Phosphorus Accumulating Bacteria and its Mechanism of Phosphorus Removal in Biological Enhanced Phosphorus Removal

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Abstract: The eutrophication of water body is the key concern of the current water environment protection work. Enhanced biological phosphorus removal (EBPR) has become an important method for ecological restoration of eutrophic water bodies due to its high efficiency, low consumption and no secondary pollution. In recent years, the research on phosphate accumulating organisms (PAOs) in EBPR system and its application in sewage treatment process have become more and more extensive. In this paper, the research progress of PAOs in EBPR system is reviewed, and several PAOs that play an important role in EBPR system are analyzed. Then, the phosphorus removal mechanism of EBPR was described from three aspects: enzymatic reaction, biochemical process and EBPR participated by extracellular polymeric substance (EPS). Finally, based on the existing problems of EBPR, the research direction of EBPR in the future is prospected in order to provide reference for improving the phosphorus removal mechanism of PAOs and EBPR process.

1. Introduction

There is a large amount of phosphorus in urban sewage. The discharge of phosphorus-containing sewage will cause eutrophication of water body, destroy ecological balance, cause water pollution, and lead to water shortage and waste of phosphorus resources. The eutrophication of water body will also lead to the reduction of dissolved oxygen in the water, which will lead to the proliferation of cyanobacteria, green algae and other algae in the water. These algae will produce algal toxins, which will lead to the death of a large number of animals and plants in the water. Direct drinking of livestock and poultry will cause poisoning and disease, which will threaten human health. In order to solve the contradiction between the increasing shortage of phosphate rock resources and the deterioration of water quality caused by the high phosphorus content in the water body, the removal and recovery of phosphorus from urban sewage has become an effective means of resource utilization. The phosphorus recovered from wastewater can meet 15-20% of the future phosphorus demand [1].

Chemical precipitation method and biological phosphorus removal method (especially enhanced biological phosphorus removal, EBPR) are usually used for phosphorus removal in urban sewage. The biological phosphorus removal method uses the characteristics of anaerobic phosphorus release and aerobic excessive phosphorus absorption of the functional microorganisms in the sludge of the phosphorus removal system to remove phosphorus from the sewage. The sewage will first pass through the anaerobic zone, and the functional microorganisms in the system will release phosphorus in the sewage under anaerobic conditions, and then the sewage will enter the aerobic zone. The functional microorganisms in the activated sludge can over-absorb phosphorus in the external sewage under aerobic conditions, Phosphorus-rich sludge produced in the aerobic zone will be discharged as surplus sludge of the system, and the phosphorus in the sewage will be finally removed [2]. Biological phosphorus removal method has high efficiency in treating low phosphorus concentration wastewater, and it is difficult to treat sludge without chemical precipitation method. Phosphorus-rich sludge can be recovered after treatment, so it is widely used in actual sewage treatment plants [3].

2. Research progress of phosphorus accumulating organisms (PAOs) in EBPR system

The EBPR system can stably remove phosphorus from sewage, which mainly depends on the integrity of microbial function and structure in the EBPR system. There are a large number of microorganisms in the EBPR system, and functional microorganisms have always been the focus of research, where *γ-proteobacteria* is the first microorganism found to have phosphorus removal function. In the 1870s, Fuhs et al. [4] obtained an Acinetobacter with phosphorus removal ability for the first time by using the pure culture method. Therefore, it was generally believed that the microorganism with phosphorus removal function was Acinetobacter. However, in later studies, it was found that the purified single Acinetobacter did not conform to the typical biological metabolism model proposed by Mino, so it was determined that Acinetobacter was not the main functional microorganism in the EBPR system.

In the study of Brodish and Joyner [5], it was found that Aeromonas can hydrolyze xylose and urea very well, and has the ability to remove phosphorus. In addition, Aeromonas can also use nitrate as an electron acceptor to carry out biological phosphorus removal process, so it is considered that Aeromonas is a functional microorganism in the EBPR system. In 1985, Suresh et al. [6] found polyphosphates stored in the cells of Pseudomonas, and the content of polyphosphates accounted for 31% of the cell dry weight, so they believed that Pseudomonas also had the ability to remove phosphorus.

