One-stage and multistage anoxic/oxic systems based on continuous plug-flow anammox process for mature landfill leachate treatment

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Abstract As a promising biotechnology, the anammox process has been increasingly investigated for landfill leachate treatment. However, its application has been limited by the long generation time of anammox bacteria (AnAOB) and insufficient nitrite supply in actual projects. To overcome these obstacles, this study innovatively established one-stage and multistage anoxic/oxic systems (S1 and S2) based on the continuous plug-flow anammox process to treat mature landfill leachate. Ultra-efficient nitrogen removal was achieved through the partial nitrification coupled anammox (PNA) and the partial denitrification coupled anammox (PDA) pathways. Over 97.0% of nitrogen was removed from the leachate (1800-2000 mg NH_4^+-N/L). Stable isotope tracing tests demonstrated that 89.8%-92.4% and 90.3%-95.0% of the nitrogen loss were contributed by the anammox pathway in S1 and S2, respectively. 16S rRNA sequencing revealed that the oxic zone, especially the oxic biofilm was the hotspot for AnAOB enrichment. *Candidatus_Kuenenia* with higher nitrite affinity and stronger tolerance to free ammonia (FA) and salinity stress outcompeted *Candidatus_Brocadia* and became the dominant anammox genus. Multiple bacteria capable of hydrolysis and acidogenesis were enriched under the in-situ FA anoxic treatment, facilitating complex organic matters degradation and volatile fatty acids production, which could be used as the carbon source during the PDA process.
Keywords: Anammox; Continuous plug-flow system; Combined process; In-situ free ammonia treatment; Landfill leachate

1. Introduction

Municipal waste production is dramatically increasing with population growth and the development of urbanization, which will reach 3400 million tons in 2050 (Srivastava & Chakma, 2021). Sanitary landfill, incineration, and composting are still the major ways for municipal waste disposition and accomplishing its innocuity, reduction, and recycling. As for developing countries, landfill with a relatively low cost is employed most frequently. However, a substantial amount of leachate is generated inevitably during waste treatment, which will threaten human health and environmental quality. The traditional nitrogen removal process falls far short of carbon neutrality due to a considerable oxygen and carbon source demand when treating high-strength ammonia wastewater (Cao et al., 2021).

The anammox-based process was regarded to be a critical step in achieving energy-neutral and its advantages will be magnified when treating landfill leachate (Yang et al., 2020). However, biomass loss, insufficient nitrite supply, and poor shock resistance restrict its application. The integrated fixed-film activated sludge (IFAS) technology was proven to be an effective way of achieving biomass retention (Kaewyai et al., 2022). The partial nitrification coupled anammox (PNA) process gradually developed into a reliable method for leachate treatment, while the partial denitrification coupled anammox (PDA) process could further remove nitrate produced in the PNA pathway (Singh et al., 2022; Zhao et al., 2022). Most of the leachate treatment plants were based on the continuous plug-flow anoxic/oxic (A/O) process. Nevertheless, the operational performance, nitrogen removal mechanism, and microorganism distribution are still unclear when applying the anammox process in one-stage and multistage A/O systems treating leachate. Therefore, this study established one-stage (S1) and multistage step feed (S2) A/O IFAS systems to treat mature landfill leachate based on the continuous plug-flow anammox process.

2. Materials and methods

2.1. Experimental apparatus and operation

The two systems (S1 and S2) were both composed of a continuous plug-flow reactor (16.5 L of working volume) and a sedimentation tank (5.7 L of working volume) and were operated over 400 days (Fig. 1). Each continuous reactor was divided into 9 compartments, and there were 4 and 6 anoxic chambers in S1 and S2. During the stable phase, the sludge reflux ratios (SRR) of S1 and S2 were 900% and 200%, respectively. The temperature of the systems and the dissolved oxygen (DO) in oxic units were maintained at 30.0±1℃ and 0-0.35 mg/L. Mature landfill

