

The external carbon source to each stages of A²/O process for simultaneously nitrogen and phosphorus removal

Kapumbe Dominick John¹, Zhang Hong¹ & Li Yongfeng^{2*}

Abstract

A novel method which could simultaneously solve the insufficient of carbon source and maximize the utilized of carbon source was investigated in this study. As methanol, glucose and ethanol were taken as respectively carbon sources for the A²/O system, getting the ethanol was the best one on simultaneously the nitrogen and phosphorus removed. Total nitrogen (TN) and total phosphorus (TP) removal efficiencies were 78.23% and 78.18%, and the time of NO_x-N removed was 20min. The study about the effect on the external carbon source shown that when dosing method was 0:1:2, the external carbon source concentration was 400mg/L and nitrate recycling ratio was 250%, the A²/O system on simultaneously total nitrogen (TN) and total phosphorus (TP) removed ran well. At this point, phosphorus concentration in anoxic was lower than phosphorus concentration in the influent, which indicated the denitrifying phosphorus accumulating organisms (DPAOs) accumulated in anoxic zones. The removal efficiencies of TN, NH₃-N and TP were 82.36%, 96.67% and 92.18%, respectively. The effluent concentrations were 11.43mg/L, 4.1mg/L and 0.45mg/L respectively, which reached the type A standard GB18918-2002 level.

Keywords : A²/O; Carbon source; Dosing method; Nitrogen and Phosphorus removal; Recycling ratio

1. Introduction

Water eutrophication had became a worldwide problem, which also known as "ecological tumor" (Kang *et al.* 2011; Zhang *et al.* 2013; Liu *et al.* 2014). Nitrogen and phosphorus in the wastewater flowing into the receiving water were the main factors which caused the water eutrophication (Kirilova *et al.* 2010; Branch *et al.* 2013). As the urban sewage concentrations of nitrogen and phosphorus were higher and higher, the environmental problems were more and more serious. The effluent concentrations of nitrogen and phosphorus in the wastewater treatment plants (WWTPs) must be limited. So, stringent legislations for wastewater were issued. With the urgent requirements for nitrogen and phosphorus discharge, new methods which could simultaneously improve nutrient removal from wastewater and reduce the cost of the WWTPs would be greatly helpful.

The A²/O process for simultaneously nitrogen and phosphorus removal had three main operational problems (Ding *et al.* 2006; Chen *et al.* 2011). In order to control the concentrations of nitrogen and phosphorus discharged had developed a variety of water treatment process (Sun *et al.* 2013). Most of the methods were to change the volume ratios of the anaerobic/anoxic/aerobic zones and the sequence of the anaerobic/anoxic/aerobic zones. The water treatment processes were not applicable, because they were designed at high carbon sources (Mino *et al.* 1998). So, how to optimize the A²/O process and develop the A²/O process for simultaneously nitrogen and phosphorus removal were very important (Zeng *et al.* 2010).

External carbon sources, such as ethanol, glucose and methanol, were usually utilized as carbon complements to improve nitrogen and phosphorus removal in WWTPs (Gao *et al.* 2011). The removal efficiencies with external carbon sources were compared in WWTPs, in order to study which could lead to a high removal efficiencies and solve the high cost of the WWTPs (Fux *et al.* 2006; Zhu *et al.* 2008). Nitrogen removal was achieved by two phases.

¹ Environmental engineering laboratory, Northeast Forestry University, 150040, Harbin, China

* Corresponding author at: College of Forestry, Northeast Forestry University, Harbin 1500040, China. Tel.: + 86 13903614476; fax: + 86 13903614476. E-mail addresses: dr_lyf@163.com (Y. Li).

One was nitrogen translating into $\text{NO}_3\text{-N}$ in aerobic zones. The other one was $\text{NO}_3\text{-N}$ translating into N_2 in anoxic zones. For the treatment of domestic wastewater, the particularly low removal efficiency of nutrient was mainly caused by the internal limit of the organic carbon source (Guerrero *et al.* 2011; Cao *et al.* 2013). As the external carbon source was flowed into aerobic zones, the denitrifying bacteria accumulated in a short time, and $\text{NO}_3\text{-N}$ through denitrification was completely removed (Estrada *et al.* 2013). The TP removal efficiency was inhibited by two factors. One was the remaining $\text{NO}_3\text{-N}$ from aerobic zones in return sludge, the other one was the internal limit of the organic carbon source in anaerobic zones. The inhibiting factors to the phosphorus release in anaerobic zones where denitrified would compete with PAOs for external carbon source, and net phosphorus release wouldn't occur until denitrification was completed (Barker & Dold 1996). The TP removal efficiency was developed and the phosphorus release in anaerobic zones was not inhibited after modifying the external carbon source into the system (Cao *et al.* 2013).

When ethanol used as the external carbon source for the A^2/O process, the nitrogen and phosphorus were removed well and nitrate could be completely removed. When methanol used as the external carbon source for the A^2/O process, it had a quicker response to the denitrification in anoxic zones, but there was some remaining methanol in the effluent. As we known, the influent concentration of the carbon source in the urban domestic wastewater was very low, which belonged to low carbon source and it aggravated the carbon source competition in the A^2/O system. In most cases, the optimization and transformation of the A^2/O process were just under various concentrations, various carbon source types, and did not study the effect on the A^2/O process for denitrification and phosphorus removed at different positions of the addition carbon sources and the addition concentrations of carbon sources.

In this study, the nutrient removed was investigated by taking consideration about various carbon sources in the A^2/O system. The rate of denitrification was used to study the time of nitrate removed completely at various carbon sources. The various dosing methods and the addition concentrations of carbon sources controlled by peristaltic pump were to optimize the nutrient removal efficiencies with low carbon sources for urban domestic wastewater in the A^2/O system. The effects of nitrate recycling ratios on simultaneously nitrogen and phosphorus removed performance in the A^2/O system were observed by treating actual urban domestic wastewater.

