The start-up of denitrifying phosphorus removal system by using nitrite as electron acceptor

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Abstract. The inoculation of short-cut denitrifying polyphosphate-accumulating organisms (DPAOs) mainly included two-phase inoculation and three-phase inoculation. The short-cut denitrifying phosphorus removal bacteria were quickly inoculated by sequencing batch reactor (SBR) to treatment domestic wastewater. The results showed that the average effluent concentration of TP was 0.85 mg/L after 132 cycles by 44 days in two-phase inoculation. The removal rates of NO2--N, TP and COD were 94.73%, 95.47% and 89.96% after 126 cycles by 42 days in three-phase inoculation, and the effluent concentrations were separately 1.31 mg/L, 0.45 mg/L and 17.07 mg/L, which reached the first A class requirement of Urban sewage treatment plant pollutant discharge standard. It was indicated that the efficiency of three-phase inoculation was higher. Anoxic phosphorus uptake was influenced seriously by anaerobic residual carbon, and it was the difference of the two inoculations.

1. Introduction

In an anaerobic / anoxic alternating operating environment, the sludge in the system can use the nitrite as electron acceptor for simultaneous nitrogen and phosphorus removal. People named this type of bacteria as denitrifying polyphosphate accumulating organisms (DPAOs). Compared with O2 and NO3-N as electron acceptor, DPAOs with nitrite nitrogen as electron acceptor has the advantages of short operating cycle, high phosphorus uptake rate, saving more oxygen consumption and energy, and shortening reaction time [1-5]. This has led some scholars’ extensive research on short-cut denitrifying phosphorus removal technology. Some scholars studied on domestication of short-cut denitrifying phosphorus removal sludge, and explored the process operating conditions, performance and practical application [6-8]. Some scholars discussed the metabolic mechanism, analyzed the influence of substrate concentration, the quality concentration of the nitrite nitrogen, SRT, pH, temperature and other environmental factors on short-cut denitrifying phosphorus removal [9-11].

Up to now short-cut denitrifying phosphorus removal technology has been reached in the laboratory, and the application and enrichment of DPAOs are the keys to short-cut denitrifying phosphorus removal technology research. This study was conducted by two acclimation methods (two-phase inoculation and three-phase inoculation) to find a method for rapid application of short-cut denitrifying. It aimed to provide support to promote the practical application of short-cut denitrification and phosphorus removal technology.
2. Materials and methods

2.1. Experimental setup
The DPAOs was quickly domesticated by SBR as shown in figure 1. It was made of double plexiglass. The outer layer was connected to the electric constant temperature water bath, so that the operation of system was in a constant temperature state. The reactor had a inside diameter of 14cm, a height of 85 cm, and the effective volume of 12 L. Sewage and sludge were mixed completely by electric mixer in the reaction process. The lower part of the reactor was equipped with microporous aeration diffusers that connected the gas flow meter. To ensure the appropriate pH range, the system was equipped with a pH on-line monitor. When exceeding the set range, the system will send an alarm signal and add acid or lye through the peristaltic pump to adjust the pH in the allowable range. The bottom of the reactor was arranged with a mud discharge port, and a plurality of outlet ports were arranged on the side. Steps of water distribution, mixing, aeration and drainage were controlled by time-control switch and solenoid valve to achieve the automatic switching.

![Experimental setup of denitrifying dephosphatation.](image)

1-inlet tank; 2-time-control switch; 3-solenoid valve; 4-water pump; 5-electric mixer; 6-Stirring paddle; 7-nitrite box; 8-microporous aeration diffuser; 9-mud discharge port; 10-gas flow meter; 11-air pump; 12-DO on-line monitor; 13-pH on-line monitor; 14-acidic buffer; 15-alkaline buffer 16- outlet ports; 17-electric constant temperature water bath; 18-Water bath inlet; 19-Water bath outlet; 20-water inlet.

