



Organic matter and concentrated nitrogen removal by shortcut nitrification and denitrification from mature municipal landfill leachate

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Abstract

An UASB+Anoxic/Oxic (A/O) system was introduced to treat a mature landfill leachate with low carbon-to-nitrogen ratio and high ammonia concentration. To make the best use of the biodegradable COD in the leachate, the denitrification of NO_x^- -N in the recirculation effluent from the clarifier was carried out in the UASB. The results showed that most biodegradable organic matters were removed by the denitrification in the UASB. The NH_4^+ -N loading rate (ALR) of A/O reactor and operational temperature was 0.28–0.60 kg NH_4^+ -N/(m^3 ·d) and 17–29°C during experimental period, respectively. The short-cut nitrification with nitrite accumulation efficiency of 90%–99% was stabilized during the whole experiment. The NH_4^+ -N removal efficiency varied between 90% and 100%. When ALR was less than 0.45 kg NH_4^+ -N/(m^3 ·d), the NH_4^+ -N removal efficiency was more than 98%. With the influent NH_4^+ -N of 1200–1800 mg/L, the effluent NH_4^+ -N was less than 15 mg/L. The shortcut nitrification and denitrification can save 40% carbon source, with a highly efficient denitrification taking place in the UASB. When the ratio of the feed COD to feed NH_4^+ -N was only 2–3, the total inorganic nitrogen (TIN) removal efficiency attained 67%–80%. Besides, the sludge samples from A/O reactor were analyzed using FISH. The FISH analysis revealed that ammonia oxidation bacteria (AOB) accounted for 4% of the total eubacterial population, whereas nitrite oxidation bacteria (NOB) accounted only for 0.2% of the total eubacterial population.

Key words: mature landfill leachate; UASB+A/O; shortcut nitrification; carbon source

Introduction

The composition and concentrations of pollutants in the municipal landfill leachate correlate with environmental conditions, landfill age etc. (Kim *et al.*, 2006). The young landfill leachate contains higher concentrations of biodegradable organic matter and ammonia, whereas the mature leachate contains relatively lower concentrations of degradable organic material but higher concentrations of ammonia. As a general rule, Barth *et al.* (1968) estimated that 4 g of BOD is needed per gram of NO_3^- -N reduced. However, the actual value will depend on operating conditions of the system and the type of electron donor used for denitrification. In general, the mature leachate contains lower concentrations of biodegradable organic matter, which results in a shortage of organic carbon source for denitrification. Therefore, it is of importance to obtain a technology that facilitates enhanced nitrogen removal from mature leachate under the conditions of low carbon source.

Denitrification via nitrite instead of nitrate (termed shortcut nitrification) saves the oxygen required for nitrite

oxidation (25% of the total oxygen demand) and 40% of the carbon demand. As reported, a biodegradable COD to NO_2^- -N ratio greater than 2.5 may ensure complete denitrification when shortcut nitrification takes place (Ruiz *et al.*, 2003; Van *et al.*, 1998; Hellinga *et al.*, 1998; Van *et al.*, 2001; Abeling and Seyfried, 1992; Mauret *et al.*, 1996). Therefore, stable shortcut nitrification and denitrification may enhance the efficiency of nitrogen removal from wastewaters with a low carbon source. In the experiment, an UASB+Anoxic/Oxic (A/O) system was introduced to treat a typical mature leachate. In the study, the shortcut nitrification with high nitrite accumulation was stably achieved at ambient temperatures. Without any physicochemical pretreatment such as chemical precipitation, ammonium stripping, and adsorption, total inorganic nitrogen (TIN) removal efficiencies ranging from 67% to 80% were achieved and effluent ammonia concentration was less than 15 mg/L.

1 Materials and methods

1.1 Experimental lab-scale reactors and characteristics of leachate

Fig.1 shows the experimental system consisting of a one-stage UASB reactor and an A/O reactor. Before this

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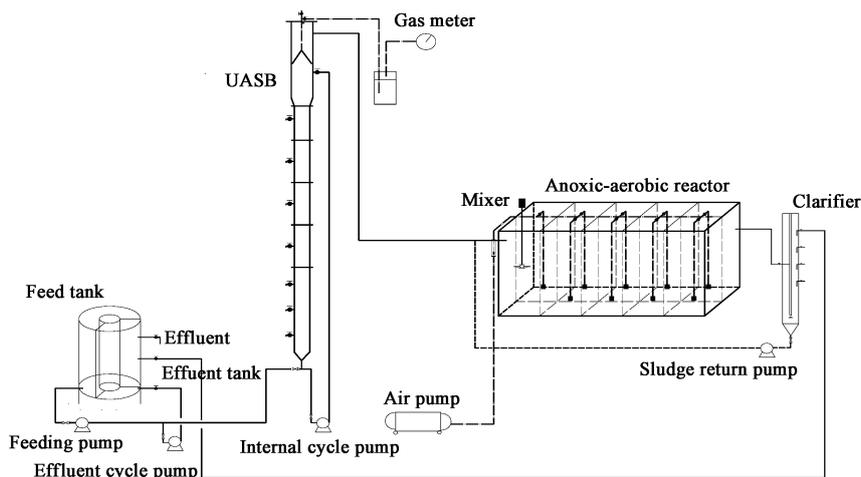


