Nutrients removal performance of a denitrifying phosphorus removal process in alternate anaerobic/anoxic–aerobic double membrane bioreactors (A2N-DMBR)

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ABSTRACT

An alternate anaerobic/anoxic–aerobic double membrane bioreactors process (A2N-DMBR) was proposed to improve denitrifying phosphorus removal efficiency. The system was operated for 70 d under different nitrogen/phosphorus (N/P) ratios with synthetic wastewater to present the performance evaluation of nutrients removal and microbial community structure in the A2N-DMBR process. The results showed that when the influent total phosphorus (TP) was 6.4 mg/L, the corresponding N/P ratio of 8.8, the high removal capacity of nitrogen and phosphorus could be achieved with the average effluent TP and total nitrogen (TN) concentration of 0.8 mg/L and 12.0 mg/L, respectively. Periodical test showed that pH and oxidation-reduction potential (ORP) could be used as control parameters for anaerobic phosphate release, and ORP was also closely related with the phosphate uptake in anoxic phase. The high-throughput sequencing analysis revealed that the Proteobacteria and Xanthomonadales-nobank related to biological nitrogen and phosphorus removal was domination bacteria at phylum and genus level in A2N-DMBR system, with the proportion of 42.5% and 39.1%, respectively. Furthermore, Dechloromonas, which was further detected as putative denitrifying phosphorus accumulating organisms (DPAOs), was enriched (9.9%) in the system.

Key words | A2N-DMBR, community structure, denitrifying phosphorus removal, nutrients removal performance, operation characteristic, removal efficiency

INTRODUCTION

It is well-known that nitrogen and phosphorus play a crucial role in water eutrophication, and should be removed from wastewater. Increasingly stringent regulations on nitrogen and phosphorus removal (NPR) from wastewater have spurred global efforts to develop and implement several cost-effective engineered biological NPR technologies at wastewater treatment plants (WWTPs) (Ahn et al. 2010). In conventional biological nutrient removal (BNR) processes, nitrogen removal was achieved by aerobic nitrification and anoxic denitrification by nitrifying and denitrifying bacteria, respectively, while phosphorus removal was accomplished under anaerobic–aerobic conditions using polyphosphate accumulating organisms (PAOs) (Kishida et al. 2006). However, PAOs compete with denitrifying bacteria for organic carbon in the influent during the process of NPR. Furthermore, the nitrifying bacteria of long sludge age and denitrifying bacteria of short sludge age were growing in the same system in conventional BNR processes. Therefore, the sludge retention time (SRT) contradiction between PAOs and nitrifying bacteria were inevitable in conventional BNR processes. Therefore, efficient nitrogen and phosphorus simultaneous removal was very difficult to be achieved for these processes when the influent carbon resource was deficient.

To solve these problems, the techniques to enrich denitrifying phosphorus accumulating organisms (DPAOs), which use nitrate/nitrite other than oxygen as the electron acceptor to remove nitrogen and phosphorus simultaneously have been developed. DPAOs can be activated under the alternate anaerobic/anoxic conditions and have metabolic characteristics similar to those of PAOs. Denitrifying phosphorus removal has been confirmed to be an
effective way retarding competition between denitrifying bacteria and PAOs for low C/N wastewater (Zhang et al. 2010). Compared to conventional enhanced biological phosphorus removal process, denitrifying phosphorus removal process can save energy (for aeration) and organic carbon source, and reduce sludge production (Kuba et al. 2016).

