Study on denitrifying dephosphatation process, influence factors and mechanism

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Abstract. Introduced the typical denitrifying dephosphatation process and compared them. Influence factors and reaction mechanism were discussed in this paper. The results could provide reliable theoretical basis for denitrifying phosphorus removal process in actual application.

1. Introduction
Denitrifying polyphosphate accumulating bacteria (DPB) under anaerobic/anoxic alternate environment used oxygen, nitrite and nitrate as terminal electron acceptor to replace the traditional phosphorus accumulation organisms, through their metabolism to complete phosphorus uptake and denitrification, it was considered as a sustainable wastewater treatment process because it can solve the problem of carbon source competition, reduce sludge production and the reaction time.

This paper comprehensive analyzed the typical process of denitrifying phosphorus removal, the characteristic of carbon source, nitrate, nitrite, pH, SRT and the reaction mechanism of denitrifying phosphorus removal. All above is to solve the problems of the typical process and also have important theoretical and practical significance to innovate and develop more reasonable technological process.

2. The typical progress of denitrifying dephosphatation
Typical denitrifying phosphorus removal processes is divided into single and dual sludge system. In single sludge system, DPB, nitrifying bacteria and other heterotrophic bacteria are stay in same reactors. In dual sludge system, DPB and nitrifying bacteria exist alone in immobilized membrane bioreactor or aerobic nitrification sequencing batch reactor [1, 2].

2.1. Single sludge system

2.1.1. UCT. In 1980s, Cape Town University in South Africa developed UCT process could improve the biological phosphorus removal, to avoid the effect of nitrate in the return sludge on anaerobic phosphorus uptake, it was based on the traditional biological nitrogen and phosphorus removal process by changing the reflux method [3, 4], but the increase of reflux system made the operation became complicated, and the operating costed also increased. Process flow of UCT was shown in figure 1. The disadvantage of UCT was the competition between DPB, nitrifying bacteria and other heterotrophic
bacteria when they stayed in the same environment [4].

Anaerobic pool: DPB used small molecular weight VFA from degrading macromolecular organic compounds to synthesize large numbers of their own cytoplasm.

Anoxic pool: DPB used nitrate in recirculation solution from aerobic pool and the organic matrix in sewage to realize denitrifying dephosphataion.

Aerobic pool: DPB used the residual carbon in sewage to decompose the PHB and sustain its growth, excessive intake dissolved phosphorus in the environment at the same time. Nitrifying bacteria converted the ammonia nitrogen of the wastewater into nitrate by nitrification.

![Figure 1. Process flow of UCT.](image)

2.1.2. BCFS. In 1996, professor Mark of Delft university in Holland developed a new process named BCFs, which was based on the Pasveer oxidation ditch and used the principle of UCT process, it could improve the biological phosphorus removal and add a chemical phosphorus removal unit in anaerobic pool [3, 5]. The BCFs process was consisted of five reactors with independent function(Anaerobic pool, Contact pool, Anoxic pool, Anoxic/aerobic pool, Aerobic pool) and three circulation systems. Circulating A makes sludge release phosphorus. Circulating B adds nitrate nitrogen into the anoxic pool. Circulating C increases the nitrification or the simultaneous nitrification and denitrification. Process flow of BCFs was shown in figure 2.

![Figure 2. Process flow of BCFs.](image)

BCFS increased a contact pool and an anoxic/aerobic pool, it is the main difference between BCFs and UCT. Returning sludge and the mixture of anaerobic pool mixed in contact pool, it could consume the residual COD in anaerobic effluent. Meanwhile, the nitrate nitrogen in sludge was also reduced to nitrogen [3]. The anoxic/aerobic pool was used to remove nitrogen, compared with the usual oxidation ditch process it was in low dissolved oxygen environment. However, the disadvantage of the BCFS was the large area of the anoxic/aerobic pool which was 1/3 of system [4].

2.2. Dual sludge system

2.2.1. Dephanox. In 1992, Wanner found that Dephanox was a typical dual sludge system for nitrogen and phosphorus removal by organic substrate, the process flow of Dephanox was shown in figure 3.
The characteristics of Dephanox were following:
- In the end of anaerobic pool, the muddy water mixture was separated; the nitrification reaction provided sufficient electron acceptor for subsequent reactions.
- After the anaerobic phosphorus released, the sludge in sedimentation tank crossed into anoxic pool used nitrate as electron acceptor to begin the denitrifying dephosphataion.
- The residual phosphorus was further removed in second oxidation tank [4, 5].

