Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2019.22900

142 (2019) 148–157
February

Simultaneous organic carbon and nitrogen removal by heterotrophic nitrifiers under oxygen-limited condition in an internal-loop fluidized membrane bioreactor

Li Yang, Qin Cai, Ping Yang*, Wei Liao, Xiao Tan

Department of Environmental Science and Engineering, School of Architecture and Environment, Sichuan University, Chengdu 610065, China, Tel./Fax: +86 18602804508; emails: sculyz@163.com (P. Yang), scuyli@163.com (L. Yang), 1823395572@qq.com (Q. Cai), Lwei0314@163.com (W. Liao), heishihe@163.com (X. Tan)

Received 20 March 2018; Accepted 29 July 2018

ABSTRACT

An internal-loop fluidized membrane bioreactor (MBR) was operated to treat low-strength municipal wastewater by simultaneous removal of organic matter and nitrogen under oxygen-limited condition. Novel porous polymer carriers were used to immobilize microbes as well as alleviate membrane fouling. The dissolved oxygen (DO) concentration was varied from 0.07 to 0.5 mg/L at the top of the reactor throughout the experiment. The running conditions for the MBR were loading ammonia 50 mg/L, chemical oxygen demand (COD) 320 mg/L, hydraulic retention time (HRT) 8 h, pH 7.8–8.2, and C/N ratio 2.6. High removal percentages of COD, total nitrogen, and NH_4^+–N were achieved as high as 88%, 76%, and 96%, respectively, with only nitrite accumulated (10–13 mg/L) in effluent at optimal DO concentration of 0.12 ± 0.06 mg/L. With a membrane flux of 8 L/m^2/h corresponding to 8 h HRT, transmembrane pressure remained below 20 KPa during 60 days of continuous operation without need for membrane chemical cleaning or backwashing. High-throughput sequencing analysis indicates that heterotrophic bacteria plays a significant role in nitrogen removal; *Rhodobacter* is the known heterotrophic nitrification–aerobic denitrification bacteria in the present system. In addition, the proliferation of filamentous bacteria in this system enhanced nitrogen and COD removal.

Keywords: Heterotrophic nitrification–aerobic denitrification; IF-MBR; Simultaneous removal of organic matter and nitrogen; Oxygen limited; *Rhodobacter*

1. Introduction

Conventional ammonium removal in domestic wastewater treatment plants (WWTPs) is most commonly and economically achieved in biological nutrient removal (BNR) [1]. In this process, autotrophic nitrifiers oxidize ammonia to nitrite, followed by nitrate under aerobic condition. And then, heterotrophic denitrifiers reduce nitrate to nitrite, finally to N_2, under anaerobic condition [2]. However, ammonia removal by traditional biological process requires not only extensive aeration energy for nitrification but also external carbon source for denitrification [3]. In addition, autotrophic nitrifiers and denitrifiers differ in physiological characteristics and survival environments. This results in certain conflicts when applying the process to treat carbon-containing wastewater. For example, autotrophs are sensitive to organic matter, and the anoxic denitrifiers are susceptible to oxygen. The process would be strongly inhibited if the C/N ratio was higher than 0.25 or completely ceased at the ratios higher than 6 [4]. Thus, a pre-denitrification process, in most cases, is required to remove excess organic carbon. Additionally, the two types of microorganisms had to be accumulated in separate bioreactors because of different requirements for chemical oxygen demand (COD) and oxygen [5], such as A/A/O, or by using different aeration intervals, such as sequencing batch reactor (SBR) activated sludge process, thus increasing the operational difficulties. These contradictions result in each of the biotransformation

* Corresponding author.

efficiency exerted insufficiently. Researchers have developed granular sludge or biofilm to culture denitrifiers on the interior and autotrophic nitrifiers on the exterior. Some studies have limited dissolved oxygen (DO) concentration to reduce its influence on anoxic bacteria. However, restricting DO will limit the activity of autotrophic nitrifiers as well as partially limit denitrifiers to obtain sufficient NO_x^-–N (NO_2^-–N and NO_3^-–N) when using a single reactor [1]. The nitrogen conversion rate in combined process is very low in contrast to separate reactor.

The first report about heterotrophic nitrifiers was published in 1970s. Verstraete first isolated *Arthrobacter sp.* strain with the capability of heterotrophic nitrification from the natural environment in 1972 [6]. This pioneer discovery revised our nitrogen cycle knowledge. Nitrification is by no means limited to chemoautotrophs. Afterward, increasing number of heterotrophic nitrifiers were isolated from various environments [2,7–10]. These special nitrogen-removing bacteria are capable of utilizing organic substrates and could directly convert NH_4^+ to gaseous nitrogen [11,12]. In other words, many heterotrophic nitrification bacteria are also aerobic denitrifying bacteria, allowing simultaneous nitrification and denitrification (SND) occurring in a single reactor. This ability is of more significance in wastewater treatment because it not only largely reduces costs for both system space and construction (eliminates the pre-denitrification process to reduce COD prior to nitrification) but also simplifies the operation which is in striking contrast to SBR. In addition, in comparison with autotrophic nitrifiers, heterotrophic nitrification bacteria have fast growth rate and can tolerate high organic loading and low DO [13]. With these advantages mentioned above, more and more heterotrophic nitrification–aerobic denitrification strains were isolated and inoculated to a single bioreactor for various wastewater treatments and obtained better results [14–16]. However, inoculating activated sludge are of great value in future practical engineering applications. In fact, heterotrophic nitrification widely existed in various kinds of environments containing activated sludge.

Due to the strictly increasing wastewater discharge standards and growth of large urban centers producing large amount of domestic wastewater, construction of treatment plants occupying the least possible physical space and cost-efficient is a better alternative. Since energy for aeration accounts for about 50% of the entire energy consumption in a WWTP, if we control DO to a low level, it would be technically feasible to save energy consumption for aeration. The attractiveness of fluidized bed bioreactors is evident from the more compact construction and high mass transfer co-efficiency, which often represent critical factors for the WWTPs. Therefore, a single continuous flow lab-scale internal-loop fluidized bed combined with membrane bioreactor (MBR), internal-loop fluidized MBR (IF-MBR), was constructed for treating domestic wastewater. Porous polymeric carriers have been successfully applied to immobilize microbes.