2. Materials and methods

2.1 Experimental system

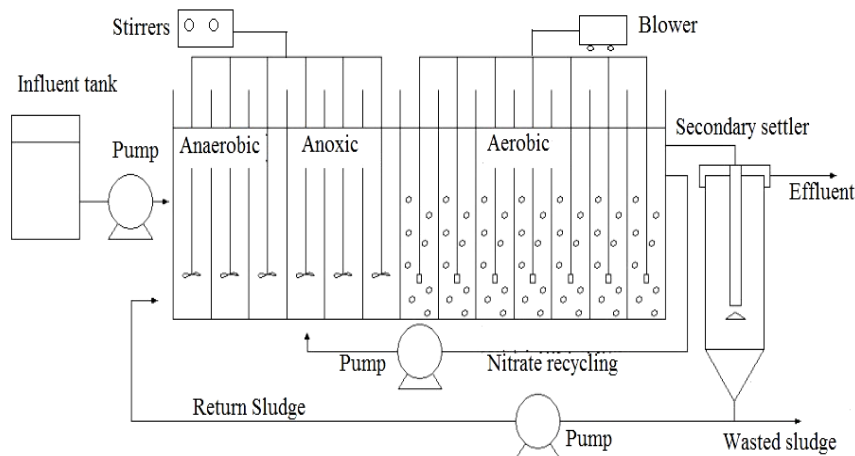


Fig1 The A^2/O process

A laboratory-scaled A^2/O system (shown in Fig. 1), was consisted of an influent tank, an A^2/O reactor and a secondary settler. It was made by transparent Plexiglas.

The volume of the A^2/O reactor was designed with 52.08L, and the secondary settler ran with a working volume of 26.18L. It had twelve compartments with a working volume of 3.3L in sequence, and was separated by baffles to create anaerobic/anoxic/oxic zones. The volume ratio of the anaerobic/anoxic/oxic zones was 1:1:2. The first three compartments were typically operated as anaerobic zones, the last six compartments were operated as aerobic zones, and the middle of the compartments were operated as anoxic zones. There was a blower via porous stone diffusers installed on the bottom of anoxic zones in order to control the dissolved oxygen (DO) to be 3 mg/L.

There were six overhead mechanical stirrers with rectangular paddles were installed over the anaerobic and anoxic zones, and there are two different recycle ratios. One was from the end of aerobic zones to the forefront of anoxic zones with 150% recycle ratio, and the other one was from the secondary settler to the forefront of anaerobic zones with 80% recycle ratio. The sludge was from Harbin Wenchang wastewater treatment plant which was A/O process was similar to the A²/O system. There were four stages, the first stage was the choice of external carbon sources (31-73days), the second stage was the dosing methods of the carbon sources (74-113days), the third stage was the addition concentrations of carbon sources (114-153days), and the last one was the optimizing at various nitrate recycling ratios (154-193days). All the fluid was controlled by the peristaltic pumps.

During the experiment period, didn't doing the strict sealing on anaerobic period which could be suitable for the actual wastewater treatment plant operation. The baffles with one hole on the edge were alternately installed in order to increase the reaction time which was to ensure the removal efficiencies of the nitrogen and phosphorus.

2.2 Experiment conditions and wastewater source

During the experiment period, the first stage was running with synthetic wastewater, and the remaining stages were running with the actual domestic wastewater which was collected from the residential area of the Northeast Forestry University. The main operating conditions of this experiment were presented in Table 1. The main characteristics of influent wastewater were presented in Table 2.

Table1. The main operating conditions of this experiment

Parameter	Value	
Flow rate (L/h)	6.51	
HRT(h)	Anaerobic	2
	Anoxic	2
	aerobic	4
	secondary settler	4
internal recycle ratio	150%	
sludge recycle ratio	80%	
DO(mg/L)	3-4	
MLSS(mg/L)	3000	
Temperature(°C)	23-25	
pH	6.9-7.5	
SRT(d)	15	

Table2. The main characteristics of influent wastewater mg/L

Parameter	Range	Average
COD	184-294	261.8
TN	56.5-71.9	68.5
TP	4.1-6.4	5.8
NH ₄ ⁺ -N	60.2-69.7	66.5
NO ₃ ⁻ -N	0-0.15	0.004
NO ₂ ⁻ -N	0.002	0.013
C/P	28.7-68.8	40.4
C/N	2.5-4.8	3.1

2.3 Analytical methods

COD, NH₄⁺-N, TN, TP and mixed liquor suspended solid (MLSS) were analyzed in accordance with standard methods (APHA–AWWA–WEF 1998). TN was detected by Alkaline Potassium Per sulfate Digestion-Ultraviolet Spectrophotometry method, TP was detected by Ammonium Molybdate Spectrophotometry method. NO₂⁻-N and NO₃⁻-N were detected by ion chromatography (Agilent). DO, pH and temperature were monitored online by using pH/oxi 340i meter (WTW, Germany). ORP was monitored online by level 2 ORP meter (WTW, Germany). All of the samples were collected from each section of the end of the zones.

2.4 Equations

The rate of denitrification, COD for phosphorus release, nitrogen removal and acidification yield could be calculated according to the following equations which were presented in Table 3.

Table 3 some equations for the A²/O system

Number	Equations	Remark
(1)	$0.3(F/M)$ Hypoxia pool +0.029	Rate of gentrification
(2)	$(\text{Into the water}_{TP} - \text{Outflow}_{TP}) \times 25$	COD for phosphorus release
(3)	$(R+r)/(1+R+r)$	Nitrogen removal
(4)	$(VFA_{\text{eff}} - VFA_{\text{in}})/(COD_{\text{eff}} - COD_{\text{in}}) \times 100\%$	Acidification yield

3. Results and discussion

3.1 The effect of various external carbon sources on A²/O process

This part (31-73days) used methanol, glucose and ethanol as the external carbon sources of the A²/O system, respectively. Fig 2 summarized the concentrations of TP in influent, effluent and each of stage removal efficiencies at various carbon sources, respectively. Fig 3 summarized the TP removal characteristics at various carbon sources during the period.