2.2.2. \(A^3N\). \(A^3N\) process was proposed by Kuba, et al [6], it placed DPB and nitrifying bacteria into different sludge systems. The process flow was shown in figure 4.

![Figure 3. Process flow of Dephanox.](image)

![Figure 4. Process flow of \(A^3N\).](image)

Return sludge and sewage were mixed completely and flew to the anaerobic pool, DPB used small molecular weight VFA to synthesize PHB and stored in the cell, at the same time to release the phosphorus. The mixture was separated in middle sedimentation tank, and then the supernatant of rich ammonia entered into the aerobic pool for nitrification. The sludge in middle sedimentation tank directly crossed into the anoxic pool and used \(NO_3^-\) as electron acceptors to denitrifying dephosphataion [2].

\(A^3N\) was suitable for low C/N sewage. The phosphorus removal was not completely for lack of electron acceptor to the high C/N sewage, so it needed to add an aerobic pool after the anoxic pool to consume the residual PHB and removed phosphorus furtherly using \(O_2\) as electron acceptor. Finally, the sludge and sewage were separated in the secondary sedimentation tank and the sludge was returned to anaerobic pool for reaction.

The disadvantages of the process were:
- The ammonia nitrogen concentration was high. Because the sludge without nitrification directly into anoxic pool would carry ammonia nitrogen and the ammonia nitrogen treatment effect was very low at later stage, so the ammonia nitrogen concentration was too high.
- The stability of system was difficult to control. When the anoxic phase lack nitrate would lead phosphorus uptake effect was poor. When nitrate was excessive would make excess nitrate added into sludge, further affected on anaerobic phosphorus released and PHB synthesized.
- The process was rather lengthy [4, 6].

2.3. Comparison of two processes
Through many years of development, the progress of denitrifying dephosphataion experienced from single sludge to dual sludge system, the single sludge system was most improved from the traditional
phosphorus and nitrogen removal process, it mostly used for theoretical and experimental research. There were several defects:

1. In low temperature (generally below 15 degrees) condition. In order to satisfy the completely nitrification reaction it needed long SBT, so the structure would be larger. (2) There were competitions among the phosphorus accumulating bacteria, DPB and nitrifying bacteria for the substrates. (3) The amount of sludge and returned flow was large, also needed higher requirements for operation [4, 5].

The dual sludge system compared with the single sludge system had following advantages: (1) With low energy requirements and oxygen demanded. (2) Solved the competitions among the phosphorus accumulating bacteria, DPB and nitrifying bacteria for the substrates, made the DPB had the best growth environment. (3) Reduced the number of sludge recirculation systems and the amount of excess sludge [2, 3, 5].

3. The influence factors of denitrifying dephosphatation process

3.1. Carbon source

3.1.1. Types of the carbon sources. Carbon source is one of the most important nutrient elements in microbial growth. In the process of nitrogen and phosphorus removal, carbon sources are most used for phosphorus release, denitrification reaction and heterotrophic bacteria metabolism. Many research results had indicated that different types of carbon sources had significant differences in phosphorus release rate, because carbon source is related to phosphorus release and intracellular storage. Generally, DPB only could use VFA for anaerobic phosphorus release, different carbon sources hydrolytic acidified into different degrees of VFA, therefore the anaerobic phosphorus release effect was also different [7]. The experimental results of Lv X M et al [8] showed that the effect of acetic acid on phosphorus release was the best using glucose, acetic acid and propionic acid as carbon source. Fu J X et al [9] found that the sewage was easily to acidification and accompany by a large number of foam floating, and the pH also decreased when glucose was used as carbon source. When acetic acid was used as carbon source, it only had bubbles generation but no acidification problem. Therefore, acetic acid could be used for phosphorus release in the anaerobic section and provided favorable conditions for the subsequent anoxic phosphorus absorption.

3.1.2. Concentration of the carbon sources. In denitrifying dephosphatation process, the carbon source concentration has effect on the maximum amount of phosphorus release directly. Li Y J et al [10] used intermittent test to study the effect of organic carbon source on nitrogen and phosphorus removal in denitrifying dephosphatation process, experimental results showed that the higher COD concentration in anaerobic zone (150 ~ 250 mg/L) the more phosphorus released, the more denitrifying phosphorus uptake, but when COD concentration exceeds 200 mg/L, the residual organic matter remained in the anoxic stage and inhibited phosphorus uptake. In anoxic zone the influence of the carbon source concentration on denitrifying phosphorus uptake mainly reflected in: (1) PHB degradation rate by DPB was lower than carbon source utilization efficiency by common nitrifying bacteria, made the denitrification preferentially, thus inhibited excessive phosphorus uptake and make the effect of denitrifying phosphorus and nitrogen removal was reduced. (2) Carbon source could reduce NO₂⁻N accumulation, and inhibitory effect of NO₂⁻N on anoxic phosphorus uptake [11].