In this study, we aimed at starting up an SND process under oxygen-limited conditions from activated sludge at a relative low C/N ratio of 2.6 and heterotrophic nitrification under low DO was preliminarily investigated. Adaptation could result either in an acclimation of inoculums to new environment or a significant change of existing bacterial population; so, the bacterial populations were also investigated through high-throughput sequencing analysis of the biomass on carriers. It would be of great theoretical significance for the future application of the heterotrophic nitrification–aerobic denitrification technology in treating low-strength domestic wastewater.

2. Materials and methods

2.1. Experimental setup and operating conditions

As shown in Fig. 1, the experimental equipment was made of Plexiglas, with an effective volume of 19 L. The reactor was 795 mm high with 200 mm internal diameter; a draught tube with internal diameter of 100 mm and height of 605 mm was installed in the center of the reactor to form up-flow zone and down-flow zone inside and outside the tube, respectively. Aeration ports were in the middle of up-flow zone in the tube and at a distance of 30 mm from the top. A submerged hollow-fiber polyvinylidenfluoride membrane module (Motian, Tianjing, China) with nominal pore size of 0.1 μm and an effective total membrane surface area of 0.3 m^2 was installed above aeration ports in the top of the draft tube. Biomass grew on carriers was driven by the aeration and recirculation flow to circulate from up-flow zone to down-flow zone. Thirty- to forty-mesh porous polymer carriers were added into the reactor with a packing ratio of about 5%. Physical parameters of the polymer porous carrier were summarized as follows: wet packing density of 1,010.00 kg/m^3, skeletal density of 1,320.00 kg/m^3, pore volume of 0.301 mL/g, and wet surface area of 5,357.00 m^2/m^3. The exterior of the reactor is wrapped up by black paper to avoid light.

The IF-MBR was started by inoculating activated sludge taken from aerobic tank at the municipal WWTP, Shuangliu, China. The initial sludge concentration was 3.0 g total suspended solids per liter. Synthetic wastewater was continuously fed to the reactor with a pump (Longer, China), and the permeate suction was done using a peristaltic pump (Longer, China) to remove permeate continuously from the MBR.

During the start-up, the hydraulic retention time (HRT) and DO concentration were set at 11 h and 0.5 mg/L. In order to develop biofilm on the packing media, the suspended sludge was discharged from the reactor and only the biological carrier was retained in the bioreactor when carriers and the suspended sludge were in full contact. After the bioreactor start-up, HRT was shortened to 8 h and DO was kept at 0.5 mg/L. The temperature was thermostatically controlled at 26°C during the whole experiment by means of a resistance wire heating.

The experiments were conducted using a synthetic wastewater to avoid any fluctuation in the feed concentration and provide a continuous source of biodegradable organic pollutants. Total COD and ammonia concentrations for municipal wastewater varying from 275.3 to 490.7 mg/L and 34.3 to 53.9 mg/L, respectively, have been reported in the literature [17]. In the study presented, the COD and ammonia concentrations were 320 and 40–50 mg/L, respectively. The composition of the synthetic wastewater was as follows: glucose,

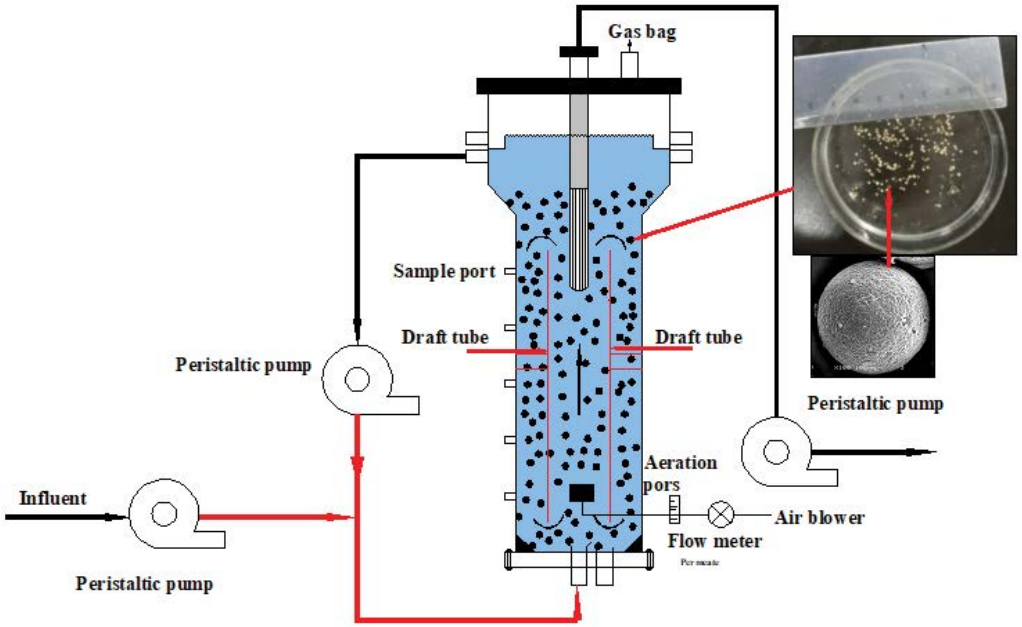


Fig. 1. Schematic diagram of the experimental setup.

