



# Analysis of the impact of reflux ratio on coupled partial nitrification–anammox for co-treatment of mature landfill leachate and domestic wastewater



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## HIGHLIGHTS

- A UASB-A/O-ANAOR combined system was used to treat a mix of landfill leachate and domestic sewage.
- Biological processing alone efficiently removed organic matter and nitrogen.
- Advanced nitrogen removal from leachate with low C/N ratio was achieved without addition of carbon sources.
- Inhibition of bacteria by free ammonia is key to partial nitrification and anammox.
- An optimum reflux ratios can improve processing and enrich anammox bacteria.

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## ABSTRACT

A combined system of anaerobic–aerobic techniques was used for deep co-treatment of mature landfill leachate and domestic sewage, mixed at a ratio of 1:5, through partial nitrification–anammox. While ensuring synchronous removal of organic matter and nitrogen, we investigated changes in several factors under different reflux ratios (0%, 100% and 300%). High nitrification efficiency and a relatively high accumulation rate of  $\text{NO}_2^-$ -N were achieved, the latter through selective inhibition of bacteria by free ammonia. The results indicated that maintaining the reflux ratio within an optimum range contributes to the enrichment of anammox bacteria. In this study, the optimum ratio, which permitted the highest removal rates of chemical oxygen demand (COD),  $\text{NH}_4^+$ -N, and total nitrogen, was 300%. The COD level in the anammox reactor (ANAOR) decreased to lower than 70 mg/L, resulting in no inhibitory effect on anammox and maximum enrichment of bacteria.

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## 1. Introduction

Landfill leachate varies greatly in water quality and amount. It contains high concentrations of organic matter and ammonia nitrogen ( $\text{NH}_4^+$ -N), and has complex constituents, such as trace heavy metals. (Corteza et al., 2010; Sun et al., 2009; Wu et al., 2011). Water quality of landfill leachate varies greatly with burial time, after being buried for at least five years, leachate becomes ‘mature leachate’ (Carlos et al., 2015; Singh and Tang, 2013; Ying

et al., 2012) and usually has concentrations of  $\text{NH}_4^+$ -N greater than 2500 mg/L, chemical oxygen demand (COD) lower than 3000 mg/L (primarily due to refractory organics), and a very low carbon-to-nitrogen (C/N) ratio (<3). These characteristics significantly impede the degradation of organic matter and biological nitrogen (Anfruns et al., 2013; Renou et al., 2008; Wang et al., 2010) resulting in poor biodegradability. Mature leachate is therefore extremely difficult to treat. A low C/N ratio not only strongly inhibits conventional biological treatment but also the denitrification process, due to the lack of organic carbon sources. The economic and efficient removal of  $\text{NH}_4^+$ -N is therefore key for effective treatment of landfill leachate, as is resolving the deficiency of organic carbon sources for denitrification (Miao et al., 2014; Peng et al., 2008).

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It is currently believed that the best processing technology for landfill leachate is the anaerobic–aerobic combined technique (Miao et al., 2015). However, conventional anaerobic biological processing has some shortcomings, such as a slow reaction rate and a high concentration of  $\text{NH}_4^+\text{-N}$  in effluent, inhibiting subsequent aerobic processing (Sun et al., 2010).  $\text{NH}_4^+\text{-N}$  is usually converted to  $\text{NO}_2^- \text{-N}$  or  $\text{NO}_3^- \text{-N}$  ( $\text{NO}_x^- \text{-N}$ ) through nitrification in the aerobic reactor. If methanogenesis and denitrification can be realized simultaneously in the anaerobic reactor, removing both carbon and nitrogen at the same time, the quality of effluent will hence be improved, alleviating the subsequent processing burden. A number of studies have been conducted examining carbon sources and nitrate concentrations for methanogenesis and denitrification in anaerobic reactors, although most existing studies have used simulated instead of real sewage. However, there have been few studies focusing on the role of the reflux ratio, which correlates directly with the removal of organic matter and nitrogen in anoxic–aerobic techniques using real wastewater. The control of free ammonia (FA) in the oxic reactor, which hinges on the adjustment of the reflux ratio, is also a crucial consideration in order to reserve carbon sources and achieve partial nitrification.

Anaerobic ammonium oxidation (anammox) is a completely autotrophic process for biological nitrogen transformation (Ma et al., 2013; Zhu and Liu, 2008). Compared with traditional denitrification, it does not require external carbon sources and can reduce energy consumption by 50%. Anammox can hence solve the problem of a lack of carbon sources for denitrification if applied to treat the low C/N ratio of mature leachate. However, anammox bacteria have a low specific growth rate, making their enrichment very difficult (Strous et al., 1998; Ni et al., 2010).

Anammox requires a relatively long solid retention time (SRT), a very small amount of biodegradable sources of COD inside the reactor, and a quantity of  $\text{NO}_2^- \text{-N}$  in the reactor. Previous studies have investigated the effects of sludge age and reactor format on anammox. Studies on the effects of the reflux ratio on anammox have also been conducted (Jin et al., 2012; Tang et al., 2011); however, these only studied recirculation to dilute influent concentration, without further research on how differences in recirculation affect anammox. They also focused on low levels of  $\text{NH}_4^+\text{-N}$  (<300 mg/L). In addition, as with conventional studies on anammox, synthetic wastewater was used.

