Improvement of mineral nutrient concentrations and pH control for the nitrite-dependent anaerobic methane oxidation process

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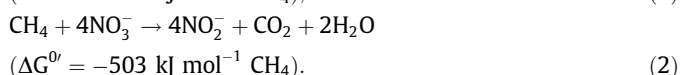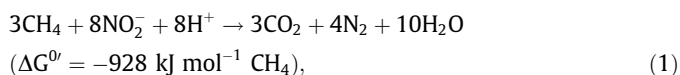
ARTICLE INFO

ABSTRACT

A novel biological nitrogen removal process can be developed based on the newly discovered nitrite-dependent anaerobic methane oxidation (n-damo) process. In this work, the short- and long-term effects of mineral nutrients on the n-damo process were investigated by single-factor and orthogonal experiments, respectively. The pH buffering capacities of different media were determined by acid–base titration. $KHCO_3$ and KH_2PO_4 greatly influenced the activity of n-damo bacteria and the pH changes of the media. An improved n-damo medium was proposed, containing $0.3 \, \text{g L}^{-1} \, CaCl_2$, $0.2 \, \text{g L}^{-1} \, MgSO_4$, $0.3 \, \text{g L}^{-1} \, KH_2PO_4$ and $0.2 \, \text{g L}^{-1} \, KHCO_3$. Moreover, the presence of 1% of CO_2 in gas could maintain the pH near neutral in the improved medium, which was first estimated in theory and then verified in practice. Finally, these results were applied in an n-damo reactor. The nitrogen removal rate was slightly increased (from 10.0 ± 1.2 to $10.8 \pm 1.4 \, \text{mg N L}^{-1} \, \text{d}^{-1}$), and the pH was quite stable after the improvement (daily changes from 0.58 ± 0.05 to 0.11 ± 0.02).

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1. Introduction

Low cost nitrate (or nitrite) removal from low C/N ratio wastewaters is a great challenge in many industries, such as fertilizer manufacturers, steel plants and explosives plants. Anaerobic oxidation of methane (AOM) coupled to denitrification is a new low-cost approach to remove nitrate or nitrite from wastewaters with methane, an end product of anaerobic digestion, as an electron donor [1–3]. AOM coupled to denitrification was first discovered in denitrifying reactors [4] and is catalyzed by NC10 bacteria and ANME-2 archaea [5]. Subsequently, it was demonstrated that NC10 bacteria independently mediate AOM coupled to nitrite reduction (Eq. (1)) [6], while ANME-2 archaea are responsible for AOM coupled to nitrate reduction (Eq. (2)) [7]. AOM coupled to nitrite reduction was also named as nitrite-dependent anaerobic methane oxidation (n-damo).

$$3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 4N_2 + 10H_2O$$
$$(\Delta G^{0\prime} = -928 \text{ kJ mol}^{-1} \text{ CH}_4), \tag{1}$$
$$CH_4 + 4NO_3^- \rightarrow 4NO_2^- + CO_2 + 2H_2O$$
$$(\Delta G^{0\prime} = -503 \text{ kJ mol}^{-1} \text{ CH}_4). \tag{2}$$

Because of the low cost of the electron donor [8], low production of excess sludge [1] and no emission of N_2O [9] (the third greenhouse gas, followed by CO_2 and CH_4), the n-damo process is a potential alternative to traditional heterotrophic denitrification [10]. However, n-damo bacteria grow very slowly (doubling time is 14–25 days [5,11]), making it difficult to obtain enough biomass required for biotechnology applications and ultimately limiting the development of the n-damo process. Previously, some work had been performed to accelerate the growth of n-damo bacteria, including improvements of inoculum sources [12], reactor configurations [3,6,13], substrate concentrations [11] and environmental conditions [14]. However, there are many problems in the n-damo process that still needed to be solved, such as low solubility of methane, low turnover rate of the bacteria and high washout rate of biomass. In earlier studies, we found some defects in the previous medium for n-damo bacteria [15], such as carbonate precipitation in the influent tank [13] and a considerable increase in pH during long-term cultivation [14], which limits the n-damo process.