Fig. 1 Flow diagram of the two-stage UASB-A/O system.

study was carried out, the system was used to treat young landfill leachate. The effective volume of the UASB was 4.25 L with an internal diameter of 5 cm and a height of 210 cm. The effective volume of A/O reactor was 15 L with a length of 50 cm, a width of 12 cm, and a height of 30 cm. The aerobic reactor was divided into 10 chambers to provide better plug flow. The system was operated at 17–30°C. First, the flow rate was 3.5 L/d from days 1 to 10, and then, the flow rate was increased to 5.8 L/d from days 11 to 60. And then the system was operated with a flow rate of 4.6 L/d from days 61 to 100. During experimental period, the final effluent return ratio was 200% and the sludge return ratio from the clarifier was 100%. The sampling points were arranged as the follows: Raw—raw leachate; MIX—UASB influent (the mixed liquid between raw leachate and recirculation effluent); Ue—UASB effluent; Ano.—the anoxic zone of A/O reactor (the first chamber of the A/O reactor); O2—O10—the individual chambers of aerobic zone of the A/O reactor (the second chamber—the tenth chamber).

Granulated anaerobic sludge from the methanogenic reactor of a beer factory in Beijing, China, was used as seeding sludge in the UASB reactors. The activated sludge from a municipal wastewater treatment plant was used as seeding sludge for the A/O reactor.

For the study, a leachate discharged from a closed municipal landfill site in Beijing City was collected on a monthly basis. The landfill age was more than 6 years and the leachate was a typical mature landfill leachate that exhibits characteristics of black color, viscous nature, and malodor. The characteristics of the leachate were shown as follows: COD (2000–4500 mg/L), $\text{NH}_4^+\text{-N}$ (1200–1800 mg/L), $\text{NO}_x^-\text{-N}$ (<10 mg/L), pH 8.0–8.3, total alkalinity (10000 mg/L), and COD/ $\text{NH}_4^+\text{-N}$ (2–2.5).

1.2 Analytical procedures

Samples were taken from the reactors at appropriate intervals. All the samples were analyzed following the standard methods (APHA, 1995, and the corresponding instrumental instruction manuals). Gas production volume was measured by liquid displacement. Methane gas was

detected using 3% NaOH (w/v) in distilled water. Temperature, pH and dissolved oxygen were monitored using a pH meter (WTWpH330) and a dissolved oxygen meter (WTW oxi 330), respectively. Biochemical oxygen demand (BOD_5) was measured by OxiTop® Control (WTW). N_2O was measured by Agilent/6890N.

1.3 Fluorescent in situ hybridization

FISH technique (Amann, 1995) was used to investigate the microbial community of nitrobacteria in the A/O reactor. Biomass samples were withdrawn from the A/O reactor on the day 90 and fixed in 4% freshly prepared paraformaldehyde solution for 2–3 h at 4°C. The fixed sludge sample was dispersed by ultrasonic treatment for 1 min, and each sample was placed on a slide coated with 0.1% gelatin in the presence of 0.01% chromium potassium sulfate and dried overnight. The specimen was dehydrated by successive 50%, 80%, and 98% ethanol washes (3 min each), air dried, and stored at room temperature. *In situ* hybridizations were performed in a hybridization incubator according to the procedure of using hybridization buffer (0.9 mol/L NaCl, 20 mmol/L Tris-hydrochloride (pH 7.2), 0.01% sodium dodecyl sulfate, and formamide at the concentrations) at 46°C for 2 h. FISH probes used in the experiments are described in Table 1. Hybridization was followed by a thorough washing step, which was carried out twice, at 48°C for 20 min with 50 ml of pre-warmed washing buffer. The slides were mounted to avoid bleaching and examined with an OLYMPUSBX52 fluorescence microscope. The quantitative analysis of the microbial community of nitrobacteria was carried out using Leica QWIN Software.

