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Achievement of high nitrite accumulation via endogenous partial denitrification (EPD)

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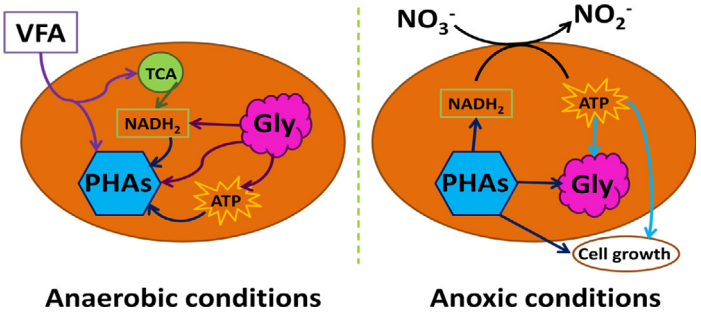
HIGHLIGHTS

- Endogenous partial denitrification was feasible to accumulate nitrite for anammox.
- Nitrate-to-nitrite transformation was kept at ~87% without nitrate in the effluent.
- Denitrifying GAOs phenotype was clearly observed.
- The presence of nitrate ensured on-going high nitrite accumulation efficiency.
- Endogenous partial denitrification combined with ANAMMOX was first put forward.

GRAPHICAL ABSTRACT



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ABSTRACT

This study proposed a novel strategy for achievement of partial denitrification driven by endogenous carbon sources in an anaerobic/anoxic/aerobic activated sludge system. Results showed that in the steady-stage, the nitrate-to-nitrite transformation ratio (NTR) was kept at around 87% without nitrate in the effluent. During the anaerobic period, exogenous carbon sources was completely taken up, accompanied by the consumption of glycogen and production of polyhydroxyalkanoates (PHAs). During the anoxic period, nitrate was reduced to nitrite by using PHAs as carbon sources, followed by the replenishment of glycogen. Thus, the phenotype of denitrifying GAOs was clearly observed and endogenous partial denitrification (EPD) occurred. Furthermore, results showed the nitrate reduction was prior to the nitrite reduction in the presence of nitrate, which led to the high nitrite accumulation.

1. Introduction

Anaerobic ammonium oxidation (anammox) has been widely accepted as the most economical and sustainable process for nitrogen removal from wastewater. Autotrophic nitrogen removal is realized by this technology, offering considerable saving in carbon source demands and low sludge production (Kuenen, 2008). For the application of anammox, ammonium and nitrite were required to be provided as substrates to enrich the anammox culture, in which ammonium was oxidized directly to dinitrogen gas with nitrite as the electron acceptor (Strous et al., 1998).

Since ammonium is the main nitrogen compound in sewage, a stable nitrite supply is a key in applying anammox process (Ma et al., 2016). Generally, two methods were used to acquire nitrite in biological nitrogen removal processes, i.e. (1) short-cut nitrification ($NH_4^+ \rightarrow NO_2^-$) and (2) partial denitrification ($NO_3^- \rightarrow NO_2^-$). Based on the short-cut nitrification, nitritation/anammox process

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has been successfully implemented for cost-efficient full-scale nitrogen removal from ammonium-rich wastewater such as anaerobic sludge digestion rejection water (Sliekers et al., 2003; Abma et al., 2010; Egli et al., 2001). However, nitritation/anammox has not been successfully applied to treat low ammonia sewage in main stream so far. The main obstacle is that it was difficult to achieve or stably sustain long-term short-cut nitrification (Liu et al., 2012). Additionally, since anammox was susceptible to the organics (Waki et al., 2013; Molinyevo et al., 2009), which was not high enough in domestic sewage to recover energy through anaerobic digestion, the organics was usually removed by aeration (Zhang et al., 2013), resulting in waste of both carbon sources and energy. Thus, in order to apply anammox process in treating domestic sewage, alternative methods to attain stable nitrite accumulation and cost-effectively alleviate the introduction of organic matters with low energy consumption are needed.

$$1NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1)$$

Recently, partial denitrification, a promising method to provide nitrite for anammox, has drawn much attention (Cao et al., 2016; Gong et al., 2013; Kalyuzhnyi et al., 2006). It was expected to produce nitrite from some industrial wastewaters containing a large amount of nitrate, such as fertilizer, explosives, metal finishing and nuclear (Cao et al., 2016; Glass and Joann, 1998, 1999), which could not be effectively and economically treated through the traditional nitritation/anammox process. Moreover, according to the stoichiometry of the anammox process presented in Eq. (1), approximately 11% of nitrogen as nitrate is contained in the effluent when applying the anammox process (Strous et al., 1998). In other words, when high strength ammonia wastewater is treated by the anammox-only process, further treatment of the effluent is required to meet the discharge standard. In this sense, partial denitrification combined with anammox process is proposed to be an energy-saving and sustainable method for advanced nitrogen removal from sewage and nitrate wastewaters. For example, Du et al. (2015) established an anammox combined with exogenous partial denitrification process in two SBRs for advanced nitrogen removal from anammox effluent containing excessive nitrate. However, partial denitrification driven by exogenous carbon sources was required to terminate the denitrification reaction in time (Du et al., 2016), which might not adequately remove the organics and might affect the normal growth of anammox bacteria. Besides, Cao et al. (2016) reported that during partial denitrification driven by exogenous carbon sources, sludge bulking occurred under high-load condition, which was caused by high content of extracellular polymeric substances (EPS).