300 mg/L; $(NH_4)_2SO_4$, 188.6–242.9 mg/L; KH_2PO_4, 43.9 mg/L; and 1 mL/L of a trace element solution. One liter of trace element solution contained 15.0 g/L $C_{10}H_{12}N_2O_8$ (EDTA), 1.1 g/L $(NH_4)_6Mo_7O_2{\cdot}4H_2O$, 0.99 g/L $MnCl_2{\cdot}4H_2O$, 4.2 g/L $CaCl_2{\cdot}H_2O$, 0.24 g/L $CoCl_2{\cdot}6H_2O$, 0.014 g/L H_3BO_4, 5.0 g/L $MgSO_4{\cdot}7H_2O$, 5.0 g/L $FeSO_4{\cdot}7H_2O$, 0.25 g/L $CuSO_4{\cdot}5H_2O$, and 0.21 g/L $NiSO_4{\cdot}6H_2O$. $NaHCO_3$ with 1 g/L was added to the influent to maintain the pH of the IF-MBR suspension between 7.8 and 8.4.

2.2. Batch test

A set of batch assays was established to study the effect of low DO on nitrogen removal in IF-MBR treating domestic wastewater at 26°C. To find the optimum DO for maximum nitrogen removal, three different DOs were used (0.5 ± 0.04, 0.07 ± 0.01, and 0.12 ± 0.06 mg/L) during the steady period. The reactor substrate loading rate was kept constant during these experiments with influent COD and NH_4^+–N concentration of 320 and 50 mg/L, respectively. COD, NH_4^+–N, NO_2^-–N, NO_3^+–N, and total nitrogen (TN) of effluents from IF-MBR treatment were analyzed. All experiments were conducted in the bioreactor, and each phase was operated for about 12 days until steady state achieved.

2.3. Analytical methods

COD of the samples was measured by COD analyzer (Lianhua, China). For soluble COD (SCOD), samples were obtained by filtering the MBR sludge (50 mL) followed by a filtration of the supernatant through a 0.45-μm filter. The pH values in the reactor were determined using a pH meter (Leici PHS-25, China). DO was measured continuously using a WTW Multi 3510 oxygen probe (Germany) under the liquid level 5 cm. NH_4^+–N, NO_2^-–N, NO_3^-–N, TN, sludge volume index, mixed liquid suspended solids, and volatile suspended solids (VSS) were analyzed according to Standard Methods [18].

2.4. Sample collection, DNA extraction, PCR amplification, and sequencing

Biomass samples from the reactor were collected at days at steady period and immediately stored at −80°C for subsequent experiments. The subsequent work including DNA extraction, PCR amplification, high-throughput sequencing (seeding sludge and carrier), and data analysis were performed by Sangon Biotech Co., Ltd. (Shanghai, China). When total genomic DNA was extracted from each sample, the bacterial primers 341F (CCCTACACGACGCTCTTCCGATCTG) and 805R (GACTGGAGTTCCTTGGCACCCGAGAATTCCA) were used to amplify the V4-V5 region of the 16S ribosomal RNA (rRNA) gene. Finally, the amplicons of bacteria were sequenced using the pair-ended method by the Illumina Miseq sequencing platform at Sangon Biotech Co., Ltd. (Shanghai, China).

3. Results and discussion

3.1. Start-up of IF-MBR

The synthetic wastewater was continuously supplied at NH_4^+–N concentration of 40 mg/L and COD of 320 mg/L during the start-up period. The evolution of ammonium, nitrite, and nitrate concentrations in the effluent can be observed in Fig. 2(a). The average removal efficiency of NH_4^+–N, TN, and COD were 25.7%, 24.9%, and 81.6%, respectively. The seeding sludge in the reactor was mainly responsible for nitrogen removal during this period, for membrane filtration did not affect retention of soluble ammonia [19]. There was hardly any biofilm on the carriers but over proliferation in the suspended sludge at the first 10 days. Given biofilm growth, the strategy of the fast washout of the inoculum biomass

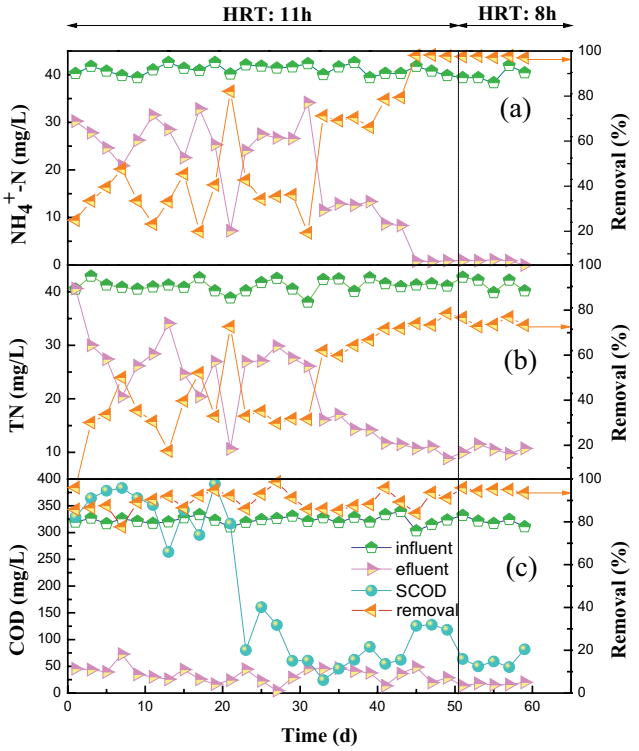


Fig. 2. Performance of the IF-MBR under different HRT. (a) NH_4^+–N removal, (b) TN removal, and (c) COD removal.

constrained the development of microorganisms in the liquid phase and strongly enhanced biofilm growth [20]. In order to weaken the competition between suspended and fixed microbes as well as accelerate the biofilm formation, we discharged the suspended sludge from the reactor. Afterward, a thin and cream colored biomembrane was shown, which meant initiation of biofilm formation and the removal efficiency of nitrogen gradually increased. As shown in Fig. 2(b), TN and NH_4^+–N removal efficiency reached about 72% and 98%, respectively, with DO concentration maintained at 0.5 ± 0.04 mg/L. It was noteworthy that a rapid formation of biofilms was obtained on carriers in this system.

