Bacterial composition and nutrient removal with a novel PIA-A$^2$/O sewage treatment

Li Dong, Luo Yahong, Cai Yanan, Zeng Huiping and Zhang Jie

ABSTRACT

A novel post intermittent aeration anaerobic–anoxic–oxic (PIA-A$^2$/O) process was developed to integrate shortcut nitrification–denitrification with denitrifying phosphorus (P) removal for domestic sewage treatment. With the transformation in configuration and phased start-up strategy, the nitrification rate and the ratio of denitrifying phosphorus accumulating organisms to phosphorus accumulating organisms (DPAO/PAO) were enhanced greatly to 88.2% and 83.9–91.7% in the PIA-A$^2$/O process, respectively. Improved total nitrogen and phosphorus removal were achieved at long sludge retention time and low aeration. High sludge activity was maintained through the periodic selection of the additional intermittent aeration zone. High-throughput sequencing revealed that Bacteroidetes (38.96%), Proteobacteria (33.59%), TM7 (4.53%), Chloroflexi (3.09%), and Firmicutes (2.49%) were the dominant phyla in the resultant bacterial community. Abundant anaerobic and facultative bacteria conducive to excess sludge reduction were generated by this process. Potential DPAOs involve the genera of Brevundimonas, Brachymonas, Steroidobacter, Haliscomenobacter, and Rhodocyclus.

Key words | anaerobic–anoxic–oxic process, denitrifying phosphorus removal, microbial community, nitritation

INTRODUCTION

In the past century, intensive human activities producing massive nitrogen (N) and phosphate (P) nutrients were held accountable for the growing deterioration of the water environment. Therefore, increasingly stringent discharge standards for wastewater treatment plants (WWTPs) were established worldwide to curb pollution at the source (Machado et al. 2009). Biological nutrient removal processes are the most common technologies currently adopted by WWTPs, although it is difficult to consistently achieve low nutrient levels in the effluent using these methods (Bekir Ersu et al. 2008). Taking the classic anaerobic-anoxic-oxic (A$^2$/O) process as an example, two limitations originating from the process itself impede its nutrient removal efficiency: the large demand for carbon sources, and the sludge retention time (SRT) conflict between N and P removal (Barker & Dold 1996; van Loosdrecht et al. 1998). Therefore, for the domestic sewage with low carbon sources, reducing chemical oxygen demand (COD) consumption and mollifying SRT contradiction during operation are the basic solutions to enhance the nutrient efficiencies in the A$^2$/O process.

Many solutions have been studied, including the addition of biological carriers for biofilm formation (Yang et al. 2010), application of a step-feed process (Ge et al. 2010), and separation of nitrifying and P removing sludge (Chen et al. 2011). However, these methods result in either limited effects or complicated management. Recently, the advent of shortcut nitrification–denitrification and denitrifying P removal technologies offers additional potential options. First, both technologies retain a large amount of carbon sources during N removal compared with the conventional process; approximately 40% COD saving for shortcut nitrification–denitrification and 50% for denitrifying P removal processes (Mulder et al. 2001). Second, the denitrifying P removal process using nitrate (NO$_3^{-}$) or nitrite (NO$_2^{-}$) as the terminal electron acceptor can remove P during relatively long SRTs (Lee et al. 2001), which effectively harmonizes the SRT of nitrification and dephosphatation in one reactor. Therefore, we speculated that simultaneously enhanced N and P removal might be achieved if the two technologies were combined successfully in the A$^2$/O process treating domestic sewage.
However, many obstacles exist in realizing the combination. First, the factors influencing ammonia-oxidizing bacteria (AOB) enrichment for partial nitrification are complex and stringent, involving pH, temperature, dissolved oxygen (DO), COD, and free ammonia concentrations (Hellinga et al. 1998; van Kempen et al. 2000; Tsuneda et al. 2006). These factors vary along the A\(^2\)/O reactor consequential to the real-time reaction in different compartments, causing AOB to have a minimal reliable chance to become the dominant nitrifiers over nitrite-oxidizing bacteria (NOB). Second, the important favourable condition of long SRT necessary for the acclimation of denitrifying phosphorus accumulating organisms (DPAOs) was never recommended in the A\(^2\)/O process because of the risk of sludge aging and denitrification in the sedimentation tank. Third, the accumulated NO\(_2\)/C\(_0\) from ammonia (NH\(_4^+\)) oxidation might be toxic to microbes involved in P removal as previous studies have indicated that the toxicity thresholds range widely from 3.0 to 93.7 mg/L (Peng et al. 2011).

