Influence of Phosphorus Release and Initial Nitrate Concentration on Anoxic Phosphorus Uptake

W. Xu, W. Qin, Z. Shao, and T. Fan

***Abstract*—Denitrifying phosphorus removal technology has advantage on beneficial for reduction of COD consumption, and the anoxic phosphorus uptake process is the key process. This study was to investigate the effect of the phosphorus release and initial nitrate concentration, which are two important infectors, on the anoxic phosphorus uptake process, by batch two Tests. The results show that the improvement of Prelease/MLSS would increase the amount of nitrogen and phosphorus removal as well as the specific phosphorus uptake rate(PUR),and the relationship between the amount of P release per MLSS was found in this paper. Nitrate would have little impact on the phosphorus uptake rate in anoxic condition if nitrate was adequate as the electron acceptor. Besides, a function formula was fitted to predict the amount of electron acceptor by the amount of P release per MLSS.**

***Index Terms*—Wastewater treatment, denitrification and phosphorus removal, anoxic phosphorus uptake.**

I. Introduction

Over the last decades, the discovery of denitrification phosphate accumulating organisms (DPAO) has been extensively reported, These organisms, as conventional poly-P bacteria, could sequester organic substrates under anaerobic condition, Instead, they utilize nitrate or nitrite as electron acceptor to accumulate phosphate [1]-[7]. Moreover, it is widely recognized that both of denitrification and phosphorus release require organism substrates as electron donor, which is a bottle-neck in resent wastewater treatment plants, especially in China with low COD/N ratio. Thus applying DPAOs in the plants has integrated nitrogen and phosphorus removal for COD is utilized simultaneously, which is beneficial for reduction of COD consumption [8], lower aeration requirement [9], and less sludge production [10]. Although many literatures reported the variety of factors that affected anaerobic phosphorus release and organism uptake, such as MLSS , SRT [11], COD/N,COD/P [12], there are few implies that all the factors would have indirectly or directly impact on the P removal efficiency through P release. No matter what had influenced the anaerobic P release, the amount of phosphorus release could be expressed all the factors affecting P removal. Furthermore, the investigation of the amount of P release should eliminate the influence of MLSS on operation of the experiment, which promoted the concept of the amount of P release per MLSS (Prelease/MLSS). Hence in this study we focused on the investigation of the effect of Prelease/MLSS on the phosphate and nitrogen removal, which will benefit for further innovative processes investigation. Furthermore, the validation of the positive role of nitrate in phosphorus uptake under anoxic condition has been highlighted in literatures [13]-[15]. Conclusion that adequate amount of nitrate would ensure the phosphorus uptake performance had been verified [16]. However, it is necessary to investigate the relation of P-uptake rate and the concentration of nitrate. Most importantly, nitrate should be controlled in a range of value to validate its sufficient requirement as electron acceptor under anoxic condition in the pilot projects to prevent secondary P release. Thus the study of the judgement to the adequate NO_3^--N supplying will be useful for the instruction to the pilot projects.

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TABLE I: Raw Wastewater Quality Parameters of the A^2N Process

quality parameters	The range of value	Raw material
COD(mg/L)	180~210	Substrates mixed with sodium acetate and glucose
TN(mg/L)	44~52	ammonia bicarbonate and urea
SP(mg/L)	4~6	potassium dihydrogen phosphate
Ammonia NH_3—N(mg/l)	25	ammonia bicarbonate

II. Methods and Material

A. *Lab-Scale A^2N Process Description*

The experimental sludge in this paper was obtained from a lab-scale A^2N process operated in the continues-flow condition, where an artificial wastewater summarised in Table I was introduced into the anaerobic tank. This setup was designed to purify the wastewater in the flow of 15L/h.

The hydraulic retention time (HRT) in anaerobic tank and anoxic one are both five hours, and eight hours in nitrification. The lab-scale plant was inoculated with an activated sludge from the sludge thickener in the municipal wastewater treatment plant of Nanjing Chengbei, which was applied with the UNI-TANK process. A well performance of denitrifying and phosphorus removal efficiency was achieved after three months of enriching DPAO. Until the anoxic phosphorus accumulation was 85% [17] of the total amount of phosphorus uptake (consist of anoxic uptake and aerobic uptake), the batch tests started.