As shown in Fig. 2(c), the reactor showed a good performance in terms of COD removal during the acclimation studies. A stable, high COD removal percentage for this system averaging 81.6% or higher was achieved initially and this increased to 88% after start-up, indicating a good biological degradation capability with consequent high effluent quality. But nevertheless, a temporary SCOD build-up (exceeding 300 mg/L) occurred initially in bulk liquid. Since a synthetic wastewater comprising of glucose was adopted, the substance contained in the wastewater was supposed to be easily decomposed, and hence, the supernatant SCOD concentration might be a pool of organic matters dealing with the bacterial adaptation for the changing environment and the remains of refractory materials contained in seeding sludge. These organic matters cannot pass through membrane except discharging out from the bioreactor. Then, SCOD concentration decreased sharply to around 75 mg/L with effluent COD concentrations below 50 mg/L. Compared with stable membrane effluent COD in Fig. 2(c), the supernatant SCOD appeared to increase after the 35th day in operation. Relatively high SCOD values, which varied in the range of 75–150 mg/L, were observed from the 35th to 50th day. The clear discrepancy between bulk liquid and permeate indicates that SCOD accumulated in bulk liquid might be attributed to colloidal material deriving from substrate metabolism (usually with biomass growth) and biomass decay [21], with a size larger than the 0.2-μm membrane pores but smaller than the pores of the 0.45-μm filter used to separate suspended and dissolved matter. As the operation time was prolonged, however, the supernatant SCOD declined, implying these matters can be partially degraded by microbes. Regarding this phenomenon, similar results have been reported in other study under different operation conditions [22,23]. Rittmann also demonstrated that the soluble microbial products were biodegradable, but at a low rate [24]. In this study, high COD removal obtained in IF-MBR is not a result of low active organisms present in the low bulk liquid (averaged 3,000 mg/L). It is the biofilm on carriers that is responsible for most of the system's bacteria (averaged 8,900 mg/L). Another factor keeping permeate COD low is membrane filtration of colloidal materials.

After start-up, the HRT was reduced to 8 h to enhance the nitrogen volume loading rate from 0.087 to 0.12 kg N/m^3 per day. The ammonium always remained below 2 mg/L in effluent. It seems that the increased nitrogen loading rate did not impair the performance of the reactor. The TN removal efficiency obtained in this system at the two HRTs were not significantly different, indicating that the TN removal rate was independent on the HRT.

3.2. Determination of preferable DO concentration for nitrogen removal

After operating for a period, we further increased influent NH_4^+–N concentration to 50 mg/L, resulting in C/N ratio of 2.6. Previous study has shown that different enzymes and species in the denitrification pathway in aerobic denitrifiers responded to different concentrations of oxygen [25]. Therefore, an optimal DO level may enhance the activity of heterotrophic nitrification and aerobic dentrification, resulting in better nitrogen removal. COD removal was not adversely affected in the IF-MBR, with stable effluent concentration of 30 ± 3 mg/L for all treatments (data not shown). This meant that substantial amounts of aerobic (facultative) bacteria were retained on the carriers and a good biological degradation capacity was obtained in our study.

In contrast, ammonia removal tended to vary with change in DO concentration (Fig. 3(b)). It was seen that in the case of 0.5 ± 0.04 mg/L DO accounted for an average TN removal of 58%, which could be attributed primarily to simultaneous heterotrophic nitrification and aerobic denitrification. However, high concentration of ammonium and nitrate in effluent reflected inefficient nitrification and denitrification rates. A previous study found that optimal shear force was essential for high removal efficiency in a circulating fluidized bed biofilm reactor, with bioparticle fluidization since it helped the bioparticles to detach less biomass by hydraulic shear and to keep higher expanded

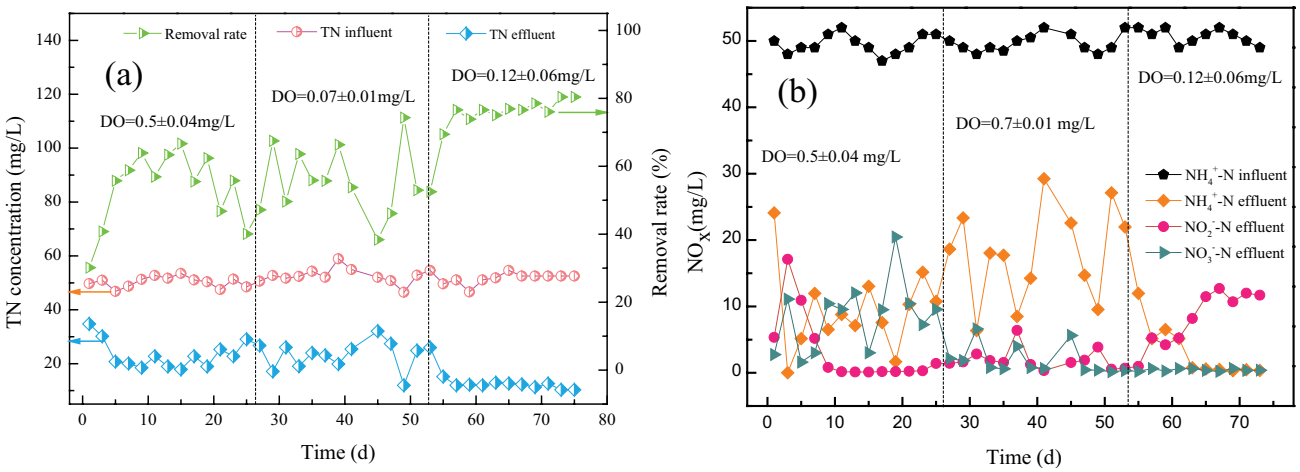


Fig. 3. Profile of the changes in nitrogen in relation to DO concentrations, where NO_x^-–N is representative of NO_2^-–N, NO_3^-–N, and NH_4^+–N; (a) TN concentration in effluent and removal rate; (b) NO_x^-–N in effluent.