In this study, the short- and long-term effects of mineral nutrients ($CaCl_2$, $MgSO_4$, KH_2PO_4 and $KHCO_3$) on the n-damo process were determined, and a set of orthogonal tests were carried out to improve the mineral nutrient concentrations of the n-damo medium. In the long-term incubation of 50 days, increases in activity and cell number of n-damo bacteria were measured. Acid–base

titration was conducted to investigate the pH buffering capacity of different media. Theoretical estimation and practical experimentation were performed to provide an efficient way to control pH in the n-damo systems. Moreover, these results were applied in a sequencing batch reactor (SBR) to obtain a better performance of the reactor.

2. Materials and methods

2.1. Biomass and medium

The biomass used in this study was taken from a previous n-damo reactor [11], which was enriched originally from freshwater sediments. The medium was composed of mineral nutrient elements and trace elements; the trace elements were prepared as previously described [15], and the mineral nutrient elements consisted of $CaCl_2$, $MgSO_4$, KH_2PO_4 and $KHCO_3$. Their concentrations were 0.3, 0.2, 0.05 and 0.5 g L^{-1}, respectively, in the previous medium [15], but were adjusted as needed in this study (see Table 1). The initial pH of the media was adjusted to 7.0–7.5.

2.2. Short-term experiments on the effect of mineral nutrients

Batch single-factor experiments were conducted in triplicate with different concentrations of mineral nutrients: 0–2.0 g L^{-1} of KH_2PO_4 and $KHCO_3$ and 0–1.0 g L^{-1} of $CaCl_2$ and $MgSO_4$. According to the mineral nutrients investigated, these tests were classified into four groups: group P (KH_2PO_4), group C ($KHCO_3$), group Ca ($CaCl_2$) and group Mg ($MgSO_4$). In group P, the short-term effect of KH_2PO_4 was investigated; the concentration of KH_2PO_4 was varied, and the concentrations of the rest of the mineral nutrients were fixed and equal to those in the previous medium. The other three groups were done in the same manner. Four concentrated solutions of KH_2PO_4 (20 g L^{-1}), $KHCO_3$ (20 g L^{-1}), $CaCl_2$ (30 g L^{-1}) and $MgSO_4$ (20 g L^{-1}) were prepared. Biomass was taken from the parent reactor and rinsed with oxygen-free deionized water, and 10 mL of washed biomass was loaded into each serum bottle (76 mL) quickly. Next, 40 mL of corresponding medium was added into the serum bottles, and the corresponding medium was prepared using the concentrated solutions of mineral nutrients and trace elements [16]. 50 μL of nitrite concentrated solution (7 g N L^{-1}) was pipetted into the serum bottles to reach the final nitrite concentration of ~7 mg N L^{-1}. The serum bottles were deoxygenated by flushing with Ar gas (99.999%) for 5 min. Then, the serum bottles were sealed and incubated on a shaking table at 30 °C and 150 rpm. After 12 h of incubation without methane, the headspace was exchanged with 5.2 mL of pure methane (99.99%). Before methane addition, 0.5 mL of liquid was sampled to determine the nitrite concentration every 6 h; after methane addition, gas in the headspace was also sampled to measure the methane concentration. The methane oxidation rate of the n-damo process was directly determined from the decrease in methane concentration over time, while the nitrite reduction rate of the n-damo process was estimated by subtracting the nitrite reducing rate without methane from that with methane in the headspace, as previously described [14].

2.3. Long-term experiments on the effect of mineral nutrients

$L_9(3^4)$ orthogonal experiments [17] and four additional tests were performed to investigate the long-term effects of mineral nutrients on the n-damo process. 76-mL serum bottles were prepared, and each was loaded with 20 mL biomass and 30 mL of the corresponding media (see Table 1). Subsequently, the serum bottles were deoxygenated and sealed, and their headspace was replaced with 13 mL methane. The serum bottles were incubated on a shaking table at 150 rpm and 30 °C. Every three days, 20 mL of medium were exchanged with fresh medium; nitrite and nitrate were measured; and nitrite stock solution (7 g N L^{-1}) was added to keep the nitrite concentrations at 7–42 mg N L^{-1}.

