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Post-anoxic denitrification via nitrite driven by PHB in feast–famine sequencing batch reactor

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HIGHLIGHTS

- Stable partial nitrification was achieved by controlling aeration time at 2.5 h.
- PHB can be used as a proper carbon source for post-anoxic denitrification.
- The faster growth rate of AOB than NOB was the main reason for achieving nitrite accumulation.
- The secondary SOP release was negligible at low ammonia loading.

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ABSTRACT

Recently, it was found that excess phosphorus removal could be induced by aerobic/extended-idle regime. In this study, an anoxic period was introduced after the aeration to realize simultaneous nitrogen and phosphorus removal. The results demonstrated that stable partial nitrification could be achieved by controlling the aeration duration at 2.5 h because it could not only obtain a desirable ammonia oxidation to nitrite but also avoid the extensive aeration converting nitrite to nitrate, and moreover, the accumulated poly-3-hydroxybutyrate still remain in a relative sufficient concentration (1.5 mmol C g^{-1} VSS), which could subsequently served as internal carbon source for post-anoxic denitrification. The nitrite accumulation ratio was observed to have relatively high correlation with biological nutrient removal. Over stages with stable high-level nitrite accumulation, the process achieved desirable and stable nitrogen and phosphorus removal efficiencies averaging 95% and 99% respectively. Fluorescence in situ hybridization analysis showed that the faster growth rate of the ammonia oxidizing bacteria than the nitrite oxidizing bacteria was the main reason for achieving nitrite accumulation. In addition, the secondary phosphorus release was negligible and the process maintained excellent nutrient removal under low influent ammonia nitrogen.

1. Introduction

Excess N and P have long been viewed as major factors causing eutrophication. As to N removal, almost all wastewater treatment plants worldwide achieve N removal by alternately exposing a population of bacteria that includes both nitrifiers and denitrifiers to oxic conditions for nitrification and anoxic conditions for denitrification (Lee et al., 2010). With respect to P removal, enhanced biological phosphorus removal (EBPR) processes which are conventionally conducted by alternating anaerobic and aerobic conditions and exploited the ability of certain microorganisms to accumulate P in excess of metabolic requirement and to store it as the intracellular biopolymer polyphosphate (poly-P), are widely applied for real wastewater treatment (Chen et al., 2004; Mullan et al., 2006). In order to achieve simultaneous N and P removal, some wastewater treatment processes such as anaerobic/anoxic/aerobic process have been developed. The EBPR systems with cyclic changes of anaerobic and aerobic (and/or anoxic) conditions have an economical advantage of lower sludge production and less use of chemicals, and play an increasingly important role in controlling eutrophication from all over the world (Oehmen et al., 2007).

However, there exist some contradictions which limit system efficiency. First of all, the circulating nitrate was reported as an inhibiting factor to anaerobic P release and could lead to reduced efficiency of biological P removal (BPR) (Barker and Dold, 1996). Secondly, the enrichment of nitrifying bacteria needs relatively long sludge retention time (SRT) and exerts negative influence on

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P removal because only relatively short SRT can lead to desirable EBPR performance (van Loosdrecht et al., 1998). On the other hand, denitrifying bacteria tend to compete with phosphate accumulating organisms (PAOs) for the limited dissolved oxygen (DO) in low strength wastewater (Ahn et al., 2002).

Fortunately, it is possible to circumvent limits exerted by traditional simultaneous N and P removal theories. Dirck et al. (2001) and Carucci et al. (2001) mentioned that since the bacteria encounter external substrates feast and famine periods. The regime can induce the bacteria to store external substrates as internal storage compounds in the feast period which hereby can take up available substrate very fast and utilize it to gain a more balanced growth. Studies on sequencing batch reactor (SBR) process for wastewater treatment have shown that the build-up of internal electron donors as storage compounds is of great importance for N removal (van Loosdrecht et al., 1997; Beun et al., 2000; Third et al., 2003). Therefore, storage compounds can be served as internal carbon sources for post-anoxic denitrification. Although the endogenous denitrification efficiency is low due to the limited carbon sources (Vocks et al., 2005), the limited internal carbon sources can be used to satisfy the need of denitrification via nitrite. In comparison to the conventional nitrate pathway, the nitrite pathway not only improved the total nitrogen (TN) removal by about 20% but also reduced aeration costs by 24% (Ma et al., 2009). Reports of stable nitritation processes from activated sludge have appeared frequently in the literatures and several process parameters, including DO concentration, temperature, SRT and aeration pattern, have been found to inhibit or wash out nitrite oxidizing bacteria (NOB) selectively to achieve sustainable partial nitrification to nitrite (Pollice et al., 2002; Ruiz et al., 2003). However, reliable termination of nitrification at nitrite (nitritation) has been proved difficult in the treatment of domestic wastewater, and controlling duration of aeration seems to be an ideal option for stable partial nitrification (Blackburne et al., 2008; Guo et al., 2009).