A novel PIA-A\(^2\)/O process based on the A\(^2\)/O process was developed in this study. The roles of the addition of an intermittent aeration zone (IAZ) and changes of sludge recycling sites in selecting functional bacteria and prolonging SRT were examined, along with the ratios of nitritation and denitrifying dephosphatation in the improved A\(^2\)/O system. The evolution of microbial communities during the experiment was also investigated. Hence, the major objectives of this study were to: (1) assess the possibility and effect of combining shortcut nitrification–denitrification and denitrifying dephosphatation via NO\(_2\) in the PIA-A\(^2\)/O process treating domestic sewage; and (2) characterise the difference in the resultant microbial communities between the PIA-A\(^2\)/O and A\(^2\)/O processes.

### METHODS

#### Experimental reactor

The laboratory-scale experimental reactor (Figure 1) with a working volume of 180 L consisted of an anaerobic zone, anoxic zone, oxic zone 1, oxic zone 2, and IAZ along the flow direction, which were divided by removable perforated flashboards. Fine diffusers were set up at the bottom of the oxic zones and IAZ using glass gas rotameters for accurate control of aeration. Specifically, a microcomputer timer-controlled electromagnetic valve was employed on the gas pipeline of IAZ for timing intermittent aeration.

The reactor was designed to operate in both processes of A\(^2\)/O and PIA-A\(^2\)/O. The internal nitrate/nitrite liquid was recycled from the IAZ or oxic zone 2 to the anoxic zone for A\(^2\)/O or PIA-A\(^2\)/O operations, respectively. Similarly, the sludge returned from the sedimentation tank or IAZ, respectively, to the anaerobic zone in the A\(^2\)/O or PIA-A\(^2\)/O process. Additionally, there were two sludge outlets at the bottoms of the sedimentation tank (outlet 1) and IAZ (outlet 2). Outlet 1 was for the routine sludge discharge of both processes, but outlet 2 was standby enabled during the phase of partial nitrification start-up to shorten SRT. Based on these improvements, the reactor could convert from the A\(^2\)/O to the PIA-A\(^2\)/O process by moving...
flashboards, manipulating valves, and timing intermittent aeration in the IAZ.

Wastewater and analytical methods

Experimental sewage was pumped from the septic tank of a residential campus in Beijing, PR China. Pollutant concentrations were described as follows: COD$_{Cr}$: 353.51 ± 57.23 mg/L; NH$_4^+$: 83.52 ± 6.24 mg/L, total Kjeldahl nitrogen (TKN): 103.5 ± 7.61 mg/L, total nitrogen (TN): 110.36 ± 6.83 mg/L, total phosphorus (TP): 5.8 ± 1.39 mg/L; pH: 7.35 ± 0.15; alkalinity (CaCO$_3$): 500 ± 25 mg/L; and C/N ratio: 3.55 ± 0.82. Seed activated sludge was taken from Gaobeidian WWTP in Beijing, which exhibited good nitrifying activity and precipitability.