Based on the theory of denitrifying phosphorus removal, several new denitrifying phosphorus removal processes, especially for low C/N wastewater treatment, have been proposed. For example, high efficiency of simultaneous phosphorus and nitrogen removal were observed in simultaneous nitrification-endogenous denitrification and phosphorus removal (SNDPR) process (Wang et al. 2015), in which the chemical oxygen demand (COD), PO43−-P and total nitrogen (TN) removal efficiency were about 81.0%, 94.0% and 77.7%, respectively, for domestic wastewater treatment at low C/N ratio (3.5) with the PAOs, DPAOs, and glycogen accumulating organisms (GAOs) ratio of 2:1:1. And Liu et al. (2016) reported a two-line BNR processes (two-line BNR), which both of the configurations performed well with average removal efficiencies of COD, NH4+ -N, TN and total phosphorus (TP) for 74.0%, 98.0%, 51.0%, 85.0% and 74.0%, 99.0%, 72.0%, 70.0% under configuration I and II, respectively. However, the rapid and stable enrichment of DPAOs could not be achieved in these process mentioned above. In addition, A2N-SBR, a two-sludge denitrifying phosphorus nitrogen removal processes, consisting of an anaerobic–anoxic SBR and a nitrification SBR, was beneficial to enrichment of DPAOs and the denitrifying phosphorus removal efficiency reached to 51.0% (Wang et al. 2008). Zhao et al. (Zhao et al. 2016) has attempted to enhance the NPR efficiencies by adjusting anoxic duration and adding post-aeration phase, which achieved a high denitrifying phosphorus removal efficiency of 96.9% and low effluent ammonia concentration with efficient utilization of limited carbon source in the pre-A2NSBR process. Nevertheless, a long cycle time, multiple precipitation and multistage reflux have been restricting the development and application of the A2N-SBR process.

Therefore, this contribution proposed a enhanced two-sludge denitrifying phosphorus removal process on the basis of A2N-SBR, namely alternate anaerobic/anoxic–aerobic double membrane denitrifying phosphorus removing process (A2N-DMBR). Compared with the previous A2N-SBR process, a buffer pool added in A2N-DMBR process may facilitate the simultaneous nitrification process and denitrifying phosphorus removal in two reactors, thus the operation cycle was shortened. Also, the SRT contradiction between nitrifying bacteria and DPAOs was solved by membrane separation other than conventional sedimentation process. In addition, the nitrification efficiency was enhanced due to the membrane unit installed in the nitrifying pond. This framework aimed to investigate some main issues as following: (1) the start-up and performance of NPR in A2N-DMBR; (2) the process characteristics of various pollutants (COD, TP, NH4+ -N, NO3−-N and NO2−-N) in the A2N-DMBR system; (3) the changes of microbial community structure of the activated sludge.

MATERIALS AND METHODS

Process setup

The setup of a laboratory-scale A2N-DMBR system, composed of four reactors, an anaerobic-anoxic activated sludge SBR (A2SBR), a nitrification-MBR (N-MBR), a separate buffer pool and a post short-time aeration tank, is illustrated in Figure 1. The A2SBR with a working volume of 9.6 L (Φ25.0 × 19.6 cm) was inoculated with activated sludge from a sewage treatment plant that employed a BNR process. The N-MBR with a working volume of 12 L (Φ28.0 × 19.6 cm) was consisted of an immersed membrane module for enhanced separation and microporous aeration for oxygen supply, as well as membrane fouling prevention. The main material of membrane module is polyvinylidene fluoride.

(PVDF), and the membrane flux and membrane area was 14 L/(m²·h) and 0.14 m², respectively. A mechanical stirrer provided mixing in the A2SBR to keep the biomass in suspension. The oxygen was supplied by an air pump through a porous stone diffuser which was installed at the bottom of these N-MBR and post short-time aeration tank. The SBR was completely automated, and all peristaltic pumps, stirrers, air pumps and phase lengths were regulated by time controllers.