B. *Experimental Sludge for Batch Tests*

The sludge for the batch tests was fetched out from the final settler as can be seen in Fig. 1 (tank 8). Before each

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experiment, the sludge rich in PAO and DPAO was fully taken up phosphorus in anoxic zone. In order to ensure its sufficient storage of polyphosphate, a post-aerobic phase in the A^2N process followed the anoxic tank was applied. Immediately, washing sludge 5 or 6 times by ultra-pure water was required followed by aeration with 30 minutes. After pre-treatment, experimental sludge was distributed to several glasses breakers with a volume of 1 liter like sequencing batch reactors. (Experiment NO.1). The vessels were applied magnetic stirring to maintain the suspension of the sludge. The details of each experiment will be described in the following. HAC was utilized as the carbon substrates, KH_2PO_4 as the phosphorus, $NaNO_3$ as the nitrate nitrogen, respectively. The pH was manually controlled by the balance of HCl (1mol/L) and NaOH (1mol/L).

C. Effect of $P_{release}$/MLSS

TABLE II: THE EXPERIMENTAL CONDITION OF THE EFFECT OF $P_{RELEASE}$/MLSS ON ANOXIC P-UPTAKE

Parameters	1#	2#	3#	4#	5#
Temperature(℃)	27.3～28.5				
MLSS(g/l)	4.14	4.20	4.11	4.13	4.10
Initial pH in anaerobic phase	7.3	7.2	7.3	7.3	7.2
Initial pH in anoxic phase	7.1	7.1	7.2	7.1	7.1
Initial soluble P concentration in anoxic phase (mg/l)	34.33	34.08	34.80	34.60	35.16
Initial nitrate concentration in anoxic phase (mg/l)	50.12	51.44	51.90	49.69	50.01

Experiment NO.1 consisted of two stages anaerobic and subsequently anoxic. In the anaerobic phase, 5 vessels acting as sequencing batch reactors were filled with the sludge treated as above, where the distributed sludge was suspended with different anaerobic reaction time to obtain different $P_{release}$/MLSS. All of them were fed with acetic as 150mg/L COD initially. The initial pH and MLSS of the anaerobic can be observed from Table II. When each vessel ended at the same time (started up at different time), 15minutes for settlement were required. Then, the supernatant liquid was leaned out carefully, some samples of which were withdrawn for later analysis. Washed 5 times by ultra-pure water immediately, the sludge was re-suspended in the five vessels again with nitrate and phosphorus added presented in Table II. The samples of the mixture in the 5 vessels were withdrawn for analysis several times during the 5h anoxic experiment.

D. Effect of Initial Nitrate Concentration on Anoxic Phosphorus Uptake

Before the experiment, the sludge from the final tank (8) was distributed to six reactors and incubated in the same anaerobic condition according to Table III. The carbon substrates were sufficiently transferred to the storage of intracellular substrates. Washed by ultra-pure water several times, the sludge was resuspended in the six reactors. In order to test the effect of initial nitrate nitrogen concentration on the anoxic phosphorus uptake, varying the nitrate from 21.27 mg/l to 69.27mg/l was operated to the six vessels. To better present the difference of P concentration in the bulk water among six reactors, if were, initial P concentration was added differently. Different initial P concentration had little impact on the specific P-uptake, which had been demonstrated in previous test (data not exhibited). The changes of the phosphorus and nitrate in the six reactors were tested.

TABLE III: THE EXPERIMENTAL CONDITION OF THE EFFECT OF INITIAL NITRATE ON ANOXIC P UPTAKE

items	1#	2#	3#	4#	5#	6#
Temperature (℃)	27.6～28.9					
MLSS(g/l)	3.55	3.54	3.57	3.57	3.54	3.54
Initial pH in anaerobic phase	7.2	7.2	7.2	7.3	7.2	7.1
Initial pH in anoxic phase	7.1	7.2	7.2	7.1	7.2	7.3
Initial soluble P concentration (mg/l)	30.06	34.04	33.69	35.97	37.37	40.37
Initial nitrate concentration (mg/l)	21.27	30.51	40.84	50.32	60.78	69.27

E. Analytical Methods

Temperature was measure form the thermal sensors of the multi-vessel equipment. The pH was taken out using a pH probe (model). The samples taken from the reactors in a volume of 30ml were immediately filtrated through filters with retention 1.2μm. Concentration was tested by means of glass fibre filters. Phosphorus, nitrate, COD, Total nitrogen, ammonia and MLSS were analysed according to the description of Standard Methods [18].

