



Partial nitrification and denitrification of mature landfill leachate using a pilot-scale continuous activated sludge process at low dissolved oxygen



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HIGHLIGHTS

- Mature landfill leachate nitrogen removal via nitrite pathway at low DO was achieved.
- Excellent $\text{NH}_4^+\text{-N}$ removal and nitrite accumulation was obtained at DO of 0.3–0.5 mg/L.
- Actual HRT of the first oxic reactor mainly decided the operational limit.
- High-throughput sequencing analysis was used for bacterial community variation.
- Genus *Nitrosomonas* was responsible for $\text{NH}_4^+\text{-N}$ removal and nitrite accumulation.

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ABSTRACT

Controlling of low dissolved oxygen (DO) levels (0.1–0.5 mg/L), a cost-effective strategy, was applied to a pilot-scale anoxic-oxic-oxic-anoxic process for partial nitrification and denitrification of mature landfill leachate. High ammonium removal efficiency, stable nitrite accumulation rate and total nitrogen removal efficiency was higher than 95.0%, 90.0% and 66.4%, respectively, implying potential application of this process for nitrogen removal of mature landfill leachate. Efficient nitrite accumulation in the first oxic reactor depended on low DO conditions and sufficient alkalinity. However, operational limit was mainly decided by actual hydraulic retention time (AHRT) of the first oxic reactor and appeared with AHRT less than 13.9 h under DO of 0.3–0.5 mg/L. High-throughput sequencing analysis demonstrated significant change of bacterial diversity in the first oxic reactor after a long-term operation and dominant bacteria genus *Nitrosomonas* was shown to be responsible for $\text{NH}_4^+\text{-N}$ removal and nitrite accumulation under low DO levels.

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1. Introduction

Mature landfill leachate is considered as one kind of typical wastewater with high ammonium and organic matter concentration (dominated by non-biodegradable compounds) (Renou et al., 2008), which presents a highly potential threat to the surroundings and should be well treated before discharge (Sri Shalini and Joseph, 2012). In order to remove pollutants in mature landfill leachate economically, especially nitrogen, biological treatment technologies are preferred compared with physicochemical technologies. However, conventional nitrification and denitrification process is

not suitable for nitrogen removal of mature landfill leachate due to high concentration of ammonium and low carbon to nitrogen (C/N) ratio (Gabarró et al., 2012), which shows importance to obtain new biological technology.

In the past decade, partial nitrification and denitrification in activated sludge process has caught much attention in the treatment of mature landfill leachate (Spagni et al., 2008; Zhang et al., 2015) because of its advantages of saving energy (low aeration consumption) and low C/N ratio required (Peng and Zhu, 2006; Ge et al., 2015). The key of partial nitrification and denitrification lies in excellent nitrite accumulation achieved by ammonia-oxidizing bacteria (AOB) accumulation and nitrite-oxidizing bacteria (NOB) inhibition or washout in the reactors (Ge et al., 2015) and studies were carried out with landfill leachate for feasible strategies. Several studies had revealed control of free ammonia (FA) or free nitrous acid (FNA) could facilitate excellent nitrite

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accumulation for landfill leachate treatment (Gabarró et al., 2012), while FA and FNA inhibitions on NOB were reversible (Han et al., 2003). High temperature was also reported (Gabarró et al., 2012), however, extra energy consumption is needed, which is not suitable for practical operation. In the literatures, dissolved oxygen (DO) concentration in the range of 0.5–5.0 mg/L was mostly applied to partial nitrification for landfill leachate (Peng et al., 2008; Wang et al., 2014a; Kulikowska and Bernat, 2013), while few presented DO lower than 0.5 mg/L (Canziani et al., 2006), which was widely proved to be practicable for the partial nitrification of ammonium-rich reject water (Zhang et al., 2011), synthetic high-ammonium wastewater (Bernet et al., 2001) and even domestic wastewater (Ma et al., 2009). Compared with previous methods, low DO concentration seems to be more available due to oxygen affinity constant difference of AOB (0.3–0.5 mg/L) and NOB (0.7–1.8 mg/L) (Guisasola et al., 2005) and easy operational controlling via manipulating air supply. Besides, low DO can reduce energy consumption and further save operational cost. Thus, it is worth to investigate whether partial nitrification and denitrification can be realized for mature landfill leachate under low DO concentration (lower than 0.5 mg/L).

In this study, a pilot-scale continuous activated sludge process was established and then carried out with mature landfill leachate under low DO levels (0.1–0.5 mg/L) for the nitrogen removal via nitrite pathway. During a long-term operation, the overall performance, including $\text{NH}_4^+\text{-N}$ removal, nitrite accumulation and total nitrogen (TN) removal, was detected and both of pollutants removal mechanisms and operational limit were investigated in order to estimate feasibility of this process for nitrogen removal of mature landfill leachate. Moreover, the microbial community structure related to partial nitrification was analyzed by Illumina high-throughput sequencing technology and bacteria community variation and possible functional bacteria were also discussed.