2.2. Test water quality
The experiment used simulated domestic sewage; the main ingredients were KH₂PO₄, NH₄Cl, CaCl₂, MgSO₄, CH₃COONa and trace element. The system used NaHCO₃ to adjust the pH in the range of 7.5 ~ 7.8.

| Table 1. Composition of simulative domestic sewage. |
|-----------------------------------------------|
| COD (mg/L) | N (mg/L) | P (mg/L) | MgSO₄ (mg/L) | CaCl₂ (mg/L) | Trace element (ml/L) |
|------------|---------|---------|-------------|-------------|---------------------|
| 170.000~220.000 | 15~20   | 9.000~12.000 | 7~9         | 20~25       | 1                   |
Trace element composition: FeCl3 1.50 g/L, H3BO3 0.15 g/L, CoCl2 0.15 g/L, CuSO4 0.03 g/L, MnCl2 0.06 g/L, Na2MoO4 0.06 g/L, ZnSO4 0.12 g/L, EDTA 10.00 g/L. Water specific composition was shown in table 1.

2.3. Analyzed items and methods
Routine testing items and analysis methods were shown in table 2. The analysis and detection methods were from the "water and wastewater monitoring and analysis methods" (fourth edition) which was promulgated by the State Environmental Protection Administration.

| Analyzed Items | Methods                      |
|---------------|------------------------------|
| pH            | PHS-25 pH on-line monitor    |
| NO3-N         | Spectrometer                 |
| TP            | Spectrometer                 |
| COD           | Spectrometer                 |
| MLSS          | Filter paper weighing method |
| Temperature   | Electric constant temperature water bath |
| DO            | HQ40d DO on-line monitor     |

2.4. Running processes
The inoculation of DPAOs mainly included two-phase inoculation (anaerobic/aerobic, anaerobic/anoxic) and three-phase inoculation (anaerobic/aerobic, anaerobic/precipitation drainage/anoxic, anaerobic/anoxic). For two-phase inoculation, the traditional polyphosphate accumulating organisms (PAOs) were enriched in anaerobic/aerobic environment; then PAOs were inoculated DPAOs in anaerobic/anoxic environment. In the anoxic phase, the nitrate solution was added to the system continuously to make nitrate become electron acceptor of phosphorus uptake. When the phosphate removal rate in sewage was high and continuous stability, it can be considered that the DPAOs were domesticated successfully. For three-phase acclimation, the operation mode was anaerobic/precipitation drainage/anoxic, sewage with residual carbon source needed to be drained at the end of the anaerobic reaction and then sewage with no external carbon source re-entered the system. It aimed to avoid the un consumed carbon source at the end of anaerobic impact on anoxic denitrifying dephosphatation, and limit the growth and reproduction of conventional denitrifying bacteria.

| Means             | Phase   | Running mode                                      |
|-------------------|---------|---------------------------------------------------|
| two-phase acclimation | Phase I | inlet—anaerobic 2 h—aerobic 2 h—sedimentation 0.5 h—outlet 0.5 h |
|                   | Phase II| inlet—anaerobic 2 h—anoxic 2 h—sedimentation 0.5 h—outlet 0.5 h |
| three-phase acclimation | Phase I | inlet—anaerobic 2 h—aerobic 2 h—sedimentation 0.5 h—outlet 0.5 h |
|                   | Phase II| inlet—anaerobic 2 h—sedimentation 0.5 h—outlet 0.5 h—second-inlet—anoxic 2 h—sedimentation 0.5 h—outlet 0.5 h |
|                   | Phase III| inlet—anaerobic 2 h—anoxic 2 h—sedimentation 0.5 h—outlet 0.5 h |
The sludge used in the experiment was taken from the aeration tank of Sambautun Sewage Treatment Plant in Fushun City, Liaoning Province. The sludge was added to two reactors named A and B in equal amounts after 48 hours of exposure. The two reactors, A and B, were domesticated in two stages of domestication and three stages of domestication. During the operation period, the water quality of the two reactors was the same. The volume ratio of sludge to water was 1:4, and MLSS is controlled at around 3500 mg/L, pH was controlled from 7.5 to 7.8, SV=30% ,SRT=24 d. The system ran three cycles per day and ran 5 to 6 hours per cycle. The specific operating mode was shown in table 3.

