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Nitrite survival and nitrous oxide production of denitrifying phosphorus removal sludges in long-term nitrite/nitrate-fed sequencing batch reactors

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ABSTRACT

Nitrite-based phosphorus (P) removal could be useful for innovative biological P removal systems where energy and carbon savings are a priority. However, using nitrite for denitrification may cause nitrous oxide (N_2O) accumulation and emissions. A denitrifying nitrite-fed P removal system ($SBR_{NO_2^-}$) was successfully set up in a sequencing batch reactor (SBR) and was run for 210 days. The maximum pulse addition of nitrite to $SBR_{NO_2^-}$ was 11 mg NO_2^--N/L in the bulk, and a total of 34 mg NO_2^--N/L of nitrite was added over three additions. Fluorescent *in situ* hybridization results indicated that the P-accumulating organisms (PAOs) abundance was 75 ± 1.1% in $SBR_{NO_2^-}$, approximately 13.6% higher than that in a parallel P removal SBR using nitrate ($SBR_{NO_3^-}$). Type II *Accumulibacter* (PAOII) (unable to use nitrate as an electron acceptor) was the main PAOs species in $SBR_{NO_2^-}$, contributing 72% to total PAOs. Compared with $SBR_{NO_3^-}$, $SBR_{NO_2^-}$ biomass had enhanced nitrite/free nitrous acid (FNA) endurance, as demonstrated by its higher nitrite denitrification and P uptake rates. N_2O accumulated temporarily in $SBR_{NO_2^-}$ after each pulse of nitrite. Peak N_2O concentrations in the bulk for $SBR_{NO_2^-}$ were generally 6–11 times higher than that in $SBR_{NO_3^-}$; these accumulations were rapidly denitrified to nitrogen gases. N_2O concentration increased rapidly in nitrate-cultivated biomass when 5 or 10 mg NO_2^--N/L per pulse was added. Whereas, N_2O accumulation did not occur in nitrite-cultivated biomass until up to 30 mg NO_2^--N/L per pulse was added. Long-term acclimation to nitrite and pulse addition of nitrite in $SBR_{NO_2^-}$ reduced the risk of nitrite accumulation, and mitigated N_2O accumulation and emissions from denitrifying P removal by nitrite.

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1. Introduction

Denitrifying phosphorus removal (DPR) is a cost effective and environmentally friendly technology for removing phosphorus (P) from wastewater. The ability to use denitrifying P accumulating organisms (DPAOs) in biological nutrient removal (BNR) is very attractive and sustainable, because nitrogen (N) and P can be removed simultaneously with reduced carbon source requirements, lower aeration costs and lower cell yields than those with P- accumulating organisms (PAOs), resulting in less sludge production (Murnleitner et al., 1997). Nitrite, as an intermediate product of denitrification, may also be a potential electron acceptor for DPR (Zhou et al., 2008a). Theoretically, oxidation costs and carbon consumption would be approximately 25% and 40% lower, respectively, than that of DPR by nitrate (Abeling and Seyfried, 1992), if stable DPR could be achieved using nitrite. Therefore, DPR by nitrite could be used for innovative BNR systems where energy and carbon savings are a priority, for example, linking nitrite pathway (i.e., partial nitrification + nitrite-based denitrification) to enhanced biological P removal (EBPR) (Guisasola et al., 2009; Vargas et al., 2011; Zhou et al., 2011; Tayà et al., 2013).