bed height which translates to larger bed voidage as well as less abrasion between bioparticles [26]. When circulation flow rate was fixed, the increase of aeration would accelerate the circulation rate of the carriers, thereby producing higher shear force. In order to promote ammonia oxidization as well as enhance denitrification rate, we further reduced the DO values to 0.07 ± 0.01 mg/L (Fig. 2(b)). The nitrification was partially inhibited, while denitrification performance of the system was influenced to a lesser degree by low DO. Since a significant amount of ammonia (averaged 27 mg/L) remained in effluent without nitrate and nitrite accumulation, nitrification becomes the rate-limiting step. Thus, oxygen is required for heterotrophic ammonia oxidation, as for autotrophic ammonia oxidation. As shown in Fig. 3(b), when DO concentration increased to 0.12 ± 0.06 mg/L, NH_4^+–N removal increased remarkably, whereas TN removal improved moderately (optimal 76%). Aeration was restricted in the riser; carriers were slowly transferred from the riser to the downer which is beneficial to full contact between microbes and ammonia with favorable mass transfer and thus enhanced BNR. Similarly, a higher suspended biomass concentration was achieved at DO 0.12 ± 0.06 mg/L by controlling sludge wastage; for example, a sludge concentration of 4,500 ± 500 mg/L which was higher than that (3,000 ± 200 mg/L) at DO 0.5 ± 0.04 mg/L was achieved, and this ultimately led to sufficient nitrification.

NO_3^-–N concentration in effluent remained low, whereas NO_2^-–N concentration (10–13 mg/L) increased when DO was 0.12 ± 0.06 mg/L. Nitrite is one of the main intermediate of aerobic denitrification and toxic to the microbes. It is assumed that nitrite is formed from ammonium by an oxidative sequence involved in some way with the metabolism of a special carbon source [27]. It is concluded that nitrite reductases in certain aerobic denitrification bacteria are still sensitive to oxygen, which is the same as traditional denitrifiers. Sun et al. improved nitrite reduction by decreased DO concentration to a low level [28]. In the present study, nitrite accumulation (about 10–12 mg/L) under low DO condition is probably attributing to be devoid of carbon since a relative low carbon (320 mg/L) was adopted. This result is roughly consistent with Liang who reported that at an insufficient carbon concentration, the electron flow is too low to provide sufficient energy for cell growth and led to accumulation of intermediate such as nitrite [29]. The better results were obtained at DO concentration of 0.12 ± 0.06 mg/L, as it demonstrated the possibility of enriching for heterotrophic nitrifiers and denitrifiers, which can be well adapted to oxygen-limited environment. This finding presents a striking contrast to traditional methods of aerobic–anaerobic nitrogen removal since it eliminates the need to reduce organic matter content preceding nitrification.

However, nitrite accumulation is a critical issue inherent in the aquaculture industry because of its toxicity to aquatic animals and even caused death by secondary pathogen infection. One possible solution would be to create an anoxic tank after the reactors. This will add to the physical space. The dosage of an external carbon source (e.g. methanol) may be required to support the removal of the residual nitrite in this zone. According to Ginige, a biomass-specific denitrification rate of 3.6–5.5 mg N/g VSS/h could be achieved by methanol addition to a post-anoxic zone [30]. This means that an HRT in the post-anoxic zone of less than 1 h is needed to achieve complete nitrite removal. Note that this would not necessarily increase the occupying space, since the anoxic tank is much smaller. The technology demonstrated in this paper is particularly suitable for N removal from wastewaters with low COD:N ratios, and hence, the occupying space can be smaller than that typically required for normal wastewater.

3.3. Evolution of TMP with time

The membrane was continuously operated at fixed flux without other membrane fouling control (such as back flushing and frequent relaxation) except porosity polymer carriers scouring the membrane surface. Carriers circulated between the up-flow and down-flow zones with recirculation flow rate of 15 m/h. Here, the MBR system (Fig. 5) was operated continuously for 105 days, using a permeate flux of 6 L/m^2/h during the start-up, increasing to 8 L/m^2/h thereafter. The

filtration operations were terminated when TMPs reached 20 KPa for the MBR system. Fig. 4 shows an ideal 'zigzag' manner of the TMP with an initially short-term rapid TMP rise followed by extended period of slow TMP rise and a transition to a quick TMP rise independent of flux. Similar patterns were obtained in other researches under different operation condition [31,32]. After start up, the permeate flux was increased to 8 L/m2/h on day 56. As illustrated in Fig. 5, a sudden increase of TMP exceeds 11 KPa, and then we decreased the flux, while the TMP did not decrease. Backwashing with membrane permeate was performed; however, this did not mitigate the membrane fouling, indicating an irreversible fouling formed. Possibly, the sudden increase in flux leads to unbalance between permeation drag and back transport velocity and thus results in local flux higher than critical flux.

The membrane module was removed from the reactor for chemical cleaning, NaOCl for 1 h and then in 500 ppm NaOH solution for 1 h after rinsing with deionization water. After the chemical cleaning, TMP decreased to less than 1 KPa. A stable operation of the MBR was achieved at a fixed flux approximately 8 L/m^2/h. For this period of operation, the TMP values were always similar in magnitude (Fig. 4), which indicates very small quantities of irreversible fouling. This flux is considered to be below the critical flux concept since the MBRs can be operated over extended periods at a fixed flux if this flux is substantially beneath the nominal critical flux [33]. However, the fouling occurred rapidly with TMP exceeding 15 KPa on day 103; backwashing was acquired to restore the TMP. However, the physical backwashing no longer restored completely the filtration behavior with the incorporation of irreversible fouling. This occurrence is evidenced by the fact that the TMP after washing remained >20 KPa, suggesting that certain clogged pores were not cleaned up. The overall TMP during long-term operation was generally well below the 20 KPa level, which has been widely considered as a maximum allowable TMP, after which chemical cleaning should be done. Without particle fluidization, the membrane would undergo severe biofouling within a few days as we observed (data not shown). The TMP of 8 L/m^2/h jump was noted until exceeded 50 days, indicating that the membrane fouling could be effectively alleviated with the presence of round particles.