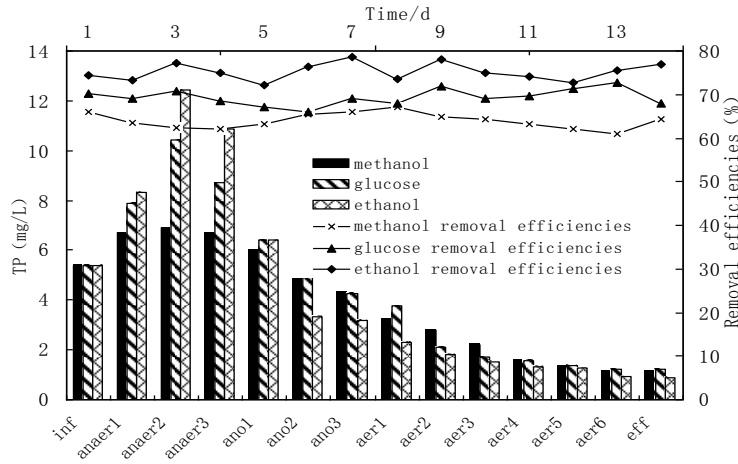


Fig2 TP removal characteristics of each stage at various carbon sources

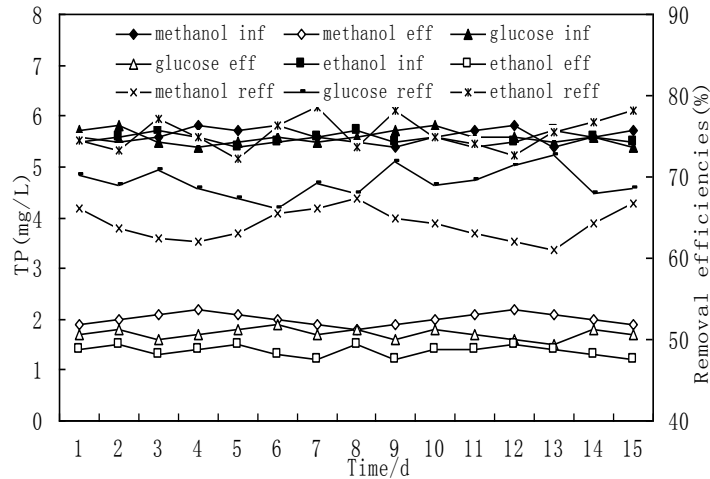


Fig 3 TP removal characteristic during the period

From Fig 2 and Fig 3, among external various carbon sources, when ethanol was taken as the external carbon source of the A²/O system, the removal efficiency of TP was the highest and the removal efficiency was 78.18%. Then the following was glucose, and the removal efficiency was 70.15%. The lowest removal efficiency was methanol, which was 66.78%. During anaerobic period, PAOs released phosphorus process could be roughly divided into two stages (Saito *et al.* 2004).

The process was the formation of polyphosphate though PAOs utilizing VFA which was used to be electron acceptor in aerobic zones. The PAOs could simultaneously absorb VFA and release phosphorus. In general, the more VAF taken-in, the more phosphorus released. And the more phosphorus released in anaerobic zones, the more phosphorus taken-in in aerobic zones. From fig2, among various external carbon sources, the concentration of phosphorus was the highest at the end of the anaerobic zones when ethanol was taken as the external carbon source of the A²/O system, and the concentration was 12.41mg/L. The concentration of phosphorus was the lowest at the end of the aerobic zones, and the effluent concentration of phosphorus was 0.9mg/L.

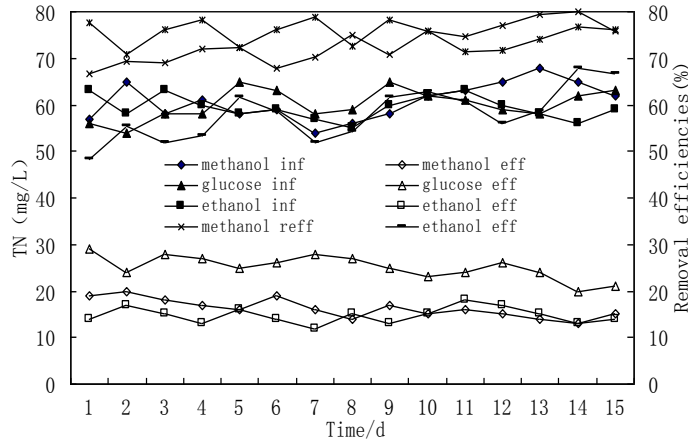


Fig4 NH₄⁺-N removal characteristic of each stage at various carbon sources

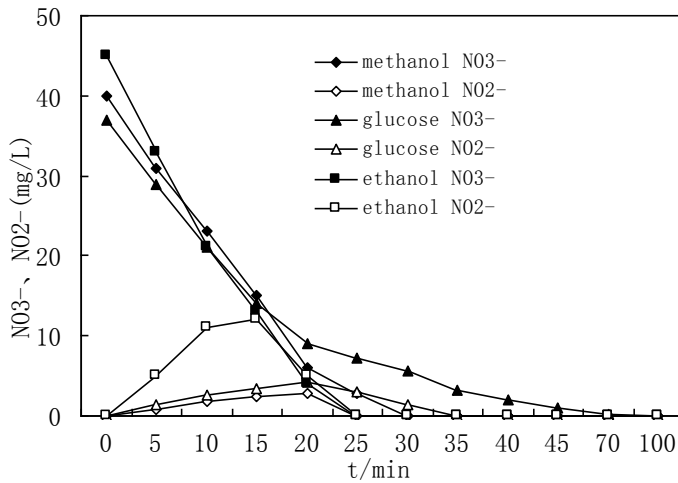


Fig5 The denitrification rates of NO₃⁻ and NO₂⁻

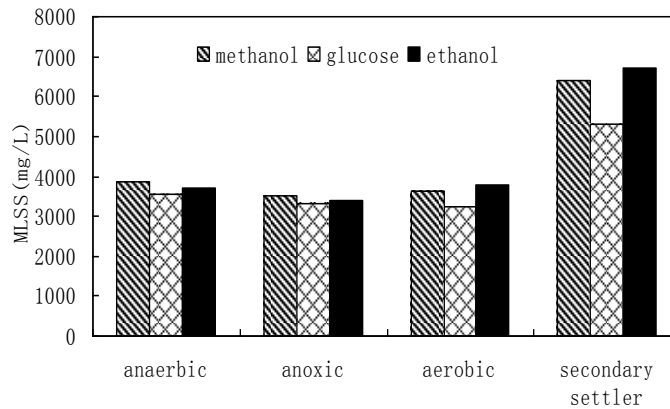


Fig6 MLSS characteristic of each stage during the period

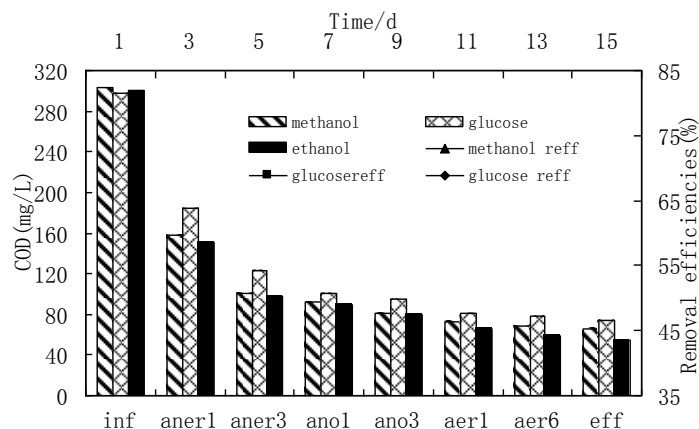


Fig 7 COD removal characteristic of each stage at various carbon sources

Fig 4 summarized the characteristic of ammonia nitrogen in influent, effluent and each of stage removal efficiencies at various carbon sources of the A²/O system, respectively. Fig 5 summarized the characteristic of denitrification during the period at various carbon sources of the A²/O system. From Fig 4, the nitrogen removal efficiencies at various carbon sources of the A²/O system were 75.81%, 66.67% and 78.23%, respectively. The concentrations of ammonia nitrogen at various carbon sources were 40.56, 37.09 and 46.15 mg/L, respectively. From the data in Fig4, when ethanol was taken as the external carbon source of the A²/O system, the nitrogen removal efficiencies and the concentration of ammonia nitrogen was the highest among various external carbon sources. From Fig 4, the ammonia nitrogen concentrations in anaerobic and anoxic period increased, mainly might be transformed from the available carbon sources.