Drawing on the above-mentioned research, this study used mature leachate from a landfill in Beijing; through a combination of an up-flow anaerobic sludge blanket (UASB), an anoxic/aerobic (A/O) reactor, and an anammox reactor (ANAOR), coupled partial nitrification–anammox processing of a mature leachate and domestic sewage mixture was conducted. UASB–A/O treatment reduces COD, while partial nitrification occurs in the A/O reactor. Advanced nitrogen removal is achieved in the subsequent ANAOR via anammox. By refluxing the nitrification liquid from the secondary settling tank effluent to the UASB, the experiment investigated the effects of different reflux ratios on the removal of organic matter and nitrogen, partial nitrification, and anammox. This study aimed to identify optimal operating conditions and reflux ratios, and to achieve coupled partial nitrification–anammox processing. Using this technology, simultaneous, deep-level removal of organic matter,  $\text{NH}_4^+\text{-N}$ , and total nitrogen (TN) can be accomplished without the addition of carbon sources.

## 2. Methods

### 2.1. Experimental apparatus and water quality

In this study, a composite UASB–A/O–ANAOR system was used to treat mature leachate. The effective capacities of the UASB and

ANAOR were 8.25 L and 4.25 L, respectively. The A/O reactor was constructed of Plexiglas and had an effective capacity of 15 L. It was divided equally into 10 cells, with the first used for anoxic treatment and the rest for aerobic treatment.

Fig. 1 shows the processing workflow, which involved a dual-reflux system. A portion of the effluent from the secondary settling tank (i.e. reflux of nitrification fluid) refluxed to the UASB. The sludge from the secondary settling tank refluxed to the anoxic sections of the A/O reactor. Inside the UASB, denitrification of  $\text{NO}_x^- \text{-N}$  in the refluxed nitrification fluid was realized using the rich organic carbon from the influent. At the same time, organic matter was further degraded through methanogenesis. The effluent from the UASB entered the A/O reactor, where partial nitrification and anammox occurred.  $\text{NO}_2^- \text{-N}$  (generated from partial nitrification) and  $\text{NH}_4^+\text{-N}$  were partially removed. The effluent of the A/O reactor first entered the secondary settling tank, then the ANAOR.

The landfill leachate used in the experiment was obtained from a ten-year-old landfill in Beijing. This had a very low carbon-to-nitrogen (C/N) ratio of 1.30–1.36, typical of mature leachate. Details of wastewater quality are given in Table 1.

### 2.2. Experimental operating mode

The duration of the experiment was 120 days, and the flow rate was 4.5 L/d. Original leachate and domestic sewage were mixed at a ratio of 1:5. The experiment included three stages, during which the effluent of the A/O reactor refluxed to the UASB; sequential reflux ratios were 0%, 100%, and 300%, respectively. The sludge from the second-stage sedimentation tank was refluxed to the A/O reactor with a reflux ratio of 100%. The UASB and ANAOR were pre-heated and maintained at 32 °C and 35 °C, respectively, through heating in a water bath; a layer of water bath casing enclosed the UASB and ANAOR and this was connected to the water bath box. The temperature of the A/O reactor was maintained at 25 °C using a heater. The pH values of the ten cells in the A/O reactor varied between 7 and 9. The amount of dissolved oxygen in the A/O reactor was controlled using an air flow meter and maintained at a level of 0.5–1.0 mg/L. During the experiment, sludge from the A/O reactor was discharged, either through natural loss or for sampling. The sludge was maintained at a SRT of 30–40 days and with mixed liquor suspended solids (MLSS) of 3200–4400 mg/L. Sampling positions included the UASB influent (raw), UASB effluent (U1e), ANAOR effluent (U2e), A/O reactor anoxic section (An1), and the different cells of the anaerobic section (O2–O10).

### 2.3. Analysis items and measurement methods

#### 2.3.1. Conventional analysis items

Conventional water quality indicators, such as COD,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^- \text{-N}$ ,  $\text{NO}_3^- \text{-N}$ , and alkalinity, were measured according to the standard method outlined by the American Public Health Association (1995). Dissolved oxygen (DO), oxidation reduction potential (ORP), and pH were measured online (WTW DO 330i, WTW ORP 340i and WTW pH 340i, respectively), along with temperature. Total organic nitrogen (TON), TN, total organic carbon (TOC), total inorganic carbon (IC), and total carbon (TC) were measured with a TN/TOC analyser (Multi N/C3000, Analytik, Jena AG, Jena, Germany).

#### 2.3.2. DNA isolation and polymerase chain reaction (PCR)

After a freeze–drying process, approximately 0.10 g of the sludge sample was used for total DNA extraction using the FastDNA SPIN kit (QBIOScience Inc., Carlsbad, CA, USA). In order to study anammox bacteria, we used an amplification method using newly designed primers of HSBeta396F/HSBeta742R for functional genes

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