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Mdodeling a nitrite-dependent anaerobic methane oxidation process: Parameters identification and model evaluation

Zhanfei He, Chen Cai, Sha Geng, Liping Lou, Xiangyang Xu, Ping Zheng, Baolan Hu *

Department of Environmental Engineering, Zhejiang University, Hangzhou 310058, China

HIGHLIGHTS

- First time identified several key kinetic parameters for n-damo bacteria.
- Methane was not a limiting factor of n-damo process.
- The optimal nitrite concentration was 1.92 mmol L^{-1}.
- A kinetic model for n-damo process was first established and evaluated.

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ABSTRACT

Nitrite-dependent anaerobic methane oxidation (n-damo) is a recently discovered process that is intermediated by n-damo bacteria that oxidize methane with nitrite to generate nitrogen gas. In this work, a kinetic model based on Monod type kinetics and diffusion-reaction model was developed to describe the bioprocess. Some key kinetic parameters needed in the model were obtained from a series of batch activity tests and a sequencing batch reactor (SBR) operation over 100 days. The growth rate, decay rate, methane affinity constant, nitrite affinity constant and inhibition constant were $0.0277 \pm 0.0022\,d^{-1}$, $0.00216 \pm 0.00010\,d^{-1}$, 0.092 ± 0.005 mmol L^{-1}, 0.91 ± 0.09 mmol L^{-1} and 4.1 ± 0.5 mmol L^{-1} for n-damo bacteria at 30 °C, respectively. The results showed that the model could simulate actual performance of the SBR in the first 76 days, that methane was not a limiting factor at atmospheric pressure for its high affinity, and that the optimum nitrite concentration was 1.92 mmol L^{-1}.

1. Introduction

Nitrite-dependent anaerobic methane oxidation (n-damo), a microbial process using nitrite as the electron acceptor to anaerobically oxidize methane, has been discovered in the last decade (Raghoebarsing et al., 2006). The stoichiometric equation is showed in Eq. (1). The process has been noticed widely (Ettwig et al., 2008; Hu et al., 2009; Shen et al., 2012), because it has several advantages over conventional wastewater treatment processes, including efficient utilization of methane (a potent greenhouse gas) and removal of nitrogenous pollutants simultaneously. Although the methane in the gas phase can be recycled and used for electric energy generation, the dissolved methane in the effluent water from a wastewater treatment plant is difficult to recover and will be released slowly into the environment contributing to the greenhouse effect (Cakir and Stenstrom, 2005). The process was suggested to treat the effluent from low-temperature anaerobic sewage treatments that contained dissolved methane and nitrogen, in which dissolved methane could be used as solo electron donor for denitrification. Kampman et al. proposed a new concept for treating anaerobic treatment effluent that consisted of a UASB-digester system, an n-damo reactor and a nitritation reactor (Kampman et al., 2012). Luesken et al. verified that n-damo bacteria and anammox bacteria could coexist experimentally, and the application of such a coculture for nitrogen removal may be feasible in the near future (Luesken et al., 2011). However, the growth of n-damo bacteria is too slow to obtain sufficient enrichment culture (Ettwig et al., 2008; Raghoebarsing et al., 2006), which has prevented research progress from being made on the n-damo process. The growth could be accelerated by breaking some "bottle necks", and several limiting factors were discussed in this work.

$$3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 4N_2 + 10H_2O \ (\Delta G^{0'} = -928\text{kJ mol}^{-1}CH_4) \quad (1)$$

Mathematical model is a useful tool to give an insight of biologic reaction system, to help design an activated sludge treatment system, and to optimize operational parameters. There have been many successful cases where mathematic models were used to

* Corresponding author. Tel./fax: +86 0571 88982340.
E-mail address: blhu@zju.edu.cn (B. Hu).

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help design and operate biologic wastewater treatment systems (Amand and Carlsson, 2012; Young et al., 2013). Despite the usefulness of the mathematic model, there is no mathematic model of the n-damo process reported to our knowledge, mainly because several key kinetic parameters have not been determined. In this work, some key kinetic parameters were identified from a series of batch activity tests and a sequencing batch reactor (SBR) operation. The main goals of this work are to develop a concise kinetic model to describe the n-damo process and to identify the key kinetic parameters of n-damo bacteria. The n-damo model is based on Monod type kinetics (Ni et al., 2009) and diffusion-reaction model (Wanner and Gujer, 1986). Moreover, the growth limiting factors of n-damo bacteria were discussed on the foundation of the results in this work.

2. Methods

2.1. Biomass and medium

The inoculum of a 1.0 L SBR was taken from a previous lab-scale SBR fed with artificial medium after 500 days. The medium contained the following components (per liter): 1.0 g $KHCO_3$, 0.05 g KH_2PO_4, 0.3 g $CaCl_2{\cdot}2H_2O$, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.014 g $NaNO_2$, 0.5 ml of an acidic trace element solution, and 0.2 ml of an alkaline trace element solution. The acidic (100 mM HCl) trace element solution contained (per liter) 2.085 g $FeSO_4{\cdot}7H_2O$, 0.068 g $ZnSO_4{\cdot}7H_2O$, 0.12 g $CoCl_2{\cdot}6H_2O$, 0.5 g $MnCl_2{\cdot}4H_2O$, 0.32 g $CuSO_4$, 0.095 g $NiCl_2{\cdot}6H_2O$, and 0.014 g H_3BO_3. The alkaline (10 mM NaOH) trace element solution contained (per liter) 0.067 g SeO_2, 0.050 g $Na_2WO_4{\cdot}2H_2O$, and 0.242 g Na_2MoO_4 (Ettwig et al., 2009). The medium was flushed by Ar–CO_2 (95:5) for 15 min and pH was control at 7.0–7.2 before feeding.