Recently, it has been reported that BPR could be achieved without specific anaerobic phase in activated sludge system if the idle period is extended properly (Wang et al., 2008, 2012). Though BPR can be well achieved during the aerobic period of aerobic/extended-idle regime, the system's denitrification capacity was comparatively weak and the TN removal efficiency was low. Therefore, an anoxic period is needed after the aerobic phase to realize thorough denitrification. The aim of this paper is to develop a process combining BPR with denitrification via nitrite driven by storage compounds to achieve simultaneous N and P removal. In this respect, an anoxic period is performed after the aerobic phase to realize thorough denitrification, and aeration duration control was used to realize sustainable partial nitrification to nitrite. The nitrite accumulation ratio and the ammonia oxidizing bacteria (AOB) and NOB population sizes were monitored. The impact of the nitrite accumulation ratio on P and N removal performance was assessed. Additionally, the mechanism of achieving nitrite accumulation was also discussed.

2. Materials and methods

2.1. Sequencing batch reactor operation

Seed sludge was inoculated into a SBR with a working volume of 42 L. Each cycle consisted of approximately 240 min aerobic period, 150 min anoxic period, 28 min settling, 2 min decanting, and 60 min idle periods. 30 L supernatant was discharged at the end of settling phase, and 30 L refresh wastewater was introduced during the first 2 min of aerobic period. The DO was supplied by an air compressor through an air diffuser inside the reactor during aerobic period and a magnetic stirrer was used to attain sound liquid mixing during anoxic phase. Temperature inside the reactor was maintained at 23 ± 3 °C with a thermostatic heater and pH was kept about 7.0. The SRT was maintained at approximately 10 d by withdrawing the sludge at the end of the anoxic period.

2.2. Wastewater and sludge

The wastewater was collected from septic tank effluent in a local residential district. It was characterized by 260–350 mg L^{-1} COD, 20–30 mg L^{-1} NH_4^+, 8–12 mg L^{-1} PO_4^{3-}. In addition, the wastewater had typical volatile fatty acid contents of 90–160 mg L^{-1} acetic acid, 80–120 mg L^{-1} propionic acid and a few other acids. Its pH level was about 7.0.

The seed sludge was obtained from the first wastewater treatment plant in Changsha, PR China. The initial concentration of mixed liquor suspended solids (MLSS) was about 4000 mg L^{-1}.

2.3. Experiment plans and operational conditions

To understand more clearly how the aeration duration affected N removal and to determine an appropriate aeration time that not only contribute to partial nitrification but also accumulate enough concentration of internal storage compounds, the experiments on the achievement, destruction and recurrence of the nitrite pathway were performed in seven stages. The duration of aeration was shortened from the normal 4 to 2.5 h over the first three stages, and was further shortened to 1.5 h during Stage IV and V. Then in Stage VI, the aeration duration was return to 2.5 h. The variations of aeration duration along with the seven phases are summarized in Table 1. During the whole experimental period, anoxic time was maintained at 2.5 h and each cycle was kept at 8 h. The idle time was correspondingly extended along with the decease of the aeration time.

2.4. Batch experiment

To examine the secondary P release during anoxic period under low influent NH_4^+, batch experimental was conducted in three identical reactors with working volumes of 2 L each. Seed sludge was taken from the parent SBR at the end of the anoxic phase but before settling. Three types of synthetic medium were added to the 3 reactors, all of the medium contained 300 mg L^{-1} COD (acetate) and 10 mg L^{-1} PO_4^{3-}, while NH_4^+ in the reactors were 15, 25 and 40 mg L^{-1}, respectively. The duration of aeration in batch experimental was maintained at 1.5 h.

2.5. Analytical methods

Sludge samples from the reactors were immediately filtered through a Whatmann GF/C glass microfiber filter (1.2 μm). The filtrate was analyzed for total phosphorus (TP), dissolved organic carbon (DOC), TN, NH_4^+, NO_2^-, NO_3^- and the filter was assayed for MLSS, mix liquor volatile suspended solids (MLVSS), poly-3-hydroxybutyrate (PHB) and sludge TN content.

TN, NH_4^+, NO_2^-, NO_3^-, soluble orthophosphate (SOP), MLSS and MLVSS were measured according to Standard methods (APHA, 1998). DO was measured by WTW Oxi 3210 SET 3 with DO probes (WTW company, Germany). DOC was determined by using a TOC analyzer (Shimadzu TOC-500, Japan) after membrane filtration (0.45 mm cellulose nitrate filter). In addition, Energy Dispersive Spectrometer (EDS, QUANTA 200, USA) has also been used to determine nitrogen content of dried activated sludge. 10 mL of activated sludge has been collected for freeze drying over 24 h and the samples were tested by EDS afterwards.

PHB was measured according to Oehmen et al. (2005) in a GC system operated with a Hewlett Packard 5890 column (30 m