From Fig 4, the efficiencies of nitrification at various carbon sources were 78.21%, 70.25% and 64.32%, respectively. From Fig5, when ethanol was taken as the external carbon source of the A²/O system, the efficiency of nitrification was the highest among various external carbon sources. The time of the nitrate nitrogen completely removed were 25, 75 and 20min, respectively. During the period, the concentration of the nitrate nitrogen slightly increased, mainly because the nitrite nitrogen converted into nitrate nitrogen. When the methanol was taken as the external carbon source, the concentration of nitrite nitrogen gradually increased until reaching 3.1 mg/L, which was completely removed at the time of 20 min. When the glucose was taken as the external carbon source, the concentration of nitrite nitrogen gradually increased until reaching 4.3 mg/L, which was completely removed at the time of 35min. When the ethanol was taken as the external carbon source, the concentration of nitrite nitrogen gradually increased until reaching 10.9 mg/L, which was completely removed at the time of 20min. From the classic equations (1), getting the response of methanol to denitrification rate was faster than the other two external carbon sources. Although it could be used as the best carbon sources receptor of denitrification among various external carbon sources, the response to nitrification was slightly slow. Either methanol or ethanol was taken as the external carbon source of the A²/O system, the rate of denitrification was faster than glucose, and both of them had reached 1.78 mg MLVSS/mg NO₃-N. The denitrification rate of glucose was the slowest among various external carbon sources, which was only 0.23 mg MLVSS/mg NO₃-N. During the period, the concentration of COD always kept highly removal efficiencies regardless of the various external carbon sources. The concentration COD decreased significantly in anaerobic zones but only slightly decreased in anoxic zones. Among various external carbon sources, the removal efficiencies of COD were 82.12%, 78.56% and 83.27%, respectively. Because of toxicity of the methanol, taking consideration of TP, TN removal efficiencies and denitrification rate, ethanol taken as external carbon source of the A²/O system was perfect.

3.2 The effect of dosing method of external carbon source on the A²/O system

During the period (74-113days), the dosing methods of external carbon source were 3:0:0, 1:1:1, 0:2:1, 0:1:2 and 0:0:3, respectively. Fig 8 summarized TP removal characteristics of each stage at various dosing methods. Fig 9 summarized TP removal characteristics at various dosing methods during the period.

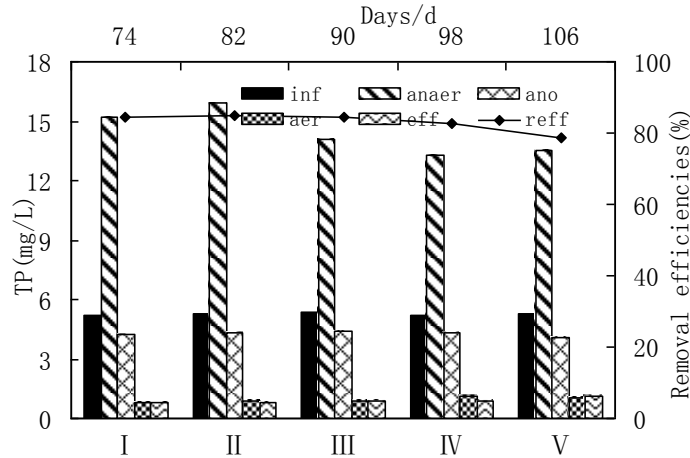


Fig8 TP removal characteristics of each stage at various dosing methods

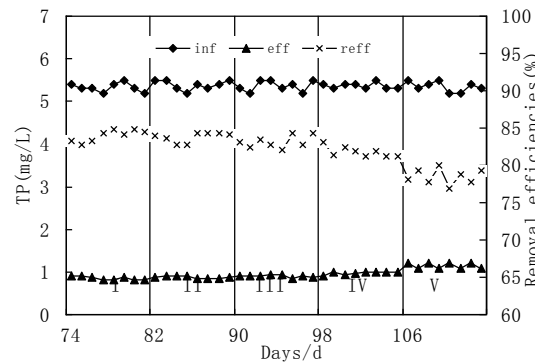


Fig 9 TP removal characteristics at various dosing methods during the period

From Fig 9, with the varied of dosing methods, TP removal efficiencies were 84.61%, 84.71%, 84.25%, 82.51% and 78.49%, respectively. With the change of the dosing methods, the removal efficiencies of TP were trend to decreasing, but the decreasing of the removal efficiencies was slight and not obvious. From Fig8, the removal efficiencies of TP decreased from 84.61% to 78.49%, and the effluent concentrations of TP increased from 0.8mg/L to 1.12mg/L. Only the C/P ratio was more than 25, TP could be better removed. The COD concentration could be calculated by the equations (2). From the equations, the influent COD concentration could satisfy a series of activities of PAOs in anaerobic zones, and the synthesis of PHA was not affected by the concentration of COD. Although the various dosing methods made the concentrations of TP at the end of the anaerobic zones different, the effluent concentrations of TP were slight different. The gap of the effluent concentration of TP was 0.32 mg/L. The dosing methods had litter effect on the TP removal efficiencies, mainly because the TP removal efficiencies relied on the synthesis and degradation of PHV, not the COD concentration during the remaining zones. And the absorption and release of phosphorus mainly relied on the transformation of PHB (Lu *et al.* 2006). As the external carbon source was ethanol, the PHB in anaerobic zones was about 80% of the PHV, and the PHA in the anaerobic zones was about 20% of the PHV.