3.4. Overview of sequencing and microbial diversity

Sequences obtained by high-throughput sequencing of the bacterial V3-V5 region were used to investigate the microbial community diversity of seeding sludge and carriers of IF-MBR bioreactor under steady operation at DO concentration of 0.12 ± 0.06 mg/L. After removing low-quality sequences and chimeras, at least 47,862 effective sequences for sample CA-1 and 50,258 for DZ-2 were obtained with an average length of 425.45 and 416.23 bp, respectively. The sequence number of each sample was normalized, and 3,738–4,703 OTUs were generated by mother clustering method with a threshold of 0.97 (Table 1). The

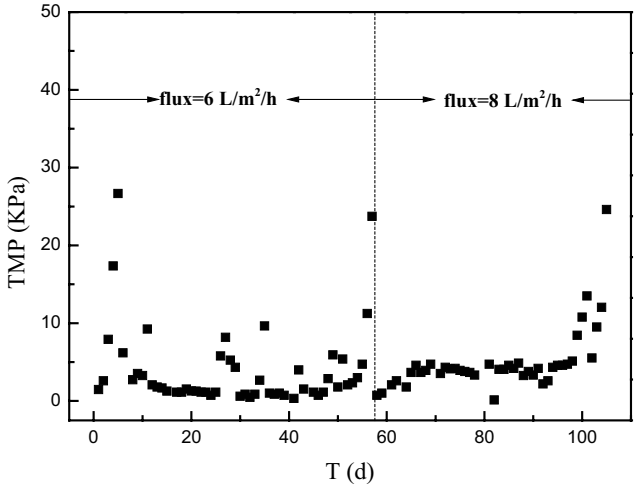


Fig. 4. Transmembrane pressure of the MBR with time.

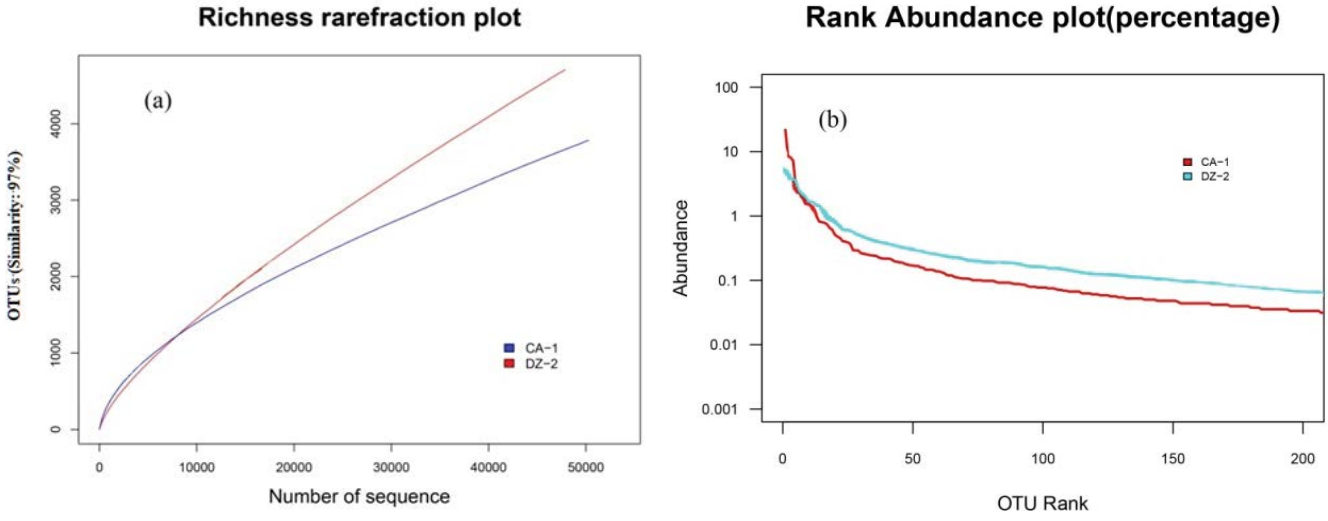


Fig. 5. Diversity of bacterial communities of seeding sludge (CA-1) and carriers (DZ-2). (a) Rarefaction curves and (b) rank-abundance curves based on bacterial OTUs at a dissimilarity level of 3%.

Table 1
Richness and diversity of the samples based on 0.03 distances

Sample	Sequences		0.03 distance					
	Raw	Effective	OTUs	Chao1	ACE	Shannon	Simpson	Coverage (%)
CA-1	52,340	47,862	4,703	26,336	54,637	4.57	0.07	92.4
DZ-2	52,202	50,258	3,738	15,461	26,508	5.62	0.01	95.0

CA-1 is the seeding sludge; DZ-2 are carriers taken from reactors under steady period at DO concentration of 0.12 ± 0.06 mg/L.

results confirmed that the sequencing was of repeatability and reliability. The rarefaction curve based on OTUs at 3% dissimilarity, as shown in Fig. 5(a), indicates there are still several minor bacterial species remain unidentified in the present study. In addition, the rank-abundance curves show that the two samples contain relatively low proportions of highly abundant bacteria, and most of the reads belong to rare organisms represented (Fig. 5(b)) [34].

In addition, the values of Ace, Chao, Shannon, and Simpson (Table 1) also indicate that the bacterial diversity of DZ-2 is higher than that of CA-1. Good's coverage (92.4% for CA-1, 95% for DZ-2) reveals that the 16S rRNA gene sequences identified in these samples represent the majority of bacterial diversity present in the two samples. Sample DZ-2 was collected from steady state under DO concentration of 0.12 ± 0.06 mg/L in our system, while sample CA-1 was collected from aerobic tank of domestic WWTP with average influent COD concentration of 100 ± 25 mg/L. Higher community richness and bacterial diversity of DZ-2 compared with CA-1 might be due to higher COD concentration (320 mg/L in the present study), which is beneficial for steady survival of more bacterial species. On the other hand, the porous polymer carrier with a larger surface area can retain most of microbes accumulating on it, and the utilization of MBR favoring the slow-growing microbes' proliferation makes the environment more complex. The high species richness of microbial community ensures the reactor's steady and a quick recovery of the consortia from the stress conditions.

