Hindawi
Journal of Chemistry
Volume 2017, Article ID 4878910, 5 pages
https://doi.org/10.1155/2017/4878910

Research Article

Decay Experiments of Effective N-Removing Microbial Communities in Sequencing Batch Reactors

Chen Lv,[1,2] Ming Li,[2] Shuang Zhong,[2] Jianlong Wang,[1] and Lei Wu[2]

[1]*Laboratory of Environmental Technology, Institute of Nuclear and New Energy Technology, Tsinghua University, Beijing 100084, China*
[2]*Key Laboratory of Songliao Aquatic Environment, Ministry of Education, Jilin Jianzhu University, Changchun 130118, China*

Correspondence should be addressed to Jianlong Wang; wangjl@mail.tsinghua.edu.cn

Received 25 April 2017; Revised 27 July 2017; Accepted 14 August 2017; Published 14 September 2017

Academic Editor: Claudio Di Iaconi

The temporal changes in the compositions of effective N-removing bacterial communities and the decay coefficients of Anammox were studied within the 120-day decay period under anaerobic or aerobic conditions at 25°C. The maximum nitrogen production rate (MNPR) was determined by measuring the temperature, pH, volatile suspended solids (VSSs), and nitrogen-removal efficiency of the microbial communities during the decay period. The decay coefficients under anaerobic and aerobic conditions at 25°C were determined through equation-based fitting to be 0.031 d^{-1} and 0.070 d^{-1}, respectively. Through molecular biological means and together with quantitative polymerase chain reaction (qPCR), the proportions of AnAOB in the microbial communities dropped from 48.70% to 3.69% under anaerobic condition and from 48.70% to 1.98% under aerobic condition during the decay period.

1. Introduction

Compared with traditional N-removal processes, the Anammox process is superior with low investment and operation costs, low sludge yield, high processing efficiency, and feasibility to wastewater with low C/N ratio and high ammonia nitrogen [1]. However, Anammox is limited by extremely low cell yield, slow cell growth, environmental sensitivity, and high requirements for temperature, pH, water, and substrate during cultivation [2, 3]. Moreover, the Anammox process is limited by the difficulty in starting, instability after start-up, and difficulty in recovery after destabilization [3–5]. These problems can be overcome if there are abundant favorable bacterial species that can be used for early-phase inoculation or anaphase fed-batch [6]. In current activated sludge models of aerobic degradation, the loss of activity and mass of activated sludge is expressed by only one process called decay [7]. And the decrease in bacterial activity in activated sludge can result from cell death and activity decay [8] and significantly affects the preservation of bacterial species and the decay coefficient [9, 10]. Moreover, the decay coefficient is one of the main variables in the mathematical modeling that is applied to biological wastewater processing. Correct estimation of the decay constant is a key factor to properly model and better understand the Anammox process; it is also a parameter that is helpful to design and manage an Anammox reactor [11].

The objectives of this study are to accurately measure the decay coefficient of N-removing functional microbes during the whole decay period, and together with quantitative polymerase chain reaction (qPCR), to analyze the compositional changes of functional microbial communities, which were maintained under anaerobic/aerobic conditions and without feeding for about 4 months.

2. Materials and Methods

2.1. Materials. All the microbe samples in the decay experiments were collected from a laboratory small-scale sequencing batch reactor (SBR) fermentation tank (4 L). The reactor ran under controlled conditions (30°C, pH 7.5 ± 0.5) and was blended and stirred at the speed of 80 rpm by the machinery stirrer in the fermentation tank. The reactor had a running period of 8 h, drainage ratio of 50%, hydraulic retention time

(HRT) of 16 h, and volume nitrogen load rate of 750 mgN/L·d. Other devices included a temperature control set installed outside the fermentation tank, the annular aeration line at the bottom, and an online data monitor for measurement of dissolved oxygen (DO), pH, temperature, NH_4^+, and NO_3^-. The composition of the inflow water was 169.7 mg/L KH_2PO_4, 751.1 mg/L $MgSO_4 \cdot 7H_2O$, 451.6 mg/L $CaCl_2 \cdot 2H_2O$, 20.0 mg/L EDTA, 5.00 mg/L $FeSO_4 \cdot 7H_2O$, 0.43 mg/L $ZnSO_4 \cdot 7H_2O$, 0.24 mg/L $CoCl_2 \cdot 6H_2O$, 0.99 mg/L $MnCl_2 \cdot 4H_2O$, 0.25 mg/L $CuSO_4 \cdot 5H_2O$, 0.22 mg/L $NaMoO_4 \cdot 2H_2O$, 0.19 mg/L $NiCl_2 \cdot 6H_2O$, and 0.21 mg/L $NaSeO_4 \cdot 10H_2O$.