With the development of molecular biology, some researchers found that in the EBPR system the abundance of microorganisms such as *β-proteobacteria* and *Actinobacteria* was higher than that of *Acinetobacter*. Bond et al. [7] found through FISH and 16s RNA technology, *Rho-docyclacae* in the *β-proteobacteria* is the main functional microorganism in EBPR system. Hesselmann et al. first named it "Candidatus Accumulactor photosphatis", which is usually abbreviated as "*Ca.Accumulibacter* ".

In 1994, it was found that the microorganisms at the gate level indicated by HGC probe had the characteristics of phosphorus accumulation. In 2000, Maszenan et al. [8] discovered two strains of PAOs that were not under *Actinobacteria* through 16 SrRNA gene sequencing, and named them *Tetrasphaera*. In 2003, Eschenhagen et al. [9] first detected two kinds of *Tetrasphaera* in two urban sewage treatment plants in Germany, and found that the proportion of *Tetrasphaera* in activated sludge was much higher than that of *Ca.Accumulibacter*. The proportion of *Tetrasphaera* and *Ca.Accumulibacter* was 10% and 3%-5% respectively. In 2005, Kong et al. [10] detected *Tetrasphaera* in the activated sludge of the sewage treatment plant in Denmark. The abundance of *Tetrasphaera* was 2-3 times that of the traditional PAOs *Ca.Accumulibacter*. Through experiments, it was proved that *Tetrasphaera* has phosphorus removal ability, and it was officially confirmed that it is a new PAOs. In 2011, Nguyen et al. [11] tested and studied the abundance of *Tetrasphaera* in a large number of actual sewage treatment plants, and further confirmed the phosphorus release and absorption capacity of *Tetrasphaera*. It was not until 2019 that the phosphorus removal contribution of *Tetrasphaera* was confirmed. Fernando et al. [12] analyzed and studied *Tetrasphaera* using Raman spectroscopy technology, and proved for the first time that *Tetrasphaera* has the same phosphorus removal contribution as *Ca.Accumulibacter*.

3. Research progress in mechanism of EBPR

3.1 Enzymatic process

Whether under aerobic or anoxic conditions, the process of microbial phosphorus accumulation is to absorb the phosphate in the environment and then store it in the cell in the form of poly-P. The above process is catalyzed by Polyphosphate kinase (PPK) [13]. PPK is the necessary enzyme for microbial phosphorus accumulation. Therefore, some researchers believe that all PAOs contain PPK [14], which is encoded by ppk gene [15]. Although PPK is widely found in bacteria, it is rarely found in fungi and archaea. After being catalyzed by PPK, the phosphate group at the end of ATP and the inorganic phosphate in the environment can be reversibly transferred to the long-chain poly-P to form a linear or cyclic polymer of orthophosphate with a length of 1000 or more. The reaction formula is as follows:

$$\mathrm{ATP} + n\mathrm{PO}_4^{3-} \xrightleftharpoons{\mathrm{PPK}} \mathrm{ADP} + (\text{poly-P})_{n+1}$$

It can be seen from the above reactions that the process of microbial phosphorus removal is closely related to the process of material metabolism and energy metabolism.

3.2 Biochemical process

Due to the synergy of nitrogen and phosphorus removal in many biological enhanced phosphorus removal processes, this paper takes the biochemical process of simultaneous nitrogen and phosphorus removal as an example to illustrate its phosphorus removal mechanism.

