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Comparison of endogenous metabolism during long-term anaerobic starvation of nitrite/nitrate cultivated denitrifying phosphorus removal sludges

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ABSTRACT

Denitrifying phosphorus removal (DPR) by denitrifying phosphorus-accumulating organisms (DPAOs) is a promising approach for reducing energy and carbon usage. However, influent fluctuations or interruptions frequently expose the DPAOs biomass to starvation conditions, reducing biomass activity and amount, and ultimately degrading the performance of DPR. Therefore, a better understanding of the endogenous metabolism and recovery ability of DPAOs is urgently required. In the present study, anaerobic starvation (12 days) and recovery were investigated in nitrite- and nitrate-cultivated DPAOs at 20 ± 1 °C. The cell decay rates in nitrite-DPAOs sludges from the end of the anaerobic and aerobic phase were 0.008 day^{-1} and 0.007 day^{-1}, respectively, being 64% and 68% lower than those of nitrate-DPAOs sludges. Nitrite-DPAOs sludges also recovered more rapidly than nitrate-DPAOs sludge after 12 days of starvation. The maintenance energy of nitrite-DPAOs sludges from the end of the anaerobic and aerobic phase were approximately 31% and 34% lower, respectively, than those of nitrate-DPAOs sludges. Glycogen and polyphosphate (poly-P) sequentially served as the main maintenance energy sources in both nitrite-and nitrate-DPAOs sludges. However, the transformation pathway of the intracellular polymers during starvation differed between them. Nitrate-DPAOs sludge used extracellular polymeric substances (EPS) (mainly polysaccharides) as an additional maintenance energy source during the first 3 days of starvation. During this phase, EPS appeared to contribute to 19–27% of the ATP production in nitrate-DPAOs, but considerably less to the cell maintenance of nitrite-DPAOs. The high resistance of nitrite-DPAOs to starvation might be attributable to frequent short-term starvation and exposure to toxic substances such as nitrite/free nitrous acids in the parent nitrite-fed reactor. The strong resistance of nitrite-DPAOs sludge to anaerobic starvation may be exploited in P removal by shortcut denitrification processes.

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1. Introduction

Owing to large fluctuations in the flow and composition of wastewater, the microorganisms responsible for biological wastewater treatment plants are frequently exposed to long-term famine conditions (days and sometimes weeks) (Lu et al., 2007). During sludge storage, by which large influent variations can be adjusted and flexible plant operation can be achieved, microorganisms may experience starvation (Morgenroth et al., 2000). Starvation significantly reduces the amount and activity of active microorganisms, and risks degrading the capacity, efficiency and robustness of wastewater treatment systems (Hao et al., 2010b; Wang et al., 2013b). Starvation is crucially important in enhanced biological phosphorus removal (EBPR) processes, since it alters the levels of intracellular storage compounds in the functional microbes (i.e., polyphosphate-accumulating organisms, PAOs) (Vargas et al., 2013). Indeed, excessive consumption of intracellular polymers (Yilmaz et al., 2007) or excessive decay of both PAOs and intracellular polymers (Miyake and Morgenroth, 2005) has been implicated in EBPR failure.

In the absence of external sustenance, starved microorganisms primarily undergo the endogenous processes consisting of cell maintenance and cell decay (Lu et al., 2007; Wang et al., 2012). The impacts of starvation on PAOs and endogenous processes have been extensively investigated (Lopez et al., 2006; Yilmaz et al., 2007; Lu et al., 2007; Hao et al., 2010a; Wang et al., 2012; Vargas et al., 2013), and effective strategies for maintaining the biomass activity have been accordingly proposed. Lopez et al. (2006) examined the effects of long-term (weeks) anaerobic and aerobic starvation on the composition and activity of PAOs. They concluded that, under aerobic starvation conditions, PAOs are notably attenuated by endogenous processes, whereas no significant PAOs decay occurs during anaerobic starvation. Lu et al. (2007) proposed an intermittent aerobic–anaerobic strategy for the long-term storage of EBPR sludge. In this strategy, the PAOs decay more slowly than in aerobic storage, and glycogen and poly-P are used at a slower rate than in anaerobic and anoxic storage. A similar recovery strategy was recommended by Yilmaz et al. (2007), who found that alternating anoxic/anaerobic and aerobic operation effectively maintains the biomass activity of activated sludge used for biological nitrogen (N) and phosphorus (P) removal, thereby enabling quick activity recovery (i.e., full recovery within 4 days).

