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Performance of denitrifying phosphorus removal of *Acinetobacteria* strain at low temperature

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ABSTRACT

In order to investigate the possibility of *Acinetobacteria* strain to realize denitrifying phosphorus removal, batch tests were carried out to study the phosphorus uptake activity under different nitrate and nitrite concentrations. Results showed that the phosphorus uptake ability of *Acinetobacteria* strain was enhanced with the increase of nitrate concentration, in the range of 0–100 mg/L. Similar trend was detected with nitrite as elector acceptor in the range of 0–20 mg/L, and 40 mg/L of nitrite could inhibit the phosphorus uptake of *Acinetobacteria* strain. Denitrifying phosphorus removal process at low temperature (15°C) showed that phosphorus uptake amount was over 30 mg/L with *Acinetobacteria* strain addition under anaerobic/anoxic condition by using 25 mg/L of nitrate as elector acceptor.

1. Introduction

Enhanced biological phosphorus removal (EBPR) is proved to be an effective and financially viable process to remove nutrients from wastewater. Simultaneous nitrogen and phosphorus removal processes, based on the activity of phosphorus accumulating organisms (PAOs) and denitrifiers, is an environmentally sustainable method of nutrient removal from wastewater. However, PAOs and denitrifiers both need organic carbon to perform phosphorus release and denitrification (Soares et al., 2010), which can result in shortage of enough organic carbon for wastewater treatment with low COD/N ratio (Kuba et al., 1996). As an alternative to conventional nitrogen and phosphorus removal, denitrifying phosphorus removal could overcome the limitation of low organic carbon (Lopez et al., 2006).

Recently, more reports have claimed that denitrifying phosphorus removal bacteria (DPB) can take up phosphorus with nitrate (NO_3^--N) as electron acceptor under anoxic conditions (Barker and Dold, 1996; Shi and Lee, 2006), and simultaneous nitrogen and phosphorus removal is achieved. Metabolic pathway of DPB leads to reduction of sludge production and oxygen demands, and a more efficient use of organic matters as well (Meinhold et al., 1999). It also has been reported that the requirement for organic substances can be further lowered with nitrite (NO_2^--N) as electron acceptor (Saito et al., 2004). Notably, high nitrite is toxic for DPB and inhibits the phosphorus uptake (Peng et al., 2011).

The denitrifying phosphorus removal is largely determined by the structure of reactor and the function of microbial community (Slater et al., 2010). It is reported that sometimes phosphorus uptake activity of sludge was close to the microbiological mechanism of DPB in EBPR system (Okunuki et al., 2004). Recently, more studies were conducted to understand and enhance the growth of DPB in EBPR system for phosphorus uptake (Nielsen et al., 2010; Zafiriadis et al., 2011). Some members of *Acinetobactearia* species isolated from sludge have the potential to realize denitrifying phosphorus removal (Bond et al., 1995), thus, culture and enrichment of DPB were deserved to be studied for enhancement of EBPR. Furthermore, the application of certain DPB for wastewater treatment should be investigated. As temperature was a vital factor in a phosphorus uptake process(Panswad et al., 2003), the EBPR process under moderate to low temperature was worth substantial attention. However, most of the previous studies were based on high temperature. Limited studies have been carried out to enhance phosphorus uptake at low temperature.

In this study, *Acinetobacteria* strain, isolated from the sludge of PAOs, was used for phosphorus uptake under different elector acceptors, such as NO_3^--N and NO_2^--N. The effect of temperature on phosphorus uptake of *Acinetobacteria* strain was studied in temperature range from 0 °C to 35 °C. The results would be helpful to understand and promote the potential application of certain DPB.

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In addition, the bioaugmentation of the *Acinetobacteria* strain associated with enhanced PAOs sludge for wastewater treatment is also discussed.

2. Materials and methods

2.1. Wastewater composition

The composition of the synthetic wastewater was (per liter): 517 mg sodium acetate (COD = 300 mg/L), 30 mg NH_4Cl (8.0 mg/L of NH_4^+-N), 39.5 mg KH_2PO_4 (PO_4^{3-}-P = 9 mg/L), 15 mg $MgSO_4$, 300 mg $NaHCO_3$, and 10 mg $CaCl_2$. The synthetic wastewater also contained a trace salt solution (0.3 mL/L) (Wang et al., 2010).

2.2. Enrichment and isolation

The inoculated sludge was taken from the secondary sedimentation tank of Wenchang municipal wastewater treatment plant in Harbin, China. The LB medium (Vonder Weid, 2007) and mineral salt medium (MSM) (Tuo et al., 2012) were used for isolation of DPB. Sludge was washed by deionized water twice, and the microbes in the supernatant were enriched in LB medium. And then, the culture was inoculated to MSM media for accumulating the phosphorus removal microbes. Finally, the phosphorus uptake microbes were isolated by plate-streaking technology. The detailed operation methods were as follows: (1) 10 ml supernatant was added into a 250 ml Erlenmeyer flask containing 90 ml LB medium, which was incubated at 25 °C with 150 rpm for 24 h. (2) the cultures were acclimated through three times of successive transfer with phosphorus concentration of 50, 100 and 200 mg/L, respectively. (3) The serial diluted suspensions (10^{-5}–10^{-8}) were spread onto the MSM plates containing 100 mg/L of phosphorus. The colonies of DPB were purified by plate-streaking technology on MSM plate. (4) Pure colonies were transferred to MSM, which contained 100 mg/L phosphorus, to investigate their ability of removing phosphorus under anaerobic and anoxic conditions. The isolated bacteria species were analyzed by sequences (Genbank : HQ891366), and the Fig. 1 showed that the isolated species belonged to *Acinetobacteria* phyla, named as *Acinetobacteria* strain K11.

