the sample taken from the outer layer of the biofilm, in which the percent of bacteria, reacting to Ntspa665 and Nit3 increased to 48 ± 5% (Fig. 6C). In addition, compared with the inner layer, the percent of PAOs also increased 50 ± 5% (Fig. 6D). As to the suspended biomass, the microbiological community and distribution was generally the same as the outer layer of the biofilm. NOB accounted for 54 ± 5% of the total nitrifying bacteria (Fig. 6E), and PAOs accounted for 48 ± 5% of the total biomass (Fig. 6F). For the inner layer of the biofilm, NOB was restrained due to the low DO concentration. Although under the sequencing operation mode, the DO maintained low level in the inner layer all the time, therefore, PAOs cannot be accumulated, either. However, for the outer layer of the biofilm and suspended biomass, in the aerobic phase, the DO concentration was suitable for both AOB and NOB. Also, the transform between aerobic condition and anaerobic condition was beneficial for PAOs accumulating. In the SBMBMBR, the final phosphorus removal was discharging the suspended biomass and the falling-off biofilm from the system as a surplus sludge.

3.6. Membrane fouling

In this study, effluent was kept at a constant flow rate by peristaltic pump. Under operation condition of invariable hydraulic retention time (HRT), trans-membrane pressure (TMP) increased

with an aggravation of membrane fouling. Therefore, membrane fouling can be investigated by TMP.

During the whole experimental period, TMP increased quite slowly which indicated that the extent of membrane fouling was very slight. Membrane cleaning had not been carried out. The previous study by Yang et al. [13] indicated that in aerobic MBMBR, membrane fouling was more serious than CMBR which resulted from the excessive filamentous bacteria inhabited in suspended biomass in MBMBR. The overgrowth of filamentous bacteria can result in a severe cake layer and induced a large quantity of extracellular polymeric substances (EPS), which deteriorated the membrane fouling [34,35]. While in the SBMBMBR, scanning electron microscope (SEM) showed that filamentous bacteria cannot be observed in the suspended biomass and the rate of membrane fouling was slowed down. It indicated that an operation of a MBR in sequencing batch mode was beneficial for restraining the overgrowth of filamentous bacteria and reducing membrane fouling. Zhang et al. [14] also reported that the sequencing batch mode operation of MBR retarded membrane fouling.

4. Conclusion

The SBMBMBR showed good performance on organic substance and nitrogen removal during the whole experiment. When COD/TN ratios were 5.6–12.9, the COD, TN and ammonium nitrogen removal efficiencies averaged at 93.5%, 82.6% and 95.6%, respectively. TP removal was closely correlated with the length of anaerobic phase and aerobic phase. When anaerobic time and aerobic time were both 2 h, the average TP removal efficiency reached to 84.1%. DO concentration in aerobic phase was an important factor affecting simultaneous nitrogen and phosphorus removal, the optimal DO concentration was about 3 mg/L. Results derived from specific phosphorus uptake rate test indicated that there existed a small amount of DPAOs in the reactor which was speculated due to the anoxic microenvironment existed in the inner of the biofilm. FISH results of microbes showed that in the inner layer of the biofilm, there existed only a small amount of NOB and PAOs. While for the outer layer of the biofilm and the suspended biomass, a large quantity of AOB, NOB and PAOs were observed. The sequencing batch operation mode was beneficial for improving membrane fouling since filamentous bacteria was restrained in the reactor.

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