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Contributions of nitrification and denitrification to N_2O emissions from aged refuse bioreactor at different feeding loads of ammonia substrates

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ABSTRACT

Nitrous oxide (N_2O) is a strong greenhouse gas, and its emissions from microbial nitrification (NF) and denitrification (DNF) are a threat to the environment. In the present study, a combined approach consisting of ^{15}N stable isotope and molecular biology (qPCR) was used to determine the contributions of autotrophic nitrification (ANF), heterotrophic nitrification (HNF), and DNF to N_2O emissions in laboratory incubations of aged refuse for different ammonia (NH_4^+-N) loads (200, 400, and 800 mg·NH_4^+-N/kg·aged refuse) and incubation times (2–144 h). Experimental results showed that the N_2O emissions increased with the increase in applied amount of NH_4^+-N substrates. Simultaneous nitrification and denitrification (SND) were demonstrated to be present in the incubations of aged refuse. The results of ^{15}N stable isotope labelling experiment indicated that NF (54.60%–68.8%) and DNF (83.38%–85.90%) contributed to majority of N_2O emissions in the incubations of 24 h and 72 h, respectively. The results of functional genes (*amoA* and *nosZ*) quantification experiments indicated that the high gene copies of *amoA* and *nosZ* were present at 24 h and 72 h, respectively. The study also demonstrated the utility of a combined stable isotope and molecular biology approach. The approaches not only provide similar inferences about the N_2O emissions, but also enable the determination of relative contributions of ANF, HNF, and DNF to N_2O emissions. The results of the study are important in providing guidance to artificially optimize the operating conditions for alleviating N_2O emissions in aged refuse bioreactors.

1. Introduction

In China, 1.5% of the total anthropogenic greenhouse gas (GHG) emissions are caused by the activities of waste treatment and management (Wang et al., 2014a). Nitrous oxide (N_2O) is a dominant ozone-depleting substance, and has a significant GHG contribution (Ravishankara et al., 2009). The global warming potential of N_2O is about 298 times higher than that of the CO_2 over a 100-year period, and has increased by approximately 20% from pre-industrial times to 2012 (IPCC, 2013). Landfilling is a cost-effective municipal solid waste (MSW) management option, and is still a mainstream MSW disposal method in China. Statistics show that N_2O emissions from landfills can be one or more orders of magnitude higher than those from the forestlands, grasslands, and farmlands (Cai, 2012). Moreover, Wang et al. (2016) reported that N_2O fluxes from active landfills (*i.e.* bioreactor landfills) were higher than those from closed landfills (*i.e.* anaerobic landfills). Traditionally, leachate-recirculation and air-introduction (*i.e.* aeration) are two important operational practices for activating landfills (Bilgili et al., 2007; Slezak et al., 2015). A previous study has shown that N_2O emissions were much higher from an active aged refuse bioreactor (ARB) landfill (with recirculation and aeration) than those from an active fresh refuse bioreactor (FRB) landfill (with recirculation and no-aeration) (Wang et al., 2014b).

Similar to the sources of N_2O production in soils, microbial nitrification (NF) and denitrification (DNF) are the main N_2O production pathways in landfills (De Boer and Kowalchuk, 2001). Fig. 1 shows that when nitrate (NO_3^--N) or nitrite (NO_2^--N) is successively reduced to N_2O and/or N_2 in heterotrophic DNF (*i.e.* NO_3^--N/NO_2^--N reduction) (III), N_2O is an intermediate-product, whereas it is a by-product during the hydroxylamine (NH_2OH) oxidation (I) and/or nitrifier-dinitrification (II) in autotrophic nitrification (ANF; *i.e.* ammonia (NH_4^+-N) oxidation) (Snider et al., 2015; Wrage et al., 2001; Zhang et al., 2016). Additionally, during the traditional NF (NH_4^+-N oxidation) process, there is also evidence that some heterotrophic microorganisms, such as *Alcaligenes faecalis*, have the ability to nitrify N-based compounds and produce N_2O in the culture (Joo et al., 2005). To date, evidence for heterotrophic nitrification (HNF) is limited to handful of studies related to cultures rich in acidic and organic pasture or forest soils where ANF can be inhibited (Islam et al., 2007). Martienssen and

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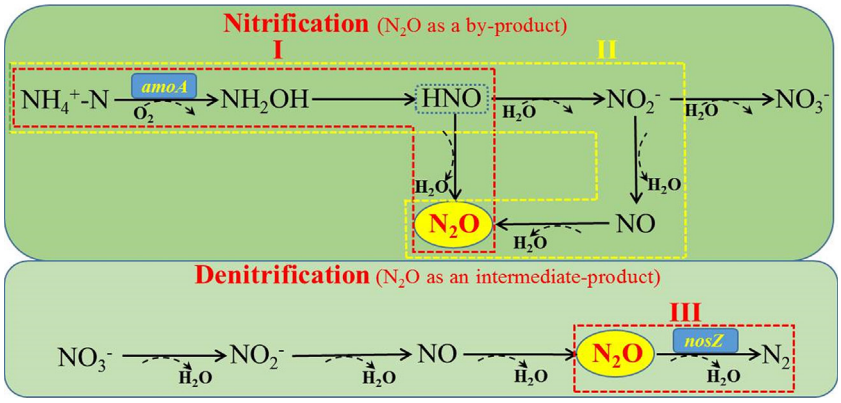

Fig. 1. Overview of simplified N_2O producing and consuming sources in nitrification and denitrification processes. I is the hydroxylamine (NH_2OH) oxidation, II is the nitrifier-denitrification, and III is the N_2O consumption/reduction process. The location of key N-related functional genes was characterized by blue background frame in this study: ammonia monooxygenase (*amoA*) in nitrification and N_2O reductase (*nosZ*) in denitrification.