COD and TP were detected using a multi-parameter water quality analyser. TKN was measured using a L6250 type automatic azotometer (Behr, Germany). NH$_4^+$, NO$_2^-$, NO$_3^-$, mixed liquor suspended solids (MLSS), and sludge volume index were all determined using the Chinese National Environmental Protection Agency standard methods (Wei 2002). TN represented the sum of TKN, NO$_2^-$, and NO$_3^-$. The pH, DO, and temperature were detected by a pH/Oxi340i meter with pH and DO probes (WTW, Germany). The ratio of DPAO/PAO in sludge was identified using the method as follows: collected the sludge from the end of the aerobic zones and washed it 2-3 times before putting into a fermentor, added acetic acid solution to dilute the sludge to 2 L with initial COD concentration of 200 mg/L. A magnetic stirrer was used to stir the mixed liquor under an anaerobic condition for sufficient P release. After COD was fully taken up, the liquor was stir the mixed liquor under an anaerobic condition for sufficient P release. After COD was fully taken up, the liquor was divided into two equal parts: one part was exposed to oxic condition, and another part was exposed to the anoxic condition that contained initial NO$_3^-$ concentration of 50 mg/L. The reactions continued until the P concentrations were constant. In this way, the value of the maximum anoxic P uptake rate to oxic P uptake rate represented the ratio of DPAO/PAO (Wachtmeister et al. 1997). All water samples were assayed in triplicate, and the mean values and standard deviations (SD) are expressed as means ± SD in the text.

The actual N and P removal amounts considering recycling dilution in the anaerobic and anoxic zones were calculated according to Equations (1) and (2) using the mean values:

$$\Delta M_{\text{anaer}} = \left[C_{\text{anaer}} - (1 + r)C_{\text{anaox}}\right] \times Q_{\text{in}} \times \frac{24}{1,000}$$  

$$\Delta M_{\text{anox}} = \left[(1 + R)C_{\text{anaer}} + rC_{\text{anaox}} - (1 + R + r)C_{\text{anox}}\right] \times Q_{\text{in}} \times \frac{24}{1,000}$$

where $Q_{\text{in}}$: influent flow, L/h; $R$: sludge recycle ratio, %; $r$: nitrate/nitrite recycle ratio, %; $C_{\text{anaer}}$: pollutant concentration in the anaerobic zone, mg/L; $C_{\text{anaox}}$: pollutant concentration in the anoxic zone, mg/L; $C_{\text{anox}}$: pollutant concentration in the recycling sludge, mg/L; and $C_{\text{anaox}}$: pollutant concentration in the recycling nitrate/nitrite liquid, mg/L.

Experimental procedures

The reactor was operated at room temperature (22 ± 3 °C) over 160 days incorporating three phases according to the operational conditions shown in Table 1. It began with an A$^2$/O process in phase I with the volume ratio of the anaerobic zone, anoxic zone, oxic zone 1, oxic zone 2, and IAZ ($V_{\text{anaerobic}}$:$V_{\text{anoxic}}$:$V_{\text{oxic1}}$: $V_{\text{oxic2}}$:$V_{\text{IAZ}}$) at 1:2:1:1:1. DO concentrations in the IAZ were maintained at 2.0 mg/L without intermittent aeration.

The reactor was transformed into a PIA-A$^2$/O process in Phase II to start up partial nitrification. The value ratio of each zone was reconfigured to be $V_{\text{anaerobic}}$: $V_{\text{anoxic}}$: $V_{\text{oxic1}}$: $V_{\text{oxic2}}$: $V_{\text{IAZ}}$ = 1:2:1:5:1:5:3 with proportionally reduced influent flow rate and A$^2$/O section with respect to that of phase I. The aim was to ensure sufficient hydraulic retention time

<table>
<thead>
<tr>
<th>Phase</th>
<th>Time (days)</th>
<th>$V_{\text{anaerobic}}$: $V_{\text{anoxic}}$: $V_{\text{oxic1}}$: $V_{\text{oxic2}}$: $V_{\text{IAZ}}$</th>
<th>Influent (L·h$^{-1}$)</th>
<th>SRT (days)</th>
<th>DO (mg·L$^{-1}$)</th>
<th>HRT (h)</th>
<th>Sludge recycling ratio (%)</th>
<th>Nitrate/nitrite recycling ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>25</td>
<td>1:2:1:1:1</td>
<td>21.95</td>
<td>11</td>
<td>11</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.2</td>
<td>85</td>
</tr>
<tr>
<td>II</td>
<td>30</td>
<td>1:2:1:5:1:5:3</td>
<td>14.6</td>
<td>7.5</td>
<td>2.0</td>
<td>2.0</td>
<td>0.5</td>
<td>85</td>
</tr>
<tr>
<td>III</td>
<td>105</td>
<td>1:2:1:5:1:5:3</td>
<td>14.6</td>
<td>26</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
<td>85</td>
</tr>
</tbody>
</table>

