Recent Advances in Characteristics and Technologies of Denitrifying Phosphorus-accumulating Organisms

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Abstract: Denitrifying phosphorus-accumulating organisms (DPAOs) have attracted continuous attention from researchers because of that they could achieve simultaneously nitrogen and phosphorus removal. So far, many new technologies about DPAOs have been developed in recent years. At present, there are some contradictions about the utilization of nitrate by DPAOs and the interaction between DPAOs and other organisms. In addition, new technologies based on DPAOs have some difficulties in practical operation. This paper reviews recent advances in characteristics and technologies of DPAOs. The influences of carbon source and electron acceptor on DPAOs are also discussed.

1 INTRODUCTION

In wastewater treatment plants (WWTPs), Enhanced biological phosphate removal (EBPR) technique is the main phosphate removal method. The key mechanism of EBPR is the excessive phosphorus absorption by phosphorus-accumulating organisms (PAOs) in active sludge (Mino et al., 1998). However, the difficulties of adjusting parameters and the sludge age contradiction between PAOs and nitrifying bacteria lead to the instability of EBPR in practical operation (Shukla et al., 2020). Longer sludge age is conducive to the growth of nitrifying bacteria, and the increase of the abundance of nitrifying bacteria can effectively improve the removal efficiency of ammonia nitrogen, but too long sludge age will reduce the abundance of PAOs, resulting in high phosphorus concentration in effluent.

In the 1990s, researchers found some PAOs could utilize nitrate as electron acceptor to achieve orthophosphate uptake in anoxic phase (Kuba et al., 1993). This kind of PAOs was named denitrifying phosphorus-accumulating organisms (DPAOs). According to the features of simultaneous denitrification and phosphorus uptake of DPAOs, researchers have developed denitrification phosphate removal (DPR) process and applied it to WWTPs . Compared to EBPR, DPR reduces the need for aeration and carbon source and less sludge production due to the low growth rate of DPAOs. Hence, compared to EBPR, DPR is more energy efficient. Recently years, many technologies based on DPR have been proposed and applied to different scenarios (Zhang et al., 2020). However, there are drawbacks to the DPR process. In DPR process, heterotrophic denitrification bacteria are always dominant in the process of carbon source competition due to different mechanisms of carbon source utilization, that is, denitrification and nitrogen removal is preferred, thus affecting the efficiency of denitrification and phosphorus removal. Therefore, in recent years, many researches have optimized DPR process and developed new technology.

This paper reviewed current studies on characteristics of DPAOs and its coupling process and provided guidance for further research.

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2 CHARACTERISTICS AND INFLUENCING FACTORS OF DPAOs

2.1 The Metabolic Mechanism of DPAOs

Since the discovery of DPAOs, researchers have done many studies on its metabolic mechanism and denitrifying phosphorus removal characteristics.

The metabolic mechanism of DPAOs can be divided into two phases as shown in Figure 1 (Mino et al., 1998). In anaerobic phase, DPAOs produce acetyl-CoA through the degradation of stored glycogen to provide reducing power ($NADH_2$) and break polyphosphates (poly-P) to generate energy (ATP) and store as polyhydroxyalkanoates (PHAs) (Ahn et al., 2001). At the macro level, this metabolic process is represented by the reduction of organic matters and the increase of orthophosphates in wastewater. In addition, DPAOs uptake carbon sources in both active and passive transportation. Hence, when the concentration of organic matter in the water is low, such as in a continuous flow reactor, DPAOs have the advantage in carbon sources uptake (Tu & Schuler, 2013). In anoxic phase, DPAOs uptake orthophosphates excessively to regenerate poly-P and synthesis glycogen by utilizing the internal PHAs. In the meanwhile, DPAOs reduce nitrate to nitrogen gas. Because DPAOs uses nitrate or nitrite as electron acceptor in the process of phosphorus absorption, the nitrogen removal effect of DPAOs is affected by phosphorus concentration. The more phosphorus released by DPAOs, the better the nitrogen removal.