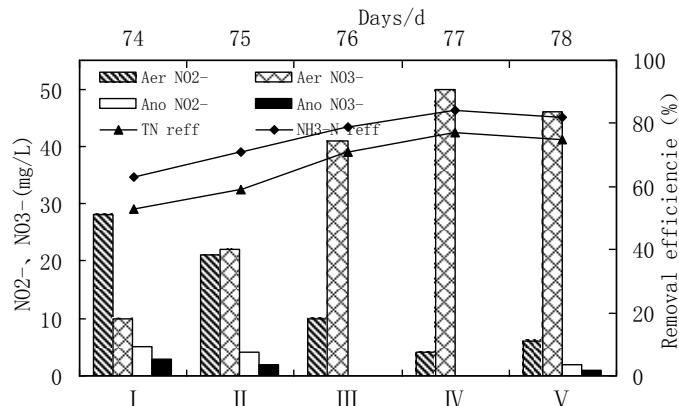


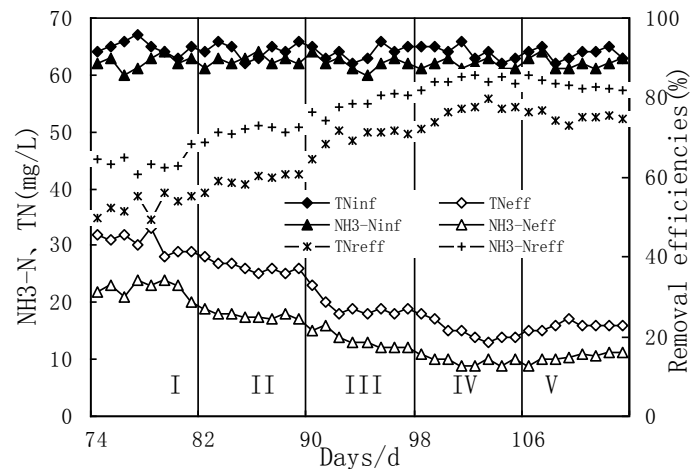
Fig10 NO_3^- 、 NO_2^- removal characteristic at various dosing methods

Fig 11 nitrogen removal characteristic at various dosing methods

The evolution of nitrogen in A^2/O system at various dosing methods of external carbon source was depicted in Fig 10 and Fig 11. From Fig 11, TN removal efficiencies were 53.22%, 60.13%, 71.32%, 77.31% and 75.38%, respectively. Ammonia nitrogen removal efficiencies were 62.32%, 71.62%, 78.74%, 84.49% and 82.46%, respectively. From the data, TN and ammonia nitrogen removal efficiencies presented a trend of increasing form stage I to stage IV, and after stage IV, both of TN and ammonia nitrogen removal efficiencies decreased. Among the five stages, TN and ammonia nitrogen removal efficiencies reached the highest when the various dosing method of external carbon source was 0:1:2, and the removal efficiencies were 77.31% and 84.49%, respectively.

From Fig 10, the concentrations of NO_3^- and NO_2^- could account for the cause of the decreasing about TN and ammonia nitrogen removal efficiencies. Compared with the NO_3^- and NO_2^- concentrations in aerobic zones at the IV and V stages, the NO_3^- concentration of aerobic zones at stage V was 4.34 mg/L lower than the NO_3^- concentration of aerobic zones at stage IV, which accounted for the inhibition for nitrification in aerobic zones. The heterotrophic bacteria's breeding was the main cause of the inhibition for nitrification in aerobic zones. Because the heterotrophic bacteria could rapidly breed at sufficient conditions of dissolved oxygen and carbon sources, which illustrated the excess carbon source was the main cause of the inhibition for nitrification in aerobic zones. From Fig 6, through the denitrification in anoxic zones at stage V, the NO_3^- and NO_2^- concentrations were 2.15 and 1.08 mg/L, respectively. And the nitrate nitrogen through the denitrification in anoxic zones at stage IV was completely removed. Theoretically, the nitrogen through the denitrification could be completely removed (Kim *et al.* 2013). The denitrification in anoxic zones at stage V did not completely be removed could be analyzed from the following summarized: (1) Contrasting the denitrification in anoxic zones at various dosing methods, the remaining nitrate was found during the period except stage IV, which mainly be caused by the insufficient of external carbon source; (2) The destruction of the nitrification conditions might result in this phenomenon, because amount of heterotrophic bacteria from aerobic zones was returned to anoxic zones with the nitrate nitrogen recycling, which made the anoxic denitrification blocked in a short time.

In conclusion, the cause of TN and ammonia nitrogen removal efficiencies decreased at stage V were the lack of external carbon source in anoxic zones and the destruction nitrating condition in aerobic zones.

3.3 The effect of the external carbon source concentration on the A^2/O system

During the period (114-153days), the concentrations of the external carbon sources were 100, 200, 300, 400 and 500mg/L, respectively. The evolution of TP in A^2/O system at various addition concentrations of external carbon sources was depicted in Fig 12 and Fig13.

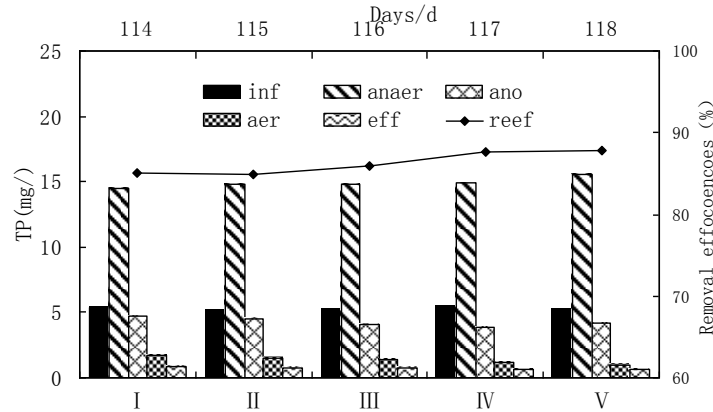


Fig 12 TP removal characteristics of each stage at various addition concentrations

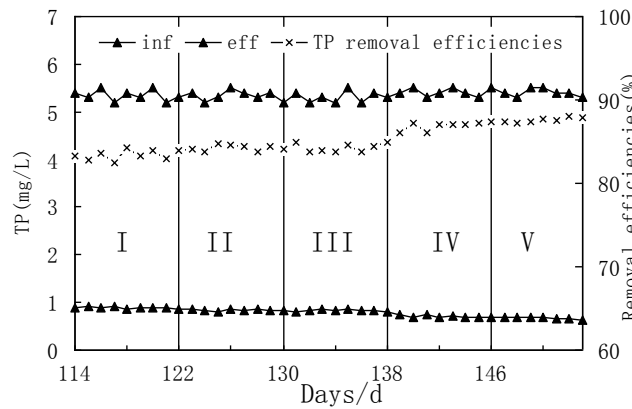


Fig 13 TP removal characteristics at various addition concentrations during the period

From Fig 13, the removal efficiencies of TP at various addition concentrations of external carbon sources were 84.11%, 84.81%, 85.85%, 87.64% and 87.74%, respectively. TP removal efficiencies presented a trend of increasing during the period, mainly because the external carbon sources flowed into the anoxic and aerobic zones, which had no effect on phosphorus release in anaerobic zones. From Fig 12, the TP removal efficiencies at the end of the anoxic zones were 31.15%, 31.23%, 32.21%, 32.85% and 33.21%, respectively. The phosphorus removal efficiencies presented a trend of increasing at the end of the anoxic zones mainly was removed by denitrifying polyphosphate accumulating organisms (DPAOs). As the TP concentration at the end of the anoxic zones was lower than the influent concentration of TP, which illustrated the denitrification polyphosphate accumulating phenomenon. From the data in Fig12, the denitrification polyphosphate accumulating phenomenon was more and more obvious with the increase of external carbon source concentration. From the stage I to V, the TP concentration at the end of the anoxic zones reduced from 4.71 mg/L to 4.28 mg/L, and the effluent concentrations reduced from 0.81mg/L to 0.65mg/L. Although the removal efficiencies of TP were increased by 3.63% and the influent concentration reduced 0.43mg/L which was not obvious for the TP removal, the ability of denitrification polyphosphate accumulating was enhanced.