2.2. Experimental Methods. The decay experiments of N-removing functional microbial communities were conducted under anaerobic or aerobic conditions at 25°C. Four groups were conducted, each in triplicate. The microbial communities taken out of the reactor were washed with the substrate-free inflow water. Each time, 250 mL of a sample was placed into a 300 mL sealed bottle, which was put under the corresponding experimental condition. At a certain interval, 25 mL of the sample was collected, pretreated, and measured in terms of $NH_4^+/NO_2^-/NO_3^-$, pH, volatile suspended solids (VSS), specific Anammox activity (SAA), and qPCR. The decay changes of N-removing microbial communities under different conditions were described quantitatively.

SAA was detected using the method from Buys [12]. This method was first applied into denitrifying bacteria, but it was also feasible to measurement of low biomass and low gas production, so it was used in this study. The principle of bacterial activity test is that an appropriate ratio of NH_4^+ and NO_2^- was added to the substrate, and then N_2 production was detected. The anaerobic condition was realized by the ventilation of nitrogen gas into the reactor. Under the anaerobic condition, the substrate (NH_4^+ and NO_2^-, each 5 mmol) was added. According to (1), if the functional microbial community was active, the generated gas should be N_2.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \longrightarrow 1.02N_2 + 0.256NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1)$$

SAA can be used to measure the newly added air pressure in the test bottle (unit: mV), or, namely, the N_2 production from the reaction. Then we could determine the nitrogen production rate n as follows:

$$PV = nRT, \quad (2)$$

where P is the overpressure (higher than normal pressure); V is the space volume in the top; R is the ideal gas constant (=0.0821 atm·mL/K·mmol); T is the temperature.

According to (2), we could determine the maximum nitrogen production rate (MNPR), which is proportional by K to the biomass solid concentration $X_{anx}(t)$. Thus, we could estimate the decay coefficient b_{AN} as follows:

$$\begin{aligned} \mathrm{MNPR}(t) &= k * X_{anx}(t) \\ \frac{dx_{anx}}{d_t} &= -b_{AN} * X_{anx} \\ X_{anx}(t) &= X_{anx(t=0)} e^{-b_{AN} * t}. \end{aligned} \quad (3)$$

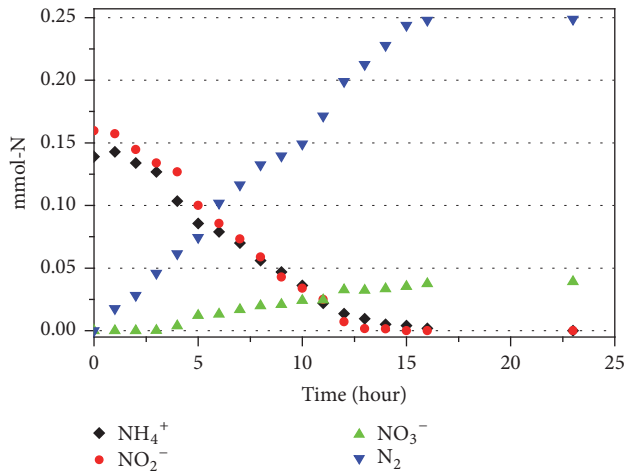

Figure 1: Initial N-removing performance of functional microbial communities.

2.3. Analytical Methods. VSS was measured by a standard method; pH was measured by a PHM210 device. N-containing particle concentration was detected by a colorimetric kit after filtration by a 0.45 μm acetic acid fibrin injector (Merck KGaA, Darmstadt, Germany).

In DNA extraction, a microbe sample after frozen drying at −50°C was weighed, and total DNA from each activated sludge sample was extracted using an MP soil DNA rapid extraction kit (Bio101, Vista, CA, USA) according to the manual. The qPCR amplification of 16rRNA functional genes was conducted with the following primers: 1055f/1391r (EUB) [13, 14], CTO 189fA/B/RT1r (AOB) [15], Nspra-675f/746r (NOB) [16], and Amx809f/1066r (AnAOB) [17, 18].

3. Results and Discussion

3.1. Initial N-Removing Performances of Functional Microbial Communities. The initial activity of each functional microbial community was measured. The results were atmospheric pressure = 18 mV and initial MNPR = 2.63 mLN_2-N/L·d. The air pressure peaked within 24 h, while the N_2 production during the whole reaction increased with time, which proves the initial microbes were highly active.