Synthetic feed

Synthetic wastewater was designed to simulate the rural domestic sewage. The synthetic wastewater contained the following (per liter): 0.24–0.32 g CH₃COONa (190–250 mg COD); 0.06–0.21 g NH₄Cl (15–55 mg N); 0.02–0.03 g KH₂PO₄ (5–8 mg P); 0.03 g MgSO₄; 0.015 g KCl; 0.01 g CaCl₂. In addition, 1 mL/L trace element solution consisted of 0.17 g H₃BO₃, 1.52 g FeCl₃·6H₂O, 1.80 g KI, 0.15 g ZnSO₄·7H₂O, 10.00 g EDTA, 0.03 g CuSO₄·5H₂O, 0.12 g MnCl₂·4H₂O and 0.15 g CoCl₂·6H₂O was added, and the right amount of KHCO₃ was added to regulate the pH of 7.0. The COD and TP was controlled at 190 mg/L and 0.11 kgBOD₅/(kgMLSS·d), respectively. Subsequently, NH₄⁺–N, NO₃⁻–N, NO₂⁻–N, NO₃⁻–N, TN and TP were analyzed according to the

Experimental methods

Start-up

The sludge was taken from a sewage treatment plant (Tongshan sewage treatment plant, Xuzhou, China), which adopted the anaerobic–anoxic–aerobic process (A²/O). The enrichment of DPAOs consisted of anaerobic/aerobic (A/O) stage and anaerobic/anoxic (A/A) stage, with operated time of 14 d and 40 d in anaerobic/anoxic SBR, respectively. PAOs was enriched in A/O stage, where the duration time of anaerobic phosphorus release and aerobic phosphorus uptake was 120 min and 180 min, respectively. Correspondingly, hydraulic retention time (HRT) and SRT were approximately maintained at 300 min and 13 d, respectively, meanwhile a mixed liquor suspended solids (MLSS) was about 3,500 mg/L in A/O mode. In addition, the mixed liquor volatile suspended solids (MLVSS) and food/microorganism (F/M) were measured about 2,400 mg/L and 0.11 kgBOD₅/(kgMLSS·d), respectively. Subsequently, DPAOs was acclimated in A/A stage with 180 min anaerobic phosphorus release and 240 min anoxic phosphorus uptake and the KNO₃ (55 mg/L) was used as an electron acceptor; meanwhile, HRT, SRT and MLSS were controlled at an average 420 min, 18 d and 4,000 mg/L, respectively. Moreover, MLVSS and F/M were detected approximately at 2,850 mg/L and 0.10 kgBOD₅/(kgMLSS·d), respectively.

Operation

For a typical cycle of the A₂N-DMBR, it can be divided into two stages, A₂SBR and N-MBR. The cycle of the A₂SBR consists of three phases, as follows. (1) Anaerobic phase. 6 L synthetic wastewater was fed into the 3 L biomass residual in previous cycle in A₂SBR during the first 30 min, resulting in a volume exchange ratio of 66.7%. In the following 120 min, volatile fatty acid (VFA) was taken up storing as intracellular polyβ-hydroxyalkanoates (PHA) and phosphate was released by DPAOs. (2) Decanting period. After the 30 min settlement, 6 L ammonia rich supernatant was transferred into the N-MBR to achieve nitrification. (3) Anoxic phase. At the first 30 min, 6 L nitrate rich supernatant recycled from the buffer pool was pumped into the A₂SBR, denitrification and phosphate uptake was performed simultaneously by DPAOs for 180 min, a 30 min post-aeration phase was added after the anoxic phase.

The cycle of the N-MBR consists of two phases. (1) Aerobic phase. 6 L ammonia rich supernatant was transferred from the A₂SBR into the N-MBR via solenoid valve, and the DO was remained above 3 mg/L to ensure complete nitrification within 210 min. (2) Membrane filtration period. At the end of the nitrification, the nitrification supernatant was pumped into the buffer pool by membrane filtration.

In general, the A₂N-DMBR was operated for 70 d with a complete cycle time of 480 min. During steady-state operation, HRT, SRT, MLSS and MLVSS were controlled approximately at 480 min, 18 d, 4,000 mg/L and 3,000 mg/L in A₂SBR, respectively, while 480 min, 40 d, 7,000 mg/L and 5,800 mg/L were maintained in the N-MBR. Further, the F/M was measured about 0.10 kgBOD₅/(kgMLSS·d) in A₂SBR. The rotation speed of mechanical mixer was controlled at 150 ± 10 rpm during non-aeration stage, and the airflow rate was controlled at 40 L/h by a gas-flow controller with a dissolved oxygen (DO) concentration of 2–4 mg/L in the aerobic phases.