TABLE IV: THE RESULTS OF THE EFFECT OF $P_{RELEASE}$/MLSS ON ANOXIC P AND N REMOVAL

Items	1#	2#	3#	4#	5#
The amount of P-release(mg/l)	8.64	12.89	21.07	25.19	28.93
$P_{release}$/MLSS(mgP/l.gMlss)	2.09	3.07	5.13	6.10	7.06
P_{uptake}/MLSS* in 5h (mgP/l.gMlss)	2.15	3.16	5.28	6.28	7.27
The amount of excess P uptake in 5h（mgP/l）	0.27	0.38	0.63	0.75	0.88
Excess of P_{uptake}/MLSS in 5h（mgP/l.gMlss)	0.06	0.09	0.15	0.18	0.22
$N_{removal}$/MLSS** in 5h (mgP/l.gMlss)	2.05	3.13	5.13	6.16	7.07
The efficiency of $N_{removal}$ in 5h (%)	17	26	41	51	58

*P_{uptake}/MLSS means the amount of phosphorus uptake per MLSS; ** $N_{removal}$/MLSS means the amount of nitrate removed per MLSS.

III. Results and Discussion

A. Effect of the Amount of $P_{release}$/MLSS

The results of P release under anaerobic condition were given out in Table IV, as well as the excess amount of P accumulation and the efficiency of nitrogen removal. Fig. 1 displayed the typical results of experiment NO.1, which illustrated the exact transformation of nitrogen and phosphorus removal respecting to time. In this experiment, the concentrations of nitrate and phosphorus in each vessel were more than 20 mg/l and 5 mg/l respectively at the end of anoxic phase, indicating that nitrate and phosphorus were not the limiting factors and the capacity of the denitrification and phosphorus removal in each vessel had performed successfully.

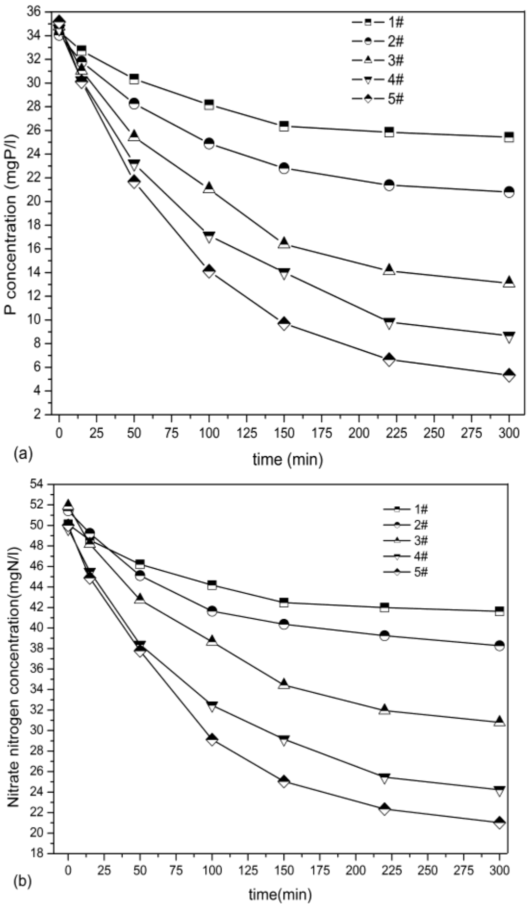

Fig. 1. Effect of $P_{release}$/MLSS on the anoxic phosphorus uptake (a) and nitrogen removal (b); “↘”1#means run1 reactor with $P_{release}$/MLSS 2.09 mgP/l.gMlss; “↩”2#means run2 reactor with $P_{release}$/MLSS 3.07 mgP/l.gMlss; “Γ”3#means run3 reactor with $P_{release}$/MLSS 5.13 mgP/l.gMlss; “⇉”4#means run4 reactor with $P_{release}$/MLSS 6.10 mgP/l.gMlss; “⌤”5#means run5 reactor with $P_{release}$/MLSS 7.06 mgP/l.gMlss