The N_2 production was measured simultaneously with sampling. The measurements of N-containing particles were converted to nitrogen molar concentrations (Figure 1). Clearly, at the 16th hour, under the action of the functional microbial communities, the newly added NH_4^+ and NO_2^- (totally 0.3 mmol-N) almost all reacted, forming 0.04 mmol-N NO_3^- and 0.25 mmol-N N_2, and the change of molar concentrations obeyed (1), which proves that the initial functional microbial communities had high N-removing ability.

3.2. Variation of VSS with Time. During the 120-day decay period, the functional microbial communities were sampled, and it was found the VSSs significantly declined under all test conditions (Figure 2). Under the anaerobic condition, VSSs dropped at a significant rate, which was slower than under the aerobic condition. The VSSs under the anaerobic condition in

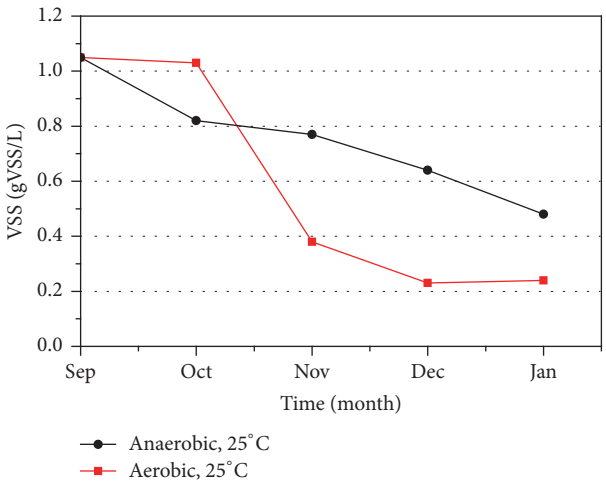

Figure 2: Variation of VSS during 120 days.

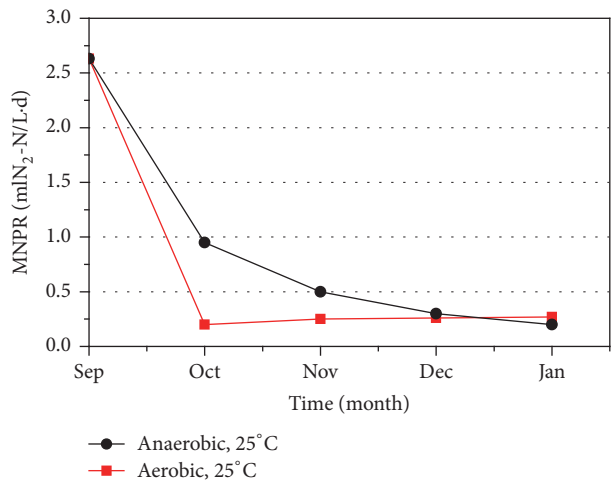

Figure 3: Variation of MNPR with time.

Oct, Nov, Dec, and Jan were 0.82, 0.77, 0.64, and 0.48 gVSS/L, respectively. Under the aerobic condition, the VSS in Oct did not change significantly, and was 1.03 gVSS/L. Nevertheless, the color changed significantly and turned light grey, which indirectly indicates the reduction of microbial activity. The VSS in Nov declined severely from the previous month and was 0.38 gVSS/L. The VSSs in Dec and Jan were 0.23 and 0.24 gVSS/L, respectively, indicating the microbes almost all decayed in the third and fourth months.

3.3. Variation of MNPR with Time. In a reactor under continuous stirring, the equation derived from the mass balance under stable conditions represents the relationship between X_{AN} and b_{AN}:

$$X_{AN} = Y_{AN} \cdot \frac{\left(NH_4^+{}_{\_IN} - NH_4^+{}_{\_OUT}\right) \cdot SRT}{HRT \times \left(1 + b_{AN} \cdot SRT\right)}$$

$$SRT \longrightarrow \infty \quad (4)$$

$$X_{AN} \longrightarrow Y_{AN} \cdot \frac{\left(NH_4^+{}_{\_IN} - NH_4^+{}_{\_OUT}\right)}{HRT \times b_{AN}}.$$

Thus, when b_{AN} and other bioreactor parameters [hydraulic retention time (HRT), sludge retention time (SRT), nitrogen-removal efficiency, and nitrogen load] are known, the Anammox biomass concentration can be easily estimated [11]. In the following equation, the relevant parameters were cited from an Anammox reaction equation [19], and the MNPR (mlN_2-N/L·d) was associated with active biomass (X_{AN}):

$$\begin{aligned} MNPR\,(t) &= \left(\frac{\mu_{max} \cdot 2.04 \cdot 22.4}{Y_{AN} \cdot 14 \cdot 2}\right) \cdot X_{ANX}\,(t) \\ &= k \cdot X_{ANX}\,(t = 0) \cdot e^{-b_{AN} \cdot t}, \end{aligned} \quad (5)$$

where b_{AN} is the decay coefficient (d^{-1}), μ_{max} is the maximum growth rate (d^{-1}), X_{AN} is the concentration of Anammox organisms (mgCOD L^{-1}), Y_{AN} is the Anammox growth yield (mg COD mg NH_4-N^{-1}), and k is the maximum specific nitrogen gas production rate (mlN_2-N L^{-1} d^{-1} mg COD^{-1}).