$^a$DO concentration during intermittent aeration.
(HRT) for nitrification and less influence of configuration change in the current reactor. The intermittent aeration cycle in the IAZ was timed at 1/59 min (on/off). The internal nitrate/nitrite liquid flowed back from the oxic zone 2 instead of the IAZ. Phase II was not over until the nitritation rate (NO₂⁻/NO₃⁻) of the effluent exceeded 80%.

Phase III was maintained as a PIA-A²/O process with a prolonged SRT. Modified spherical polypropylene carriers (diameter 25 mm) were added into the oxic zone 1 at the volume ratio of 40% to facilitate AOB growth.

### DNA extraction and high-throughput sequencing

Sludge samples were, respectively, collected on days 25 and 150 from the oxic zone 2 of the reactor representing stable A²/O (sample 1) and PIA-A²/O sludge (sample 2). Then, the two samples were centrifuged at 10,000 rpm for 15 min to gain concentrated sludge precipitates for convenient storage at −20°C prior to DNA extraction. A soil biological genomic extraction kit (Sangon Biotech Co., Ltd, Shanghai, China) was used for genomic DNA extraction according to the manufacturer’s protocol. The products were detected by electrophoresis on an 0.8% (w/V) agarose gel, and purified using a DNA purification kit (Sangon). Finally, the purified DNA was submitted to the commercial service provider Sangon Biotech for high-throughput sequencing on the Illumina MiSeq platform (San Diego, CA, USA). Briefly, after removing the non-target sequences and chimeras, the RDP Classifier tool was used to assign the available sequences to different operational taxonomic units by matching the data to that in the Ribosomal Database Project at 97% identity.

### RESULTS AND DISCUSSION

#### Partial nitrification start-up

The average pollutant removal performance during the whole experimental period is shown in Figure 2. The COD, TP, NH₄⁺, and TN removal rates increased gradually after inoculation and finally stabilized at 86.61%, 82.57%, 97.63%, and 92.02%, respectively, on day 22 in phase I. This indicated that the predominant eradication of the pollutants and rapid recovery of seed sludge in the A²/O process. However, for the effluent concentrations of these pollutants, the TP of 1.01 mg/L was still too high to meet the class A level (≤ 0.5 mg/L) requirement of the National Discharge Standard of Pollutants for WWTPs (GB18918-2002, China), which called for a further improvement.

In the following Phase II, the effluent COD remained below 50 mg/L with the clearer effluent, and the maximum removal rate of 88.18% was obtained for the entire experiment. Meanwhile, the TP removal rate rose to 90.67%. This could be due to the decreased SRT that benefited the proliferation of PAOs in the system, and then the enriched PAOs improved the turbidity and COD in the effluent (Schuler et al. 2000).

Nevertheless, NH₄⁺ removal started to constantly deteriorate, giving rise to its effluent concentration up to 33.46 mg/L until phase completion. In contrast, the nitritation rate gradually increased to 89.08%. The phenomena were the results of the shortened SRT that washed out abundant nitrifiers. However, more NOB were eliminated than AOB because of its longer generation time, which caused the increased nitritation rate. Note that the favourable nitritation was not impacted by the aeration in the oxic zones, perhaps suggesting some NOB was left in the system. By the end of phase II, effluent TN concentration reached 49.88 mg/L, severely affecting the treatment effect. However, the purpose of starting up partial nitrification was accomplished successfully.