3.2. pH

The permeability of the cell membrane and the surface charge of the microorganism are affected by pH, the growth and reproduction of microorganism are also inseparable with the pH, and therefore, it was very important to find a suitable pH for the functional bacteria. The pH has affected anaerobic phosphorus release and anoxic phosphorus uptake. Under anaerobic conditions, pH increased would lead the amount of phosphorus released raised, because pH related to cell membrane charge could affect activity of enzymes in metabolic process. At the same time, the phosphorus accumulation
organisms and the glycogen accumulation organisms compete with each other, inappropriate pH would make phosphorus accumulation organisms lose their advantage in flora. Under anoxic conditions, low pH would lead the amount of anaerobic phosphorus released reduced, and also inhibited the anoxic phosphorus uptake. When pH was increased, the amount of anoxic phosphorus uptake also raised, but when pH was too high ineffective phosphorus release would happen, it had adverse effect on PHB synthesized, the amount of PHB as electron donor was reduced, and thus it had a negative effect on anoxic phosphorus absorption [12]. The test results of Li Y F et al [13] showed that phosphate was probably precipitated on the surface of micelle with the raise of pH, it inhibited the absorption of carbon source and the anaerobic phosphorus release. But too low pH would have inhibitory effect on DPB. In conclusion pH too high or too low both had an adverse effect on denitrifying phosphorus and nitrogen removal process.

The domestic and foreign scholars’ research results on the optimal range of pH can been seen in table 1.

| Author                  | The test method | the optimal range of pH |
|-------------------------|-----------------|-------------------------|
| Li Yafeng [11]          | The static test | 7.0 ~ 8.0               |
| Hu Xiaomin [10]         | The method of SBR | 6 ~ 8                  |
| Ren Nanqi [24]          | The method of SBR | 7.0 ~ 8.0              |
| Xu Wei [25]             | The method of SBR | 7 ~ 8.5                |
| Adeline S M Chua [17]   | The method of SBR | 7.0 ~ 8.0              |

### 3.3. The NO\textsubscript{3}\textsuperscript{-} and NO\textsubscript{2}\textsuperscript{-} mass concentration

#### 3.3.1. NO\textsubscript{3}\textsuperscript{-}

The results showed that NO\textsubscript{3}\textsuperscript{-} and organic carbon source coexisted in anaerobic stage would make the denitrification preferentially, inhibited the release of phosphorus and the synthesis of PHB and then further affected the phosphorus uptake [11, 14].

On the other hand, in the case of sufficient carbon source, the NO\textsubscript{3}\textsuperscript{-} concentration would determine whether the phosphorus uptake completely. Because the denitrification rate of using intracellular storage by DPB was much lower than the denitrification rate of degrading COD by conventional denitrifying bacteria, therefore, only when the NO\textsubscript{3}\textsuperscript{-} concentration was higher than the demand of ordinary denitrifying bacteria, the DPB could make full use of NO\textsubscript{3}\textsuperscript{-} to grow, otherwise would be suppressed [15, 16].

Zhu W T et al [16] found that when NO\textsubscript{3}\textsuperscript{-} concentration was too low (5 mg/L), the DPB would lack electron acceptor to uptake phosphorus; When the NO\textsubscript{3}\textsuperscript{-} concentration was too high (20 mg/L), it would lead the reduction of nitrogen removal rate. The experiment results [17] showed that in anaerobic section the nitrate nitrogen concentration should be below 10 mg/L and in anoxic section the nitrate nitrogen concentration should be controlled at about 40 mg/L, the effect of denitrifying phosphorus removal was best.