Under anaerobic conditions, the poly-P in the cells of PAOs is hydrolyzed, and the inorganic phosphate (PO_4^{3-}-P) produced will be released into water. At the same time, PAOs can absorb easily degradable low molecular fatty acids (volatile fatty acids, VFAs), such as acetic acid, propionic acid, *n*-butyric acid, valeric acid and isovaleric acid, into the body by using the ATP produced by poly-P hydrolysis and the reducing power provided by glycogen decomposition $NADH_2$, which is activated to generate Acetyl-CoA (AcCoA), and finally stored in the body in the form of PHA, which is the anaerobic phosphorus release process of PAOs. It is worth noting that PHA are biodegradable carbon polymers, mainly including poly-β-hydroxybutyrate (PHB) and polyhydroxyvalerate (PHV), which can not only serve as the carbon source for biochemical reactions under aerobic conditions to provide ATP for cell growth and metabolic activities, but also can be directly decomposed as energy under anoxic conditions to form proton driving force and provide electrons for electron transmission chain [16]. Under anoxic conditions, PAOs utilize NO_3^--N/NO_2^--N to be an electron acceptor. The PHA stored in the cell during anaerobic phosphorus release is decomposed into AcCoA. AcCoA can enter the tricarboxylic acid cycle. The generated electrons are transferred by electrons to generate ATP to supply energy for their own growth and life activities. Moreover, the released H^+ forms a proton driving force, and excessive absorption of extracellular PO_4^{3-}-P. PO_4^{3-}-P is stored in the cell in the form of poly-P, and NO_3^--N/NO_2^--N is reduced to gaseous nitrogen after receiving electrons, where simultaneous nitrogen and phosphorus removal is realized [17]. Under aerobic conditions, PAOs can use O_2 as the electron acceptor to generate CO_2 from the PHA stored in the anaerobic phosphorus release stage of the cell through a series of oxidation reactions, and produce more ATP than under anoxic conditions, which are respectively used for microbial growth, glycogen synthesis and excessive uptake of PO_4^{3-}-P in the environment is stored in the cell in the form of poly-P. At the same time, the denitrification process can also use PHA as carbon source, which will provide energy and electrons for the of reduction NO_3^--N/NO_2^--N to gaseous nitrogen [18]. To sum up, the nitrogen and phosphorus removal mechanism of PAOs is shown in Figure 1.

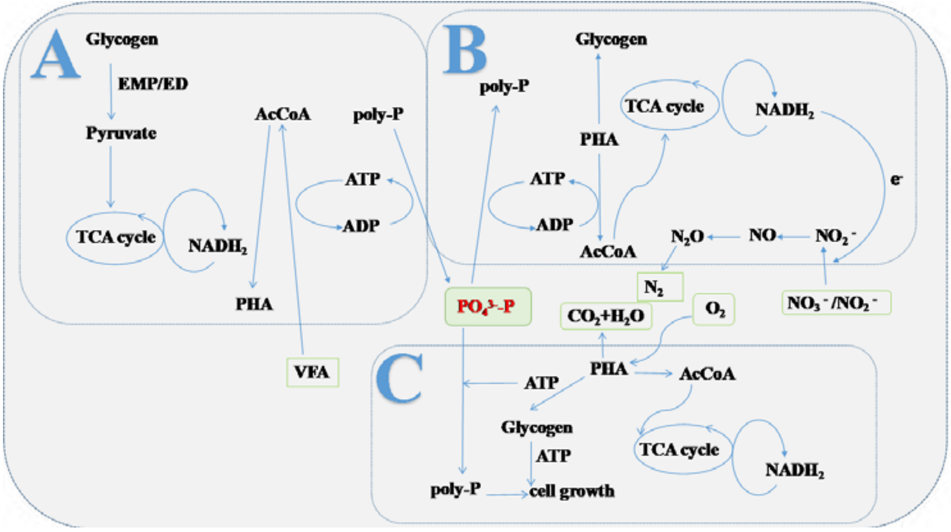

Figure 1 Mechanistic diagram of nitrogen and phosphorus removal by DPAOs (A: anaerobic B: anoxic C: aerobic).

3.3 Role of Extracellular polymeric substances (EPS) in EBPR system

Extracellular polymeric substances (EPS) is an important component of biological flocs, mainly derived from microbial secretion, cell lysis and organic substances in wastewater [19,20]. Its structure concept diagram is shown in Figure 2. A large number of studies have shown that EPS in EBPR activated sludge contains a large amount of phosphorus, and its role in EBPR cannot be ignored. It is necessary to re-examine the existing biological phosphorus removal theory [21].