#### Pollutant removal during the PIA-A²/O process

With the SRT extension during phase III, the effluent COD concentrations were still around 46.56 mg/L. The persistent high efficiency in COD removal was principally due to the biodegradability and low C/N ratio of the domestic sewage. A notable trend of TP removal was observed in this phase. The TP removal rate first underwent an abrupt decrease to 30.4%, and then began to rise on the eleventh day (day 65), until it eventually reached 93.03% with the effluent concentration of 0.46 mg/L that satisfied the class A standard. The decrease of the TP removal at the beginning was due to the inactivity of PAOs at the long SRT; afterward, with the acclimatization and growth of the DPAO with long SRT, the TP removal restored gradually. It can be seen in Figure 3 that the DPAO/PAO ratio in this phase achieved 91.7%, significantly higher than that in the A²/O process.

In addition, NH₄⁺ removal improved steadily over time, and its effluent concentration reduced to less than 1 mg/L within 18 days because of the gradual reproduction of AOB in the long SRT system. Nevertheless, the effluent NO₃⁻ and NO₂⁻ concentrations never increased and remained at 1.42 mg/L and 10.59 mg/L, respectively. Potential reasons include washout of NOB during the last phase, low DO inhibition to NOB, or reduction of the generated...
NO$_3$ and NO$_2$ to N$_2$ by the gradually recovered denitrifiers in phase III. Eventually, the effluent TN concentration fell to 13.08 mg/L with the nitritation rate at approximately 88.2% on day 74. Stable shortcut nitrification–denitrification was realized in the continuous PIA-A$^2$/O reactor, and the enhancement of TP removal was achieved.

**Analysis of the mechanism of pollutant removal**

Comparing the distributions of COD, TP, and N pollutant (NH$_4^+$, TN, NO$_3$ and NO$_2$) concentrations throughout the stable A$^2$/O and PIA-A$^2$/O systems (Figure 4), two characteristics were found in the latter system: little COD was removed in the oxic zones, and a higher NO$_2$ composition presented in each compartment of the process.

As shown in Figure 4, COD exhibited 4.8 mg/L reduction in concentration upon passage through the anoxic and oxic zones in the PIA-A$^2$/O process, but about 20 mg/L decrease in the A$^2$/O process. Since the effective NH$_4^+$ oxidization in the novel process, the failure in aerobic COD degradation was speculated to be the existence of few aerobic COD-degrading bacteria. Heterotrophic COD-degrading bacteria have a stronger capability to compete for oxygen than do nitrifiers, so sufficient nitrification could not happen prior to COD removal under the aeration (Bodelier et al. 1996). This possibility is theoretically plausible because heterotrophic
aerobic bacteria usually grow at a quick rate and with loose structure, and are therefore unfit to live abundantly in the improved system with long SRT and low DO. Furthermore, the periodic intermittent aeration in the IAZ might consistently eliminate these bacteria because of their relatively light weight.

Calculations indicated that the actual degrees of anoxic P removal in the PIA-A2/O and A2/O processes were, respectively, 11.97TP and 7.79TP g/d, which accounted for approximately 83.9% and 50.3% of the TP removal amount in the total zones after the anaerobic zones of the two processes. The percentages were found to be close to the DPAO/PAO ratios of 91.7% and 61.9% determined in Figure 3, which further verified the above-mentioned conclusion that DPAOs were enriched and contributed to the recovery of P removal in phase III. The errors between the two results may arise from the omission of the denitrifying P removal that occurred in the anoxic microenvironment.
in the oxic zones of the reactor using the calculation method. In fact, the PIA-A²/O system was designed with many advantages for DPAOs’ cultivation including long SRT, residual COD in the oxic zones, limiting DO, intermittent aeration (Guglielmi & Andreottola 2011), and high nitrate/nitrite recycling.

NO₂⁻ was persistently the primary oxidation product of NH₄⁺ with concentrations within 3.5–11.2 mg/L along the PIA-A²/O process and provided the main electron acceptor for DPAOs.

Therefore, a preliminary conclusion was drawn that the nutrients were simultaneously removed by shortcut nitrification–denitrification and denitrifying P removal. The COD removal occurred mainly through the means other than aerobic decomposition. Consequently, more evidences will be present in the analysis of bacterial community composition.