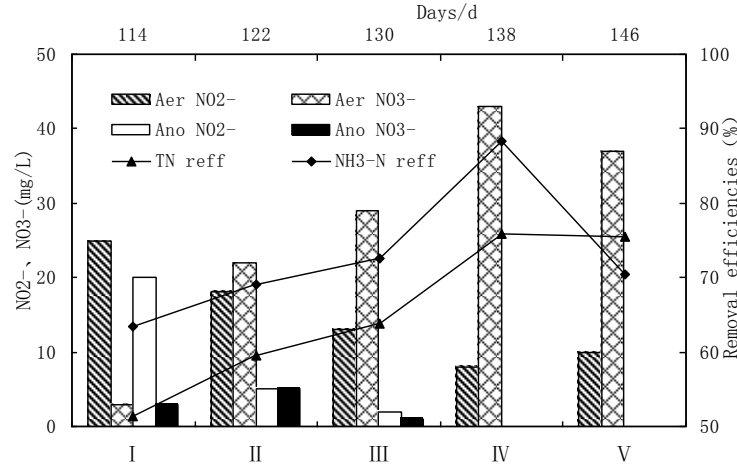


Fig14 NO₃⁻、NO₂⁻ removal characteristics at various addition concentrations during the period

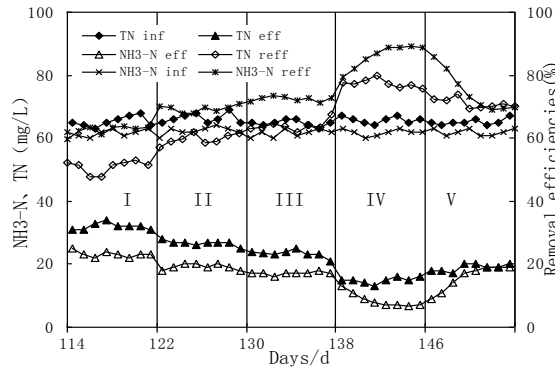


Fig15 Nitrogen removal characteristic at various addition concentrations

The evolution of nitrogen in A²/O system at various addition concentrations of external carbon source was depicted in Fig 14 and Fig 15. From Fig 15, TN removal efficiencies at various addition concentrations were 51.41%, 59.48%, 63.89%, 75.91% and 75.41%, respectively. Ammonia nitrogen removed efficiencies at various addition concentrations were 63.51%, 68.98%, 72.54%, 88.41% and 70.49%, respectively. From the data in Fig 15, both TN and ammonia nitrogen removal efficiencies presented a trend of increasing form stage I to IV. After the stage IV, both TN and ammonia nitrogen removal efficiencies deceased. The removal efficiencies characteristics of TN and ammonia nitrogen could be proved through the characteristics of the nitrate nitrogen in Fig 14. Through the equations (2), the COD concentration of the external carbon source was sufficient (Zhang *et al.* 2005). From Fig 14, the concentration of nitrate nitrogen at the end of the aerobic zones presented a similar removal characteristic to TN and ammonia nitrogen. Although the NO₃⁻ concentration in aerobic zones at stage V was 5.64 mg/L lower than the NO₃⁻ concentration in aerobic zones at stage IV, both of the nitrate nitrogen in anoxic zones at stage IV and V were completely removed. To sum up, as the external carbon source concentration was 500mg/L at stage V, the effect of nitrification in aerobic zones was inhibition and the denitrification in anoxic zones was not inhibition. Contrasting the sludge sedimentation performance and sludge concentration which revealed the sludge presenting a puffy state testified the inhibition of the nitrification in aerobic zones.

3.4 The effect of various nitrate recycling ratios on the A²/O system

During the period (154-193days), the various nitrate recycling ratios were 100%, 200%, 250%, 300% and 400%, respectively. The evolution of TP in A²/O system at various nitrate recycling ratios was depicted in Fig 16 and Fig 17.

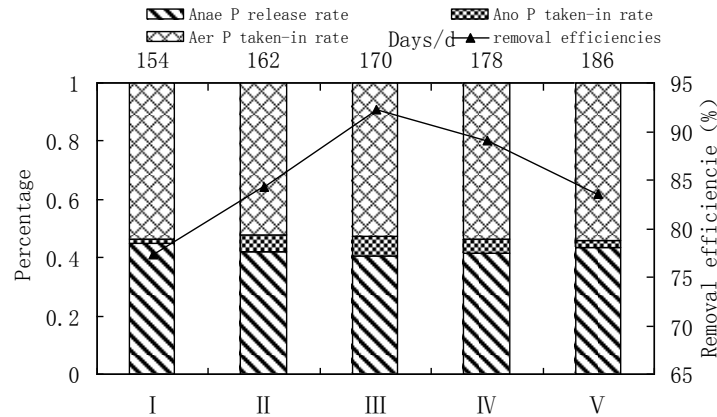


Fig 16 The percentage of the anaerobic phosphorus release and anoxic/aerobic phosphorus absorption

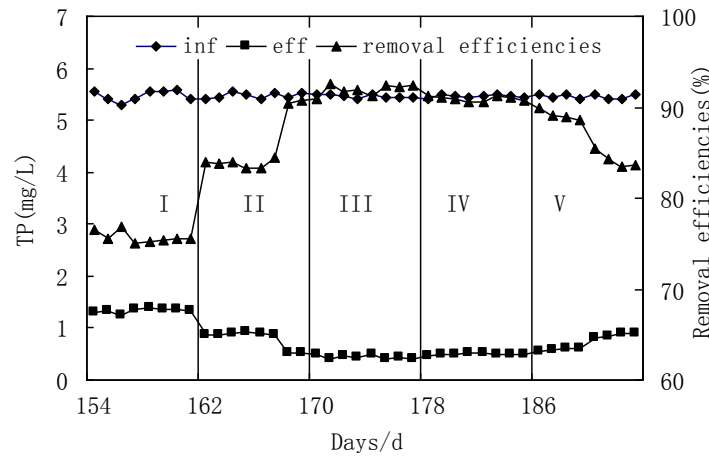


Fig 17 TP removal characteristics at various nitrate recycling ratios during the period

