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Biological nitrogen removal with enhanced phosphate uptake in $(AO)^2$SBR using single sludge system

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Abstract: Simultaneous biological phosphorus and nitrogen removal with enhanced anoxic phosphate uptake *via* nitrite was investigated in an anaerobic-aerobic-anoxic-aerobic sequencing batch reactor($(AO)^2$ SBR). The system showed stable phosphorus and nitrogen removal performance, and average removals for COD, TN and TP were 90%, 91% and 96%, respectively. The conditions of pH 7.5—8.0 and temperature 32℃ were found detrimental to nitrite oxidation bacteria but favorable to ammonia oxidizers, and the corresponding specific oxygen uptake rates(SOUR) for phase 1 and 2 of nitrification process were 0.7 and 15 $mgO_2/(gVSS \cdot h)$ in respect, which led to the nitrite accumulation in aerobic phase of $(AO)^2$ SBR. Respiratory tests showed that 40 $mgNO_2$-N/L did not deteriorate the sludge activity drastically, and it implied that exposure of sludge to nitrite periodically enabled the biomass to have more tolerance capacity to resist the restraining effects from nitrite. In addition, batch tests were carried out and verified that denitrifying phosphorus accumulation organisms (DPAOs) could be enriched in a single sludge system coexisting with *nitrifiers* by introducing an anoxic phase in an anaerobic-aerobic SBR, and the ratio of the anoxic phosphate uptake capacity to aerobic phosphate uptake capacity was 45%. It was also found that nitrite (up to 20 $mgNO_2$-N/L) was not inhibitory to anoxic phosphate uptake and could serve as an electron acceptor like nitrate, but presented poorer efficiency compared with nitrate.

Keywords: denitrifying phosphorus accumulation organisms(DPAOs); shortcut nitrification and denitrification; anoxic phosphate uptake; sequencing batch reactor(SBR); nitrite; electron acceptor

Introduction

Biological phosphorus and nitrogen removal process has provided significant benefits to ameliorate eutrophication of surface water without exacerbating salination. Recent researches on nitrogen removal are mostly either towards improvement of performance and energy savings in traditional processes or towards development of new processes/microorganisms that are able to convert ammonium/oxidated nitrogen into harmless forms. Shorter nitrification and denitrification, i.e. partial oxidation of NH_4^+ to NO_2^- and subsequent reduction of the latter to molecular nitrogen, was regarded as a favorable short-cut, especially for treatment of wastewaters with low C/N ratio (Yuan, 2000; Pollice, 2002). In comparison with complete nitrification, several advantages of shortcut nitrification and denitrifation were reported to be lower oxygen consumption (up to 25% energy saving during aeration), reduced organic substrate requirements for heterotrophic denitrification (up to 40%), lower biomass production (up to 300%), and increased denitrification kinetics(Turk, 1987).

Biological phosphorus removal(BPR) is based on the activities of phosphorus accumulating organisms (PAOs). In anaerobic phase, the PAOs take up simple organics and store them as polyhydroxyalkanoates (PHAs) at the cost of hydrolysis of intracellular polyphosphate and glycolysis of glycogen. In the subsequent aerobic phase, PAOs use the PHAs to generate energy for growth, glycogen synthesis, and phosphate uptake, where the uptake amounts of phosphate by PAOs are more than the released one in anaerobic phase (Mino, 1998). Although it was initially thought that PAOs lacked the ability to denitrify and could not grow and accumulate phosphate under anoxic conditions, it has been demonstrated experimentally that a fraction of the PAOs (denitrifying PAOs, or DPAOs) could do so, using either oxygen or nitrate/nitrite as electron acceptors to uptake phosphate(Meinhold, 1999; Lee, 2001; Wachtmeister, 1997). The use of DPAOs in BPR system can be attractive because the same organic substrate is efficiently used both for nitrogen and phosphorus removal, which is significant since organics availability is often a limiting factor in nutrient removal process in China. Additionally, other advantages associated with DPAOs activity include a reduction in aeration energy and sludge production owning to the lower energy production with nitrate compared to that with oxygen (Kuba, 1994).

In recent years, sequencing batch reactor(SBR) has been proposed as an alternative for conventional activated sludge process for its operational flexibility, lower operating costs, improved phosphorus and nitrogen removal and less sludge bulking(Lee, 2001). In the single sludge system (DPAOs coexist with *nitrifiers*) of SBR, simultaneous phosphorus and nitrogen removal usually requires an aerobic and anoxic phase to generate electron acceptors and oxygen-free conditions for DPAOs, respectively.