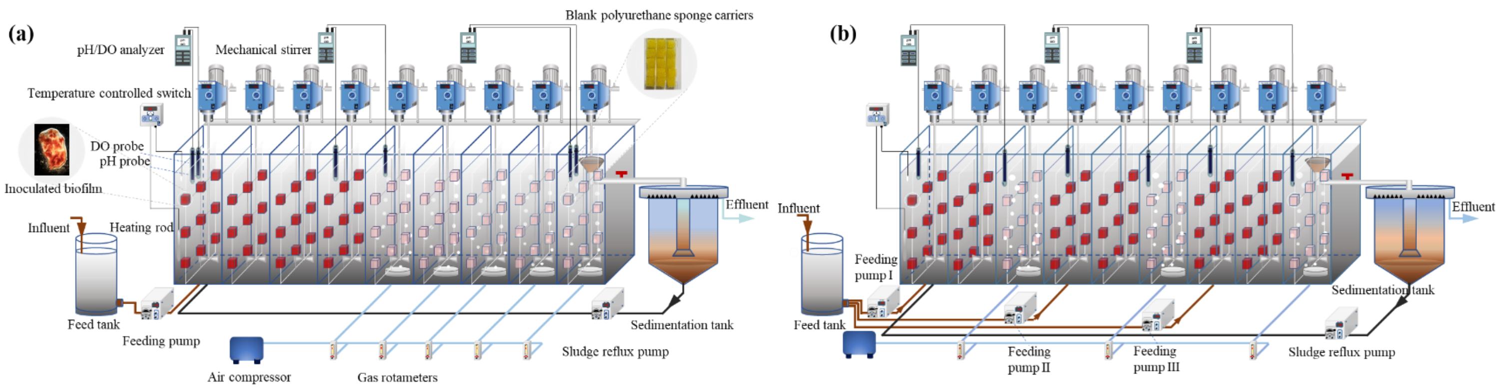

Figure 1. One-stage (a) and multistage (b) A/O systems based on the continuous plug-flow anammox process.

leachate was used as the influent, in which the contents of total inorganic nitrogen (TIN), chemical oxygen demand (COD), and five-day biological oxygen demand (BOD5) were shown in Table 1.

Table 1. The characteristics of the landfill leachate used in this study.

Parameters	Concentrations
TIN	1750-2000 mg/L
COD	3150-3600 mg/L
BOD_5	1100-1600 mg/L
NH_4^+-N	1740-1980 mg/L
pH	7.9-8.3
Salinity	16-19 g/L

2.2. 16S rRNA gene sequencing

The composition and long-term variation of the microbial community in S1 and S2 were investigated through the 16S rRNA gene sequencing technology. The specific methods of bioinformatics analysis used in the current study were summarized in Table 2.

Table 2. The methods of bioinformatics analysis used in this study.

Items detection and analysis	Materials and methods
DNA extraction	Fast DNA Spin kits for soil (MP Bio 101, Vista, CA, USA) based on the manufacturer's instructions
Concentration determination of the extracted DNA	TBS-380 mini fluorometer (Turner Biosystems, Sunnyvale, CA, USA)
Purity determination of the extracted DNA	NanoDrop2000 spectrophotometer (Nano Drop, Wilmington, DE, USA)
Quality examination of the extracted DNA	Agarose gel electrophoresis technique (1% agarose gel)
16S rRNA gene high-throughput sequencing	The V3-V4 gene region was amplified through PCR with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Purified amplicons pooled in equimolar were paired-end sequenced on the Illumina MiSeq PE300 platform (Illumina, San Diego, USA).

3. Results and discussion

3.1. Advanced nitrogen removal via the anammox pathway

Both S1 and S2 were run over 400 days and the whole operation was divided into five stages including partial nitrification initiation, IFAS establishment, AnAOB activity recovery, nitrogen removal performance enhancement, and the stable phase (Fig. 2). The partial nitrification was achieved after about 100-days operation under the in-situ free ammonia (FA) treatment. Then the IFAS process was established by adding and fixing polyurethane sponge carriers in S1 and S2. The anoxic zone was inoculated with anammox biofilm whereas blank carriers without biomass were added into the oxic zone since the anoxic compartments were the critical zone for AnAOB enrichment (Li et al., 2021; Li et al., 2019). The nitrogen loading rate (NLR) was decreased in phase III and then increased during phase IV to recover AnAOB activity and enhance nitrogen removal capacity, respectively.