From Table IV, it is worth pointing out that the improvement of the $P_{release}$/MLSS under anaerobic condition was responsible for the high amount of P_{uptake}/MLSS in anoxic phase, which coincided well with other literatures [19], [20]. Because DPAO is able to uptake readily bio-degradable carbon source like acetic and other volatile fatty acids (VFAs) and synthesis to polyhydoxyalkanoates (PHA). Interestingly, the storage substrate PHA will be consumed if the bacteria are exposure to the electron acceptor like oxygen or nitrate. Obviously, the amount of phosphorus release is directly relative to the accumulation of carbon substrates in anaerobic phase, which will be utilized in anoxic P-uptake. Thus, the more the amount of P release is, the better effective of anoxic P removal perform. Accordingly, Fig. 1 illustrated that these curves were deeper with an increase of $P_{release}$/MLSS. In addition, it also explained that the phosphorus uptake rate under anoxic condition was increased due to the higher amount of $P_{release}$/MLSS. On the other side, it was easily to conclude that the more the amount of $P_{release}$/MLSS was, the higher the nitrogen removal rate could be, as well as the amount of $N_{removal}$/MLSS, so the main function of the improvement of $P_{release}$/MLSS is also to increase the efficiency of nitrogen removal. That is to say, the efficiency of nitrogen removal will be promoted under the same condition of MLSS and initial nitrate concentration. It is well recognized that nitrogen gas will be transformed from nitrate by using the stored substances, PHA, as the electron donor. Thus from the view of kinetics methods, the higher storage of PHA per MLSS (presuming no presentation of glycogen accumulation organisms) will induce to a higher rate of denitrification. While by stoichiometry methods, it is obviously to reveal that the high amount storage of PHA, caused by sound $P_{release}$/MLSS, may lead to high efficiency of $N_{removal}$/MLSS.

Obviously, Table IV suggested a relationship between $P_{release}$/MLSS and excess of P_{uptake}/MLSS in five hours, from which we were able to conclude a formula (1).

$$\mu_{P\text{-}c}=0.0301\mu_{P\text{-}r}(R^2=0.9985) \quad (1)$$

$\mu_{P\text{-}c}$ is the amount of excess P_{uptake}/MLSS (mgP/l.gMlss)
$\mu_{P\text{-}r}$ is the amount of $P_{release}$/MLSS (mgP/l.gMlss)

As discussed previously, the formula also indicated that the amount of anoxic P uptake will increase with increasing the amount of anaerobic P release, however, the extent of which accelerated slightly.

B. Effect of Nitrate on Anoxic Phosphorus Uptake

In the beginning of the test, Fig. 2(a) indicated that all of the six curve lines were in parallel, which implies that specific anoxic P-uptake rate was relatively the same. Subsequently, run1 and run2 started to decrease their P-uptake rate, especially run1.

Obviously, the rate of run3-run6 was almost the same during anoxic reaction time, which coincided well with the investigation[21] that the anoxic PUR in initial 30min was 153mg/(g Mlss d) and 146 mg/(g Mlss d) with nitrate concentration of 25mg/l and 60mg/l, respectively. It was probably because the high initial nitrate concentration might lead to a nitrite built-up, which inhibited the specific PUR in the investigators' experiments. However, the sludge incubated in our A_2N system could adapt to 5-10mg/L nitrite concentration. Thus, the built-up of nitrite caused by high initial nitrate concentration was not an inhibitor to anoxic PUR. So run6 with the highest concentration of nitrate did not inhibit the specific PUR. In short, the concentration of nitrate would not affect the specific PUR, shown in Fig. 2. Moreover, A metabolic model (formula 2 suitable for denitrification and dephosphation named ASM2D [22], was introduced in this literature.

In formula 2, there is a section of switch function,

$\frac{S_{NO_3^-}}{K_{NO_3^-}+S_{NO_3^-}}$ The value of $K_{NO_3^-}$ in the function is 0.1~0.2mg(NO_3^--N)/l. Therefore, common denitrification process is 0 order of reaction depending on nitrate concentration. Furthermore, it was predicted that in the process of specific denitrification and dephosphation, $K_{NO_3^-}$ was similar to the common denitrification, since the unique distinguish of the two processes was the type of the electron donor. Common one is external carbon source, and the specific one is intracellular carbon source, mainly PHA. Consequently, the specific denitrification is also zero order of reaction depending on nitrate concentration. However, from Fig. 2, when nitrate had been exhausted in run1, it performed a worse efficiency of P-uptake. While run2 exhibited a better performance than run1, a lower amount of accumulation than the others. Furthermore, if nitrate as electron acceptor is sufficient, nitrate will have little impact on the phosphorus uptake in anoxic condition.