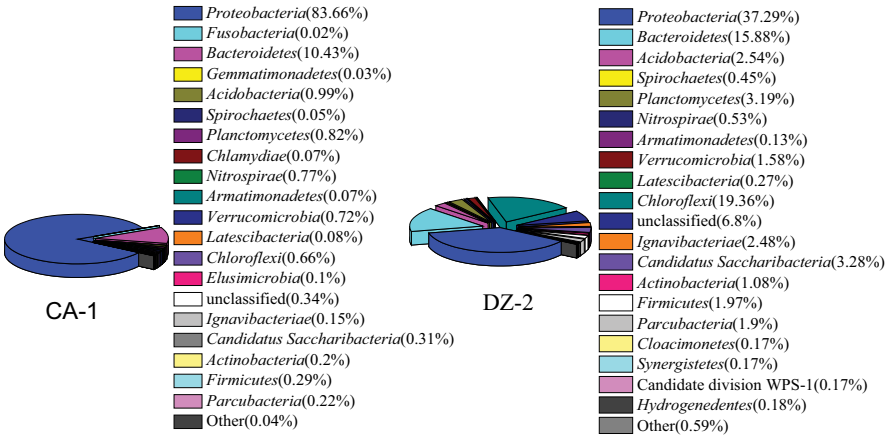
3.5. Microbial community analysis

The behavior of the reactor is closely related to the microbial community structure; microbial population of seeding sludge and carriers during steady period was investigated by high-throughput sequencing. The taxonomic classification and bacterial diversity at the phylum, order, and genus levels are presented in Fig. 6, demonstrating distinct bacterial community compositions. From the phylum assignment result (Fig. 6(a)), it was found that discrepancy of bacterial diversity was present in DZ-2 and DX-2. In total, two domain bacterial phyla were detected in the seeding sludge, which mainly included *Proteobacteria* and *Bacteroidetes* accounting for 83.66% and 10.43%, respectively. Most of the bacterial phyla decreased sharply and eventually vanished in sample DZ-2, especially for *Proteobacteria*. The abundance of *Proteobacteria* significantly decreased to 37.29%, while the abundance of *Chloroflexi* underwent a significant increase to 19.36%. Other represented phyla underwent a slight increase, such as *Candidatus Saccharibacteria* (3.28%), *Planctomycetes* (3.19%), *Acidobacteria* (2.54%), and *Ignavibacteriae* (2.48%) in DZ-2.

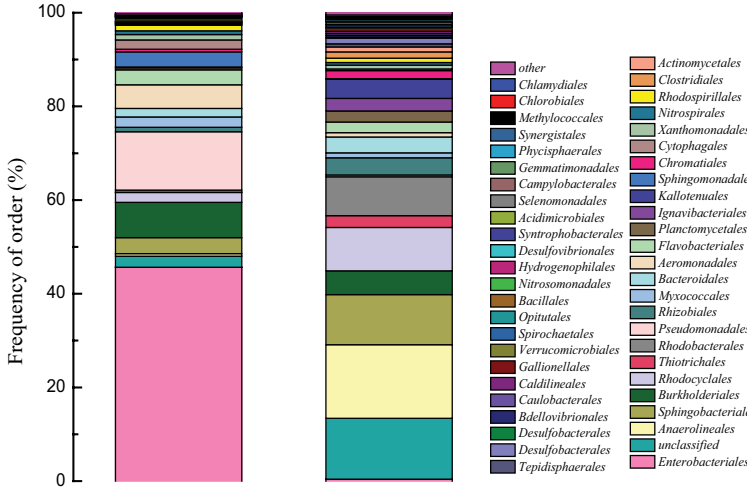
At order level, domination of the system belongs to *Anaerolineae*; these have been enriched efficiently in the carrier after the start-up with relative abundance of 15.64%, while only 0.52% in seeding sludge (Fig. 6(b)). Within Phylum *Bacteroidetes*, the abundance of *Sphingobacteriales* rose highly from 3.52% to 10.47% contrast to *Flavobacteriales* whose abundance decreased little from 3.23% to 2.24 % during the steady period. Additionally, it is interesting to find that the dominant orders in Phylum *Proteobacteria* presented a dramatical difference between the seeding sludge and the biomass from stable phase, that is, *Rhodobacterales* (8.31%), *Rhizobiales* (3.67), *Rhodocyclales* (8.82%), and *Burkholderiales* (4.96%) dominated in stable phase while only *Enterobacteriales* (45.67%), *Pseudomonadales* (12.44%), *Burkholderiales* (7.64%), and *Aeromonadales* (5.05%) prevailed in the biomass from seeding sludge. Members of *Sphingobacteriales* have been reported to undergo anaerobic/anoxic phosphorous removal with utilization of nitrite as terminal electron acceptor [35]. And members of *Anaerolineales* have been detected in Anammox bioreactors, and the role is rarely studied [36,37], but the phylum *Chloroflexi* they belong to was reported to be easily enriched in MBR system and could preferentially degrade carbohydrates and consequently prevent membrane fouling [38]. The distinct differences in bacterial communities between CA-1 and DZ-2 indicate that the change of microbial environment in the reactor can lead to a significant variation of microbial community diversity.

In order to have a deeper understanding of the bacterial community function in the microbial ecosystem, the genus level was analyzed (Fig. 6(c)). From Fig. 6(c), we can observe that there was a large proportion of sequences (27.57% in sample DZ-2) not assigned to any genera, indicating that many taxa remain unknown in the reactor. These novel microbes might have significant effects on wastewater treatment processes and worth comprehensive evaluation [39]. Among the dominant bacteria in the inoculum, such as *Citrobacter* (21.76%), *Acinetobacter* (11.25%), and *Raoultella* (10.16%), almost all of them were eliminated in the reactor, whereas a wide range of bacterial genera was enriched in the reactor, such as *Rhodobacter* (6.2%), *Longilinea* (6.59%), *Ornatilinea* (3.87%), *Haliscomenobacter* (4.0%), *Saccharibacteria_genera_incertae_sedis* (3.28%), *Thiothrix* (2.37%), *Ignavibacterium* (2.22%), and *Bellilinea* (2.07%).