Table 1 16S rRNA-targeted oligonucleotide probes

Probe	FA (%)	Fluorescence tag	Targets
EUB _{mix}	/	FITC	Eubacteria
NSO1225	35	Cy3	Ammonia-oxidizing β -Proteobacteria
NIT3	40	Cy3	Nitrobacteria
Ntspa662	35	Cy3	<i>Nitrospira</i>

EUB_{mix}: EUB338/EUB338II/EUB338III=1:1:1, FA: free ammonia.

2 Results and discussion

2.1 Removal of organic matter

The influent of UASB was a mixture of the raw leachate and the final effluent using the different mixing ratios. The NO_x^- -N from the recirculation effluent was denitrified in the UASB, and nitrification of high concentrations of NH_4^+ -N took place in the A/O reactor. The influent COD and COD loading rate of the UASB was 2000–4500 mg/L and 3.6–6.0 kg COD/($\text{m}^3 \cdot \text{d}$), respectively. The COD removal efficiency in the system was 50%–70% with effluent COD of 1000–1500 mg/L. The residual NO_x^- -N was already present when the effluent return ratio was 200%. The gas produced from the UASB was measured by Gas chromatography. The results indicated that the main content of the biogas was nitrogen gas and methane yield might be omitted. The above results showed that the biodegradable organic matters were mainly used as carbon source for denitrification of the NO_x^- -N from the return effluent. Highly effective nitrification of NH_4^+ -N took place in aerobic zone as COD was almost removed.

2.2 Shortcut nitrification and denitrification

The typical COD and nitrogen variation in the flow path is shown in Fig.2. After denitrification of NO_x^- -N (NO_2^- -N or NO_3^- -N) in the recirculation effluent in the UASB, its effluent COD was almost nonbiodegradable. According to the report by Police *et al.* (2002), the influent 1534 mg/L NH_4^+ -N can inhibit activity of microorganisms under high-pH conditions. In this experiment, the UASB effluent NH_4^+ -N was reduced to 560 mg/L by dilution of the recirculation effluent. Because of the further dilution of return sludge from the clarifier, the influent NH_4^+ -N of A/O reactor was already decreased to 256 mg/L. With continuous nitrification of NH_4^+ -N in the A/O reactor, the effluent NH_4^+ -N concentration was only 14 mg/L and the NH_4^+ -N removal efficiency was approximately 99%. Meanwhile, the effluent NO_3^- -N and NO_2^- -N concentrations were below 30 mg/L and 480 mg/L, respectively. The highly effective and stable shortcut nitrification with 94% NO_2^- -N accumulation was obtained in the study. Under

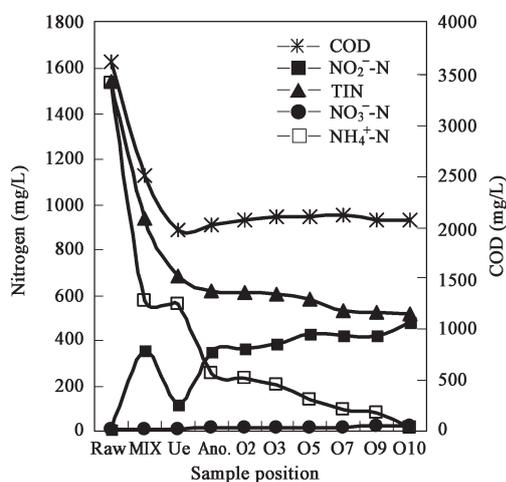


Fig. 2 COD and nitrogen variation in the flow path.

the conditions of COD/ NH_4^+ -N=2.4, effluent recirculation ratio of 200%, and sludge return ratio of 100%, the TIN removal efficiency was 67% in the system. The effluent TIN concentration was 518 mg/L at the influent TIN of 1545 mg/L.

In the aerobic zone, TIN was reduced from 623 mg/L to 518 mg/L. The above results showed that simultaneous nitrification and denitrification took place in the aerobic zone. The partial TIN was removed in the aerobic reactor and nitrous gas in the waste air released from the A/O reactor was analyzed using gas chromatography. In this study, NH_4^+ -N loading rate (ALR) of aerobic reactor was 0.28–0.60 kg NH_4^+ -N/($\text{m}^3 \cdot \text{d}$). When ALR was below 0.45 kg NH_4^+ -N/($\text{m}^3 \cdot \text{d}$), the nitrification efficiency was more than 98%. But when ALR was higher than 0.45 kg NH_4^+ -N/($\text{m}^3 \cdot \text{d}$), the nitrification efficiency was decreased to approximately 90%.

