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Kinetic study on nitrogen removal performance in marine anammox bacterial culture

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Kinetics of anaerobic ammonium oxidation (anammox) reaction in marine anammox bacterial culture was first investigated. The nitrogen removal performance of the reactor was compared with prediction of Monod model, modified Stover–Kincannon model, first-order and the Grau second-order substrate removal models. Based on calculations, Monod model, modified Stover–Kincannon model and the Grau second-order model proved to be more appropriate to describe the nitrogen removal kinetics of the reactor than first-order model with high determination coefficients of 0.993, 0.993 and 0.991, respectively. According to the modified Stover–Kincannon model, the maximal substrate removal rate (r_m) and saturation rate constant (K_B) were suggested as 7.37 and 6.41 g N/L/d, respectively. In addition, in light of the Monod model, the saturation concentration (K_s) and the maximal specific substrate removal rate constant (R_m) were determined to be 0.107 g/L and 0.952 g N/g MLVSS/d, respectively. Moreover, model evaluation was carried out by assessing the linear correlation between measured and predicted values. Both kinetics study and model evaluation showed that Monod model, modified Stover–Kincannon model and the Grau second-order substrate removal models could be used to describe the kinetic behavior or design of the marine anammox reactor.

[**Key words:** Marine anammox bacteria; Marine anammox reactor; Kinetics; Model; Nitrogen removal]

Anaerobic ammonium oxidation (anammox) is a novel, cost-effective and promising alternative to the traditional biological nitrogen removal process (1). The anammox process was carried out via oxidizing ammonia to nitrogen gas with nitrite as an electron acceptor under anoxic conditions, meanwhile, the growth of anammox bacteria was supported by carbon dioxide fixation (2). Almost a decade ago, anammox reaction in marine sediments was first detected (3). Later observations from the anoxic water column of the Black Sea also demonstrated the evidence of anammox reaction in oceanic environment (4). Since their discoveries, marine anammox bacteria have caught much attention. It brought extremely important novelty and expansion to the anammox process, not only, for its high nitrogen removal potential under high-salinity conditions, but also for contribution to direct elucidation of the marine nitrogen cycle in nature. However, the widespread occurrence but low diversity of marine anammox bacteria was another great obstacle along with slow growth rates for the implementation of marine anammox process. So far, very limited work has been reported in the peer-reviewed literature related to the start-up and process control of marine anammox process.

Kinetic models are widely used in fundamental research of biodegradation processes to examine the hypotheses, to control and predict the operation performance in bioreactors and to optimize the reactor design (5–7). The process kinetics also provides convincing basic recipe for dealing with the operational and environmental factors affecting substrate removal performance and bioreactor operation, which could promote and guarantee successful enrichment cultures of anammox bacteria. There are a large number of mathematical models cited in the literature on wastewater treatment, such as first-order substrate removal model (8), Grau second-order substrate removal model (9), Stover–Kincannon model (8), and Monod model (10). For instance, first-order and second-order substrate removal models are popular models to determine kinetic constants for anammox processes (5,11,12). Moreover, Monod model is initially used to describe the growth of microorganisms (13). At that time, some researchers found that it was difficult to apply this model (14–16). Recently, Monod model was commonly used to describe the biodegradation kinetics (12,17). Furthermore, Stover–Kincannon model is one of the most widely used mathematical models for determination of kinetic constants in immobilized bioreactors (5). Stover–Kincannon model has been applied to continuously operated mesophilic- and thermophilic-upflow anaerobic filters for the treatment of soybean wastewater treatment (6), paperpulp liquors (18), simulated starch wastewater (19), and determination of kinetic constants in a packed bed reactor for decolorization (20). However, these models have not so far been applied for the

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determination of nitrogen removal kinetics in marine anammox reactor.

Microbiological studies have convincingly demonstrated that marine anammox bacteria and freshwater anammox bacteria belong to different genera (21). For instance, the optimal temperature for freshwater anammox bacteria proved to be 30–40°C (22,23), but the temperature optima for marine anammox bacteria was suggested as 25°C (24). So there are probable huge differences in character between marine anammox bacteria and freshwater anammox bacteria, such as the growth rate of bacteria, nitrogen removal ability, and so on. Consequently, the process kinetics of marine anammox processes and freshwater anammox processes are highly likely to be different. So far, kinetics of freshwater anammox processes has been studied in the last decade (7,11,17). However, to the best of our knowledge, there are no public reports related to the kinetic study on marine anammox processes, and none was done on kinetics of nitrogen removal performance in marine anammox bacterial culture.

