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Nitrogen removal pathway of anaerobic ammonium oxidation in on-site aged refuse bioreactor

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HIGHLIGHTS

- Pollutants were effectively removed by on-site aged refuse bioreactor in landfill.
- *amoA*, *nirS* and anammox 16S rRNA gene were found to coexist in every bioreactor.
- Ratios of functional genes and pollutants removal performance were closely related.
- Anammox provides an alternative pathway for nitrogen management in landfill.

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ABSTRACT

The nitrogen removal pathways and nitrogen-related functional genes in on-site three-stage aged refuse bioreactor (ARB) treating landfill leachate were investigated. It was found that on average 90.0% of COD_{Cr}, 97.6% of BOD_5, 99.3% of NH_4^+–N, and 81.0% of TN were removed with initial COD_{Cr}, BOD_5, NH_4^+–N, and TN concentrations ranging from 2323 to 2754, 277 to 362, 1237 to 1506, and 1251 to 1580 mg/L, respectively. Meanwhile, the functional genes *amoA*, *nirS* and anammox 16S rRNA gene were found to coexist in every bioreactor, and their relative proportions in each bioreactor were closely related to the pollutant removal performance of the corresponding bioreactor, which indicated the coexistence of multiple nitrogen removal pathways in the ARB. Detection of anammox expression proved the presence of the anammox nitrogen removal pathway during the process of recirculating mature leachate to the on-site ARB, which provides important information for nitrogen management in landfills.

1. Introduction

Sanitary landfilling is currently recognized as the most common ultimate disposal method for municipal solid waste (MSW) worldwide. The sanitary landfill method not only presents economical advantages but also permits the decomposition of waste under controlled conditions until the MSW is eventually transformed into relatively inert, stabilized material (Renou et al., 2008). However, landfill leachate, which is generated during the biodegradation of MSW and the percolation of rainwater during landfilling, is a heavily polluted wastewater. It is generally enriched in numerous organic, inorganic, ammonium, and toxic constituents, and its characteristics vary significantly with increasing disposal time (Foo and Hameed, 2009). Mature leachate, which is achieved in landfills after operation for more than 5 years, is characterized by high ammonium (NH_4^+) content and low biodegradability of organics (low ratio of biochemical oxygen demand (BOD_5)/chemical oxygen demand (COD)), making its treatment challenging, especially *in situ* treatment (Cortez et al., 2010; Kurniawan et al., 2006).

An aged refuse bioreactor (ARB), filled with 8- to 10-year-old refuse excavated from a landfill as a filter medium, has been shown to be a highly efficient method for treating landfill leachate, both at the lab-scale and the field-scale (Zhao et al., 2002; Xie et al., 2010). The primary pollutant removal pathways in ARB treatment of landfill leachate involve degradation by aerobic microorganisms in the surface layer and anaerobic microbes beneath the surface, adsorption of refuse particles, ion exchange, and chelation (Long et al., 2008). Previous studies have also verified that the high level of nitrogen pollutant removal in ARB treatment is achieved through a wide range of numerous microorganisms present in the aged refuse (Li et al., 2010; Song et al., 2011; Xie et al., 2012). With regard to nitrogen removal, it is a common notion that nitrogen-removing functional microbes such as ammonia-oxidizing bacteria (AOB) and denitrifying bacteria play a vital role in both nature and

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engineered systems. However, considering the characteristics of mature leachate, the removal rate of total nitrogen (TN) in ARB treatment of mature leachate will be restricted by the low biodegradable organic matter content of the leachate according to the traditional nitrification/denitrifaction pathway. Thus, multiple nitrogen removal pathways may exist in ARBs.

Recently, different nitrogen removal mechanisms such as autotrophic denitrification and anaerobic ammonium-oxidation (anammox) have been demonstrated in the nitrogen cycle and have received widespread attention because they provide an alternative nitrogen removal pathway in environments with a low carbon to nitrogen ratio (Daverey et al., 2013; Sliekers et al., 2002). The anammox process, which combines ammonium as an electron donor and nitrite as an electron acceptor to form nitrogen, is universally believed to require approximately equimolar concentrations of ammonium and nitrite (Jetten et al., 2001). Previous studies have reported contradictory results on the presence of the anammox process in landfills that conduct leachate recirculation. Price et al. (2003) suggested that the existence of the anammox process remains uncertain in the conditions of leachate recirculation to the bioreactor after the conversion of ammonium to nitrate in an ex situ aerobic reactor. However, Valencia et al. (2011) confirmed that the introduction of small quantities of oxygen promotes the growth of anammox bacteria, and the presence of the anammox process contributes to nitrogen removal in landfill bioreactors. In addition, our previous work also verified the presence of anammox bacteria in a lab-scale ARB and revealed that multiple nitrogen removal pathways coexist when leachate was pretreated using shortcut nitrification and the influent ratio of ammonium and nitrite was approximately 1 (Xie et al., 2013). Although simultaneous partial nitrification, anammox, and denitrification (SNAD) were found in a single liquor bioreactor for nitrogen removal (Chen et al., 2009; Kumar and Lin, 2010), for an on-site ARB in which the environment is aerobic at the surface and gradually becomes anaerobic deeper within the reactor (Han et al., 2013; Wang et al., 2013), the existence of anammox bacteria and the anammox nitrogen removal pathway remain uncertain when nitrogen compounds exist primarily in the form of ammonium in influent leachate. Nonetheless, it will be helpful to understand the different nitrogen removal pathways that occur in landfill processing if anammox bacteria are present in on-site ARBs.