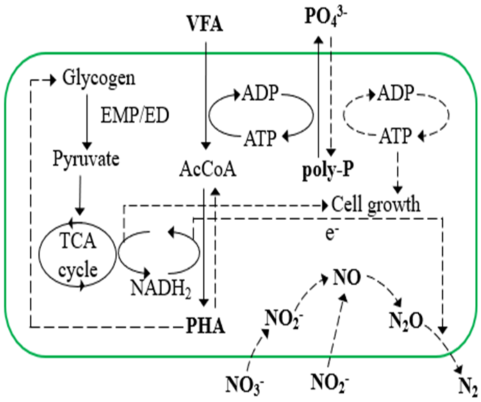

Figure 1: The metabolic mechanism of DPAOs (The solid line presents the metabolism in anaerobic condition and dotted line presents the one in anoxic condition).

The general metabolic pathways of DPAOs are described above. However, different carbon sources have a significant impact on PHAs storage of DPAOs and the distinction of electron acceptors utilization is also visible between different strains under anoxic condition.

2.2 Carbon Source

Since DPAOs can only use volatile fatty acids (VFAs) as energy sources, researchers generally use propionate and acetate to grow DPAOs. DPAOs cultured with acetate and propionate as a single substrate or mixed substrate show different denitrification and phosphorus removal performance and substrate absorption rate (Lu et al., 2006). It was found that acetate-based DPAOs collapsed after the aerobic phase was cancelled, while propionate-based DPAOs remained stable. By analyzing the types of PHA and their contents, acetate-based DPAOs synthesized more polyhydroxybutyrate (PHB) and propionate-based DPAOs mainly synthesized polyhydroxyvalerate (PHV) and 3-hydroxy-2-methylvalerate (3H2MV) but not PHB. The glycogen content also showed differences between acetate-based DPAOs and propionate-based DPAOs. The former was usually higher than the latter (Carvalho et al., 2007). The glycogen required to store acetate as PHA was higher than that required for propionate, which means acetate-based DPAOs need to produce more glycogen for VFA uptake and the elimination of aerobic phase limited the amount of glycogen stored in acetate-based DPAOs. Hence, propionate maybe is a better substrate for DPAOs than acetate.

Using acetate and propionate simultaneously is more favourable to DPAOs enrichment and does not need to adjust pH, while a pH above 7.5 is required for DPAOs cultivation when using acetate . It may be related to the increasing concentration of the acetic acid when pH below 7.5 because the acetic acid form of the acetic acid/acetate acid−base pair may negatively affect the growth and metabolism of some organisms (Tu & Schuler, 2013). Another strategy of DPAOs enrichment is to alternate the sole carbon sources between acetate and propionate, which achieved up to 90% biological abundance of DPAOs in a lab-scale reactor (Lu et al., 2006).

Although there are many studies on the effects of carbon source types on DPAOs metabolism, most carbon sources used in these researches are simple short-chain organic compounds. However, the carbon source in actual sewage is mostly long-chain organic matter, and the utilization process of carbon source by DPAOs is more complicated. Therefore, future research should pay more attention to the utilization of organic matter in actual sewage by DPAOs.

2.3 Electron Acceptor

In early study, researchers argued DPAOs could only utilize nitrate in anoxic phase and nitrite was considered as an inhibitor for denitrifying phosphorus removal of DPAOs. However, later researchers successfully cultured nitrite-based DPAOs in the lab, showing a great phosphorus uptake performance at the nitrite concentration of 45mg/L (Zhang et al., 2010). This contradiction may be due to the free nitrous acid (FNA) which restrains DPAOs uptake orthophosphates in anoxic phase (Pijuan et al., 2010). Moreover, the presence of FNA can inhibit PHA oxidation, glycogen regeneration and cell growth. The concentration of FNA is related to the concentration of nitrite and pH value of water, and a small amount of FNA can seriously inhibit the metabolic growth of DPAOs. Therefore, when nitrite acts as the electron acceptor of DPAOs, the pH must be strictly controlled to ensure that the concentration of FNA is kept at a very low level. With the development of metagenomics, DPAOs have been classified more carefully depending on the denitrification capability.