Schöps (1997) indicated that HNF may be one of the microbial processes during the biological treatment of landfill leachate. However, the contributions of HNF to N_2O emissions from bioreactor landfills, especially the ARBs with recirculation of leachate rich in NH_4^+-N, are still unknown. Similar to the application of N-fertilizers in agricultural soils, the increasing leachate recirculation load of NH_4^+-N in ARB is an important reason for N_2O emissions resulting from the aggravation or impediment of microbial NF and DNF (Parkes et al., 2007; Zhang et al., 2009). During the operation of ARBs, especially with leachate-recirculation and/or aeration, it is important to track N_2O production pathways and differentiate N_2O production source from various microbial processes (*i.e.* NF and DNF) for devising operational strategies to minimize N_2O production and emission. However, dynamic shifting between NF and DNF raises confusion regarding the differentiation of N_2O sources. Therefore, it is necessary to devise an approach for evaluating the relative contributions of NF and DNF in N_2O emissions in the application of ARBs.

Some studies have employed stable isotope tracers to examine N_2O production in the incubations of soils, which facilitate the direct measurement of ^{15}N-N_2O and allow a more accurate determination of N_2O sources in different microbial processes (Bateman and Baggs, 2005; Snider et al., 2015). Furthermore, Bateman and Baggs (2005) demonstrated the usefulness of a combined approach consisting of stable isotope and acetylene (C_2H_2; 0.01%, vol.%) inhibition to quantify N_2O emissions of N-fertilized soil from different microbial processes. Additionally, molecular methods have been used as independent approaches to study the mechanism of N_2O production in soils (Huang et al., 2014; Németh et al., 2014), such as quantitative polymerase chain reaction (qPCR), which was used to examine the nitrifier and denitrifier communities and make relative comparisons of gene abundance. In this study, two key functional genes, which code bacterial ammonia monooxygenases (*amoA*, NH_4^+-N → NH_2OH) in N_2O production process (I and II; Fig. 1) (Ni et al., 2014) and N_2O reductase (*nosZ*, N_2O → N_2) in N_2O consumption process (III; Fig. 1) (Henry et al., 2006) were selected. Recent studies indicated that the assessment of functional gene abundance in tandem with stable isotope measurements can be used to study the complex mechanism of N_2O production in soil ecosystems (Butterbach-Bahl et al., 2013; Snider et al., 2015).

In this study, a novel combination technique consisting of stable isotope (^{15}N-labelled), molecular biology (qPCR), and ANF inhibition by C_2H_2 (0.01%, vol.%) was used in laboratory incubations of aged refuse for different NH_4^+-N loads. The main objectives of the study are to: 1) identify the effect of different NH_4^+-N loads and incubation times on N_2O production; 2) determine the respective contributions of HNF, ANF, and DNF to N_2O emissions; and 3) evaluate the utility of combined stable isotope and molecular biology approach to determine the short-term dynamics of N_2O production pathway in ARBs.

2. Materials and methods

2.1. Aged refuse samples

Aged refuse samples stabilized for 10–12 years were excavated from an abandoned MSW sanitary landfill in Qingdao, China (Fig. S1). The samples had a uniform particle size, high porosity, low water content, and typical organic content (mainly humic substances, which are not easy to be used by microbes), similar to the description given in Han et al. (2011) and Zhao et al. (2007). Large particles (such as, plastic bags, glass bottles, stones, and rubber) were removed manually. The samples were air-dried for 48 h at room temperature, and screened through a 2-mm sieve for microbial acclimation experiment.

2.2. Microbial acclimation experiment

Two Plexiglas columns with an inner diameter of 150 mm and a height of 1000 mm were used to simulate the ARB (Fig. S2). 50-mm gravel was placed at the bottom of each ARB to facilitate the outlet of effluent leachate, followed by 800-mm aged refuse (loading density: 991 kg/m^3). Then, 50-mm fine sand was placed at the top to promote an even distribution of the influent leachate. A leachate recirculation valve was installed at the top of each ARB. Each column had 50 mm of free space at the top of fine sand for gas storage and 50 mm of space at the bottom for leachate storage. A perforated pipe with a height of 200 mm was installed at the center of each ARB, which was used to spread the incoming air, while the base of the airway pipe was connected to an air pump which was used for periodic intermittent aeration.

For the two ARBs, the methods of incremental volume load and incremental hydraulic load were used to acclimate microorganisms, respectively (Han et al., 2011). The leachate used in the acclimation was taken from the regulating tank of a leachate treatment plant at XiaoJianXi MSW sanitary landfill in Qingdao, China. The process flow diagram of the leachate treatment plant is shown in Fig. S3. The leachate had a chemical oxygen demand (COD), NH_4^+-N, and pH of 11850–12000 mg/L, 3500–4000 mg/L, and 7.42–7.53 respectively, and a COD/TN (*i.e.* total nitrogen) ratio greater than 3.0. By comparing the two acclimation methods, the method of achieving higher COD and NH_4^+-N removal rate (>90%) and longer

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