In this study, the technical feasibility of concurrent phosphorus and nitrogen removal was investigated in a single sludge system by enhancing anoxic phosphate uptake *via* nitrite. To achieve such a goal, an anoxic phase was introduced into the middle of aerobic phase of an anaerobic-aerobic sequencing batch reactor (AO SBR) to form $(AO)^2$SBR for enriching DPAOs over a long period time, and ammonium oxidation was maintained at the first stage of nitrification, i.e. nitrosification, by adjusting pH and temperature (T) during the aerobic phase. In this research, two issues as follows were the major contents to be discussed: (1) the validity of pH 7.5—8.0 with 32℃ to accumulate nitrite during ammonium oxidation; (2) the possibility of nitrite/nitrate to serve as an efficient electron acceptor for anoxic phosphate uptake in comparison with oxygen.

1 Materials and methods

1.1 Experimental equipment

A cylindrical vessel with a 7 L effective volume was used for the $(AO)^2$ SBR which was operated in a fill-and-draw mode(Fig.1) and consisted of 2 h anaerobic, 1.5 h aerobic, 2.5 h anoxic, 1 h aerobic and 1 h settling and fill/draw phases. In each operational cycle, 4 L of clarified supernatant was displaced by a same volume of raw wastewater during fill/draw stage. Sludge retention time(SRT) was maintained at 20 d by wasting mixed liquid at the end of aerobic stage and the aerobic reaction *T* and pH were controlled at 32℃ and 7.5—8.0 with a thermostat and by addition of 0.5 mol/L NaOH/0.5 mol/L HCl, respectively.

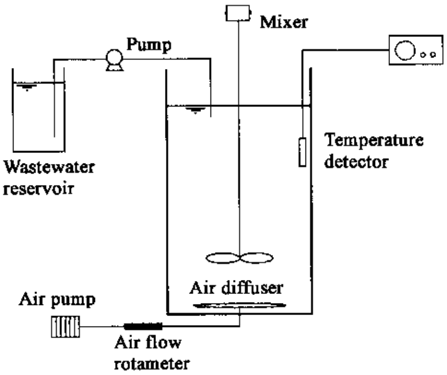

Fig.1 Schematic diagram of SBR system

The test wastewater was collected from a septic tank in the 2nd Campus of Harbin Institute of Technology(HIT) and its characteristics are summarized in Table 1.

Table 1 The quality of the raw domestic wastewater

Parameter	Range	Mean
pH	6.9—7.9	7.2
SS, mg/L	32—152	76
COD, mg/L	187—327	248
BOD_5, mg/L	89—165	108
NH_3-N, mg/L	19.8—39.5	30.3
TN, mg/L	22.6—45.6	37.8
TP, mg/L	4.4—6.1	4.7

1.2 Sludge characterization under aerobic and anoxic conditions

Batch experiments were carried out to investigate anoxic phosphate uptake in more details. A stirred tank-type glass vessel of 1 L was used as the reactor in the batch experiment. The activated sludge at the end of anaerobic phase was transferred into the reactor and further kept in the anaerobic conditions to ensure the end of phosphate release. Then aerobic or anoxic conditions were applied to the reactor and the amount of phosphate uptake was measured under different conditions. There are two main purposes in the tests: (1) sludge characterization and (2) study of the effects of nitrate and nitrite on anoxic phosphate uptake. In the experiment for the sludge characterization, two batch tests were conducted using the same sludge under anoxic and aerobic conditions. In the anoxic conditions, 35 mg/L of NO_3-N was dosed in the reactor at the beginning, and with the process of experiment, nitrate was continuously added to supplement the consumed one. In the tests of the effects of nitrate and nitrite on the anoxic phosphate uptake, three different concentrations of two kinds of electron acceptor(10 mgNO_3-N/L, 10 mgNO_2-N/L, 20 mgNO_2-N/L) were supplied in anoxic conditions. The pH was strictly controlled around 7.0 with 0.5 mol/L NaOH/0.5 mol/L HCl to avoid phosphate precipitation throughout the experiments.

1.3 Activities of *nitrifiers*

For a more complete evaluation of the nitrification process, the specific oxygen uptake rates(SOUR) of microorganisms for particular nitrification phases were estimated, since the method of SOUR assessment of particular *nitrifiers* groups appeared to be very useful in the case of municipal wastewater nitrification(Joanna, 1996). The test sludge was taken from the reactor at the end of aerobic phase, and the temperature and pH were similar to that in the bioreactor.