3. Results and discussion

3.1. Accumulation of traditional PAOs

![Figure 2](image1.png)  ![Figure 3](image2.png)

**Figure 2.** Concentration variation of TP of A reactor in first stage.  **Figure 3.** Concentration variation of TP of B reactor in first stage.

The system was running for 14 days at this stage and the average influent concentration of TP was 10 mg/L. The concentrations variation of TP in reactor A and B were shown in figures 2 and 3, concentration variation of COD was shown in figure 4. The water quality and running mode of the two reactors were the same, so the variation tendency was similar. PAOs hydrolyzed the polyphosphate (poly-P) in the cells to produce energy by using the nutrients in the sewage, so that they could convert the external carbon source into internal carbon source poly-β-hydroxybutyrate (PHB) and store in cells. This process removed most of the organic in the sewage in the form of phosphorus-release; and in the aerobic stage PAOs used PHB to absorb phosphorous excessively to achieve phosphorus removal. As shown in figures 2 and 3, the initial phosphorus removal of the system was not good. Figure 4 showed that the external carbon source was mainly consumed at the anaerobic phase. After the end of the first day, the mass concentrations of TP in the two reactors were 5.61 mg/L and 6.50 mg/L, respectively. With the system running, TP removal efficiency gradually increased. Figure 3 showed that the anaerobic phosphorus release decreased in the 10th day of a reactor, which is due to the trouble with solenoid valve. The problem caused water outflow from solenoid valve, which affected anaerobic phosphorus release owing to system’s lack of nutrients. The system ran smoothly at the next 4 days, and the phosphorus release and uptake were steadily rising. On the 14th day, the maximum anaerobic phosphorus release in the two systems reached about 19.00 mg/L, and TP mass concentration in the effluent was less than 0.50 mg/L, which reached the "pollutant discharge standard for urban sewage treatment plants" (GB18918-2002) level 1 A standard. The results showed that the traditional PAOs
became the dominant species in the two systems, that is, the enrichment of the PAOs with O₂ as the electron acceptor provided favorable conditions for the domestication and enrichment of DPAOs.

Figure 4. Concentration variation of COD of A and B reactors in first stage.

Figure 5. Concentration variation and removal rates of COD of A reactor in second stage.

3.2. Domestication and enrichment of DPAOs
There were already a large number of traditional PAOs in the two systems after the domestication of the first stage. Then change the system operating conditions, namely to stop the aerobic aeration and use the nitrite as electron acceptor of anoxic dephosphorus, to achieve DPAOs enrichment. High concentration of nitrite solution had certain toxicity to microbial growth, which lead to the deterioration of biological dephosphorization system [12-14]. If the concentration of nitrite was too low, the electron acceptor of the denitrifying phosphorus removal was deficient, which made the system could not achieve the optimum state of phosphorus removal [15]. Therefore, nitrite nitrogen solution was dropped to the reactors. In the anoxic phase, the concentration of nitrite nitrogen increased from 10.00 mg/L to 25.00 mg/L, which gave the microbial a buffer adaptation process. The results were discussed in terms of two-phase acclimation and three-phase acclimation.