Analytical methods

After filtering through 0.45 μm filter paper, the COD, NH₄⁺–N, NO₂⁻–N, NO₃⁻–N, TN and TP were analyzed according to the
American Public Health Association (APHA) Standard Methods (Rice 2012), and the weight method was used to measure the MLVSS. The oxidation-reduction potential (ORP) and pH were measured using a pH/ORP 3,210 meter (WTW, Germany), DO was measured using a DO 3,210 meter (WTW, Germany). The relative phosphorus removal activity of the DPAOs to the PAOs under anoxic and aerobic conditions was measured using the method proposed by Wachtmeister et al. (Wachtmeister et al. 1997), who employed the DPAOs of enrichment cultures from laboratory sequencing batch reactor (SBR) systems operated under anaerobic–aerobic (AO) or anaerobic–anoxic (A2) conditions. Additionally, the DNA sequence of microorganism was measured by high-throughput sequencing, and the DPAOs was analyzed for this relied on sequence similarity comparisons against already identified DPAOs.

The batch experiments, using nitrate and nitrite as an electron acceptor were investigated in order to study the performance of denitrifying phosphorus removal. The sludge mixture of 1.6 L from anaerobic phase (90 min) was divided into 8 fractions, corresponding the MLSS was about 3,500 mg/L. And the KNO3 and KNO2 of 1.63 mg, 3.26 mg were investigated in order to study the performance of denitrifying phosphorus removal. The sludge mixture was beneﬁted by high-throughput sequencing, and the DPAOs was analyzed for this relied on sequence similarity comparisons against already identified DPAOs.

The start-up process consisted of anaerobic/aerobic (A/O) stage and anaerobic/anoxic (A/A) stage. The TP removal performance of the biomass acclimation was shown in (Figure 2). In the A/O stage, the removal efﬁciency of phosphorus was improved from 36.4% to 95.7% and the effluent TP concentration was dropped below 0.5 mg/L, which was consistent with the results obtained by Lu et al. (Lu et al. 2017). In the A/A stage, the nitrate concentration of 35.0 mg/L as the electron acceptor was added into tank at the beginning of anoxic phase. After 40 d of acclimation, the maximum amount of anaerobic phosphorus release reached 25.6 mg/L, and the effluent TP concentrations was only 0.8 mg/L with efﬁciency of phosphorus removal of over 89.1%.

According to the measured ratio of DPAOs/PAOs in the end of A/O stage and A/A stage, the ratio at the end of A/A stage was 94.4%, which was much higher than 21.9% of A/O stage and coincided with results of 93.0% and 94.8% reported by Lu et al. (Lu et al. 2017) and Liu et al. (Liu et al. 2015), which was also used the method of A/O stage and A/A stage. It could be found that the A2SBR process was beneﬁcial to the enrichment of DPAOs with synthetic wastewater. The DPAOs were successfully enriched in A2N-DMBR system within 54 d.

**RESULTS AND DISCUSSION**

**Experimental start-up**

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**Nutrients removal performances**

The A2N-DMBR was operated for 213 cycles to further improve the NPR. The inﬂuent COD concentration was remained at 190–250 mg/L and the whole operation period was divided into three phases based on the different N/P ratio. The effluent COD concentration was gradually
dropped to 15 mg/L, the corresponding COD removal efficiency was increased to 94.3% in the whole operation period (Figure 3(b)).

In phase I (1–62 cycles), the influent TP concentration was 6.8–7.6 mg/L, the corresponding N/P ratio of 4.7, with anaerobic P release amount of 28.4 mg/L (Figure 3(a)). The NH$_4^+$-N and TN removal efficiencies were 97.4% and 91.3%, respectively (Figure 3(c) and 3(d)). However, the TP removal efficiency was only 71.2%, with effluent TP concentration of 2.1 mg/L and anoxic effluent TP concentration of 4.1 mg/L, revealing that only 43.8% removal efficiency of TP was attributed to the role of DPAOs. The nitrate concentration of anoxic effluent was lower than 0.1 mg/L. Therefore, insufficient electron acceptor for phosphorus uptake under the lower N/P ratio might be responsible for the higher effluent TP concentration.