The Candidatus Accumulibacter phosphatis (Accumulibacter) are the most common PAOs in study. Accumulibacter Type I and Accumulibacter Type II differ in the use of electron acceptors. The former can utilize both nitrate and nitrite whereas the later can only reduce nitrite due to the lack of gene responsible for the encodification of the respiratory nitrate reductase (Martín et al., 2006). However, recent research showed that in a highly enriched Accumulibacter Type I bioreactor a small amount of phosphorus uptake occurred in anoxic phase when using nitrate as electron acceptor (Rubio-Rincon et al., 2017). Accumulibacter Type I were cultured with nitrite as electron acceptor in a reactor, and mixed carbon source was used to eliminate glycogen-accumulating organisms (GAOs) which competed with PAOs for carbon source in anaerobic phase. After successful enrichment of Accumulibacter Type I, the electron acceptor was replaced with nitrate and almost no phosphorus uptake occurred. On the contrary, when denitrifying glycogen-accumulating organisms (DGAOs), which could reduce nitrate to nitrite, coexisted with Accumulibacter Type I in another reactor, orthophosphates and nitrate were obviously removed in anoxic phase. This difference implied that when DPAOs and DGAOs coexisted, DGAOs first converted nitrite into nitrite and then DPAOs used nitrite to achieve phosphorus uptake. Hence, the relationship between DPAOs and DGAOs cannot be simply concluded as a competitive relationship. The relationship between them may change from competition to co-existence with the change of environmental conditions. Figure 2 illustrates the possible nitrogen metabolic patterns in the coexistence of DPAOs and DGAOs.

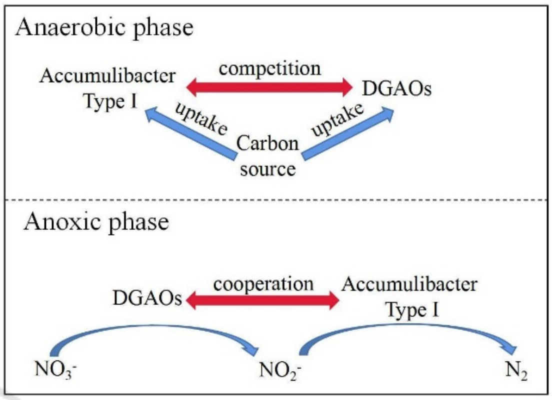

Figure 2: The potential nitrogen metabolic pathways of DPAOs and DGAOs system.

3 RECENT TECHNOLOGIES OF DPAOs

Many new technologies of DPAOs have been developed for the past few years with the deep understanding of DPAOs characteristics. In order to achieve deep removal of the nutrient, Fu et al. enriched DPAOs and heterotrophic nitrifying bacteria in a single-stage biofilter to treat secondary effluent (Fu et al., 2019). The abundance of heterotrophic nitrifying bacteria decreased with the increase of reactor height, while DPAOs increased gradually. Therefore, heterotrophic denitrification mainly occured in the middle and lower part of the reactor, while denitrification phosphorus removal occured in the upper part. Nevertheless, this treatment effect was based on the effluent COD up to 120mg/L, which means additional carbon sources need to be added. And whether an excessively long anoxic period (40 hours) affects DPAOs metabolism remains to be studied in a longer term.