The purpose of this study was to investigate the nitrogen removal efficiency of on-site ARBs at the Shanghai Laogang landfill and the corresponding expression patterns of nitrogen-removing functional genes (*amoA*, *nirS*, and anammox 16S rRNA) using quantitative polymerase chain reaction (qPCR). It is expected that the results will reveal a potential alternative nitrogen removal pathway in *in situ* landfill bioreactors.

2. Methods

2.1. On-site ARB

The Shanghai Laogang MSW plant (31°00′N, 120°52′E), located north of Hangzhou Gulf and south of the mouth of the Yangtze River in China, is the largest landfill in Asia. It receives 10,000 tons of MSW and produces nearly 3000 m^3 leachate per day (Ding et al., 2007; Li et al., 2009). Aged refuse was excavated from a single landfill compartment that has been closed for nearly 10 years and sieved to less than 40 mm before use as the packing material (Zhao et al., 2002). Some basic characteristics of the aged refuse were: volatile suspended solids (VSS) of 10–12%, moisture content of 12–15%, and density of 1.60–1.75 g/cm^3. Three-stage horizontal and tower ARBs were constructed on-site to treat the landfill leachate.

In the three-stage horizontal ARB, the surface area of the first aged refuse bed (bioreactor) was 2300 m^2, and that of the additional two beds was 2000 m^2. The vertical height of each bed was about 3 m. The leachate was sprayed over the bed through holes in the influent tube, and the effluent was collected and pumped to the second and third beds. Effluent from each bed was collected in corresponding collecting basins approximately 30 m^3 in volume. In the three-stage tower ARB, which was constructed above ground, the first bed was on the top and the third one was on the bottom. The vertical height of each tower ARB was approximately 1 m, which was less than that of the horizontal ARB. The leachate was pumped and sprayed over the first tower bed and then infiltrated into the sequenced beds by gravity. (Images of the horizontal and tower ARBs are shown at Fig. S1(a) and (b), respectively, in the Supplementary Material). The characteristics of the leachate from Shanghai Laogang MSW were as follows: COD_{Cr} of 2322.9–2754.4 mg/L, BOD_5 of 276.5–361.9 mg/L, NH_4^+–N of 1237.2–1506.2 mg/L, NO_2^-–N of 0.5–4.7 mg/L, NO_3^--N of 13.7–46.1 mg/L, TN of 1251.3–1580.5 mg/L, pH between 7.9 and 8.2, and oxidation/reduction potential (ORP) of −300 to −340 mV.

The ARBs were operated under a hydraulic loading rate (HLR) between 10 and 20 L/m^3 d, and the field temperature varied between 20 °C and 30 °C during the study period. The wastewater samples were collected over 10 weeks for water quality analysis, and at the end of the experiment, aged refuse was sampled for microbiological analysis. For the horizontal ARB, the influent, first effluent, second effluent, final effluent, and triple aged refuse samples of each bioreactor (1 m below the surface using screw drilling, Fig. S2 of Supplementary Material) were sampled. For the tower ARB, because the space between the first and second stage was very small, and it is not always possible to get samples from the second bed, only the influent, third effluent, and duplicate aged refuse samples of first and third beds were collected (0.20 m below the surface).

2.2. Analytical methods

2.2.1. Wastewater quality

The influent and effluent parameters such as COD_{Cr}, BOD_5, NH_4^+–N, NO_2^-–N, NO_3^-–N, and TN were measured according to the standard methods (APHA, 1998).

2.2.2. Microbiological analysis

2.2.2.1. DNA extraction and plasmid standard preparation. Samples of 0.6 g aged refuse were used for total DNA extraction. Genomic DNA was extracted using MoBio Ultra-Clean™ soil DNA isolation kits (MoBio Laboratories Inc., CA, USA) according to the manufacturer's protocol. Total 16S rRNA gene, *amoA*, *nirS*, and anammox 16S rRNA gene were amplified using the following PCR procedure. Each 50-μL PCR reaction mixture was composed of 5 μL 10 × Buffer, 4 μL (25 mmol/L) Mg^{2+}, 1 μL (5 mmol/L) dNTP, 0.5 μL (5 U) Taq polymerase, 1 μL of each primer, 1 μL DNA template, and 36.5 μL ddH_2O. The PCR products were analyzed using 1% agarose gel electrophoresis.

The V3 region of 16S rRNA of bacteria was amplified with the outer 27F (AGAGTTTGATCMTGGCTCAG)/1492R (TACGGYTACCTT GTTACGACTT) (Xu et al., 2008) and inner F357 (CCTACGGGAGGC AGCAG)/R518 (ATTACCGCGGCTGCTGG) (Muyzer et al., 1993) primers. The PCR program for each 27F/1492R primer pair was: initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 30 s, and annealing for 30 s. The first cycle was at 65 °C, with the temperature in each of the next 11 cycles decreasing by 0.5 °C. The next 23 cycles were carried out at 59 °C, followed by extension at 72 °C for 1 min, and final extension at 72 °C for 7 min. The PCR