**Function of IAZ**

Seen from Figure 4, considerable COD, TN, and TP from the A²/O section were further removed in the IAZ, specifying that the IAZ has the capacity for pollutant removal.

Furthermore, through comparing the morphologies between the discharged sludge and the bottom sludge in the IAZ by microscope (Supplementary Figure S1, available with the online version of this paper) after the intermittent aeration, it was found that there were many crisscrossed filaments and immobile, dead microorganisms in the discharged sludge. Conversely, rich zoogloeae with a mean diameter of 200 μm were common in the bottom sludge, and various bioflocculant-producing protozoan and metazoan microorganisms were observed to be moving among the zoogloeae. Theoretically, filamentous and aged sludge have lighter weights than the highly active sludge with abundant zoogloeae. Therefore, the IAZ also acted as a biological selector that improved sludge activity and prevented bulking.

Finally, because of the selection for sludge with good settleability in the IAZ constantly, the MLSS rose significantly and reached as high as 6.02 g/L in the phase III (Figure 5), which benefited the gross pollutant removal of the system.

No interference from the intermittent aeration was found during the experiment, which was due to the very short aeration time and the quick recovery of the MLSS of the recycled sludge. Supplementary Figure S2 demonstrates that the MLSS of IAZ recycled sludge on days 60, 70, 80, and 150 varied with the settling time after intermittent aeration. The MLSS showed an increasing trend and achieved a steady maximum value of 18.6 g/L on day 70 that was maintained through day 150. Meanwhile, at each time point the MLSS made a quicker recovery to its previous maximum concentration, within about 10 min, 8 min, 4 min, and 4 min, respectively, which facilitated the negligible impact of intermittent aeration on the MLSS of the main reaction section.

**Microbial community comparison between the A²/O and PIA-A²/O systems**

A significant shift in bacterial abundance distribution could be found by comparing samples 1 and 2 across different taxonomic levels, revealing the microbial evolution during the two processes (Supplementary Figure S3).

At the phylum level, **Proteobacteria** were found to be the most abundant for sample 1, contributing to 44.24% of the total sequences reads, followed by **Actinobacteria** (10.58%), **Firmicutes** (7.81%), **Bacteroidetes** (3.62%), and **Nitrospira** (3.31%). However, **Bacteroidetes** had grown to become the primary phylum in sample 2 (38.96%), and the additional dominant phyla were **Proteobacteria** (33.59%), **TM7** (4.53%), **Chloroflexi** (3.09%), **Firmicutes** (2.49%), and **Armatimonadetes** (1.73%). Among these phyla, the increase of **Bacteroidetes** (4.96 to 38.96%) and reductions of **Actinobacteria** (10.58 to 0.54%) and **Nitrospira** (3.31 to 0%) from sample 1 to 2 were the most substantial.

At the class level, **Sphingobacteria** (2.61 to 18.79%), **Bacteroidetes** (0.53 to 2.01%), and **Flavobacteria** (0.09 to 1.01%) showed the largest increase from sample 1 to 2 during the process transition within the **Bacteroidetes** phylum. These classes contributed to the organics...
degradation and sludge reduction of the PIA-A²/O system (Lu et al. 2012; Ma et al. 2013). However, the class of Actinobacteria was reduced obviously between samples and included the families of Acidimicrobiaceae (2.69 to 0.07%), Intrasporangiaceae (1.24 to 0.06%), and Acidimicrobinae (1.16 to 0.2%), which mainly belong among the oxic bacteria that are related to organic compound degradation and P removal (Kong et al. 2009). This shift verified the speculation that rare aerobic COD-degrading bacteria existed in the PIA-A²/O system, and that COD removal was increasingly owing to the anaerobic and facultative bacteria.