In this study, different mathematical models including first-order substrate removal model, Grau second-order substrate removal model, modified Stover–Kincannon model, and Monod model were conducted to investigate the reaction kinetics of marine anammox reactor and kinetic coefficients were determined. The objectives of this study were to evaluate different mathematic models for describing the nitrogen removal kinetics in the marine anammox reactor and to compare the applicability of different models.

MATERIALS AND METHODS

Reactor configuration Schematic diagram of the marine anammox reactor was shown in Fig. 1. The cylindrical column reactor composed of plexiglass with a total volume of 0.80 L (an effective volume of 0.65 L) was used as the marine anammox reactor. A porous polyester non-woven fabric (Japan Vilene Co. Ltd., Japan) was used as a biomass carrier. The reactor was enclosed with a thermostatic jacket to maintain a constant temperature of 25°C, and also was covered with black cloth to avoid growth of phototropic bacteria.

Synthetic medium Ammonium and nitrite in the forms of $(NH_4)_2SO_4$ and $NaNO_2$ were supplemented to the synthetic medium in the required amounts, respectively. The composition of synthetic medium was: NaCl 30 g/L, $MgSO_4 \cdot 7H_2O$ 5 g/L, $MgCl_2 \cdot 6H_2O$ 6 g/L, $CaCl_2 \cdot 2H_2O$ 1 g/L, KCl 700 mg/L, NaBr 100 mg/L, $SrCl_2 \cdot 6H_2O$ 20 mg/L, H_3BO_3 20 mg/L, NaF 2 mg/L, KI 0.08 mg/L, K_2HPO_4 54 mg/L, $KHCO_3$ 250 mg/L, and 0.5 mL Micro Fe/EDTA solution, and 1 mL Micro nutrient solution. The Micro Fe/EDTA solution contained: $FeSO_4 \cdot 7H_2O$ 18 g/L and EDTA·2Na 10 g/L. The Micro nutrient solution contained: $CuSO_4 \cdot 5H_2O$ 250 mg/L, $ZnSO_4 \cdot 7H_2O$ 430 mg/L, $CoCl_2 \cdot 6H_2O$ 240 mg/L, $MnCl_2 \cdot 4H_2O$ 990 mg/L, $Na_2MoO_4 \cdot 2H_2O$ 220 mg/L, $NiCl_2 \cdot 6H_2O$ 190 mg/L, Na_2SeO_4 110 mg/L, H_3BO_3 14 mg/L and EDTA·2Na 15 g/L. The synthetic medium was always deoxygenated by sparging with Argon gas for 20 min before feeding to the reactor and pH was adjusted at 7.5.

Inoculum Seed sludge was taken from a running non-woven anammox bioreactor. The mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) of the inocula were 1.94 g/L and 1.36 g/L, respectively. The bioreactor was initially inoculated with anaerobic sludge taken from a sea-based waste disposal site located at the North Port of Osaka Bay in Japan. More details about the partial 16S rRNA sequences of the marine anammox bacteria were described by Kawagoshi et al. (25).

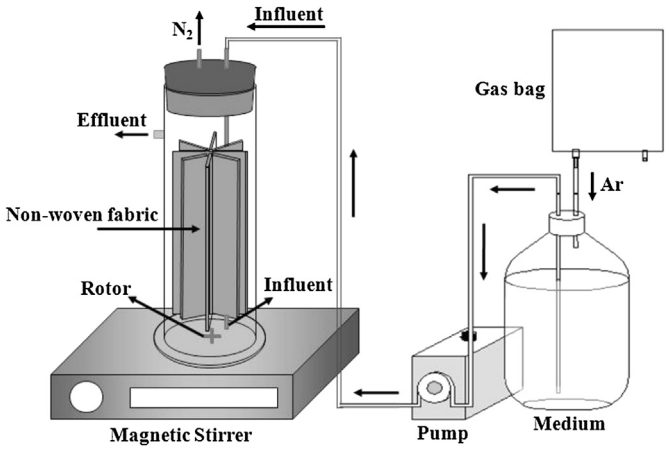

FIG. 1. Schematic diagram of the marine anammox reactor.