1.4 Analytical procedures

COD, BOD_5, NH_3-N, NO_2-N, NO_3-N, TN, TP, PO_4-P, MLSS and MLVSS were determined using the standard methods issued by the National Environmental Protection Agency(NEPA) of China(1989). pH was measured using glass electrodes connected to a PHS-3C pH meter. DO was detected with a YSI(MODEL 50B) dissolved oxygen meter. SOUR of sludge was monitored by the method described by Joanna *et al*. (Joanna, 1996).

2 Results and discussion

2.1 Performance of $(AO)^2$SBR

Fig.2 demonstrates the change of PO_4-P, NO_2-N and NO_3-N concentrations in the effluent along with PO_4-P concentration at the end of anaerobic and anoxic phases during the operation period of SBR. As shown in Fig.2, the dropping trend of the profile of PO_4-P anoxic accompanied by a reduction of phosphorus concentration in the effluent indicated the enhancement of DPAOs step by step in the system with the prolongation of $(AO)^2$ SBR and it in turn improved the phosphorus removal. As for the removal of nitrogen, low concentration of nitrite and nitrate in the effluent gave a satisfying removal performance in terms of TN.

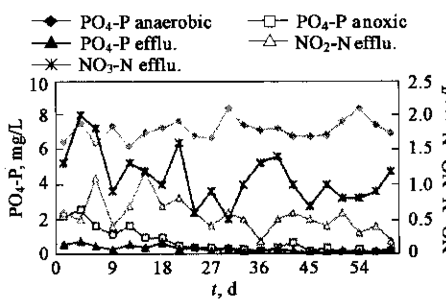

Fig.2 Daily variation of PO_4-P, NO_2-N, NO_3-N concentrations in the effluent and PO_4-P concentration at the end of anaerobic and anoxic phases

Fig.3 shows the typical profiles of COD, PO_4-P, NH_3-N, NO_2-N and NO_3-N concentrations in an operational cycle when a pseudo-steady state was achieved after 20 operation days. During the first 1 h after the anaerobic phase started, COD was rapidly removed and phosphate was sharply released, then the variation grew more gently, which was consistent with the activity of the PAOs under the anaerobic conditions. In the subsequent aerobic phase, over half of the released phosphorus was taken up by PAOs, and NH_3-N was already converted to nitrite and nitrate completely with the ratio of nitrite nitrogen(NO_2-N) to total oxidated nitrogen(NO_2-N + NO_3-N) at about 85%, which meant that it was effective to remove ammonium via nitrosification by controlling the reaction at 32℃ and pH between 7.5—8.0. In the beginning of the anoxic phase, the ammonium was further removed due to the residual amounts of oxygen in the reactor, but the COD curve showed a slight upward. And the concentrations of nitrite and nitrate started to decrease slowly along with the uptake of released phosphate, which clearly indicated that anoxic phosphate uptake via nitrite occurred. It was in accordance to the results of Meinhold *et al*. and Lee *et al*., but contradictory to that of Comeau *et al*. (Meinhold, 1999; Lee, 2001; Comeau, 1998). Although the required long aerobic time for nitrification was reported unfavorable for DPAOs (Kuba, 1996a), some amounts of intracellular PHA was oxidized in DPAOs using nitrite and nitrate as electron acceptors in this system, which was probably ascribed to the shorter nitrification time due to the achievement of nitrosification. Consequently, the system showed an excellent removal performance and the removals for COD, TN and TP were 90%, 91% and 96% respectively.

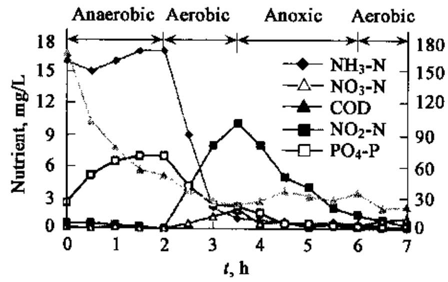

Fig.3 Typical profile of COD, PO_4-P, NH_3-N, NO_2-N and NO_3-N in the $(AO)^2$ SBR

2.2 Activities of *nitrifiers*

For a more complete evaluation of the nitrification process, the sludge SOUR values for two particular nitrification phases were estimated, as shown in Fig.4. If both phases of nitrification have the same rate, due to higher oxygen demand, the SOUR for phase 1 is three times of that of phase 2 according to the equations as follows:

Phase 1 Phase 2

$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O$, $NO_2^- + 0.5O_2 \rightarrow NO_3^-$.