From Fig 17, TP removal efficiencies at various nitrate recycling ratios were 77.31%, 84.31%, 92.18%, 89.15% and 83.56%, respectively. From the data in Fig17, the TP removal efficiencies presented a trend of increasing from stage I to III. After stage III, TP removal efficiencies presented a trend of decreasing. The TP removal efficiencies of stage III reached the highest among the five stages, and the TP removal efficiencies was 92.56%, while the effluent TP concentration was 0.45 mg/L, reaching the type A standard GB18918-2002 level. When the nitrate recycling ratio was higher than 250%, although the nitrate recycling ratio was increased, the TP removal efficiencies decreased. The removal characteristics could be analyzed from the following summarized: (1) with the nitrate recycling ratios increasing, the effect of nitrate in anaerobic zones on phosphorus release was weakened which could be testified in the Fig 16. From Fig 16, the percentages of phosphorus release in anaerobic zones presented a trend of increasing with the nitrate recycling ratio increasing, but the percentages of phosphorus release in anaerobic zones didn't increase when the nitrate recycling ratio reached 250%, because phosphorus release in anaerobic zones was not only effected by the nitrate recycling ratios, but also effected by temperature, organic load and pH. (2)with the nitrate recycling ratios increasing which enhanced the phosphorus absorption in aerobic zones, but the denitrifying phosphorus absorption weakened in anoxic zones, the data in the Fig 16 proved the phenomenon which occurred in the aerobic and anoxic zones. From Fig 16, the percentages of phosphorus absorption in aerobic zones were 75.13%, 82.17%, 89.54%, 90.43% and 90.51%, and the percentages of the denitrifying phosphorus absorption in anoxic zones were 2.74%, 10.43%, 13.41%, 8.47% and 3.75%, respectively. If the reaction was too long, it might lead to a second release of phosphorus (Podedworna & Żubrowska-Sudoł 2012). With the nitrate recycling ratios increasing, lengthening the reaction time resulted to a second release of phosphorus, which illustrated the effluent concentration at the stage V was higher than which at the stage III.

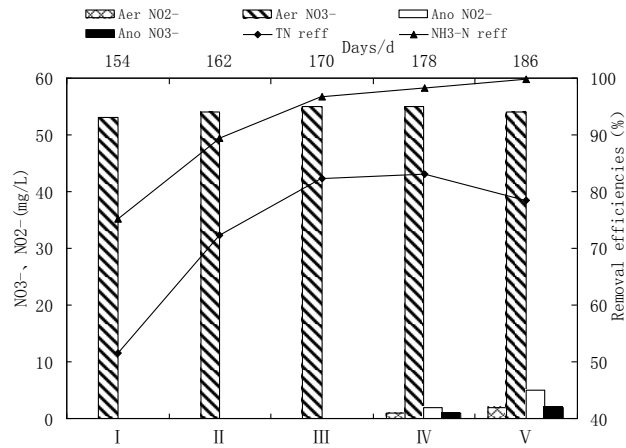


Fig18 NO₃⁻、NO₂⁻ removal characteristics at various nitrate recycling ratios during the period

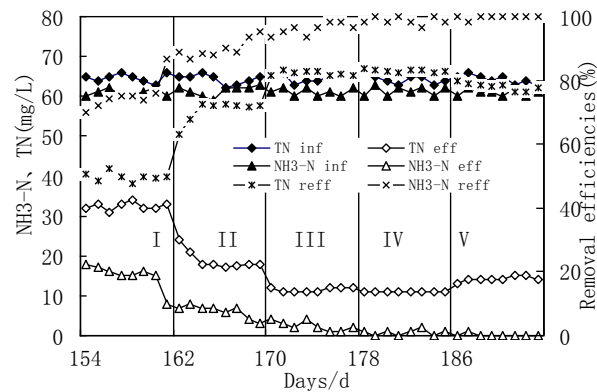


Fig19 Nitrogen removal characteristics at various nitrate recycling ratios

The evolution of nitrogen in A²/O system at various nitrate recycling ratios was depicted in Fig 18 and Fig 19. From Fig 19, TN removal efficiencies at various recycling ratios were 51.52%, 72.36, 82.36%, 83.13% and 78.51%, respectively, and TN removal efficiencies presented a trend of increasing form stage I to IV. After stage IV, TN removal efficiencies presented a trend of decreasing. Ammonia nitrogen removal efficiencies at various recycling ratios were 75.17%, 89.42%, 96.67%, 98.30% and 99.9%, respectively, which presented a trend of increasing at various recycling ratios. As the nitrate recycling ratio was 300% at stage IV, the TN removal efficiencies reached the highest at various recycling ratios which was 83.13%, and the effluent concentration was 11.43mg/L. According to the theoretical equation (3) for nitrogen removal (Volokita *et al.* 1996), the higher the nitrate recycling ratio, the better nitrogen removed. But the actual removal efficiency was not like this, the cause of the difference could be analyzed from the following summary: (1) the theoretical equation of nitrogen removal was based on anoxic zones, the crux of the nitrogen removal was the condition in anoxic zones. (2) the ability of the denitrification in anoxic zones was related to denitrification bacteria biomass, when the biomass had enough electron acceptors, even increasing nitrate recycling ratio could not strengthen the effect on denitrification. On the contrary, it might destroy denitrification in anoxic zones. The data from Fig18 could illustrate this phenomenon. At stage IV and V, some of NO₃⁻ and NO₂⁻ existed at the end of the anoxic zones. The concentrations of NO₃⁻ at stage IV and V were 1.32 and 2.14mg/L, and the concentrations of NO₂⁻ at stage IV and V were 2.12 and 5.04mg/L, which accounted for the electron acceptors for denitrification were sufficient. The cause of the remaining NO₃⁻ and NO₂⁻ was the recycling of nitrate with much of dissolved oxygen which not only weaken the ability of the denitrifying polyphosphate absorption, but also destroyed the conditions in anoxic zones. As the removal efficiencies of TN at stage III and IV were 82.36% and 83.13%, and the effluent concentrations were 12.48 and 11.73mg/L, which were slight difference and conformed to the nitrogen discharge standard. Besides, considering the energy consumption, choosing the nitrate recycling ratio was 250% was a wise choice

4. Conclusions

The main findings from this study were summarized as the following: (1) The A²/O system operated perfectly as the external carbon source was ethanol, and the removal efficiencies of TN and TP were respectively 78.23% and 78.18%, while the time for nitrate nitrogen removed was 20min. (2) As the dosing method was 0:1:2, the addition carbon source concentration was 400mg/L and nitrate recycling ratio was 250%, the A²/O system operated perfectly, and the removal efficiencies of TN, NH₃-N and TP were 82.36%, 96.67% and 82.18%, while the effluent concentrations were 12.3, 4.1mg/L and 0.45mg/L, Which reached the type A standard GB18918-2002 level. (3) Denitrifying phosphorus absorption phenomenon was occurred at anoxic zones. (4) The novel method solved simultaneously shortage of carbon source and maximized the utilized of carbon sources.