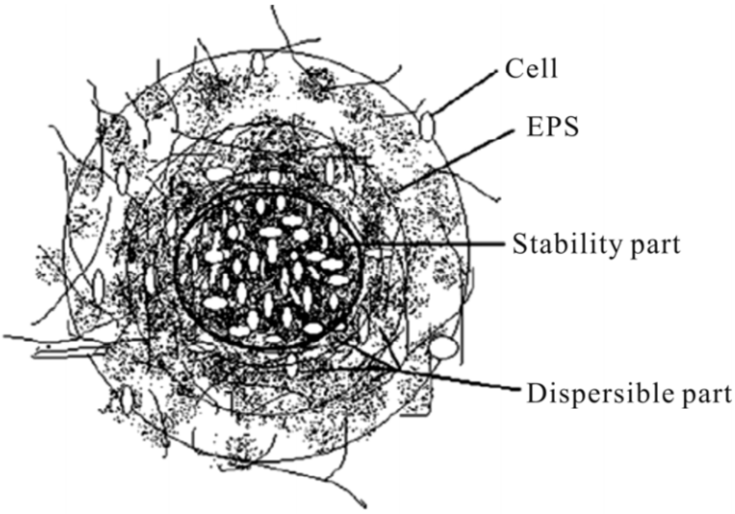

Figure 2 Conceptual model of EPS

More and more evidences show that EPS plays an important role in maintaining the stability of EBPR system. Generally speaking, EPS may promote the removal of P in EBPR process in the following ways. First, EPS, as a "bridge" connecting bacterial cells and the main liquid phase, can protect PAOs from the pollution of toxic substances (such as heavy metals). It is well known that high concentrations of heavy metal ion, such as Cu^{2+} and Ni^{2+}, will inhibit the metabolism of PAOs [22]. EPS can intercept these toxic chemicals to ensure the physiological activity of PAOs. Mu et al. [23] reported that the negatively charged residues in EPS can bind Zn^{2+}, and the chelating property of EPS can reduce the toxicity of Zn^{2+} to internal microorganisms. Therefore, when there are toxic substances in the EBPR system, the microbial cells in the sludge flocs will tend to produce more EPS to protect themselves from the adverse environment [24].

Secondly, during the operation cycle of EBPR system, ACP sediment will gradually form in EPS [25], which will be beneficial to phosphorus removal. It is worth noting that the formation of inert phosphate precipitation in EPS will affect the metabolic process of PAOs, because a large amount of "inert" phosphate precipitation accumulated in EPS will hinder the migration and transformation of PO_4^{3-} in the anaerobic/aerobic process. Zhang et al. [26] pointed out that when the concentration of Ca^{2+} in influent water is too high, a large amount of HAP precipitation will be synthesized in EPS, and the metabolic pathway of PAOs may change from polyphosphate (PAM) metabolism to glycogen metabolism (GAM). However, some studies have shown that although the synthesis of "inert" phosphate in EPS can inhibit the EBPR process, it can still achieve stable and effective removal of P, which is due to the high content of EPS can significantly promote P adsorption/precipitation [27].

In other words, phosphorus removal by EBPR system includes microbial metabolism phosphorus removal and EPS induced precipitation phosphorus removal. If a large amount of phosphorus can be accumulated in EPS, even if more GAOs proliferate than PAOs, phosphorus removal will not be weakened. The above research will be more conducive to the management of EBPR process. More importantly, the formed HAP precipitation is very stable and can be easily dehydrated and used as an available fertilizer [28], which further increases the attraction of EPS induced phosphorus precipitation. Therefore, in the actual EBPR operation, it is necessary to balance the effects of biological factors and chemical factors [29].

4. Conclusion

EBPR has become an important biotechnology with high efficiency, low consumption, energy conservation and emission reduction in the application of repairing eutrophic water. Clarifying the phosphorus removal mechanism of PAOs can not only promote the understanding of the interaction between different metabolic pathways, but also help improve the existing phosphorus removal process, which is of great significance for the bioremediation of eutrophic water and the treatment of another phosphorus-containing wastewater. Based on previous research reports, this paper systematically analyzed the research progress and phosphorus removal mechanism of PAOs, especially the role of EPS in EBPR. However, there are still many areas that have not yet been clarified. In the future, research can be carried out from the following aspects.