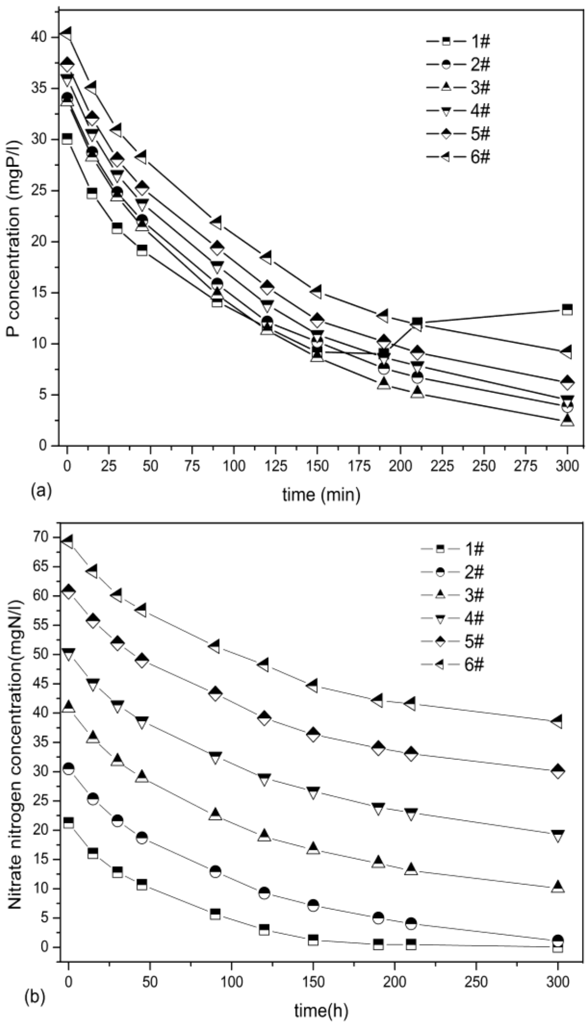

Fig. 2. Effect of nitrate on anoxic phosphorus uptake (a) and nitrogen removal (b); 1#means run1 reactor with initial nitrate 21.27 mg/l; 2#means run2 reactor with initial nitrate 30.51 mg/l; 3#means run3 reactor with initial nitrate 40.84 mg/l; 4#means run4 reactor with initial nitrate 50.32 mg/l; 5#means run5 reactor with initial nitrate 60.78 mg/l; 6# means run6 reactor with initial nitrate 69.27 mg/L.

Moreover, Fig. 2a implied that run1 had a P release course at the point of 150min, when nitrate had consumed up (Fig. 2b). The phenomena called secondary phosphate release, is detrimental to the biological nutrient removal process. This was discovered by several literatures [23], [24]. Therefore, avoiding the secondary P-release is important for the process. So, if nitrate is inadequate, shorten the hydraulic retention time in anoxic zone properly is necessary to solve the problem.

IV. Conclusions

This study investigated the impact of Prelease/MLSS, MLSS, and initial nitrate concentration on specific denitrification and dephosphation. The main conclusions are summarized below.

When the amount of phosphorus release per mixed liquor suspended solid (MLSS) was increase from 2.09 mgP/(L g Mlss) to 7.06 mgP/(L g Mlss) ,the improvement of Prelease/MLSS would increase the amount of nitrogen and phosphorus removal as well as the specific phosphorus uptake rate(PUR),and the results suggested a relationship between $P_{release}$/MLSS and excess of P_{uptake}/MLSS in five hours presented as$\mu_{P-c}=0.0301\mu_{P-r}$($\mu_{P-c}$: the amount of excess P_{uptake}/MLSS; μ_{P-r} is the amount of $P_{release}$/MLSS). Moreover, it was drawn from this study that one important meaning of the improvement of $P_{release}$/MLSS is to increase the efficiency of nitrogen removal.

When nitrate concentration ranged from 21.27to 60.78mg/L), nitrate would have little impact on the phosphorus uptake rate in anoxic condition if nitrate, which acts the electron acceptor, was adequate, and if nitrate is inadequate, shorten the hydraulic retention time in anoxic zone properly is necessary.

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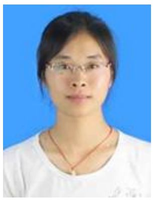

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