In full-scale wastewater treatment plants (WWTPs), biological P removal is typically combined with nitrogen removal, both requiring organic carbon that is often limiting (Pijuan et al., 2010). Nitrite may accumulate (e.g., to several mg/L) in the denitrification process under limited carbon conditions (Lemaire et al., 2008; Ma et al., 2009). To reduce the requirement for carbon, great effort has been devoted in recent years to achieve nitrogen removal through nitrite pathway (Pijuan et al., 2010). However, there is limited knowledge about how PAOs populations evolve in long-term nitrite-DPR systems, mostly because of the fact that nitrite accumulation has a negative effect on aerobic and anoxic P uptake (Meinhold et al., 1999; Saito et al., 2004). In general, nitrite is very toxic and inhibits the growth and respiration of bacteria (Yarbrough et al., 1980), such that elevated nitrite concentrations are expected to negatively affect the DPR process. Zhou et al. (2008a) and Pijuan et al. (2010) found that free nitrous acid (FNA), the protonated species of nitrite, inhibits aerobic and anoxic P uptake. 50% inhibition was observed for DPAOs sludge when the FNA concentration was 0.7–1.0 μg HNO_2-N/L (equivalent to 3–4 mg NO_2^--N/L at pH 7) (Zhou et al., 2008a), and for EBPR sludge when the FNA concentration was approximately 0.5 μg/L HNO_2-N/L (equivalent to 2.0 mg NO_2^--N/L at pH 7.0) (Pijuan et al., 2010). Nitrite/FNA is therefore harmful to PAOs and thus has limited the application of DPR process using nitrite. However, Guisasola et al. (2009) operated an anaerobic/anoxic/aerobic (A^2/O) SBR successfully for more than 6 months, even with a maximum concentration of 60 mg NO_2^--N/L. Vargas et al. (2011) also reported that, after acclimation for a period, the EBPR system can operate steadily under anaerobic-anoxic conditions using nitrite as the sole electron acceptor. Furthermore, Tayà et al. (2013) demonstrated that conventional anaerobic-aerobic EBPR system can be directly adapted to an anaerobic-anoxic pattern with nitrite as electron acceptor. Clearly, many aspects of the nitrite-DPR process like the adaptation strategy and the nitrite-PAOs endurance to FNA are still subject of debate.

It is generally acknowledged that greater nitrite/FNA accumulation tends to stimulate nitrous oxide (N_2O) production by DPAOs (Yarbrough et al., 1980; Zhou et al., 2008a,b, 2011; Zeng et al., 2011; Ye et al., 2010). Zeng et al. (2003) was the pioneer to report that DPR by nitrate or nitrite could trigger N_2O production and emissions, and that N_2O, instead of N_2, was the main denitrification product. N_2O is a powerful greenhouse gas with a global warming effect that is approximately 300 times greater than that of carbon dioxide (CO_2) (IPCC, 2013). N_2O emissions reached 0–14.6% of the nitrogen load in full-scale wastewater systems (Kampschreur et al., 2009). N_2O can be produced during both microbial nitrification and denitrification, and emissions from both processes can increase under suboptimal conditions (Burgess et al., 2002), e.g., in the presence of toxic substances such as nitrite/FNA (Zhou et al., 2008a, 2008b, 2011; Pijuan et al., 2010). Zhou et al. (2008a) explained that FNA can react with N_2O reductase (copper-containing enzymes) and that it can cause competitive inhibition to N_2O reduction, contributing to accumulation of N_2O. To date, however, studies have mainly focused on the impact of short-term nitrite/FNA exposure on N_2O production during anoxic P uptake processes. It remains to be seen if nitrite/FNA endurance can be improved, or if N_2O accumulation can be mitigated in the long term when DPAOs are cultivated in a nitrite-based system.

Two sequencing batch reactors (SBRs) were run for 500 days with nitrate and nitrite as the sole electron acceptors. In this contribution, we compared the treatment performance and N_2O accumulation in nitrite- and nitrate-fed DPAOs SBRs. We also highlighted the need to consider greenhouse gas accumulation and emissions when implementing this technology. Fluorescent *in situ* hybridization (FISH) was used to assess the composition of the populations and their influence on the systems' ability to perform denitrification and P uptake with nitrite/nitrate. Batch tests were carried out using enriched nitrite and nitrate-DPAOs biomass. A series of batch experiments were conducted at various levels of nitrite and nitrate, during which anoxic P uptake, N_2O accumulation, PHA consumption and glycogen production were monitored and compared between enriched nitrite- and nitrate-DPAOs. The aim of this study was to identify the nitrite endurance capacity and N_2O production characteristics of long-term nitrite-fed DPR systems, and to provide an insight into developing strategies for stable DPR performance (using nitrite or nitrate) with the lowest N_2O emissions.

2. Materials and methods

2.1. Reactor set-up and operation

2.1.1. Reactor set-up and operating conditions

DPAOs sludge was cultivated using nitrite and nitrate as electron acceptors in two identical SBRs ($SBR_{NO_2^-}$ and $SBR_{NO_3^-}$) with a working volume of 7.5 L (an internal diameter of 16 cm and a height of 50 cm) (Wang et al., 2011). Both SBRs were fed with synthetic wastewater (Section 2.4) with alternating anaerobic-anoxic-aerobic operating conditions. The SBRs