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Reduction of greenhouse gases emissions during anoxic wastewater treatment by strengthening nitrite-dependent anaerobic methane oxidation process

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HIGHLIGHTS

- Co-existence of n-damo bacteria and methanogens was achieved by using UASB.
- Introduction of n-damo process in anoxic environment could reduce GHG emissions.
- Carbon source had significant influence on diversity of n-damo bacteria.
- *In situ* production and consumption of CH_4 led to unique n-damo bacteria community.

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ABSTRACT

Nitrite-dependent anaerobic methane oxidation (n-damo) is a recently discovered process performed by NC10 phylum, which plays an important role in greenhouse gases (GHG) reduction. In this study, co-existence of n-damo bacteria and methanogens was successfully achieved by using upflow anaerobic sludge blanket (UASB) reactor. Reactor with inorganic carbon source (CO_2/H_2) showed the highest abundance of n-damo bacteria and the highest n-damo potential activity, resulted in its highest nitrogen removal rate. Significant reduction in GHG was obtained after introduction of n-damo process, especially for N_2O. Furthermore, GHG emissions decreased with the increase of n-damo bacteria abundance. Community structure analysis found carbon source could influence the diversity of n-damo bacteria indirectly. And phylogenetic analysis showed that all the obtained sequences were assigned to group B, mainly due to *in situ* production and consumption of CH_4.

1. Introduction

As is well known, wastewater treatment plant (WWTP) is considered to be a significant emission source of anthropogenic greenhouse gases (GHG), including methane (CH_4) and nitrous oxide (N_2O) (Daelman et al., 2013). Generally, CH_4 emission mainly occurs during the anaerobic decomposition of organic pollutants in wastewater or primary and secondary sludge, activated by methanogenic bacteria (Hwang et al., 2016), while most of N_2O emission was attributed to nitrification and denitrification occurred in the aerobic or anaerobic/anoxic condition (Mannina et al., 2016b). It is reported that anaerobic/anoxic environment would account for 23.78% of CH_4 and 47.12% of N_2O emissions in WWTP (Hwang et al., 2016). Thus, it is necessary to investigate mitigation strategies to reduce GHG emissions from WWTPs, especially from anaerobic/anoxic condition.

Many studies have been conducted to reduce GHG emissions from WWTPs, and currently, the main methods are focused in the following aspects: (1) Biogas recovery. Presently, the biogas recovery and utilization technologies are used on a large scale for power and heat production (Tilche and Galatola, 2008). However, high infrastructure investment, operation cost, and technical requirements restricted the application of biogas recovery in WWTPs (Thien Thu et al., 2012). (2) Wastewater treatment process optimization. Fortunately, GHG emissions could be significantly reduced through optimization of many process parameters, such as dissolved oxygen (DO) concentration, water temperature, sludge retention time (SRT), salinity and etc. (Flores-Alsina et al., 2011; Sweetapple et al., 2014; Mannina et al., 2016a). But the reduction of GHG emissions through process optimization was often accomplished at the cost of lower pollutants removal efficiencies or higher operation cost (Sweetapple et al., 2014). In addition, most

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of the present studies were conducted in laboratory scale, great distance exist before its practical usage. (3) Novel wastewater treatment technology. In recent year, many researchers had reached a consensus that anammox processes have significant impact on reduction of GHG emissions (Bani Shahabadi et al., 2009; Ren et al., 2015), since it could reduce gaseous N emissions and sequesters CO_2. Furthermore, recent study of Winkler et al. (2015) showed that the implementation of n-damo in anammox process had great advantage in reducing GHG emissions. The application of novel wastewater treatment technology is considered to be the most promising method to reduce GHG emissions, although complete and elaborated economic feasibility study is needed to evaluate the profit of such solution.

N-damo process was first found and confirmed in a laboratory enrichment culture by Raghoebarsing et al. (2006). This process is performed by "*Candidatus* Methylomirabilis oxyfera" (*M. oxyfera*) bacteria (Ettwig et al., 2010), which is affiliated with the uncultured NC10 phylum (Ettwig et al., 2009). At present, many studies have proved that n-damo bacteria are widespread in natural environment and play an important role in reducing global CH_4 emissions. Deutzmann et al. (2014) showed that n-damo process could mitigate CH_4 emission mostly or even completely in the 274 km^2 of lake sediment. And Hu et al. (2014b) estimated that wetlands would consume roughly 2–6% of current worldwide CH_4 flux. More importantly, it is believed that n-damo process could establish a unique link between carbon cycle and nitrogen cycle and had great potential in minimizing GHG emissions from WWTPs (Raghoebarsing et al., 2006). In such a way, methane originated from anaerobic digestion could be used for denitrification purposes which avoid the possible N_2O emission during conventional denitrification process and waste of carbon source during anammox process. However, to date, thorough investigation on reduction of GHG emissions in WWTP by applying n-damo process is still lacking.