#### 3.3.2. NO\textsubscript{2}\textsuperscript{-}

The results showed it had no inhibition on anoxic phosphorus uptake and it could be used as electron acceptor, when NO\textsubscript{2}\textsuperscript{-} concentration was low, but if NO\textsubscript{2}\textsuperscript{-} concentration reached a certain degree would inhibit the reaction [6, 11]. Until now there has been no consistent conclusion about nitrite inhibition concentration, but most scholars believed that the upper limit value was related to the characteristics of phosphorus accumulating sludge [1, 18]. Yang Y Y, et al [18] suggested there were three kinds of theories about the inhibition of nitrite: (1) Free nitrous acid (FNA) had the function of uncoupling. The existing research believed that nitrite was an uncoupler, it could increase the permeability of the membrane to destroy the proton driving force. If the proton driving force as motivity for ATP synthesis was inhibited, then the energy produced by oxidation cannot be used for ADP phosphorylation, finally the coupling process of oxidative phosphorylation was separated. (2)
FNA inhibited the phosphorus absorption and the microorganism growth, reducing the energy requirements of microorganism. (3) FNA inhibited nitrite reductase.

Li W, et al [19] used anoxic sludge in A2O process to study the effect of NO$_3^-$ as electron acceptor on anoxic phosphorus uptake found that when nitrite concentration was too low, it couldn’t provide enough electron acceptor for denitrifying phosphorus accumulating reaction; But when nitrite concentration was too high, most of denitrifying bacteria in the system became dominant strain and had poor effect in the whole process. The experimental results of Xu R [20] and Li Y F, et al [13] showed that under anaerobic-anoxic-aerobic running mode when NO$_3^-$ concentration was 30 mg/L, the effect was the best. The study of Kang T T, et al [21] showed that under anaerobic-anoxic-aerobic running mode, gradually increased or reduced NO$_3^-$ concentration could induce the DPB to tolerate high NO$_3^-$ concentration, and considered the long carbon source adding time was beneficial to carbon source consumption in anaerobic section. The NO$_3^-$ sudden input whether caused inhibition, it depended on the adaptability of DPB to nitrite, the continuous NO$_3^-$ injection method was beneficial to relieve the inhibition of the reaction.

3.4. Sludge retention time
Sludge retention time (SRT) reflects the basic characteristics of microbial growth, growth conditions, generation cycle in the activated sludge system. It is an important technical parameter in the denitrifying nitrogen and phosphorus removal process [3]. Denitrifying phosphorus and nitrogen removal process can be divided into single sludge system and dual sludge system, because they have different nitrification section set mode and different requirements on SRT. In the single sludge system, DPB, nitrifying bacteria and other heterotrophic bacteria exist in the same environment, there are competitions between different strains. The SRT can be controlled in narrow range which can meet the needs of nitrogen and phosphorus removal. The longer the SRT, the more obvious nitrification; on the other hand, the shorter the SRT, the better effect of phosphorus removal [11]. In dual sludge system, the denitrifying bacteria and the DPB exist independently, the SRT can be selected according to their own respective conditions and they don't affect each other.

Li Y J, et al [10] used SBR experimental studied the effects of three different SRT on denitrifying phosphorus and nitrogen removal found that when the SRT was 15 d, the reaction effect was best, and SRT had a significant effect on the COD, nitrogen and phosphorus removal in SBR. The study of Fu J X, et al [9] showed when the SRT was too long would make the sludge renew slowly and the sludge mass concentration decrease gradually, which would lead serious influence on the sludge activity; When the SRT was too short, the small amount of denitrifying sludge would eliminate the phosphorus accumulating bacteria in the reactor, and excessive sludge discharged would lead to a serious reduction of sludge mass concentration.

3.5. Glycogen accumulation organisms
The PAOs and GAOs metabolic patterns are similar, the main difference is that the energy of PAOs is derived from Poly-p hydrolyze, while the energy of GAOs is derived from glycolysis [22]. In anaerobic stage, there is a competition between PAOs and GAOs for organic substrates, resulting in unstable operating system, and GAOs can't absorb phosphate in matrix like PAOs, the effect of phosphorus removal reduced. Therefore, it is necessary to control the competition between GAOs and PAOs on the carbon sources, maintain the efficient and stable phosphorus removal capacity [23].

Ren L, et al [23] believed that the influent C/P was a significant factor to competition between PAOs and GAOs. PAOs metabolic process needed more carbon sources to provide the energy, so the influent with low carbon sources could affect the phosphorus removal capacity. The high carbon source concentration was not conducive to PAOs growth, because the dephosphorus particles of PAOs were reduced and inhibited its growth in condition of high C/P, but the GAOs needed less phosphorus to grow. Wang Y Y, et al [1] studies showed that the temperature was also an important factor affecting the competition between PAOs and GAOs. At the temperature of 20 degrees, PAOs was the dominant strain in the phosphorus removal system. But when the temperature rose to 30 degrees, GAOs replaced
PAOs as the dominant strain in the system.