To alleviate the FA inhibition of anammox bacteria (AnAOB), a higher sludge reflux ratio (SRR, 900%) was required in S1, whereas a lower SRR (200%) in S2 was feasible under the step feed strategy (Fig. 2). The maximum concentrations of FA in S1 and S2 were effectively controlled at 20.2 and 14.4 mg N/L through the

SRR enhancement and step feed strategies, respectively. During the stable phase, over 97.0% of total inorganic nitrogen (TIN) was removed from the landfill leachate, when the hydraulic retention time (HRT) was controlled at 5.50 d and 6.35 d in S1 and S2, respectively (Fig. 2).

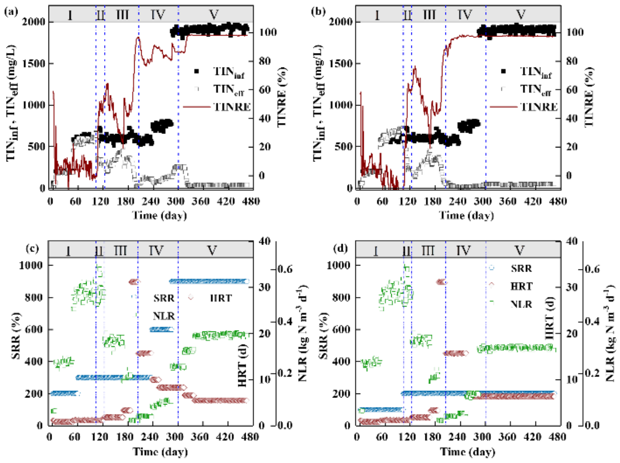

Figure 2. Nitrogen removal performance and operational parameters of the one-stage (a), (c) and multistage (b), (d) systems.

The biodegradable organics were mainly removed in anoxic zones with the simultaneous decrease of ammonia and nitrate concentrations, indicating the existence of the PDA phenomenon. In oxic zones, ammonia and TIN concentrations declined dramatically whereas the COD concentration was barely reduced, exhibiting a substantial PNA potential. Stable isotope tracing tests demonstrated

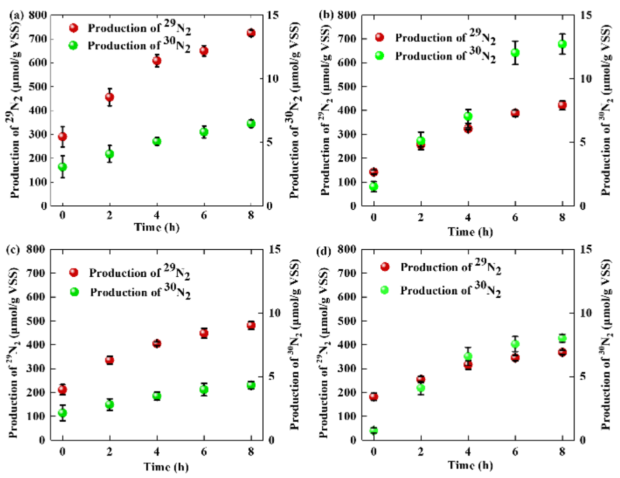

Figure 3. Verification of the nitrogen removal pathway in one-stage (a), (b) and multistage (c), (d) systems by stable isotope tracing tests (NH_4^+-^{14}N, NO_2^--^{15}N, and NaAc (a) (c), NH_4^+-^{14}N, NO_3^--^{15}N and NaAc (b) (d)).

that anammox nitrogen removal contributions of S1 and S2 could reach 89.8%-92.4% and 94.3%-95.0%, respectively (Fig. 3). Volatile fatty acids (VFAs) were only detected (4.0-20.0 mg/L acetic acid) in anoxic chambers, which could be used as the carbon source during the PDA process.