References

- Barker P. S. & Dold P. L. 1996 Denitrification behavior in biological excess phosphorus removal activated sludge system. *Water Research*, 30 (4), 769–780.
- Branch G. M., Bustamante R. M. & Robinson T. B. 2013 Impacts of a ‘black tide’ harmful algal bloom on rocky-shore intertidal communities on the West Coast of South Africa. *Harmful Algae*, 24, 54–64.
- Cao G., Wang S., Peng Y. & Miao Z. 2013 Biological nutrient removal by applying modified four step feed technology to treat weak wastewater. *Bioresource Technology*, 128, 604–611.
- Cao S., Wang S., Peng Y., Wu C., Du R., Gong L. & Ma B. 2013 Achieving partial denitrification with sludge fermentation liquid as carbon source: The effect of seeding sludge. *Bioresource Technology*, 149, 570–574.
- Chen Y., Peng C., Wang J., Ye L., Zhang L. & Peng Y. 2011 Effect of nitrate recycling ratio on simultaneous biological nutrient removal in a novel anaerobic/anoxic/oxic(A²/O)-biological aerated filter (BAF) system. *Bioresearch Technology*, 102 (10), 5722–5727.
- Ding Y., Lin W., Wang B. Zheng W. 2006 Removal of nitrogen and phosphorus in a combined A²/O-BAF system with a short aerobic SRT. *Journal of Environmental Sciences*, 18 (6), 1082–1087.
- Estrada J. M., Quijano G., Lebrero R. & Muñoz R. 2013 Step-feed biofiltration: A low cost alternative configuration for off-gas treatment Original Research Article. *Water Research*, 47(13), 4312–4321.
- Fux C., Velten S., Carozzi V., Solley D. & Keller J. 2006 Efficient and stable nitrification and denitrification of ammonium-rich sludge dewatering liquor using an SBR with continuous loading. *Water Research*, 40 (14), 2765–2775.
- Gao Y., Peng Y., Zhang J., Wang S., Guo J. & Ye L. 2011 Biological sludge reduction and enhanced nutrient removal in a pilot-scale system with 2-step sludge alkaline fermentation and A²/O process. *Bioresource Technology*, 102, 4091–4097.
- Guerrero J., Guisasaola A. & Baeza J. A. 2011 The nature of the carbon source rules the competition between PAO and denitrifiers in systems for simultaneous biological nitrogen and phosphorus removal Original Research Article. *Water Research*, 45(16), 4793–4802.
- Kang X. S., Liu C. Q., Zhang B., Bi X. J., Zhang F. & Cheng L. H. 2011 Application of reversed A²/O process on removing nitrogen and phosphorus from municipal wastewater in China. *Water Science and Technology*, 63(10), 2138–2142
- Kim H. S., Moon T. S., Kim Y. J., Kim M. S., Piao W. H., Kim S. J. & Kim C. W. 2013 Evaluation of rule-based control strategies according to process state diagnosis in A²/O process. *Chemical Engineering Journal*, 222, 391–400.
- Kirilova E. P., Cremer H., Heiri O. & Lotter A. F. 2010 Eutrophication of moderately deep Dutch lakes during the past century: flaws in the expectations of water management? *Hydrobiology*, 637(1), 157–171.
- Liu Y., Wang Y., Sheng H., Dong F., Zou R., Zhao L., Guo H., Zhu X. & He B. 2014 Quantitative evaluation of lake eutrophication responses under alternative water diversion scenarios: A water quality modeling based statistical analysis approach. *Science of the Total Environment*, 469(468), 219–227.
- Lu H., Oehmen A., Virdis B., Keller J. & Yuan Z. 2006 Obtaining highly enriched cultures of *Candidatus Accumulibacter phosphatus* through alternating carbon sources. *Water Research*, 40(20), 3838–3848.
- Mino T., Van Loosdrecht M. C. M., Heijnen J. J. 1998 Microbiology and biochemistry of the enhanced biological phosphate removal process. *Water Research*, 32, 3193–207.
- Podedworna J. & Żubrowska-Sudol M. 2012 Nitrogen and phosphorus removal in a denitrifying phosphorus removal process in a sequencing batch reactor with a forced anoxic phase. *Environmental Technology*, 33 (2), 237–245.
- Saito T., Brdjanovic D. & Van Loosdrecht M. C. M. 2004 Effect of nitrite on phosphate uptake by phosphate accumulating organisms. *Water Research*, 38 (17), 3760–3768.

- Standard methods for the examination of water and wastewater 1998 20th edn, American Public Health Association/American Water Works Association/Water Environment Federation (APHA–AWWA–WEF), Washington DC, USA.
- Sun F., Wang X. & Li X. 2013 An innovative membrane bioreactor (MBR) system for simultaneous nitrogen and phosphorus removal. *Process Biochemistry*, 48, 1749–1756.
- Volokita M., Belkin S., Abeliovich A. & Soares M. I. M. 1996 Biological denitrification of drinking water using newspaper. *Water Research*, 30(4), 96–103.
- Zeng W., Li L., Yang Y., Wang S. & Peng Y. 2010 Nitritation and denitritation of domestic wastewater using a continuous anaerobic–anoxic–aerobic (A²/O) process at ambient temperatures. *Bioresource Technology*, 101, 8074–8082.
- Zhang H., Hu W., Gu K., Li Q., Zheng D. & Zhai S. 2013 An improved ecological model and software for short-term algal bloom forecasting. *Environmental Modeling and Software*, 48, 152–162.
- Zhang T., Liu Y. & Fang H. H. P. 2005 Effect of pH change on the performance and microbial community of enhanced biological phosphate removal process. *Biotechnology and Bioengineering*, 92(2), 173–182.
- Zhu G. B., Peng Y. Z., Li B.K., Guo J. H., Yang Q. & Wang S. Y. 2008 Biological removal of nitrogen from wastewater. *Rev. Environ. Contam. Toxicol*, 192, 159–195.