Operation conditions In this study, the marine anammox reactor was initially adopted to enrich the marine anammox bacteria. After the enrichment culture, some biomass was taken out to study the high-rate nitrogen removal by marine anammox process and the reactor was further used for kinetics study. At that time, the biomass concentration in the reactor was 1.56 g VSS/L. The reactor was operated until a pseudo-steady-state was reached which was indicated by constant high nitrogen removal efficiencies along with the increasing of nitrogen loading rate. And the vessel was stirred at 150 rpm by a four-bladed rotor determined by a magnetic stirrer.

Two series of experiments were conducted to study on the performance of the reactor, and the post-experimental data of the same reactor were used to test the model validity. In Phase 1, in order to obtain the kinetic parameters of Monod model, the reactor was run with different influent concentrations at a constant hydraulic retention time (HRT) of 0.5 day. The influent concentrations of ammonium and nitrite were gradually increased from 20.0 and 20.0 mg N/L to 188.0 and 183.5 mg N/L, respectively, corresponding to an increase of nitrogen loading rate (NLR) from 0.08 to 0.74 g/L/d. In Phase 2, aiming to investigate the nitrogen removal performance of the marine anammox reactor, the NLR was increased stepwise through raising influent concentrations of ammonium and nitrite or by shortening HRT. The operation parameters were not changed until a pseudo-steady-state was reached before proceeding to the next condition. Data based on arithmetic means of three or more measurements obtained at pseudo-steady-state were adopted for kinetic study.

Analysis The influent and effluent samples were analyzed immediately or stored in a refrigerator at 4°C until the analyses were carried out. Measurements of ammonium, nitrite, and nitrate were performed according to the Standard methods (26). The MLSS and MLVSS of the inoculum were determined by weighing after being dried at 105°C and ignited at 600°C. The biomass concentration was determined as MLVSS. The nitrogen removal performance was evaluated by nitrogen removal efficiency (NRE, %) which was defined as follows:

$$\mathrm{NRE} = 100(C_{n0} - C_{ne})/C_{n0} \quad (1)$$

where C_{n0} is nitrogen concentration in influent (mg N/L), C_{ne} is nitrogen concentration in effluent (mg N/L).

First-order substrate removal model Assuming the first-order substrate removal model was prevailed in the marine anammox reactor, the substrate removal rate is expressed as Eq. 2.

$$\frac{dS}{dt} = -k_1 S \quad (2)$$

where dS/dt is the substrate removal rate (g/L/d), k_1 is first-order substrate removal rate constant (1/d), S is substrate concentration in a reactor (g/L). In a complete stirred-tank reactor (CSTR), mass balance under pseudo-steady-state is expressed as following Eq. 3 or 4 by introduction of the Eq. 2.

$$\frac{Q}{V}(S_0 - S) = -k_1 S \quad (3)$$

or

$$\frac{(S_0 - S)}{\theta_H} = -k_1 S \quad (4)$$

where Q is the inflow rate (L/d), V is the effective volume of the reactor (L), S_0 is substrate concentration in influent (g/L) (11), and θ_H is HRT (d). The value of k_1 is obtained from the slope of the approximate curve by plotting $(S_0 - S)/\theta_H$ against S.

Grau second-order substrate removal model The common equation of a second-order model is given as follows (9):

$$-\frac{dS}{dt} = k_2 X\left(\frac{S}{S_0}\right)^2 \quad (5)$$

where k_2 is Grau second-order substrate removal rate constant (g substrate/g MLVSS/d), and X is the biomass concentration in a reactor (g MLVSS/L).

The following Eq. 6 is obtained via integration of Eq. 5 within the boundary conditions of $S = S_0$ to S and $t = 0$ to θ_H, X = constant, and linearization,

$$\frac{S_0\theta_H}{S_0 - S} = \theta_H + \frac{S_0}{k_2 X} \quad (6)$$

where θ_H is HRT. As $(S_0 - S)/S_0$ can be expressed as substrate removal efficiency and S_0/k_2X is a constant, Eq. 6 is modified as follows (23):

$$\frac{\theta_H}{E} = a + b\theta_H \quad (7)$$

where a is S_0/k_2X and b is a constant, E is substrate removal efficiency.

The values of a, b and k_2 are easily derived by plotting θ_H/E against θ_H.