The SAAs during the 120-day decay period were measured and used to determine the MNPR according to (5). As shown in Figure 3, the MNPR (mlN_2-N/L·d) is 2.63 at first and then declines significantly with time. Under the anaerobic condition, the MNPRs in Oct, Nov, Dec, and Jan are 0.95, 0.5, 0.3, and 0.2 mlN_2-N/L·d, respectively. Under the aerobic condition, MNPRs decline significantly and are 0.25, 0.27, and 0.26 mlN_2-N/L·d, in the first three months, respectively. In the fourth month, nearly no air pressure could be detected, which indicates the inactivity of the functional microbial community. The above results suggest that MNPRs under the anaerobic condition decline regularly and in a gradient way with the prolonging of time. MNPR under the aerobic condition drops rapidly in the first month but does not change severely in the following three months, indicating that the O_2 concentration largely affects the decaying process.

3.4. Decay Coefficient b_{AN} of Functional Microbes. The decay coefficient (b_{AN}) is commonly used in mathematical modeling of biological wastewater processing. Specifically, for the majority of Anammox process reactors, the balanced concentration of active biomass is largely dependent on b_{AN}.

b_{AN} can be determined by fitting NMPR according to (5) on software (Figure 4). b_{AN} of the microbes under the anaerobic and aerobic conditions at 25°C are 0.031 d^{-1} and 0.070 d^{-1}, respectively, indicating, at the same temperature, b_{AN} under the aerobic condition is two times larger than the anaerobic condition. Strous et al. studied the effects of oxygen on Anammox by using SBR [19]. In a reactor, the anaerobic and aerobic conditions were alternated, and Anammox reaction occurred only after the stop of oxygen supply but not during oxygen supply. Thus, the experiments prove that oxygen supply could inhibit the activity of Anammox, which can be restored after oxygen supply. Nevertheless, the inhibitory effect of oxygen concentrations on the Anammox activity should be further studied. When the oxygen concentration was 0.5%–2.0% of the saturated oxygen concentration in air,

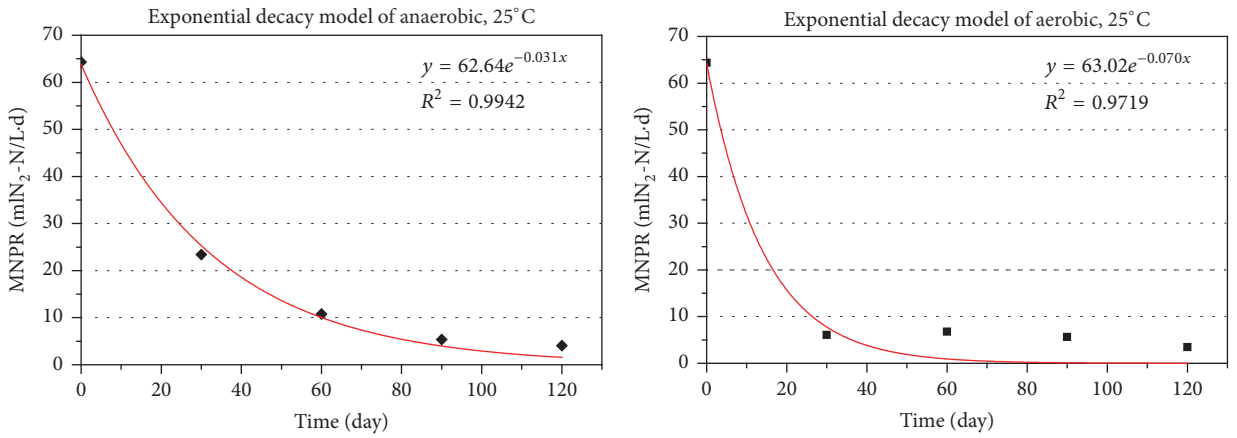

Figure 4: Fitting curves of NMPR during the decay period under different conditions.