Wang et al. enriched DPAOs and DGAOs in an anaerobic/anoxic/short micro-aerobic sequencing batch reactor (SBR) and achieved 75.3% nitrate-to-nitrite transformation rate and 92.3% phosphorus removal efficiency (Wang et al., 2019a). In this system, DPAOs and DGAOs stored PHA anaerobic and in the anoxic phase DPAOs completed denitrification and phosphorus uptake while DGAOs achieved partial denitrification. In the meanwhile, the excess organic matter was used for denitrification by heterotrophic denitrifying bacteria. In the micro-aeration stage, PAOs utilised oxygen to achieved excessive phosphorus uptake, reducing the remaining phosphorus concentration. On this basis, they combined anammox with this technology to reduce the total nitrogen and total phosphorus to 6mg/L and 0.2mg/L, respectively (Wang et al., 2019b). On the one hand, this process can save the aeration, on the other hand, it can realize the deep removal of nitrogen and phosphorus under the condition of low organic matter. However, there are still many difficulties in realizing the mainstream anammox technology in WWTPs.

Some researchers enriched DPAOs/AOB and anammox in an anaerobic/anoxic/aerobic continuous flow reactor and a moving bed biofilm reactor, respectively (Zhang et al., 2020). The effluent total nitrogen and phosphorus were 10.26mg/L and 0.21mg/L, respectively. In addition, this process reduced carbon source requirement by 16% and oxygen requirement by 15%. This indicates that phosphorus removal via nitrite can maximize carbon sources than nitrate, which is very important for many urban sewage plants with low organic matter concentration influent.

Recently, a new technology was proposed, called SNADPR, for simultaneous nitrification, denitrification and phosphorus removal, which enriched anammox, DPAOs and ammonium oxidizing bacteria (AOB) in a single SBR (Xu et al., 2019). The main mechanism of SNADPR is shown in Figure 3. The SNADPR could remove 89.15 ± 2.19% of total nitrogen and 92.93 ± 0.60% of total phosphorus. Interstingly, the SNADPR process used a filler in the reactor and anammox mainly grew on the filler while DPAOs and AOB were principally located in suspended sludge. The strategy of adding fillers solved the problem of sludge retention time contradiction between anammox and DPAOs, which allowed anammox, DPAOs and AOB to coexist in a single SBR. The technology of combining activated sludge with carrier at the same time may solve the problem of different microbial sludge age conflict and provide the possibility of mainstreaming anammox in sewage plants. But in the meanwhile, this approach will also bring about the carrier crushing and aging sludge treatment efficiency decline.

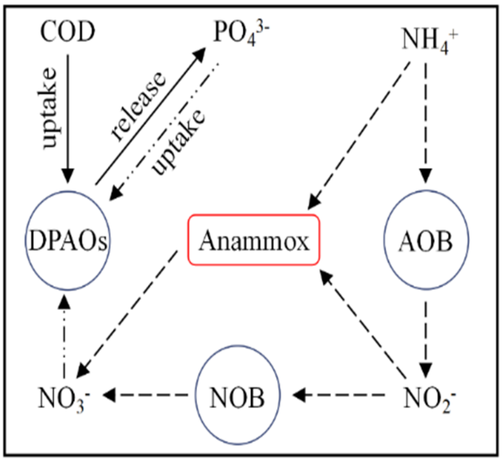

Figure 3: The mechanism of SNADPR (The solid line presents the metabolism in anaerobic condition, dotted line presents the one in aerobic condition and chain-dotted line presents the one in anoxic condition).

Although these technologies can achieve good treatment effect, most of them are in the laboratory stage, and the difficulty of sludge acclimation, complexity of operation and process instability limit their practical application. In addition, WWTPs tend to operate in a continuous flow mode, and the SBR model used in most studies also hinders the application of these technologies in practice. Therefore, future studies on these new processes should pay more attention to their performance in pilot tests, and the comprehensive evaluation should be carried out in combination with the treatment effect, economy and ease of operation..

4 CONCLUSIONS

Although the metabolic mechanism of DPAOs is generally clear, there are still some confusions about the utilization of nitrate and the classification of DPAOs. In addition, few studies focused on the interaction between DPAOs and other organisms, such as DGAOs, which need to be studied in the future. The combination of anammox technology and DPR can effectively reduce the energy consumption of wastewater treatment and the need for influent organic matter, so it is also an important direction in the future. In terms of process research, most used SBR mode, and the future research should focus on continuous flow process which is easier to be applied in WWTPs.

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