Batch tests were carried out before and after 50 days of incubation to determine the n-damo activity. The corresponding media were used, and the method used to test activity was the same as that in Section 2.2.

The number of n-damo bacteria was estimated by quantitative PCR (qPCR) as previously described [18,19]. Briefly, 2 mL biomass was collected before and after incubation; DNA was isolated using a PowerSoil DNA Isolation Kit (Mo Bio Laboratories, United States); and qPCR was performed in triplicate by an iCycler iQ5 thermocycler and a real-time detection system (Bio-Rad, United States), using qP1F (5′-GGG CTT GAC ATC CCA CGA ACC TG-3′)/qP1R (5′-CGC CTT CCT CCA GCT TGA CGC-3′) [15] as primer pair.

2.4. Experiments of pH buffering and controlling

Acid–base titration was carried out to determine the pH buffering capacities of different media. 0.25 M NaOH or 0.25 M HCl was added into 50 mL medium at 30 °C, and the pH values with different dosages of acid/base were recorded. To verify that 1% of CO_2 in gas can control the pH near neutral in the improved medium, the medium (50 mL) was continually flushed with 1% CO_2 (99% Ar) or pure Ar at ~150 mL min^{-1} and 30 °C. The pH values over time were recorded.

2.5. Operation of an SBR reactor

An SBR was operated to apply the results of the improved concentrations of mineral nutrients in the medium and the pH control method of adding 1% CO_2. The reactor consisted of 0.3 L of enrichment culture, 0.6 L of medium and 0.4 L of headspace, and its configuration was described previously [13]. The operation period of the reactor could be divided into three phases: phases I, II and III. In phase I (day 1–5), the previous medium was used; in phase II (day 5–9), the improved medium was adopted; in phase III (day 9–13), the improved medium was used and 1% of CO_2 was added into the headspace of the reactor. The reactor was operated at 35 °C and the solid–liquid phase (0.9 L) was mixed by a magnetic stirrer at 500 rpm. Every day, 0.4 L of supernatant liquid was exchanged with the same volume of the corresponding fresh

Table 1
Designs and results of the $L_9(3^4)$ orthogonal experiments (Tests 1–9) and the additional experiments (Tests A1–A4).

Test	$CaCl_2$ (g L^{-1})	$MgSO_4$ (g L^{-1})	KH_2PO_4 (g L^{-1})	$KHCO_3$ (g L^{-1})	Increase in activity (times)	Increase in cell number (times)
1	0.15 (1)[a]	0.1 (1)	0.05 (1)	0.2 (1)	4.65	9.5 ± 0.9
2	0.15 (1)	0.2 (2)	0.125 (2)	0.6 (2)	3.87	3.4 ± 1.0
3	0.15 (1)	0.3 (3)	0.2 (3)	1.0 (3)	3.99	1.9 ± 0.6
4	0.3 (2)	0.1 (1)	0.125 (2)	1.0 (3)	3.21	2.5 ± 0.7
5	0.3 (2)	0.2 (2)	0.2 (3)	0.2 (1)	7.25	14.8 ± 0.5
6	0.3 (2)	0.3 (3)	0.05 (1)	0.6 (2)	3.70	4.1 ± 0.1
7	0.45 (3)	0.1 (1)	0.2 (3)	0.6 (2)	4.98	5.9 ± 1.0
8	0.45 (3)	0.2 (2)	0.05 (1)	1.0 (3)	2.33	3.2 ± 0.1
9	0.45 (3)	0.3 (3)	0.125 (2)	0.2 (1)	5.09	9.9 ± 1.0
A1	0.3	0.2	0.3	0.2	7.17	ND[b]
A2	0.3	0.2	0.5	0.2	5.90	ND
A3	0.3	0.2	0.2	0.1	5.98	ND
A4	0.3	0.2	0.2	0.05	6.22	ND

[a] The numbers in round brackets were the levels of factors in the orthogonal experiments.
[b] ND means "not determined".