2.3. Anaerobic/Anoxic-SBR operation

SBR reactor with a working volume of 4.0 L was operated in anaerobic/anoxic conditions to study the phosphorus uptake capability of *Acinetobacteria* strain. The operation procedure of SBR was as follows: 120 min anaerobic phase, 180 min anoxic phase, 30 min settling, 20 min decanting and 30 min idle. The temperature, hydraulic retention time (HRT) and sludge retention time (SRT) were controlled at 25 °C, 7 h and 20 d, respectively.

2.4. Batch experiment

Batch experiments were performed with different nitrate and nitrite concentration under anoxic condition in order to examine the effect of nitrate, nitrite and temperature on anoxic phosphate uptake in more detail. Magnetic stirring conical flasks were used as reactors in the batch experiments. About 150 mL of 1000 mg/L bacterial suspension taken from SBR at the end of anaerobic stage was transferred to the batch reactor. Immediately 10 mL sodium nitrate solution or sodium nitrite solution was added to the batch reactor, to make sure the mixture nitrate concentrations were controlled at 0, 25, 50 and 100 mg/L, and nitrite concentrations were 0, 10, 20 and 40 mg/L, respectively. Thereafter the magnetic stirring apparatus was started and each anoxic phosphorus uptake batch experiment lasted for 3 h at temperature of 25 °C.

The effect of temperature on the phosphorus uptake was performed by batch test similarly; the fixed initial nitrate and phosphorus concentrations were 100 mg/L and 40 mg/L, respectively. The temperature was ranged from 0 °C to 35 °C.

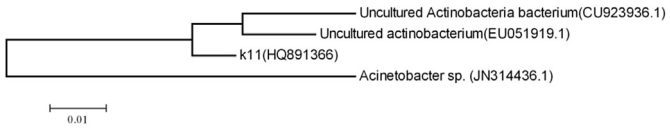

Fig. 1. Phylogenetic tree of 16S rRNA sequences represented the relationship of the isolation of bacteria species. The scale bar represents two substitutions per 100 nucleotide positions.

2.5. Phosphorus uptake at low temperature

Test was conducted to study the ability of the *Acinetobacteria* strain for enhancing EBPR of wastewater at low temperature (15°C). Sludge of SBR (120 mL, 2000 mg/L) and the *Acinetobacteria* strain (30 mL, 1000 mg/L) was mixed and fed into SBR for wastewater treatment. 25 mg/L of NO_3^--N as electron acceptor was added into reactor at time of 120 min and 210 min during anoxic period.

2.6. Analytical methods

PO_4^{3-}-P, NO_2^--N, NO_3^--N and MLSS were analyzed according to the Standard Methods (APHA, 1998). All the samples were filtered through 0.45 μm filter papers before analysis. The pH and DO was monitored using pH/oxi 340i meter (WTW, Germany). All measurements were conducted in triplicate.

3. Results and discussions

3.1. Effect of nitrate on denitrifying phosphorus removal

The profiles of phosphate and nitrate with different concentrations of nitrate feeding in anoxic conditions in the batch experiment were shown in Fig. 2. The phosphorus concentration decreased dramatically from 35.7 to 7.72 mg/L with the time of 180 min when nitrate concentration increased from 0 to 25 mg/L. It is also observed that there is no obvious change of phosphorus concentration with nitrate concentration continuously increasing to 100 mg/L. Simultaneously, the corresponding nitrate concentrations decreased in all reactors. Especially, the nitrate was completely consumed at 150 min with initial concentration of 25 mg/L, indicating that phosphorus uptake amount might be affected by limited nitrate under anoxic condition. The above results indicated that nitrate can be used as electron acceptor for phosphorus uptake in anoxic conditions, and the simultaneous phosphorus uptake and nitrate denitrification could be achieved by *Acinetobacteria* strain.

3.2. Effect of nitrite on phosphorus uptake

The phosphorus uptake potential of *Acinetobacteria* strain using nitrite as electron acceptor was shown in Fig. 3. The phosphorus concentrations decreased to 20.06 mg/L and 15.71 mg/L from 40 mg/L, respectively, when initial nitrite concentrations were 10 and 20 mg/L at the end of anoxic phase. Corresponding nitrite concentrations were decreased distinctly and consumed completely after 60 and 90 min, respectively. The highest phosphorus uptake rate was observed around 20 mg/L of nitrite feeding. This observation indicated that the optimal concentration of nitrite for phosphorus uptake was 20 mg/L. It is demonstrated that *Acinetobacteria* strain also could use nitrite as the electron acceptor for