Notably, glycogen accumulating organisms (GAOs), which could absorb and store organic substances as poly-β-hydroxy-alkanoates (PHAs) in the anaerobic period and use the accumulated PHAs for glycogen re-synthesis and cell growth in the following aerobic period, were found to show the denitrification capabilities (Wang et al., 2008) and play an important role in endogenous denitrification (ED) (Miao et al., 2015). What's more, the ED process could make full use of organic compounds in the raw wastewater compared with the traditional denitrification (Miao et al., 2016). Therefore, partial denitrification driven by endogenous carbon sources would not only provide anammox with nitrite, but also make the most use of the carbon sources in sewage and alleviate the negative effect of organics on anammox. Further, this process might greatly reduce the risk of sludge bulking caused by the EPS thanks to the alternating anaerobic/anoxic operational mode. To the best of our knowledge, little effort has been made to realize high nitrite accumulation driven by endogenous carbon sources in partial denitrification process. It is extremely significant to advancedly remove nitrogen from the effluent of anammox process or the industrial wastewaters containing high nitrate and solve the problems mentioned above when applying the anammox process to treat domestic sewage.

In this study, a novel partial denitrification driven by endogenous carbon sources (PHAs) was established in anaerobic/anoxic/aerobic activated sludge system to demonstrate the feasibility of this strategy through a long-term experiment. The research also aimed to investigate the variation of nitrogen and carbon source in a typical SBR cycle to verify the denitrification pathway. Furthermore, to assess the endogenous partial denitrification (EPD) ability of the sludge, anoxic batch tests with the addition of only nitrate were performed using the sludge from the system at the end of the anaerobic period.

2. Materials and methods

2.1. Wastewater and seeding sludge

The substrates fed into the EPD system were divided into anaerobic substrate and anoxic substrate. The anaerobic substrate, only consisting of sodium acetate, was continuously pumped into the EPD reactor during anaerobic stage. The chemical oxygen demand (COD) was 1500 mg/L until Day 60 and decreased to 1200 mg/L subsequently. The anoxic substrate was the effluent of a 10 L laboratory-scale pre-denitrification SBR fed with real domestic wastewater. The main characteristics of the anoxic substrate were: NO_3^--N 22.03–45.76 mg N/L, NH_4^+-N < 1.5 mg N/L, NO_2^--N < 1.0 mg N/L, PO_4^{3-}-P 4.53–8.01 mg P/L, and COD 33.01–51.12 mg/L. The seeding sludge was taken from a 10 L laboratory-scale sequencing batch reactor (called the parent SBR), which clearly showed the denitrification GAOs phenotype (Fig. 1). The cycle time of the parent SBR was 8 h, consisting of 180 min anaerobic reaction (including 167 min anaerobic substrate feeding period), 30 min settling, 10 min decanting, 60 min anoxic reaction (including 10 min anoxic substrate feeding period), 120 min aerobic reaction, 30 min further settling, 10 min decanting and 40 min idle phase. During the anaerobic period, 0.5 L anaerobic substrate (the concentration of COD was 4000 mg/L) was continuously added to the reactor. At the beginning of the anoxic period, 5 L anoxic substrate was pumped into the reactor in 10 min. During the two decanting period, 0.5 L and 5 L supernatant was respectively discharged from the reactor.

2.2. Experimental set-up and operation process

A laboratory-scale SBR (working volume of 10 L) made of methyl methacrylate (PMMA) was used for endogenous partial denitrification system (termed as EPD-SBR) (Fig. S1). The system was operated for 110 days applying alternating anaerobic/anoxic/aerobic operational mode. The cycle time of the EPD-SBR was 6 h, consisting of 120 min anaerobic reaction (including 117 min feeding period), 30 min anoxic reaction (including 10 min feeding period), 30 min settling, 10 min decanting, 30 min post aerobic reaction and 140 min idle period. During the anaerobic period, 0.5 L anaerobic substrate was continuously added to the EPD-SBR. At the end of the anaerobic period, 4.5 L anoxic substrate was pumped into the EPD-SBR in 10 min. During the decanting period, 5 L supernatant was discharged from the reactor with a hydraulic retention time (HRT) of 6 h. The EPD-SBR reactor was operated without sludge discharge during the 110-day operation period. The mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentrations were about 2800 mg/L and 2400 mg/L, respectively.

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