Journal of Environmental Sciences 26 (2014) 264–273

Available online at www.sciencedirect.com

Journal of Environmental Sciences

www.jesc.ac.cn

Effect of temperature on anoxic metabolism of nitrites to nitrous oxide by polyphosphate accumulating organisms

Zhijia Miao, Wei Zeng*, Shuying Wang, Yongzhen Peng*, Guihua Cao, Dongchen Weng, Guisong Xue, Qing Yang

Key Laboratory of Beijing for Water Quality Science and Water Environment Recovery Engineering, Engineering Research Center of Beijing, Beijing University of Technology, Beijing 100124, China. E-mail: miaozhijia@emails.bjut.edu.cn

ARTICLE INFO

Article history:
Received 04 March 2013
revised 22 April 2013
accepted 26 April 2013

Keywords:
polyphosphate accumulating organisms
temperature
nitrite
denitrifying phosphorus removal
N_2O
kinetics
stoichiometry

DOI: 10.1016/S1001-0742(13)60406-4

ABSTRACT

Temperature is an important physical factor, which strongly influences biomass and metabolic activity. In this study, the effects of temperature on the anoxic metabolism of nitrite (NO_2^-) to nitrous oxide (N_2O) by polyphosphate accumulating organisms, and the process of the accumulation of N_2O (during nitrite reduction), which acts as an electron acceptor, were investigated using 91% ± 4% *Candidatus Accumulibacter phosphatis* sludge. The results showed that N_2O is accumulated when *Accumulibacter* first utilize nitrite instead of oxygen as the sole electron acceptor during the denitrifying phosphorus removal process. Properties such as nitrite reduction rate, phosphorus uptake rate, N_2O reduction rate, and polyhydroxyalkanoate degradation rate were all influenced by temperature variation (over the range from 10 to 30°C reaching maximum values at 25°C). The reduction rate of N_2O by N_2O reductase was more sensitive to temperature when N_2O was utilized as the sole electron acceptor instead of NO_2, and the N_2O reduction rates, ranging from 0.48 to 3.53 N_2O-N/(hr·g VSS), increased to 1.45 to 8.60 mg N_2O-N/(hr·g VSS). The kinetics processes for temperature variation of 10 to 30°C were (θ_1 = 1.140–1.216 and θ_2 = 1.139–1.167). In the range of 10°C to 30°C, almost all of the anoxic stoichiometry was sensitive to temperature changes. In addition, a rise in N_2O reduction activity leading to a decrease in N_2O accumulation in long term operations at the optimal temperature (27°C calculated by the Arrhenius model).

Introduction

The enhanced biological phosphorus removal (EBPR) system has been confirmed to be an economical and sustainable process, and plays an increasingly important role in wastewater treatment. A group of bacteria known as polyphosphate accumulating organisms (PAOs) dominate in the EBPR system (Crocetti et al., 2000). The PAOs are able to take up volatile fatty acids and store them as polyhydroxyalkanoates (PHAs), which is attributed to the release of phosphorus in the anaerobic phase. In the subsequent aerobic phase, PHAs are used for growth and phosphate uptake, leading to a net phosphate removal from the wastewater. In most previous studies, one group of PAOs called denitrifying PAOs were shown capable of oxidizing their intracellular PHAs to fulfill their energy requirements and take up phosphorus under anoxic conditions, while utilizing nitrite or nitrate as electron acceptors instead of oxygen (Ahn et al., 2001; Meinhold et al., 1999; Zeng et al., 2003). In this process, the same carbon source can be used to simultaneously remove N and P, requiring 20%–30% less microorganisms, and reducing plant operational costs due to the savings in the consumption of both oxygen and carbon sources (Kishida et al., 2006).

* Corresponding author. E-mail: pyz@bjut.edu.cn (Yongzhen Peng); zengwei_1@263.net (Wei Zeng)

Nitrous oxide (N_2O), a significant greenhouse gas, has 300 times greater warming potential than carbon dioxide (CO_2) (IPCC, 2001). Most studies on nitrification, denitrification, and phosphorus removal pathways have demonstrated that N_2O could be accumulated in wastewater treatment systems. There is an increasing amount of evidence showing that ammonia oxidizing bacteria, heterotrophic denitrification organisms, and denitrifying glycogen accumulation organisms are the major contributors to N_2O emissions from wastewater treatment plants (Tallec et al., 2006; Ahn et al., 2010; Zeng et al., 2003b). Meanwhile, the PAOs, namely *Candidatus Accumulibacter phosphatis*, are often dominant in both lab-scale EBPR reactors (Crocetti et al., 2000; Hesselmann et al., 1999; Lu et al., 2006) and full-scale wastewater treatment plants (Pijuan et al., 2008). PAOs may have contributed to N_2O emission during anoxic metabolism in WWTP operation, which cannot be ignored. The production of N_2O is affected by many parameters such as low dissolved oxygen concentrations, accumulation of nitrite, rapidly changing conditions, types of organic carbon sources, pH, and temperature (Kampschreur et al., 2009; Yoshida, 1988). It has been reported that the accumulation of nitrite leads to an increase in N_2O emission rather than nitrogen (N_2) as the major end-product in denitrifying phosphorus removal processes (Lemaire et al., 2006; Zeng et al., 2003). Indeed, it was reported that free nitrous acid (FNA), rather than nitrite and pH, is likely the real inhibitor of N_2O reduction by denitrifying PAOs (Wang et al., 2011; Zhou et al., 2008). The concentration of free nitrous acid (FNA, HNO_2-N) was calculated by using the following formula:

$$\mathrm{FNA} = S_{(NO_2^-\text{-}N)}/(K_\alpha \times 10^{\mathrm{pH}}) \quad (1)$$

where, $K_\alpha = e^{-2300/(273+T)}$, T (K) is temperature (Anthonisen et al., 1976). Temperature, being an important factor for FNA, should have a major influence on denitrifying phosphorus removal and N_2O metabolism processes. However, most previous studies focused on the aerobic metabolism of PAOs or the influence of temperature on the competition between PAOs and glycogen-accumulating organisms (GAOs). Panswad et al. (2003) investigated the competition between PAOs and GAOs and found that PAOs were dominant at low temperature (e.g., 10°C, and they considered that PAOs were constituted of lower range mesophiles or possibly psychrophiles. Brdjanovic et al. (1997) performed a systematic study concerning the effects of temperature on the aerobic metabolism of PAOs and the dependence of different processes at 5 to 30°C. However, a systematic study on the effects of temperature in N_2O metabolism by a PAO culture under anoxic conditions, with respect to the utilization of nitrite as an electron acceptor, has not been reported yet.

In this study, a series of batch tests were carried out using a highly enriched culture of *Candidatus Accumulibacter phosphatis*. We aimed to find the effects of temperature on PHA oxidation, nitrite reduction, phosphorus uptake, and N_2O metabolism during the denitrifying phosphorus removal process. This study also evaluated the stoichiometry and kinetics of PAOs in combination with N_2O metabolism during this anoxic process.

1 Materials and methods

1.1 Reactor and operation

A laboratory-scale sequencing batch reactor (SBR) with a working volume of 8 L was operated for 240 days under anaerobic and aerobic conditions. The SBR was fed with acetate or propionate, switching at a frequency of one to two sludge ages. The cycle time was 6 hr and consisted of the following: a 150 min anaerobic period, a 180 min aerobic period, 25 min settle/decant period, and a 5 min idle period. In each cycle, 2 L of synthetic wastewater was fed to the reactor in the first 6 min of the anaerobic period, resulting in a hydraulic retention time of 24 hr. At the end of the cycle, 200 mL of sludge was removed to achieve a solids retention time of 10 days and a mixed liquor suspended solid level of 2.5–3.5 g/L. The dissolved oxygen concentration was maintained at 2.0±0.2 mg/L in the aerobic period by using an online on/off controller switch. The pH was controlled during both the anaerobic and aerobic phases at a range of 7.2–8.0 by doses of 0.5 mol/L HCl and 0.5 mol/L NaOH solutions. The temperature was maintained at 20°C.

1.2 Synthetic wastewater

Synthetic wastewater (2 L) described by Lu et al. (2006) was composed of 0.3 L solution A and 1.7 L solution B. The mixed feed of solutions A and B contained 800 COD/L and 40 mg P/L. Solution A contained 3.41 g of acetate and 1.76 mL of propionic acid per liter of solution. In addition, solution A also contained (per liter) 1.02 g NH_4Cl, 0.01 g peptone, 0.01 g yeast extraction, 1.20 g $MgSO_4{\cdot}7H_2O$, 0.19 g $CaCl_2{\cdot}2H_2O$, 7.94 mg allyl-N thiourea (a nitrification inhibitor), and 4.00 mL of a trace elements liquid. Solution B contained 173 mg $K_2HPO_4{\cdot}3H_2O$ and 104 mg KH_2PO_4 per liter of solution. For the propionic acid feed, 10.47 mL of 5 mol/L NaOH was used to adjust the pH to 7.5.

1.3 Batch experiment 1

The sludge sample for testing in batch experiment 1 was taken from the SBR fed with acetate during the normal cycle of operation. At the end of the anaerobic stage, 5 L of mixed liquor was divided into five parts and put into a 1.25 L batch reactor. A nitrite stock solution (sodium nitrite at a concentration of 10 g NO_2-N/L) was added to the batch reactors at the beginning of each experiment, which resulted in initial concentrations of 20 mg NO_2-