2.2. Operation of SBR

The previous lab-scale SBR (2.0 L) was operated for 500 days, and then another SBR (1.0 L) was operated continually for 100 days and shut down for 25 days. The inoculum of the new SBR was taken from the previous SBR after 500 days. The biomass used in the batch tests for parameters identification (affinity constant and inhibition constant) was taken from the previous lab-scale SBR on day 420. The growth rate of n-damo bacteria was obtained from the 100 days operation period of the new SBR and the decay rate coefficient was measured over the 25 days shutdown period. Moreover, the biomass from the new SBR was sampled on day 1, 45 and 94 to measure the n-damo activity.

The new reactor owned 1.0 L of working volume and 0.3 L of headspace, and the inoculum sludge volume was 0.3 L. The operation cycles contained 22 h of reaction, 1.8 h of settling and 0.2 h of influent inflow and effluent discharge. The exchange volume was 0.5 L (exchange ratio was 0.5), and the hydraulic retention times was 48 h. The reactor was supplied continually by methane (99.99%) at 10 ml min^{-1} and stirred by a magnetic stirring son at 150 rpm, and the operating temperature was 30 ± 0.5 °C.

2.3. N-damo activity measurement

N-damo activity measurement was conducted in 70 ml serum bottles containing 10 ml of biomass (sediment after 1.8 h of settling), 30 ml of medium and 30 ml of gases. The biomass was washed with a nitrite-free medium and transferred immediately into serum bottles with 30 ml medium. Subsequently, the serum bottles were flushed by Ar–CO_2 (95:5) for 10 min and sealed rapidly. These bottles were divided into two groups equally, one of which was the experimental group, and the other was the control group. Every group has three replicates. Methane (99.99%) was only replaced into the experimental group to obtain certain partial pressures. The bottles were incubated in a shaking table at 30 ± 0.5 °C, and 0.5 ml sample was taken and centrifuged (5 min, 7440*g*) every 1.5 h from 0.5 h to 8.0 h. The concentrations of nitrite in the supernatant were determined. The n-damo activity was calculated by subtracting the nitrite conversion rate in the control group from that in the corresponding experimental group.

2.4. Analytical methods, data treatment and modeling software

The measurements of nitrite and volatile suspended solids (VSS) were performed according to standard methods (APHA, 2005), while pH was measured using a PHS-9V acidimeter (Hangzhou Huaguang Radio Factory, China) and DO was measured by a JPB-607 DO meter (Shanghai Rex Instrument Factory, China). The experimental data were combined, processed, and analyzed using MS Excel 2007, the figure of contour lines was constructed by Matlab 7.0, the other figures were plotted by Origin 8.0, and the model was simulated by Matlab 7.0.

3. Model development

3.1. Fundamental assumptions

To mathematically solve the process of biologic reaction and methane diffusion, some simplifying assumptions were made as follows: (1) the biomass was in solid dominantly and the rest in liquid could be ignored; (2) the biomass was constant in the batch experimental time (about 8 h); (3) the reaction system reached a quasi-steady-state quickly; (4) the methane transfer coefficient ($K_L a$) was constant; (5) the effects of sampling were ignored; (6) the medium and sludge were mixed completely; and (7) the differences of methane solubility and mass transfer coefficient between medium and distilled water were neglected.

3.2. Biologic reaction kinetics

In this model, multiple Monod kinetic type was applied to describe the dependency of the n-damo bacteria growth on nitrite and dissolved methane concentrations. Notably, the inhibitory effect of nitrite was also described by Monod type. Hence, the conversion rates of methane and nitrite can be presented as Eqs. (2) and (3), respectively.

$$r_{CH_4} = \frac{\mu_{max} X_{DA}}{Y_{DA}} \frac{S_{CH_4}}{K_{CH_4} + S_{CH_4}} \frac{S_{NO_2}}{K_{NO_2} + S_{NO_2}} \frac{K^I_{NO_2}}{K^I_{NO_2} + S_{NO_2}} \quad (2)$$

$$r_{NO_2} = \frac{8\mu_{max} X_{DA}}{3Y_{DA}} \frac{S_{CH_4}}{K_{CH_4} + S_{CH_4}} \frac{S_{NO_2}}{K_{NO_2} + S_{NO_2}} \frac{K^I_{NO_2}}{K^I_{NO_2} + S_{NO_2}} \quad (3)$$

where r_{CH_4} is the conversion rate of methane, r_{NO_2} is the conversion rate of nitrite for n-damo process, μ_{max} is the maximum growth rate of n-damo bacteria, Y_{DA} is the yield coefficient for n-damo bacteria growth on methane, S_{CH_4} is the concentration of dissolved methane, S_{NO_2} is the concentration of nitrite, K_{CH_4} is the methane affinity constant for n-damo bacteria, K_{NO_2} is the nitrite affinity constant for n-damo bacteria, $K^I_{NO_2}$ is the nitrite inhibition constant for n-damo bacteria, and X_{DA} is the active biomass of n-damo bacteria. Notably, all the parameters of n-damo in this work were used for a group of bacteria that intermediate the n-damo process.