In order to compare both phases of nitrification, the value of the SOUR for phase 2 should be multiplied by 3. It was found from Fig.4 that the activities of nitrite oxidizing bacteria were inhibited drastically, and the corresponding SOUR of phase 2 was only 0.7 $mgO_2/(gVSS \cdot h)$ which was only about 5% of that of phase 1. It as a result led to the accumulation of nitrite in aerobic phase of $(AO)^2$ SBR. So it could be concluded that the conditions of 32℃ and pH 7.5—8.0 was favorable for ammonia oxidizing bacteria to profilerate, but had an adverse effect on nitrite oxidizers, which could be explained as follows: (1) Under the conditions of 32℃ and pH 7.5—8.0, ammonia oxidizers proliferate much faster than nitrite oxidizers which otherwise act vigorously at 25—30℃ and pH 7.0—7.5. In addition, high pH also inhibited the former bacteria through free ammonia (FA), which was produced from the movement of ionization-balance-equation existing between FA and ammonium under higher pH. In this study, the concentration of FA in aerobic phase varied from 0.25 to 1.9 mg/L calculated by the equation proposed by Anthonisen *et al*. (Anthonisen, 1976), which was in the range of FA to inhibit the nitrite oxidizers (0.1—1.0 mg/L), but significantly lower than that of ammonia ones (10—150 mg/L) (Yuan, 2000); (2) $(AO)^2$ SBR itself was a system with a trend to form shortcut nitrification, due to fact that ammonia oxidation capacity of activated sludge under anaerobic conditions could remain constant, while the nitrite oxidation capacity would decrease measurably, which suggested that the decay rate of ammonia oxidizing bacteria was zero in the absence of oxygen and nitrate/nitrite, whereas that of nitrite oxidizing ones was the same under anaerobic and anoxic conditions (Nowak, 1995).

A disadvantage of shortcut nitrification is the accumulation of nitrite and its presumed toxic effect on the biomass, however, the inhibition is not always consistent, which makes it interesting to study the effect of nitrite on the sludge activity in the bioreactor. Based on it, sludge respiratory tests with different nitrite concentrations were therefore carried

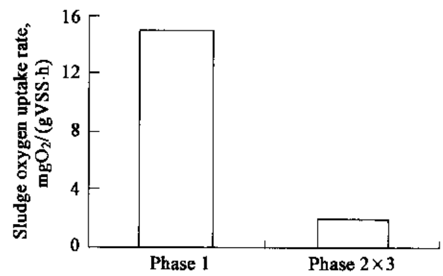

Fig. 4 Sludge oxygen uptake rate in phase 1 nitrification and that in phase 2 nitrification multiplied by 3

out, and it was found from Fig.5 that nitrite had little detrimental effect on the biomass activity even when the concentration of nitrite in the bulk was 40 $mgNO_2$-N/L, shown by the slight drop of SOUR from 36 $mgO_2/(gVSS \cdot h)$ at 0 $mgNO_2$-N/L to 31 $mgO_2/(gVSS \cdot h)$ at 40 $mgNO_2$-N/L. It was contrary to the results drawn by Muller *et al*. (Muller, 1995). In this system, the SBR sludge in the bioreactor was always exposed to nitrite (about 10 $mgNO_2$-N/L), which probably made itself adaptable for such unfavorable conditions.

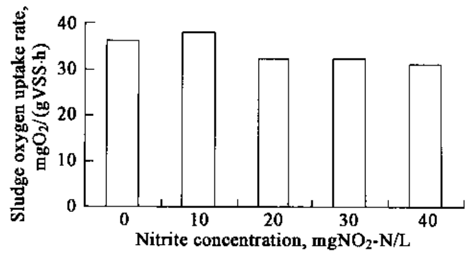

Fig.5 Effect of nitrite on sludge oxygen uptake rate at different concentration of 0—40 $mgNO_2$-N/L

2.3 Sludge characterization under aerobic and anoxic conditions

It has been reported that the contribution of phosphate removal by DPAOs to the total phosphate removal could be calculated from the ratio of the anoxic phosphate uptake rate to the aerobic phosphate uptake rate (Wachtmeister, 1997). This is based on the fact that the DPAOs can take up phosphate at the nearly same rate under both aerobic and anoxic conditions, whereas aerobic PAOs are inactive under the anoxic conditions. In viewpoint of this, phosphate uptake rates under anoxic and aerobic conditions were measured in separate batch reactors for the $(AO)^2$ SBR sludge. After the end of phosphorus release was confirmed in the anaerobic phase, the sludge was then divided into two parts. One was exposed to the anoxic conditions, and the other was to the aerobic conditions. Fig. 6 shows the phosphate release and uptake by the sludge, and the rates of anoxic and aerobic phosphate uptake were calculated as 0.32 and 0.71 $mgP/(gVSS \cdot h)$, respectively. According to the data, the contribution of the anoxic phosphate uptake capacity to aerobic (total) phosphate uptake capacity was therefore estimated at 45%, which directly demonstrated to some extent that DPAOs had been enriched in $(AO)^2$ SBR system.