In phase II (63–131 cycles), the influent TP concentration was dropped to 5.9–6.5 mg/L, while the N/P ratio increased to 7.0 for sufficient electron acceptor to enhance anoxic phosphorus uptake efficiency. Hereafter, TP removal efficiency was improved, with effluent TP concentration gradually decreased to 1.3 mg/L at 129 cycle. Specifically, during 75 cycle to 93 cycle, due to the damage of aeration equipment, the worse nitrification efficiency was found and the effluent NH$_4^+$-N concentration reached to 5.2 mg/L, at the same time the effluent TP concentration increased to 3.0 mg/L. When the aeration equipment trouble relieved, the system performance was gradually resumed, the corresponding the NH$_4^+$-N, TN and TP removal efficiencies rose to 94.7%, 82.0%, and 79.0%, and the average effluent TP and NO$_3^-$-N was about 1.3 mg/L and 0.4 mg/L, respectively. In addition, the anoxic effluent TP concentration declined to 2.4 mg/L, and the TP removal efficiency by DPAOs increased from 45.8% to 61.3%, nevertheless, this result was lower than that of A/A stage (94.4%). It indicated that there was not sufficient nitrate acting as the electron acceptor for anoxic phosphorus uptake at the N/P ratio of 7.0.

In phase III (132–213 cycle), the influent TP concentration was 5.9–6.5 mg/L, corresponding N/P ratio was 8.8. After 60 cycles of operation, the removal efficiencies of TP, NH$_4^+$-N and TN was 91.7%, 94.5% and 77.3%, respectively, with the effluent decreased to 0.8 mg/L, 3.2 mg/L and 12.0 mg/L, respectively. Moreover, the anoxic and final effluent TP concentration was both 0.8 mg/L, demonstrating that most of TP concentration was removed by DPAOs and the TP removal efficiency by DPAOs was reached to 91.7% in anoxic stage. The results showed that when the influent TP concentration was 6.4 mg/L, with N/P ratio of 8.8, the removal capacity of nitrogen and phosphorus achieved by 0.12 kg N/(m$^3$d) and 0.018 kg P/(m$^3$d) with the average effluent TP and TN concentration were 0.8 mg/L and 12.0 mg/L, respectively. Thus, high N/P ratio was favorable for NPR as well as for the enrichment of DPAOs in A$_2$N-DMBR process. The similar removal efficiency of COD, NH$_4^+$-N and TN was presented by Wang et al. (Wang et al. 2008) in the A$_2$NSBR system, while the enhanced denitrifying phosphorus removal performance was obtained in A$_2$N-DMBR process. Gao et al. (Gao et al. 2010) also report that when influent N/P raised, anoxic phosphorus uptake rate increased under high N/P ratio (N/P = 7.7–10.7) condition.
Figure 3 | TP (a), COD (b), NH₄⁺-N (c) and TN (d) removal performance of the A₂N-DMBR system over optimizing period.
The process characteristics of A2-N-DMBR

The periodic variation of different pollutants was shown in Figure 4(a), and the variations of pH and ORP clearly correlated with nutrient removal performance was shown in Figure 4(b).