4. Denitrifying phosphorus removal mechanisms
Under anaerobic/anoxic alternate operating conditions, DPB used NO$_3^-$-N or NO$_2^-$-N as electron acceptor for phosphorus and nitrogen removal, it can be seen from figure 5. Compared with the traditional biological nitrogen and phosphorus removal process, it had advantages like low running cost, low energy consumption and less oxygen demand.

![Figure 5. Metabolic model of denitrifying phosphorus removal.](image)

Under anaerobic conditions, acetic acid entered DPB by active transport to make it multiply. This process consumed the proton motive force, so it was necessary to reconstruct or restore the proton motive force by decomposing the intracellular polyphosphate and releasing in the form of inorganic phosphate [14, 22, 24]. Acetic acid entered the cell and stored in the form of PHB. PHB was a kind of high molecular polymer which was a straight chain lipid compound consisted of beta hydroxybutyrate monomer. PHB required reducing power and energy to synthesize, in which the degradation of polyphosphate could provide the ATP, the glycogen degradation and TCA cycle could produce reduction force. Polyphosphate is a linear chain or cyclic polymer which was orthophosphate monomer formed by ester bonds. In anaerobic conditions, the polyphosphate in DPB was hydrolyzed, part of the energy made ADP translate to ATP which was provided to PHB synthesized. And another made ADP translate to ATP, the orthophosphate formed by hydrolyzing was excreted. Some scholars [25] believed that reducing power was produced by intracellular glycogen degraded. Under anaerobic conditions, the glycolytic pathway degraded glycogen to pyruvate and at the same time produced the NADH. A part of pyruvate directly entered into the TCA cycle and eventually converted to carbon dioxide, another was converted to acetyl coenzyme A for the synthesis of PHB. Some scholars [26] believed that the reducing power was generated under anaerobic conditions by the TCA cycle. However, some researchers have suggested that under anaerobic conditions DPAOs had only a part of TCA cycle been used. The TCA cycle generated NADH through a series of oxidation reaction and produced acetyl coenzyme A through part of the reduction reaction [26].

Under anoxic conditions, DPAOs used NO$_3^-$ or NO$_2^-$ as electron acceptor to oxidize PHB which was stored in cell to produce acetyl coenzyme A as carbon source for microbial growth, and produced the ATP, a part of it was used for its own synthesized and sustain life activities, another part was used for phosphorus uptake and polyphosphate synthesized. In order to stabilize the proton flow driven produced by the consumption of PHB, DPAOs excessive absorbed inorganic phosphate to synthesize Poly-p and store in cells, at the same time NO$_3^-$ or NO$_2^-$ was reduced to N$_2$ and discharged from system, finally achieve the effect of phosphorus and nitrogen removal [14, 20, 24]. Although NO$_2^-$ can be used as electron acceptor but it was also an inhibitor for microbial growth and metabolism, so it was very important to control the NO$_2^-$ concentration in denitrifying phosphorus removal process.
5. Conclusions

- Denitrifying phosphorus removal dual sludge system had low energy needs and oxygen requirement. Solve the competition on matrix and nutrient between species, and reduced the number of sludge return system, compared with single sludge system.
- Under anaerobic conditions, add acetic acid as substrate can provide favorable conditions for the subsequent anoxic phosphorus uptake. When pH was controlled between 7.0 and 8.0, it was suitable for the growth of DPB. Under anaerobic conditions, the NO₃⁻ mass concentration should be less 10 mg/L. Under anoxia conditions, the NO₃⁻ mass concentration was about 40 mg/L. When NO₃⁻ was used as electron acceptor, the NO₃⁻ mass concentration was about 30 mg/L, the effect of denitrifying phosphorus removal was best. SRT of 15 days was favorable for denitrifying phosphorus removal.
- In the anaerobic phase, DPB absorbed and degraded carbon source to synthetize PHB and store it in cell. In the anoxic phase, the excessive phosphorus absorption was accomplished by PHB oxidation, and the NO₃⁻ or NO₂⁻ was restored, which made the two opposing processes phosphorus absorption and denitrification occur together.

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. LJ2016014). In addition, the study was sponsored by National Nature Science Foundation of China (No. 51508342), National Water Pollution Control and Governance Scientific and Technological Special Project (2014ZX07202-011). The authors declare that they have no conflict of interest.

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