2.3 Effect of pH and FA on shortcut nitrification

In the study, FA (free ammonia) concentration (C_{FA}) calculated according to Equation (1) (Anthonisen *et al.*, 1976).

$$C_{\text{FA}} = \frac{C_{\text{NH}_4^+-\text{N}} \times 10^{\text{pH}}}{\exp(6334/(273 + T)) + 10^{\text{pH}}} \quad (1)$$

High FA inhibits not only nitrite oxidizing bacteria (NOB) but also ammonia oxidizing bacteria (AOB). NOB is known to be more sensitive to FA in the range of 0.1–1.0 mg/L, whereas AOB can be inhibited in the range of 10–150 mg/L. As a result, nitrite (NO_2^- -N) or even ammonium can accumulate if the free ammonia level in a reactor is high (Turk and Mavinic, 1989; Fdz-Polanco *et al.*, 1994; Yoon and Kim, 2003; Welander *et al.*, 1998). In addition, high pH can increase the FA concentration and inhibit nitrifying activity especially when the NH_4^+ -N level was high.

In the experiment, the typical FA and pH variation in the A/O reactor is shown in Fig.3. With NH_4^+ -N being oxidized continuously till the end of nitrification, pH decreased gradually to the lowest point—the “ammonia point”—indicating the completion of the NH_4^+ -N oxidation. Excessive aeration not only increases aeration cost but also damages stable shortcut nitrification unless aeration was stopped when “ammonia point” occurred. Accordingly, if pH is used as control parameter and “ammonia point”

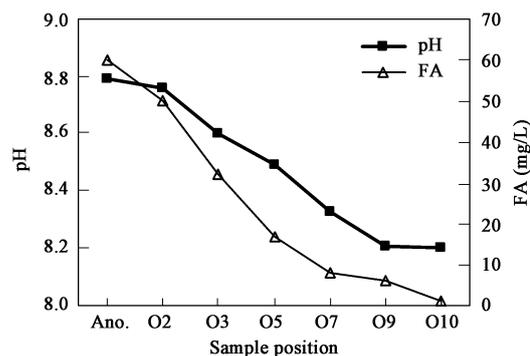


Fig. 3 FA and pH variation in the A/O reactor.

as control point, the completion of $\text{NH}_4^+\text{-N}$ oxidation may be indicated conveniently and exactly. When “ammonia point” is reached, aeration should be stopped to facilitate the formation and maintenance of stable shortcut nitrification.

As $\text{NH}_4^+\text{-N}$ and pH decreased gradually, FA concentration also reduced correspondingly. As a result, FA concentration was gradually decreased from 60 mg/L to approximately 1 mg/L in the flow path of the A/O reactor. Because pH was always higher than 8.2, FA concentration in the last chamber was still approximately 1.0 mg/L, which was in the range of FA inhibiting NOB activity. In the experiment, the FA in the range between 60 mg/L and 1 mg/L intensively inhibited NOB growth, whereas the growth of AOB was generally not influenced. The feasible FA concentration was realized by controlling both effluent return ratio and sludge return ratio. When either of the two returning ratios was increased, the FA concentration in the A/O reactor would be lowered accordingly.

2.4 FISH analysis

The stable shortcut nitrification with approximately 90–99% nitrite accumulation was carried out in the study. The effluent $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ concentrations were less than 15 mg/L and 150–400 mg/L, respectively, at the influent $\text{NH}_4^+\text{-N}$ of 1200–1800 mg/L. At nitrite accumulation efficiency of 98%, the sludge samples from A/O reactor were analyzed using FISH to determine the bacterial composition, especially AOB and NOB composition. The results are shown in Fig.4. The FISH analysis shows that AOB accounted for 4% of the total eubacterial population, whereas NOB only for less than 0.2%. The results indicated that NOB was actually washed out from the system so as to make possible the occurrence of shortcut nitrification in the system.

2.5 Denitrification and carbon source

The ratio between biodegradable COD (bCOD) and $\text{NH}_4^+\text{-N}$ should be greater than 4.0 for the completion of nitrate denitrification. The relative ratio for nitrite denitrification is only greater than 2.5, which is attributed to the 40% carbon source saved in the shortcut nitrification denitrification. However, the actual ratio was also decided by operational conditions and electron-donor types. In the experiment, the raw COD was 2000–4500 mg/L, and at

least 1000 mg/L raw COD was nonbiodegradable. The average ratio of bCOD to $\text{NH}_4^+\text{-N}$ was only 1.45, which meant that there was severe shortage of carbon source for denitrification with respect to the raw leachate used in the experiment, even if shortcut nitrification took place.