3.2.1. Two-phase Acclimation. After completing the operation of the first stage, reactor A worked with the next phases of anaerobic/anoxic directly, which lasted 30 days. In the anaerobic, DPAOs used the energy generated by phosphorus release to absorb the external carbon source and stored it in the form of PHB. Meanwhile the resedual carbon source entered the anoxic stage. At the anoxic stage, DPAOs used PHB to absorb phosphorous excessively without carbon source, therefore, excessive carbon source would be used by denitrifying bacteria for denitrification, which inhibited the growth, reproduction and other life activities of DPAOs, and prevented DPAOs to become a systemic dominant bacteria. The concentration and removal rate of COD in this stage were shown in figure 5. In the first day, the COD mass concentration of the influent was 215.37 mg/L, and the COD concentration in the system remained at 60.83 mg/L at the end of anaerobic stage and 25.63 mg/L in the end of anoxic stage. It was concluded that the supply of carbon was much larger than that of the absorption in the anaerobic influent, so that a large amount of the external carbon source was not used, which led to the high COD concentration of effluent after the anaerobic stage. The nitrite nitrogen solution added in the anoxic stage was denitrified by denitrifying bacteria using the remaining external carbon source, so that the COD content was further reduced. The concentration of COD in the influent was reduced to about 170.00 mg/L, which made the concentration of COD was basically stable at about 32.00 mg/L at the end of anaerobic reaction and 20.00 mg/L after the anoxic stage. Analysis suggested that part of the carbon source met the needs of microbial growth and metabolism, and the
other part was used by the regular heterotrophic denitrifying bacteria in the system. The average removal rate of COD was 87.57% at this stage.

Figure 6 showed the variation on concentration and removal rate of TP and nitrite nitrogen in the second stage of A reactor. The initial mass concentration of nitrite nitrogen was 10.00 mg/L. As shown in figure 6, nitrite nitrogen removal rate was 89.61% in first day, whereas the removal rate of nitrite nitrogen in third day dropped to 32.33%, and after the fourth day, the removal rate increased gradually. This was because in first day, there was an excess carbon source at the end of anaerobic, and most of the nitrite nitrogen was removed by denitrification. Figure 5 showed that the amount of carbon source dosage began to be reduced after two days. In the anoxic stage, the mass concentration of COD was only 32.17 mg/L. Therefore the nitrite nitrogen could only be removed by a small amount of DPAOs at this point, and the removal rate dropped sharply. As the reaction progressed, the DPAOs gradually became a dominant strain in the system, and nitrite nitrogen was used as an electron acceptor for phosphorus removal, and its removal rate increased. The mass concentration of nitrite nitrogen increased to 25.00 mg/L, the concentration of nitrite nitrogen in effluent was 1.71 mg/L, and the removal rate was steady above 92.00%. The amount of phosphorus release decreased in the initial four days, and began to rise gradually five days later. This was because DPAOs were non-dominant species in the system, and the traditional PAOs could not play a good role in removing phosphorus due to the lack of O2 electron acceptor. As the domestication progressed, the number of DPAOs in the system gradually increased. DPAOs became the dominant bacteria of the system, and the removal rate of TP increased above 92.00% from the 28th day. After 30 days’ domesticated cultivation, The mass concentration of COD, nitrite nitrogen and TP of effluent were 19.86 mg/L, 1.99 mg/L, 0.79 mg/L respectively, and the removal rate of them reached 88.32%, 92.03% and 92.15% respectively, which reached the "pollutant discharge standard for urban sewage treatment plants" (GB18918-2002) level 1 B standard.

![Figure 6](image1.png)  ![Figure 7](image2.png)