(1) The research on PAOs mainly focuses on the phosphorus metabolism process at the biochemical level, and the key genes and enzymes that regulate the phosphorus metabolism process of PAOs have not yet been identified. Therefore, the phosphorus removal mechanism of PAOs can be more comprehensively understood through the collaborative analysis of genomics, transcriptome, proteome and metabolome.

(2) The response mechanism of the influencing factors in the EBPR process has not been clarified. Some key regulatory factors can be studied by combining reaction kinetics, spectral scanning analysis and other technical methods to build the response model of the influencing factors.

(3) The efficiency of phosphorus removal can be improved by constructing genetically engineered bacteria with high phosphorus removal efficiency. Its significant advantage is that it can regulate the phosphorus metabolism process of microorganisms through gene recombination or gene editing and other technical means. However, the construction of genetic engineering PAOs for efficient phosphorus removal also depends on the excavation of efficient functional genes of wild strains.

References

[1] Mihelcic J R, Fry L M, Shaw R. Global potential of phosphorus recovery from human urine and feces. Chemosphere, 2011, 84(6): 832-839.

[2] Ma Juan, Wang Fangjun, Tian Wenqing, Cai Yuqi, Zhong Jingchao, Deng Ying, Chen Yongzhi, Wang Gang. Effects of long-term exposure to ciprofloxacin on the performance of an enhanced biological phosphorus removal (EBPR) and its microbial structure. Journal of Environmental Chemical Engineering, 2022, 10(3).

[3] Hao Tianwei, Lin Qingshan, Ma Jie, Tang Wentao, Xiao Yihang, Guo Gang. Microbial behaviours inside alternating anaerobic-anoxic environment of a sulfur cycle-driven EBPR system: A metagenomic investigation. Environmental research, 2022, 212.

[4] Fuhs G W, Chen M. Microbiological basis of phosphate removal in the activated sludge process for the treatment of wastewater. Microbial Ecology, 1975, 2(2): 119-138.

[5] Brodisch K E U, Joyner S J. The role of micro-organisms other than Acinetobacter in biological phosphate removal in activated sludge processes. Water Science and Technology, 1983, 15(3-4): 117-125.

[6] Suresh N, Warburg R, Timmerman M, et al. New Strategies for the Isolation of Microorganisms Responsible for Phosphate Accumulation. Water Science &Technology, 1985, 17(11-12): 99-111.

[7] Bond P L, Keller J, Blackall L L. Anaerobic phosphate release from activated sludge with enhanced biological phosphorus removal. A possible mechanism of intracellular pH control. Biotechnology and Bioengineering, 1999, 63(5): 507-515.

[8] Maszenan A M, Seviour R J, Patel B, et al. Three isolates of novel polyphosphate-accumulating gram-positive cocci, obtained from activated sludge, belong to a new genus, Tetrasphaera gen. nov, and description of two new species, Tetrasphaera japonica sp. nov. And Tetrasphaera australiensis sp. nov. International Journal of Systematic and Evolutionary Microbiology, 2000, 50(2): 593-603.

[9] Eschenhagen M, Schuppler M, Röske I. Molecular characterization of the microbial community structure in two activated sludge systems for the advanced treatment of domestic effluents. Water research, 2003, 37(13): 3224-3232.

[10] Kong Y, Nielsen J L, Nielsen P H. Identity and ecophysiology of uncultured actinobacterial polyphosphate-accumulating organisms in full-scale enhanced biological phosphorus removal plants. Applied and environmental microbiology, 2005, 71(7): 4076-4085.

[11] Nguyen H T T, Le V Q, Hansen A A, et al. High diversity and abundance of putative polyphosphate-accumulating Tetrasphaera-related bacteria in activated sludge systems. FEMS Microbiology Ecology, 2011, 76(2): 256-267.

[12] Fernando E Y, Mcilroy S J, Nierychlo M, et al. Resolving the individual contribution of key microbial populations to enhanced biological phosphorus removal with Raman-FISH. The ISME Journal, 2019, 13(8): 1933-1946.