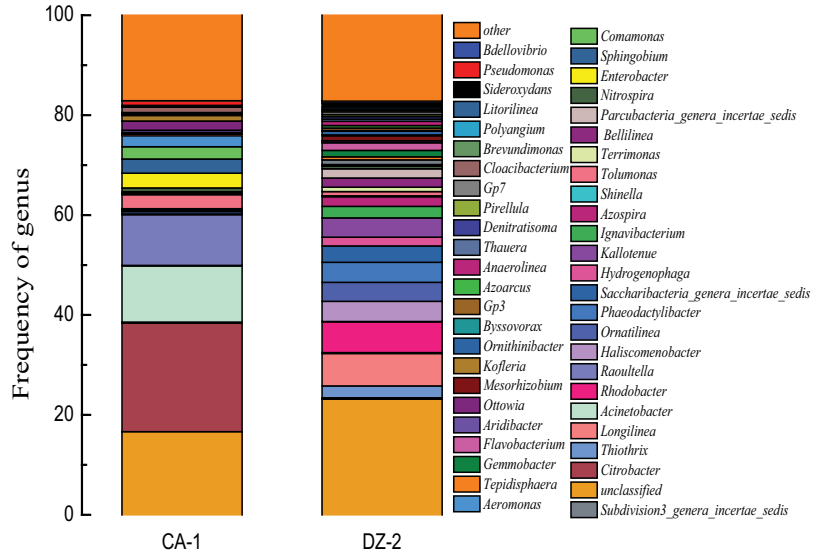
Of all the sequences above, *Rhodobacter* can heterotrophically consume ammonia for cell growth [40,41] as well as oxidize ammonia to nitrate [42] and denitrify nitrate to nitrogen gas, exhibiting better nitrification and denitrification capacity in aerobic-dark [43]. It is the main heterotrophic



(a) Phylum



(b) Order



(c) Genus

Fig. 6. Taxonomic classification of the bacterial communities at (a) phylum, (b) order, (c) genus. Phylum, class, and genus making up less than 0.5% of total composition in all samples were classified as "other."

nitrification and aerobic denitrification microbe correlated to nitrogen loss. *Longilinea* was detected in most annamox reactor and strongly related with denitrification and COD removal [44]. *Ignavibacterium sp.* have been proposed as a strictly anaerobic and heterotrophic, fermentative bacterium [45]. *Ornatilinea* is an obligate anaerobic bacterium and have the capacity of fermenting proteinaceous substrates and sugars [46]. *Bellilinea* is obligate anaerobic microorganism and belongs to class *Anaerolinea* [47]. However, high abundance of *Chloroflexi* (19.36%) in the reactor makes filamentous bacteria also become the dominant population on the carriers. The presence of *Chloroflexi* could utilize or degrade the recalcitrant soluble microbial production derived from biomass decay, thus reducing membrane fouling potential [38]. In addition, Guo increased TN removal efficiency from 45% to 75% under low DO by filamentous bacteria which can degrade lower remaining substrate concentrations due to their special characteristics [48].

In spite of the wide bacterial diversity in the MBR system, scarcely any sequences of ammonia oxidation bacteria (AOB) (0.13% for CA-1, 0.15% for DZ-2) and nitrite oxidation bacteria (NOB) (0.77% for CA-1, 0.53% for DZ-2) were obtained in the present study, which are commonly reported as the main contributors to classic ammonia oxidation, of which *Nitrosomonas* and *Nitrosospira* are the most essential genera [24]. The autotrophic microbial result is consistent with the result of Ye, who observed that very low percentages (0.05% and 1.01%, respectively) of the microbes were AOB and NOB in a full-scale WWTP in Hong Kong [49]. Such low abundance of autotrophic bacteria probably is correlated to the C/N ratio exceeding 2 in influent. High concentration of COD provides excess growth substrates for the potential heterotrophic nitrifiers such as *Rhodobacter* aforementioned, resulting in the dilution of AOB and NOB. They may outcompete autotrophic bacteria and contribute more to ammonium removal. It seems that the high concentration of nitrite in effluent may be contradictory with the bacteria abundance result. However, there are a large proportion of sequences (27.57%) not assigned to any genera. Ma et al. analyzed the major unidentified sequences by BLAST using the 16S rRNA sequence database and postulated that these novel bacteria (likely uncultured bacteria) might play important roles in wastewater treatment processes [39]. It implies there are still some undiscovered microbial resources for N removal in the genus obtained from this study and required comprehensive evaluation in future. Nevertheless, the specific roles and nitrification mechanism of heterotrophic microbes needed further study. Microbiological analysis shows great differences between the seed sludge and the reactor, indicating DO is a key factor for selection of diversity heterotrophic nitrifiers.

4. Conclusion

This study demonstrated the feasibility of internal circulation fluidized bed membrane bioreactor (IF-MBR) with porous polymer carriers for the treatment of domestic wastewater by simultaneous organic and nitrogen removal under oxygen-limited condition. Batch tests reveal that the removal rates of COD, NH_4^+–N, and TN can be achieved as high as 88%, 96%, and 76%, respectively, at optimal DO concentration of 0.12 ± 0.06 mg/L. For around 60 days of operation at a fixed flux of 8 L/m^2/h, no membrane fouling control, such as chemical cleaning or backwashing, was needed other than that resulting from the souring effect of the fluidized porous polymer carriers. High-throughput sequencing result indicates that heterotrophic nitrifiers such as *Rhodobacter* play significant roles in nitrogen removal; in addition, some novel microbes are also responsible for N removal, and their nitrification mechanism needs further study. The IF-MBR system has good potential as a high-efficiency low-energy cost-effective domestic wastewater treatment system. Further study should focus on pilot scale of reactor system and investigation on the feasibility of the small carriers in application.

Acknowledgements

This study was supported by Grants from the science and technology project of SiChuan (No. 17ZDYF3097).

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