2.4 Nitrite as an electron acceptor on anoxic phosphate uptake

Theoretically, nitrite can play a role as an electron acceptor in the denitrification reaction by DPAOs. However, no clear answer regarding the effect of nitrite on anoxic phosphate uptake has been reported in literature. Comeau *et al*. (Comeau, 1998) reported that anoxic phosphate uptake did not occur with nitrite as electron acceptor, but only one concentration (10 $mgNO_2$-N/L) was examined in his study. On

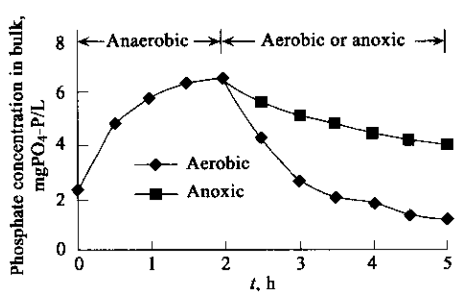

Fig.6 Phosphate uptake tests under aerobic and anoxic conditions(VSS = 2.5 g/L)

the contrary, Meinhold *et al*.(Meinhold, 1999) and Lee *et al*.(Lee, 2001) found that the low concentration of nitrite did not inhibit anoxic phosphate uptake but could serve as an electron acceptor, and the quoted concentration range were both around 10 mg/L.

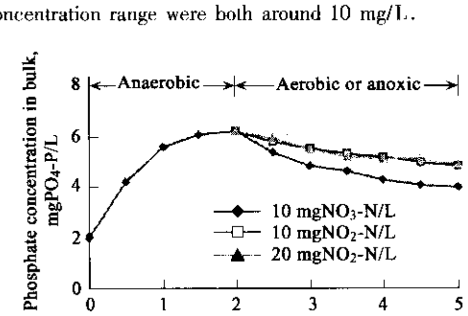

Fig.7 Comparison between effects of nitrite and nitrate on the anoxic phosphate uptake(VSS = 2.6 g/L)

In this work, anoxic phosphate uptake utilizing nitrite as electron acceptor has been clarified in 2.1. In order to further confirm the effect of nitrite on the anoxic phosphate uptake and whether nitrite had the same efficiency as an electron acceptor in comparison with nitrate, the sludges obtained from the$(AO)^2$ SBR were respectively exposed to 10 $mgNO_3$-N/L, 10 $mgNO_2$-N/L and 20 $mgNO_2$-N/L. As can be seen in Fig.7 that both nitrate and nitrite could serve as electron acceptors for DPAOs to take up phosphate, however it seemed that DPAOs showed more capacity to take up phosphate using nitrate in contrast with nitrite, which was inconsistent with the opinion of Lee *et al*.(Lee, 2001). Additionally, it was found that at least 20 $mgNO_2$-N/L of nitrite was not detrimental to anoxic phosphate uptake, which was indicated by the almost same phosphate uptake rate compared with that under 10 $mgNO_2$-N/L. It was a rather broader range of nitrite for DPAOs anoxic phosphate uptake than the reported values(Meinhold, 1999; Lee, 2001), which was possibly depended on the operational conditions and sludge types. In this study, the long exposure of sludge in the bioreactor to relatively high nitrite concentration generated in the shortcut nitrification process maybe the explanation.

3 Conclusions

An $(AO)^2$SBR aiming at removing phosphorus and nitrogen simultaneously was operated to investigate shortcut nitrification and anoxic phosphate uptake using nitrite as an electron acceptor by DPAOs. The system showed a very stable organics and nutrient removal efficiencies, i.e. 90% for COD, 91% for TN and 96% for phosphate, respectively. The conditions of pH 7.5—8.0 and 32℃ were proved reliable to maintain the nitrification process at the first phase by means of SOUR measurement, which consequently led to the nitrite accumulation in the aerobic phase of$(AO)^2$ SBR, and the ratio of nitrite nitrogen to total oxidated nitrogen at the end of aerobic phase was nearly 85%. It in turn enabled the biomass and DPAOs has more tolerance capacity for nitrite. Results of batch experiments showed that nitrite, like nitrate, could serve as electron acceptors for anoxic phosphate uptake, but showed lower uptake rate. In addition, a rather higher concentration of nitrite(up to 20 $mgNO_2$-N/L) was found no detrimental to DPAOs for anoxic phosphate uptake. By calculation, the ratio of the anoxic phosphate uptake capacities to aerobic phosphate uptake capacities was 45%.

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