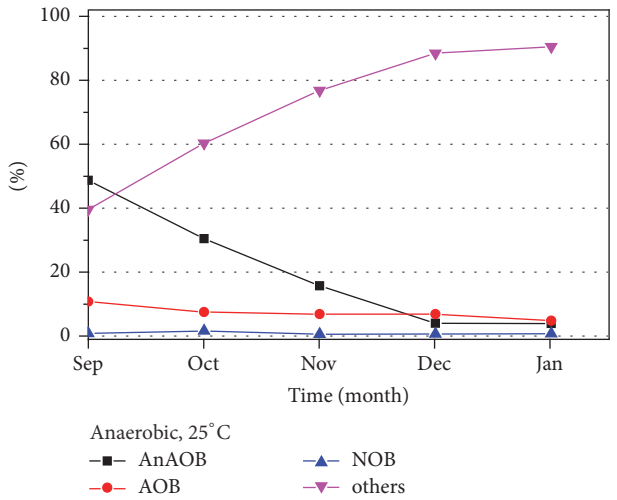

Figure 5: Proportion variation of species in the total functional microbial communities under anaerobic condition.

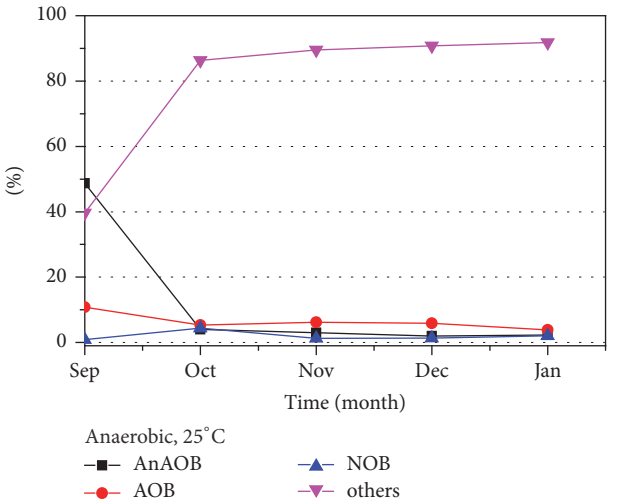

Figure 6: Proportion variation of species in the total functional microbial communities under aerobic condition.

the activity of Anammox was completely inhibited, indicating that the inhibitory oxygen concentration on the activity of Anammox is 0.5% of saturated oxygen concentration in air. Through our experiments, we not only determined the decay coefficient but also validated that again the oxygen concentration could inhibit the activity of Anammox.

3.5. Molecular Biology Analysis. Fluorescence qPCR can be used to detect the copy number of a gene in an unknown sample. Namely, a known concentration of the gene was diluted to a series of gradient concentrations, which were detected through one qPCR trial. The results determined from this gradient of concentrations were used to plot a standard curve, from which we could deduce the concentration of an unknown sample. The detected microbes were divided into four classes: AOB, NOB, AnAOB, and others. Based on qPCR, the measured data were used to estimate the proportion of each bacterial species in the overall biomass. When the proportion declines, the decaying speed of this species surpasses the average rate of other species, and vice versa. The functional microbial communities were initially composed of AOB (10.80%), AnAOB (48.70%), NOB (0.87%), and others (39.63%).

Under the anaerobic condition, the functional microbial communities consisted of AnAOB (30.50%) and others (60.37%) (Oct); AnAOB (15.70%) and others (76.86%) (Nov); AnAOB (4.04%) and others (88.44%) (Dec); AnAOB (3.92%) and others (90.53%) (Jan) (Figure 5). Under the aerobic condition, the functional microbial communities consisted of AnAOB (3.98%) and others (96.31%) (Oct); AnAOB (2.98%) and others (89.51%) (Nov); AnAOB (1.98%) and others (90.80%) (Dec); AnAOB (2.30%) and others (91.79%) (Jan) (Figure 6). Clearly, under the aerobic condition, the decaying rate of AnAOB surpassed those of other species throughout the experiments, indicating that oxygen is extremely unfavorable for the survival of AnAOB and the decay of AnAOB is severe under aerobic conditions. The results of qPCR are consistent with the results of VSS, MNPR, and b_{AN}.

4. Conclusions

Correct evaluation of decay coefficient helps to better understand the Anammox process. The decay of functional microbial communities in 120-day experiments was monitored. MNPR was determined by measuring the temperature, pH, VSS, and nitrogen-removal efficiency of microbial communities during the decay period. The decay coefficients under anaerobic and aerobic conditions at 25°C were determined through fitting to be 0.031 d^{-1} and 0.070 d^{-1}, respectively.

Through molecular biology means, AnAOB communities decreased faster under aerobic than anaerobic condition. The proportion of AnAOB in the microbial community is proportional to the N-removing efficiency.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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