[13] Zhang MY, Pan LQ, Liu LP, et al. Phosphorus and nitrogen removal by a novel phosphate-accumulating organism, Arthrobacter sp. HHEP5 capable of heterotrophic nitrification-aerobic denitrification: Safety assessment, removal characterization, mechanism exploration and wastewater treatment. Bioresource Technology, 2020, 312: 123633.

[14] Zheng XL, Sun PD, Lou JQ, et al. The long-term effect of nitrite on the granule-based enhanced biological phosphorus removal system and the reversibility. Bioresource Technology, 2013, 132: 333-341.

[15] Shivani S, Ankita R, Hema S, et al. Simultaneous nitrification-denitrification by phosphate accumulating microorganisms. World Journal of Microbiology & Biotechnology, 2020, 36(10): 151.

[16] Miao XN, Cheng C, Zhu L, et al. Research progress on the coupling process of short-cut nitrification and denitrification for phosphorus removal. Technology of Water Treatment, 2020, 46(12): 12-16, 24 (in Chinese).

[17] Li W, Wang YQ, Hou YH, et al. Enrichment and agent preparation of Gordonia for denitrifying phosphorus removal. Microbiology China, 2022, 49(6): 2022-2036 (in Chinese).

[18] Zhao WH, Zheng SH, Wang K. Research progress of the mechanism and process of the wastewater denitrifying phosphorus removal. Technology of Water Treatment, 2020, 46(7): 1-5, 25 (in Chinese).

[19] Zhang H L, Fang W, Wang Y P, et al. Phosphorus removal in an enhanced biological phosphorus removal process: roles of extracellular polymeric substances. Environmental Science & Technology, 2013, 47(20): 11482-11489.

[20] Long X, Tang R, Fang Z, et al. The roles of loosely-bound and tightly-bound extracellular polymer substances in enhanced biological phosphorus removal. Chemosphere, 2017, 189: 679-688.

[21] Wangwang Yan, Tingting Qian, Liang Zhang, Li Wang, Yan Zhou. Interaction of perfluorooctanoic acid with extracellular polymeric substances - Role of protein. Journal of Hazardous Materials, 2021, 401.

[22] Fang, J., Sun, P.D., Xu, S.J., Luo, T., Lou, J.Q., Han, J.Y., Song, Y.Q. Impact of Cr(VI) on Premoval performance in enhanced biological phosphorus removal (EBPR) system based on the anaerobic and aerobic metabolism. Bioresour. Technol., 2012,121(0): 379-385

[23] Mu, H., Zheng, X., Chen, Y., Chen, H., Liu, K. Response of anaerobic granular sludge to a shock load of zinc oxide nanoparticles during biological wastewater treatment. Environ. Sci. Technol., 2012, 46(11): 5997-6003.

[24] Tao G J, Long X Y, Tang R, et al. Comparison and optimization of extraction protocol for intracellular phosphorous and its polyphosphate in enhanced biological phosphorus removal (EBPR) sludge. Science of the Total Environment, 2020,699(c): 134389.

[25] de Kreuk, M., Heijnen, J.J., van Loosdrecht, M.C.M. Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge. Biotechnol. Bioeng., 2005, 90 (6): 761-769.

[26] Zhang H L, Sheng G P, Fang W, et al. Calcium effect on the metabolic pathway of phosphorus accumulating organisms in enhanced biological phosphorus removal systems. Water Research, 2015, 84:171-180.

[27] Barat, R., Montoya, T., Borras, L., Ferrer, J., Seco, A. Interactions between calcium precipitation and the polyphosphate-accumulating bacteria metabolism. Water Res. , 2008,42 (13):3415-3424.

[28] Wang R, Peng Y, Cheng Z, et al. Understanding the role of extracellular polymeric substances in an enhanced biological phosphorus removal granular sludge system. Bioresource Technology, 2014, 169: 307-312.

[29] Sarvajith M., Nancharaiah Y.V.. Concurrent tellurite reduction, biogenesis of elemental tellurium nanostructures and biological nutrient removal in aerobic granular sludge sequencing batch reactor. Journal of Environmental Chemical Engineering, 2022, 10(6).