**Figure 6.** Concentration variation, removal rate of TP and removal rate of NO2−-N of A reactor in second stage.

**Figure 7.** Concentration variation of COD of B reactor in third stage.

### 3.2.2. Three-phase Acclimation

The three-phase acclimation method lasted for 28 days, of which the second phase lasted 18 days and the third stage lasted for 10 days. Figure 7 showed the variation on concentration of COD in the reactor B for the second phase and the third phase. It could be seen that in the first day, the COD concentration was 215.37 mg/L in influent and was still 60.84 mg/L after anaerobic stage. Analysis suggested the content of residual carbon was too much, so the mass concentration of COD in influent was reduced to as low as 170.00 mg/L. No extra carbon source was added to the anoxic stage. The concentration of COD in the system was lower than 12.00 mg/L.
8 showed the variation on concentration and removal rate of TP, and removal rate of nitrite nitrogen in the reactor B for the second phase and the third phase. The initial removal rate of TP and nitrite were 50.34% and 34.70% respectively. The reason for this analysis was that the number of DPAOs in the system with nitrite nitrogen as electron acceptor was very small, so the denitrifying dephosphatation was poor. The conventional anti-nitrification could not take place because of the deficiency of the external carbon source and only a small part of nitrite was used by DPAOs. So the removal rates of TP and nitrite nitrogen were not high. According to the test results, DPAOs with nitrite nitrogen as the electron acceptor were present in the system after the domestication of first phase. As the reaction proceeded, the removal rate of TP and nitrite nitrogen increased gradually. The graph showed that the removal rates of nitrite nitrogen and TP were highly relevant, it was indicated that there were already a significant amount of DPAOs in the system. The removal rates of TP and nitrite nitrogen were more than 94.00% as the reaction was progressing into its fifth day. DPAOs accumulated a large number of PHB as an electron donor for phosphorus-uptake after phosphorus-release in anaerobic stage, at the same time, using nitrite nitrogen as electron acceptor to remove phosphate and nitrite in the system successfully.

![Figure 8. Concentration variation, removal rate of TP and removal rate of NO\textsubscript{2}-N of B reactor in second and third stages](image)

Figure 8. Concentration variation, removal rate of TP and removal rate of NO\textsubscript{2}-N of B reactor in second and third stages

Through the domestication of the second phase, DPAOs had become the dominant population in the system. In the third phase, the secondary influent part of the anoxic was canceled, and the concentration ratio of COD and TP in the anaerobic influent was controlled to 17. Under these conditions, the system steadily ran for 10 days, and the removal rates of TP, COD and nitrite nitrogen reached 95.47%, 94.73% and 94.73% respectively, and the mass concentration of them were 0.45 mg/L, 1.31 mg/L and 17.07 mg/L, which reached the "pollutant discharge standard for urban sewage treatment plants" (GB18918-2002) level 1 A standard. The stable running of the system illustrated the success of DPAOs domestication.

3.3. Analysis of result

The results of the two acclimatization methods showed that DPAOs can be successfully acclimated by methods of anaerobic/anoxic, and the three-phase acclimation method was shorter and more efficient, and the better sewage treatment effect can be achieved. Analysis suggested that the remaining external carbon source after the anaerobic stage was discharged in the second stage of the three-phase
acclimation and add no external carbon source water, which made the process of phosphorus uptake was not affected by the external carbon source. It could be considered that the concentration of exogenous carbon source had a great influence on the effect of denitrifying phosphorus. If the amount of residual external carbon source was too much at the end of anaerobic stage, it would be used by the oxygen-depleting organic presented in the later of anaerobic stage. In that case, it was beneficial to the denitrification reaction of the traditional denitrifying bacteria in the anoxic stage, which formed the competition with the DPAOs on the electronic receptor, and resulted in inhibition of phosphorus absorption. Ultimately, the growth and reproduction of DPAOs got damaged. Under the condition of low COD concentration, DPAOs would use internal carbon sources for denitrification while removing phosphorus, so it could be conclude that low concentration of COD environment would benefit DPAOs. This might be the main reason for the better results of the three-phase acclimation method.

4. Conclusion

- In the process of periodic culture, the domestication of sludge was carried out. First cultivated the traditional PAOs and made them be the dominant species in the system; then domesticated and enriched DPAOs by continuous dropping nitrite nitrogen solution. The short-cut denitrifying phosphorus removal system could be start up successfully.
- The results of the two reactors showed that the three-stage acclimation method was more suitable for the domestication of DPAOs. The difference between the two methods was that the influent quality in the second stage of the three-stage acclimation method, and it was the critical stage. The concentration of COD in the system after the end of the anaerobic stage was the main factor affecting the enrichment of DPAOs.

Acknowledgments

This work was supported in part by the Doctoral Scientific Research Foundation of Liaoning Province, China (No. 201501069), and Foundation of Liaoning Educational Committee (No. LJZ2016014). In addition, the study was sponsored by National Nature Science Foundation of China (No. 51508342). National water pollution control and control scientific and technological special project (2014ZX07202-011). The authors declare that they have no conflict of interest.

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