Unlike PAOs in traditional EBPR processes, the impacts of starvation on denitrifying polyphosphate-accumulating organisms (DPAOs) have been little reported. Denitrifying phosphorus removal (DPR) by DPAOs is a viable and sustainable technology, as N and P can be simultaneously removed with lower carbon source requirements, aeration costs and cell yields (Murnleitner et al., 1997). In particular, since DPAOs can use nitrite as an electron acceptor, DPR is naturally amenable to shortcut nitrification. By replacing nitrate with nitrite, the oxygen cost and carbon consumption of DPR can be reduced by approximately 25% and 40%, respectively (Abeling and Seyfried, 1992). Therefore, DPR by nitrite could be used for innovative biological nutrient removal (BNR) systems where energy and carbon savings are a priority, for example, linking nitrite pathways (i.e., partial nitrification + nitrite-based denitrification) to EBPR (Guisasola et al., 2009; Marcelino et al., 2011; Zhou et al., 2011; Tayà et al., 2013). Moreover, as nitrite-enriched PAOs need less intracellular carbon source (i.e., poly-β-hydroxyalkanoates (PHA)) for P-uptake, and eventually they might have higher PHA accumulation which can be used to speed up their anoxic metabolism after the endogenous period.

Recently, identifying the inhibitory effects of nitrite and the feasibility of nitrite-based DPR has received increasing attention (Guisasola et al., 2009; Marcelino et al., 2011; Zhou et al., 2011). However, DPAOs metabolism, especially their endogenous metabolism, has not been properly elucidated. To our knowledge, the endogenous characteristics of nitrite-DPAOs have not been assessed. A better understanding of the mechanism of the impact of starvation on nitrite- and nitrate-DPAOs, and endogenous metabolism of DPAOs may favor the development of strategies for improvement of the robustness and performance of DPR processes, the resuscitation of DPR systems after famine scenarios, and the storage of DPAOs sludge.

Increasing evidence shows that extracellular polymeric substances (EPS) can serve as carbon and energy sources for active biomass growth under starvation conditions (Zhang and Bishop, 2003; Wang et al., 2005, 2007; Liu et al., 2007; Flemming and Wingender, 2010). Wang et al. (2005) found that most biodegradable EPS, especially polysaccharides, are located in the core of aerobic granular sludge, and that this fraction of EPS can be depleted after long-term starvation (20 days), as evidenced by the void structure in the core of starved granules. Since most previous starvation investigations of EBPR sludge did not involve EPS, the contribution of EPS to the maintenance of metabolic activity of PAOs/DPAOs remains unclear.

The purpose of this study is to identify the differences between the endogenous characteristics of nitrite- and nitrate-DPAOs sludges during 12-day anaerobic starvation, and to better understand the endogenous metabolism of nitrite-DPAOs. The transformation of intracellular polymers and post-starvation activity recovery are also compared between nitrite and nitrate-DPAOs sludges. We highlight the different transformation pathways of intracellular polymers in nitrite- and nitrate-DPAOs biomass. We also attempt to clarify the role of EPS (especially polysaccharides) in nitrite/nitrate-DPAOs under anaerobic starvation conditions.

2. Materials and methods

2.1. Set up and long-term operation of parent reactors

DPAOs sludge was enriched in two identical laboratory-scale sequencing batch reactors (SBR_{NO3^-} and SBR_{NO2^-}, using nitrate and nitrite as electron acceptors, respectively) with a working volume of 7.5 L as outlined by Wang et al. (2011). Both SBRs were independently operated in a cyclical anaerobic-anoxic-aerobic pattern with a cycle time of 8 h (15-min filling period, a 120-min anaerobic period, a 210-min anoxic period, a 30-min aerobic period, a 20-min settling period, a 15-min effluent discharging period, and a 70-min idle period).