During the anaerobic period, the average influent COD was around 220.0 mg/L, dropped to 60.0 mg/L within 90 min, and then was degraded to 42.0 mg/L at the end of anaerobic phase, where organic substance was utilized by PAOs to be used as cell depoitor for phosphorus uptake. Normally, the more COD was consumed, the more PHA was stored, meaning a stronger P-uptake potential for the subsequent anoxic phase (Chen et al. 2011). About 3.8% of the COD removed at anoxic phase, and the less consumption of COD is beneficial for the growth of denitrifying phosphorus removal bacteria. Moreover, the low anaerobic effluent COD concentration was considered to be an advantage in the followed nitrification. In the first 30 min of anaerobic phase, the P-release rate achieved to the maximum value of 5.4 mg P/(g MLSS·h), as well as the COD utilization rate was 44.5 mg COD/(g MLSS·h) and pH sharply declined from 7.8 to 7.5. After 90 min, the COD consumption rate was decreased, the P-release was almost completed with a TP concentration of about 29.7 mg/L and pH tended to be stable. The pH decreased steadily throughout the anaerobic phase for which H⁺ was produced (Equation (1)), which coincided with P-release.

\[
2C_2H_4O_2 + (HPO_3) + H_2O \rightarrow (C_2H_4O_2)_2 + PO_4^{3-} + 3H^+ \quad (1)
\]

Meanwhile, the ORP values sharply dropped to a plateau of -179.4 mV in the anaerobic phase of A2SBR, indicating an anaerobic environment for P-release. Clearly, the pH and ORP profiles could exhibit a specific point identifying the end of the anaerobic P-release and the anaerobic time was controlled by the inflection point of pH and ORP curve.

In aerobic stage, the NH₄⁺-N concentration was reduced from 39.9 mg/L to 0.6 mg/L, and followed by NO₃⁻-N concentration increased to 37.6 mg/L after membrane filtration with the nitrification rate of 2.5 mg NO₃⁻-N/(g MLSS·h), while the NO₂⁻-N concentration was negligible and no significant phosphorus removal was observed in the N-MBR during nitrification phase, indicating that enrichment of nitrifying bacteria in N-MBR was successfully gained due to membrane complete retain role, promoting the performance of nitrification.

In anoxic process, the NO₃⁻-N was sharply consumed with the anoxic P-uptake. After 30 min, the TP concentration decreased from 26.0 to 12.0 mg/L, correspondingly the NO₃⁻-N concentration dropped from 26.3 to 13.1 mg/L, which indicated that the TP removal was positively correlated with nitrate utilization. Zhao et al. (Zhao et al. 2016) was also found that the TP removal was positively correlated with nitrate utilization in the pre-denitrification anaerobic/anoxic-aerobic nitrification sequence batch reactor (pre-A2NSBR). In addition, ORP curve jumped quickly to -98.3 mV at 30 min of anoxic phase due to nitrate recycling, and the anoxic P-uptake and denitrification rates were 8.6 mg P/(g MLSS·h) and 8.2 mg NO₃⁻-N/(g MLSS·h), respectively. Then ORP declined as the TP consumption slowly with the ratio of 9.1 mV ORP/(mg P) within the following 90 min, which indicated the end of anoxic P-uptake, and thus ORP could be used as an index parameter for anoxic P-uptake. Nevertheless, pH value has been slightly increased as a result of anoxic P-uptake and denitrifying nitrogen removal by residual COD during which OH⁻ was produced (Equation (2)).

\[
NO_3^- + 5H^+ \rightarrow 0.5N_2 + 2H_2O + OH^- \quad (2)
\]

Therefore, the pH could not be used as a control parameter for anoxic P-uptake. It is indicated that the profiles variation of real-time online indicator such as ORP and pH were demonstrated closely related with the nutrient removal performance, and based on this, it is possible or expected to establish the real time control strategy in the future.