At the genus level, the methane-oxidizing bacteria belonging to the class of Alphaproteobacteria were found to have boomed in the PIA-A²/O sludge, such that the total sum of these bacterial sequences was about 6% (Methyllocapsa 4.43%; Methylcocystis 0.92%; Methyloversatilis 0.35%; and Methyllocella 0.27%), far more than that of 0.62% in A²/O sludge (Methyllocapsa 0.33%; Methyloplia 0.18%; and Methylibium 0.11%). Correspondingly, many novel genera within Bacteroidetes that were expert in hydrolysis and acidification were found in sample 2, including Haliscomenobacter (4.22%), Sediminibacterium (3.59%), and Ohtaekwangia (2%). This phenomenon reflected the perfect fermentation performance of the system, which supplied sufficient energy to remove nutrients. Recent research has also suggested that the methane oxidation process is typically coupled with N removal (Conrad 2009).

The total percentages of AOB and NOB were found to be reduced from 3.51% (Nitrobitactor 0.07%, Nitrospirea 0.11%, Nitrosomonas 0.02%, and Nitrospira 3.31%) in sample 1 to only 0.17% (0.08%, 0.06%, 0.03%, and 0%, respectively) in sample 2; however, this reduction did not appear to affect successful NH₄⁺ oxidation in the PIA-A²/O system. Similar phenomena have been observed by Zhao et al. (2013). In fact, NH₄⁺ oxidation can be driven by various bacteria, such as AOB, NOB, heterotrophic nitrifiers, and ammonia-oxidizing archaea. Overall, regardless of the decrease in the total AOB and NOB, the ratio of AOB to (AOB + NOB) was greatly enhanced, from 3.7% in sample 1 to 53% in sample 2, which intuitively proved that nutrients were substantially removed by shortcut nitrification–denitrification, and the observed NO₂⁻ concentrations (3.5–11.2 mg/L) did not influence the P removal in the novel process. With regard to the practical nitritation rate of over 80% in the effluent, it might be due to the inhibition of NOB by limiting DO.

Many potential genera of PAOs could be found in the two sludge samples but with different abundance distributions. Among them, the PAO-related genera that showed sharp increases from sample 1 to 2 should represent the potential DPAOs. The indicated genera included Brevundimonas (0.02 to 2.47%) and Brachymonas (0.33 to 1.33%) within Alphaproteobacteria, Steroidobacter (0.91 to 1.89%) within Gammaproteobacteria, and Rhodocyclus (0.15 to 0.21%) within Betaproteobacteria. Furthermore, in sample 2, the fourth largest genus, Haliscomenobacter belonging to Bacteroidetes, has been reported to be abundant in many enhanced biological P removal reactors. However, Bacteroidetes seems to have little association with denitrification (Mehlig et al. 2013); therefore, the question of whether the genus of Haliscomenobacter contains DPAOs should be further investigated.

CONCLUSIONS

Here, effective removal of COD, NH₄⁺, TN, and TP in the PIA-A²/O process by shortcut nitrification–denitrification and denitrifying denitrophation could be stably achieved, and that the average removal rates reached 87.71%, 95.2%, 98.72%, and 92.27%, respectively. Anaerobic and facultative bacteria were dominant, which was conducive to the excess sludge reduction and aeration reduction in the system. The genera of Brevundimonas, Brachymonas, Steroidobacter, Haliscomenobacter, and Rhodocyclus might contain potential DPAOs. Overall, the exploration and understanding of the PIA-A²/O process in this study might be valuable for the simultaneous improvement of TN and TP removal in the A²/O process with a new approach.

Because of shortcut nitrification, it is noted that the effluent NO₂⁻ increased in the novel system instead of NO₃⁻ inevitably, which might lead to concern about the toxicity on aquatic ecosystem. Therefore, PIA-A²/O process is suggested to operate in cooperation with a supplementary treatment process like other short-cut nitrification–denitrification processes (i.e. SHARON). In consideration of the instability and relatively low level of NO₂⁻, the supplementary process could be brief and natural, such as constructed wetlands and an oxidation pond. Future research will focus on the exploitation of an integrated process based on PIA-A²/O for the conventional and advanced treatment of sewage at minimal cost.

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REFERENCES


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