N-damo bacteria, similar as anammox bacteria, are famous for its long doubling time, which results in relatively low reaction rate (Ren et al., 2015; Ettwig et al., 2009). To data, the highest n-damo reaction rate reported was 76.9 mg NO_2^--N/L·d (Hu et al., 2014a), and the low reaction rate of n-damo bacteria restricted its wide application. So far, many researchers have done a series of attempts to increase the n-damo reaction rate, including optimization of n-damo bacteria growth conditions (temperature, pH, salinity, and etc.) (He et al., 2015a) and modification of reactor design to enhance sludge retaining capability (Hu et al., 2014a), both of which had successfully increased the n-damo reaction rate. Another obstacle before the practical application of n-damo process is the low dissolubility of CH_4. Although this could be solved by using a single reactor with high CH_4 partial pressure and dedicated only for n-damo process, high operation cost and sophisticated technique requirement are need. It would be much more efficiency if CH_4 could be utilized *in situ* it was produced. Although the coexistence of denitrifies and methanogens have been reported (Andalib et al., 2011), few studies have showed the coexistence of methanogenesis and n-damo process. In addition, methanogens is significantly influenced by carbon source (Kundu et al., 2013). However, the effect of carbon source on the growth of autotrophic *M. oxyfera* bacteria is unknown. And the introduction of carbon source would also led to undesired process (e.g., denitrification), which may endanger the coexistence of methanogenesis and n-damo processes. Thus, it is essential to study the effect of carbon sources on n-damo process.

Thus, the objective of the present study was to thoroughly evaluate the feasibility of reducing GHG emissions from anoxic wastewater treatment environment by strengthening n-damo process. Effect of carbon source on coexistence of methanogens and n-damo bacteria was investigated and nitrogen transformation was revealed by using molecular biotechnology and stable isotope analysis.

2. Materials and methods

2.1. Experimental setup and operation

The schematic diagram of the experiment setup is shown in Fig. 1. Three double-walled cylindrical column gastight upflow anaerobic sludge blanket (UASB) reactors, with effective volume of 7.5 L, were operated side by side for 318 days. Sludge taken from anaerobic tank of a full-scale A^2/O municipal WWTP in Jinan, China, was used as inoculum. At the beginning of the study period, the sludge concentration in all reactors were maintained around 15 ± 0.5 g/L in accordance with mixed liquor suspended solids (MLSS), the ratio of mixed liquor volatile suspended solids (MLVSS) concentrations and MLSS was 75 ± 2%.

Each reactor was continuously fed with synthetic wastewater consisting of different carbon sources, i.e., sodium acetate (R1), methanol (R2), and CO_2/H_2 (R3). For R1 and R2, chemical oxygen demand (COD) concentration of influent was maintained 150 ± 10 mg/L to reach the final C/N ratio of 1.2–1.3. For R3, 3.18 ml/min of CO_2/H_2 mixture (1:3.76, vol/vol; purity, >99.999%) was sparged gently through diffuser at the bottom of the reactor to perform a complete conversion (Burkhardt et al., 2015). Besides carbon sources, the other composition of synthetic wastewater is shown in Table S1, and the acidic/alkaline trace element solution was prepared according to previous description (Ettwig et al., 2009). To avoid nitrite toxic effect, NO_2^- concentration in the influent was increased step by step, from 2 mgN/L to 120 mgN/L. Thus, the whole study period was divided into five phases in accordance with the NO_2^--N concentration of influent. To maintain strict anoxic environment in the reactor, influent was stripped with high purity N_2 before entering into the reactors. The influent rate was 3.5 ml/min, resulted in hydraulic retention time (HRT) of 1.5 days. Throughout the study period, water temperature was maintained at 33 ± 1 °C, and the influent pH was controlled at 7.3 ± 0.2 by manual injection of 1 M HCl.

2.2. Stable isotope tracer batch experiments

At the end of study period, isotope tracer batch experiments using ^{15}N-labeled NO_2^- were conducted to determine the n-damo potential activity. Sludge samples collected from each reactor were firstly washed three times with anaerobic water in order to remove the residual NO_x^- (NO_2^- and NO_3^-) and organic compounds, and then transferred to Ar-flushed 1L glass bottle. Three treatment groups with the same sludge concentration (10 g/L) were conducted: (a) CH_4 (CH_4 at 99%, blank group I), (b) $^{15}NO_2^-$ (^{15}N at 99.9%, blank group II), (c) $CH_4 + {}^{15}NO_2^-$ (experiment group). Blank group I and II served as controls to rule out the possible influences of other biological reactions except for n-damo. The initial CH_4 and NO_2^- concentrations were maintained around at 1.0 ± 0.01 mmol/L and 1.4 ± 0.01 mmol/L, respectively. Batch experiments were run for six hours and CH_4 and NO_2^- concentrations were determined through sampling 3 ml of liquid and 10 ml of gas every hour. All the liquid and gas samples were measured in 24 h. The potential methane and nitrite consumption rates and nitrogen production rates were evaluated by linear regression of CH_4 and NO_2^- decrease, and N_2 increase in the experimental groups.

2.3. Analytical methods

Water samples of influent and effluent were collected every three days after feeding and were analyzed immediately for COD,