In addition, some previous report that the volume exchange ratio of A2-N-SBR had a great negative influence on the NH₄⁺-N removal efficiency (Zhao et al. 2016). In this study, the volume exchange ratio was about 63.2%, resulting in a higher concentration of anoxic effluent NH₄⁺-N. Nevertheless, in spite of the existence of volume exchange ratio, a higher removal efficiency of NH₄⁺-N was achieved in A2-N-DMBR, where the short post-aeration was proposed for further ammonia and phosphorus removal, indicating that this process solved the bottleneck of treating high ammonia in low C/N domestic wastewater. It was worth mentioning that the A2-N-DMBR had a significant efficiency with the denitrifying phosphorus removal ratio of 85.0%, which was much higher than that (51.0%) of A2-N-SBR process (Wang et al. 2008).
Sludge characteristics

Performance of denitrifying phosphorus removal

In order to study the performance of denitrifying phosphorus removal, the batch experiments, using nitrate and nitrite as an electron acceptor was investigated. The relation between the consumption of NO₃⁻N and P uptake was shown in Figure 5(a) and indicated that there was a linear positive relationship between the change of NO₃⁻N and P concentration, and a ratio of 1.2 mgPO₄³⁻/mgNO₃ was observed, which was similar to the ratio of 1.3 mgPO₄³⁻/mgNO₃ obtained in
A2NSBR process by the study of Zhao et al. (Zhao et al. 2016). However, the PO$_4^{3-}$/NO$_3^{-}$ ratio in this study was lower than the result reported by Kern-Jespersen & Henze (Kern-Jespersen & Henze 1995) which was 2.0 mgPO$_4^{3-}$/mgNO$_3^{-}$ in a fixed biofilm reactor, and it was higher than the result (1.0 mgPO$_4^{3-}$/mgNO$_3^{-}$) obtained in SBR process by the study of Liu et al. (Liu et al. 2017). Obviously, the different research had the district difference on the ratio of P-uptake to NO$_3^{-}$/N removal, which may be explained by the mode and condition of operation. Thus, the improvement of the ratio of P-uptake to NO$_3^{-}$/N removal was crucial for the enhancement of denitrifying phosphorus removal efficiency.

According to the relationship between the consumption of NO$_2^{-}$/N and P uptake (Figure 5(b)), it was found that the change concentration of NO$_2^{-}$/N and P had good linear relationship with the ratio of 1.0 mgPO$_4^{3-}$/mgNO$_2$ when the initial NO$_2^{-}$/N concentration was 20.0 mg/L, which was higher than the ratio of 1.2 mgPO$_4^{3-}$/NO$_3^{-}$ in this study. This result was similar to those reported by Guisasola et al. (Guisasola et al. 2009), who found that the anoxic P/N ratio obtained with nitrite was lower than the values with nitrate. Meanwhile, it was also lower than the result (2.1 mgPO$_4^{3-}$/mgNO$_2$, 1.6 mgPO$_4^{3-}$/mgNO$_2$) obtained by Wang et al. (Wang et al. 2014) and Wang et al. (Wang et al. 2015), which was researched by using nitrite as an electron acceptor. Apparently, the anoxic P-uptake efficiency using nitrite as an electron acceptor was correlated with domestication conditions, process characteristics, operation conditions and so on. However, Zeng et al. (Zeng et al. 2011) reported that the phosphorus uptake by DPAOs was seriously inhibited by the presence of a relative high level of nitrite (20.0 mg/L NO$_2^{-}$/N). Nevertheless, in this experiment, the high concentration of NO$_2^{-}$/N had no significant inhibition effect on anoxic P-uptake, and on the contrary, the high performance of denitrifying phosphorus removal using nitrite as an electron acceptor was also observed. It was considered that in anoxic period, short-term denitrification was conducted by denitrifying bacteria, which was capable of reducing nitrate to nitrite by the residual COD, and thus the DPAOs using nitrite as an electron acceptor was enriched. In addition, no significant inhibition and enriched DPAOs using nitrite as an electron acceptor obtained in SBR process by Peng et al. (Peng et al. 2011) indicated that the DPAOs adapted to the 20.0 mg/L nitrite conditions by long-time domestication, which was similar to the previous results from Guisasola et al. (Guisasola et al. 2009).

**Microbial community structure**

The classification of the sequences at the phylum and genus levels are shown in Figure 6(a) and 6(b).