2. Materials and methods

2.1. Experimental reactor set-up and operation

The pilot-scale reactors with a total working volume of 720 L was divided into 4 equal chambers (each with a volume of 180 L), which were set as anoxic zone 1 (A1), oxic zone 1 (O1), oxic zone 2 (O2) and anoxic zone 2 (A2) orderly (Fig. 1). The cylindrical secondary settler had a working of 150 L. A1 was used for denitrification of nitrite/nitrate from the internal recycle. O1 and O2 were applied for nitrification and A2 for further denitrification if necessary. Mechanical mixers and pH meters were equipped in every chamber and DO probes were put in O1 and O2. Aeration controlled by an airflow rotameter was provided in O1 and O2 to create desired DO concentration. The inflow rate, internal recycle and sludge recycle were controlled by peristaltic pumps. Temperature was kept as followed: A1 ($32 \pm 1^\circ\text{C}$), O1 ($27 \pm 1^\circ\text{C}$), O2 ($27 \pm 1^\circ\text{C}$), A2 ($27 \pm 1^\circ\text{C}$) by thermostatic heaters, respectively. The sludge retention time (SRT) was controlled at 15–25 days by discharging an appropriate amount of settled sludge. The mixed liquor suspended solid (MLSS) concentration was controlled about 3500 ± 500 mg/L.

2.2. Wastewater and sludge

The mature landfill leachate was obtained from a municipal landfill plant which was closed at 2004 in South China. Characteristics of leachate are shown in Table 1. Mean COD_{Cr} to nitrogen ratio (C/N) is only about 2, which means that the organic carbon source of this leachate is typically limited.

Seed sludge was taken from Membrane Bio-Reactor (MBR) reactor in that plant, which is applied for nitrification and denitrification of the leachate. After a month of sludge acclimation and nitrification operation, the experiments were begun.

2.3. Experimental procedure

After stable nitrification operation, the effect of DO concentration on nitrite accumulation ratio in O1 was studied with continuous feeding firstly and it was confirmed that O1 can be operated under low DO (0.1–0.5 mg/L) for stable nitrite accumulation (data no shown). The AOOA process was under operation with continuous feeding for 188 days divided into five phases (see Table 2). Phase I (day 1–58) was purposed to build up stable partial nitrification under low ammonium loading at a DO concentration of 0.1–0.3 mg/L. Then the process was stopped in phase II (day 59–69), and restarted in phase III (day 70–80) to study the recovery of partial nitrification. Phase IV (day 81–132) was carried out with an increasing influent ammonium concentration at a DO of 0.1–0.3 mg/L and phase V (day 133–188) was under operation with an increasing inflow rate (60, 72, 84, 90, 100, 110, 120 L/d) at a DO of 0.3–0.5 mg/L to investigate ammonium removal limit of this process. The pollutants removal, potential mechanism for partial nitrification and nitrogen removal, and partial nitrification kinetics analysis were also studied in phase V.

2.4. Analytical methods

Chemical oxygen demand (COD_{Cr}), ammonium ($\text{NH}_4^+\text{-N}$), nitrite ($\text{NO}_2^-\text{-N}$), nitrate ($\text{NO}_3^-\text{-N}$), total nitrogen (TN), alkalinity were respectively analyzed daily by potassium dichromate reflux method, Nessler's reagent spectrophotometer method, N-(1-Naphthalene)-Ethylenediamine dihydrochloride spectrophotometer, ultraviolet spectrophotometry, potassium supersulphate oxidation-ultraviolet spectrophotometry and potentiometric titration according to Chinese standard examination of water and wastewater (China, 2002). MLSS, mixed liquor volatile suspended solid (MLVSS) and sludge volume index (SVI) were measured according to gravimetric method (China, 2002). The COD_{Cr} value was corrected according to the fact that nitrite exerts a COD_{Cr} of 1.0 mg $\text{O}_2/\text{mg NO}_2^-\text{-N}$. DO was measured using a digital DO meters (HQ30d, HACH, USA) and pH was measured by pH meter (PHS-3C, INESA Scientific Instrument Co. Ltd, China).

In order to evaluate the substrate utilization, the proliferation of AOB and further explained partial nitrification mechanism of O1 within its $\text{NH}_4^+\text{-N}$ removal limit, kinetics analysis of O1 in phase V was carried out by ammonium specific utilization rate kinetic and Lawrence-McCarty model equation (Koch et al., 2000). Ammonium specific utilization rate kinetic in O1 can be expressed by equations as follow:

$$v = v_{\max} \frac{S_e}{K_s + S_e} \quad (1)$$

$$\frac{1}{v} = \frac{K_s}{v_{\max}} \times \frac{1}{S_e} + \frac{1}{v_{\max}} \quad (2)$$

where v is the ammonium specific utilization rate ($\text{kg NH}_4^+\text{-N kg}^{-1} \text{MLVSS day}^{-1}$); v_{\max} is the maximum ammonium specific utilization rate ($\text{kg NH}_4^+\text{-N kg}^{-1} \text{MLVSS} \cdot \text{day}^{-1}$); S_e is the effluent ammonium concentration (mg/L) and K_s is the half saturation value constant (mg/L).

Under steady-state, Lawrence-McCarty model equation can be expressed by equation as follow:

$$\frac{1}{\text{SRT}} = Y \frac{S_0 - S_e}{X_d t} - K_d \quad (3)$$

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