3.2. Microbial community succession

Candidatus_Brocadia was the dominant anammox genus in the inoculated biofilm (17.9%-18.1%) (Fig. 4). However, during phase V, *Candidatus_Kuenenia* outcompeted *Candidatus_Brocadia* as the predominant AnAOB, which might be ascribed to its higher nitrite affinity and stronger tolerance to FA and salinity stress (Huang et al., 2021; van der Star et al., 2008; Van Tendeloo et al., 2021). *Nitrosomonas,* a typical ammonia-oxidizing bacteria (AOB) genus was mainly enriched in floc sludge and the oxic biofilm. Nitrite-oxidizing bacteria (NOB) were eliminated under the long-term in-situ FA treatment. AnAOB (*Candidatus_Kuenenia*), AOB (*Nitrosomonas*), partial denitrification bacteria (PDB, *OLB14 and Thauera*), and multiple heterotrophic bacteria capable of denitrification, hydrolysis, and acidogenesis were enriched and coexisted in the two systems, which ameliorated the microenvironments for AnAOB and alleviated the inhibition by organic matters or oxygen (Fig. 4). 16S rRNA sequencing tests revealed that the oxic zone, especially the oxic biofilm was the hotspot for AnAOB enrichment because there were more nitrite and less FA in oxic chambers. In addition, uncultured species possessing the anaerobic ammonia oxidization ability were also detected in S1 and S2 deserving further investigations.

3.3. Significance

This study established novel anammox-based systems, in which 61.7% and 87.5% of oxygen and carbon source requirements were reduced theoretically compared with the traditional nitrification-denitrification process. The installation employed was simple for upgrading and reconstruction of current plants treating leachate. Moreover, the quality and efficiency of the leachate treatment would be remarkably enhanced by applying the integrated PNA and PDA process. Therefore, this study could provide significant guidance for leachate treatment in engineering and contribute to achieving energy-efficient wastewater treatment.

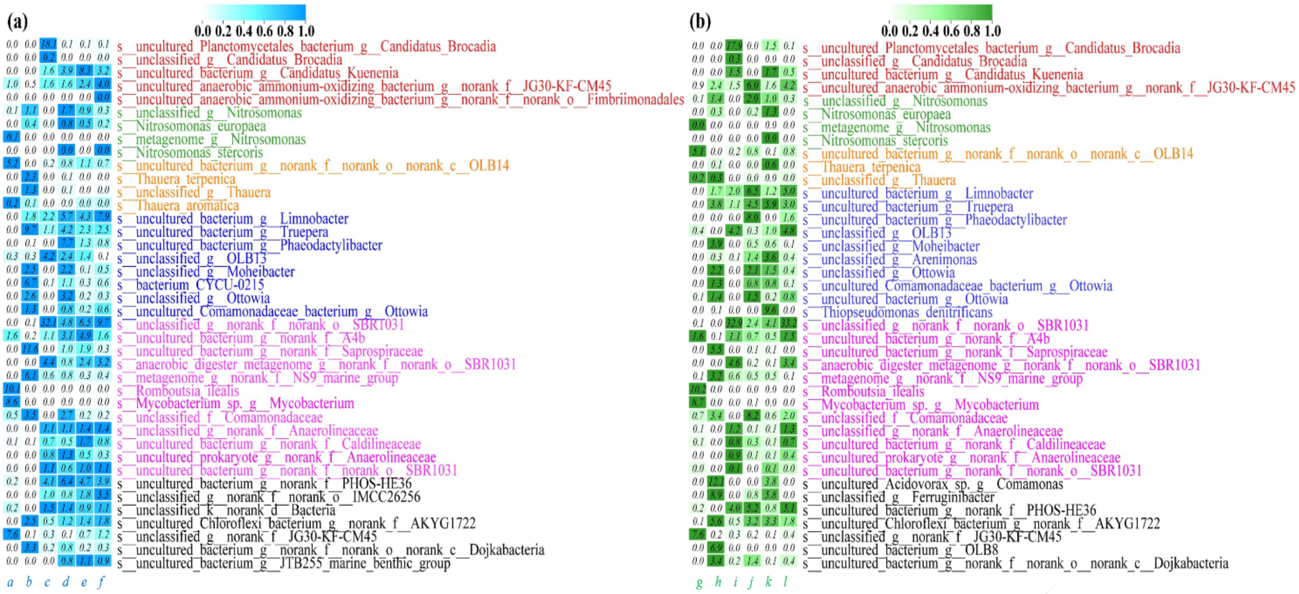

Figure 4. Functional bacteria enriched in the one-stage (a) and multistage (b) systems on species level (samples a and g, b and h, c and I, d and j, e and k, f and l represented inoculated floc, floc at the end of phase II, inoculated biofilm, floc, oxic biofilm, and anoxic biofilm during the stable phase, respectively).

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