As shown in Figure 6(a), the two kinds of seed sludge from anaerobic tank and anoxic tank had the similar community structure at the phylum level. The bacteria domain was represented by 17 different phyla, with *Proteobacteria*, *Chloroflexi* and *Bacteroidetes* ranking as the top three most abundant ones in all of this sludge, which was reached above 90.3%. This was in accordance with the result reported by others microbial community structure (Lv et al. 2015). Among them, *Proteobacteria* was the most abundant phylum for the seed sludge and system sludge, accounting for 68.3% and 42.5% of the total bacterial sequences, respectively. Furthermore, abundances of
Chloroflexi, which has been reported to be tightly correlated with propagation of filamentous organisms, was obviously reduced (from 14.8% to 8.3%) compared with the seed sludge, indicating that the sludge bulking was effectively inhibited under alternating anaerobic/anoxic conditions (Saunders et al. 2013). In addition, *Nitrospira*-related with nitrifying bacteria only dominated in seed sludge with an abundance of 2.3%, while its abundance in the system sludge was only 0.4%, perhaps due to the absence of aeration limitation.

The phylogenetic classification of functional community at genus level was performed in Figure 6(b). There was great difference in relative abundance of *Xanthomonadales-norank* between seed sludge (3.7%) and system sludge (39.1%). *Xanthomonadales-norank* belongs to γ-Proteobacteria, which was confirmed as PAOs (He et al. 2016), and was the most dominant class in the system sludge. Also Xia et al. (Xia et al. 2014) reported that β-Proteobacteria was the most dominant class in the sludge samples from denitrifying phosphorus removal process. *Anaerolineaceae_uncultured* was most abundant in seed sludge samples, accounting for 22.0%, while a substantial decrease was observed in system sludge samples, with the proportions of 4.7%, perhaps due to the elimination of strictly anaerobic sludge. The relative abundance of *Dechloromonas* was considerably higher in the system sludge (9.9%) than any other seed sludge...
sludge (1.8%). Currently, Candidatus Accumulibacter (Ca. Accumulibacter), a proposed genus phylogenetically closely related to Propionibrio, Dechloromonas or Rhodocyclus within family Rhodocyclaceae was highlighted as one of the most important phosphorus removal bacteria candidates. Lv et al. (Lv et al. 2014) reported that the Dechloromonas-related organism was further detected as putative DPAOs in an independent environment, and was able to tolerate long-term strict A-A operation, which was attributed to its competitive advantage over ‘Candidatus Accumulibacter’ (Kim et al. 2015). It was speculated that the high efficiency of denitrifying phosphorus removal was related to the increased relative abundance of DPAOs.

CONCLUSIONS

The DPAOs was successfully enriched by anaerobic/oxic condition and anaerobic/anoxic condition, and the ratio of DPAOs/PAOs increased from 21.9% to 94.4% after operation of 162 cycles during acclimation. The excellent COD and NH\textsubscript{4}\textsuperscript{+}-N removal performance was achieved stably in A\textsubscript{2}N-DMBR under different N/P ratios, the different N/P ratio had a great influence on the denitrifying phosphorus removal performance. When the N/P ratio was about 8.8, the higher denitrifying phosphorus removal efficiency was monitored in A\textsubscript{2}N-DMBR process, where the TP and TN removal efficiencies was 91.7% and 77.3%, and the effluent TP and TN was 0.8 mg/L and 12.0 mg/L, respectively. While under the lower N/P ratio (4.7), despite the high TN removal efficiency in the system, the TP removal efficiency was limited due to the insufficient electron acceptor in the anoxic stage. In addition, the profiles variation of real-time online indicator such as pH and ORP were demonstrated closely related with the nutrient removal performance. Furthermore, the Proteobacteria and Xanthomonadales-nobank related to NPR, were the dominant bacteria in A\textsubscript{2}N-DMBR, which was likely responsible for the higher NPR efficiencies. Therefore, this study provide a guidance that can be used to two-sludge denitrifying phosphorus removal process combined with membrane bio-reactor (MBR) for treatment of municipal wastewater.

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REFERENCES


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