Fig.5 shows the variation of COD to $\text{NH}_4^+\text{-N}$ ratio and TIN removal efficiency during the experimental period. Because of the lack of carbon source for denitrification, the residual $\text{NO}_x^-\text{-N}$ already occurred in the UASB effluent at effluent return ratio of 200%. The TIN removal efficiency was 70%–80% at the COD (containing nonbiodegradable COD)/ $\text{NH}_4^+\text{-N}$ of 2–2.5. The results suggested that shortcut nitrification could save 40% of carbon source for denitrification, and the raw biodegradable COD was nearly all used as carbon source in the UASB. Similar results could be found in the report of Harmsen and Birgitte (1996). Moreover, the results of this study showed that simultaneous nitrification and denitrification took place in the aerobic zone. The denitrification also occurred in the anoxic zone of the A/O reactor and microorganism assimilation also some amounts of $\text{NH}_4^+\text{-N}$. The above results meant that the technology used in this experiment resulted not only in the reduction of carbon source for denitrification by shortcut nitrification but also in the use of almost all biodegradable COD as carbon source by effluent recirculation to UASB for denitrification.

The raw leachate alkalinity and $\text{NH}_4^+\text{-N}$ were approximately 10000 mg $\text{CaCO}_3\text{/L}$ and 1200–1800 mg/L, respectively. The nitrification of 1 g $\text{NH}_4^+\text{-N}$ consumed 7.14 g CaCO_3 , so that a shortage of alkalinity for nitrification could occur unless highly effective denitrification was carried out. In this study, the highly efficient denitrification took place in the UASB by effluent recirculation to the UASB. The denitrification resulted in the reclamation of approximately half of the alkalinity consumed in the oxidation of ammonia, and the recovered alkalinity provided enough inorganic carbon for high ammonia nitrification. Meanwhile, enough alkalinity could also maintain higher pH and help carry out stable shortcut nitrification and denitrification.

3 Conclusions

In the system, nitrification and denitrification were accelerated equally and organic matter degradation and

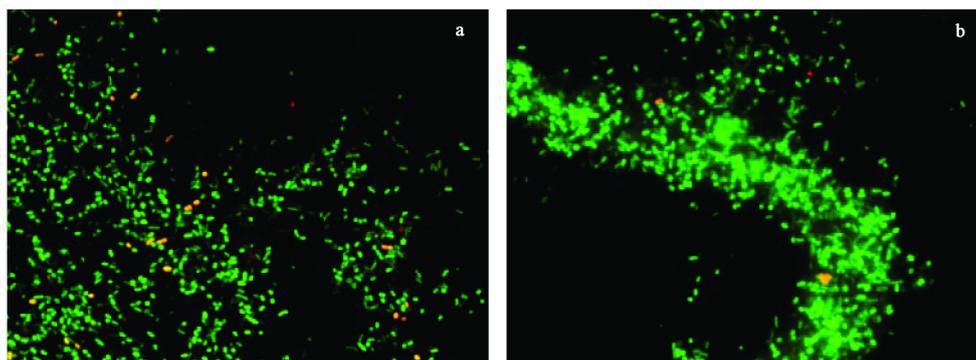


Fig. 4 Results of FISH analysis for AOB and NOB.

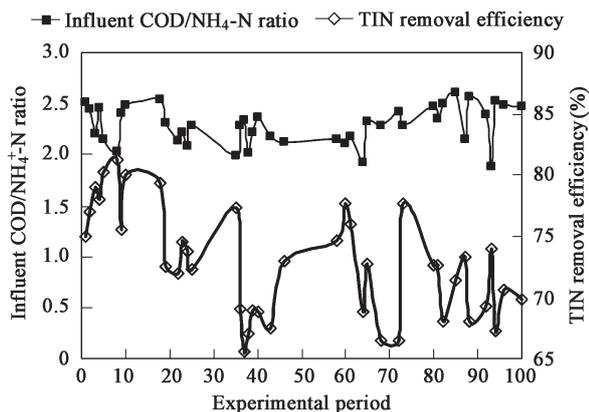


Fig. 5 Relationship between raw COD/NH₄⁺-N and TN removal efficiency.

nitrogen removal were closely correlated. By the effluent return, biodegradable organic matter was used as carbon source for denitrification and simultaneous organic matter and nitrogen removal was obtained.

Without any physicochemical pretreatment, 1200–2000 mg/L of the raw NH₄⁺-N was decreased to less than 15 mg/L, and approximately 99% NH₄⁺-N removal efficiency was achieved by shortcut nitrification with 90%–99% nitrite accumulation. FA is the main factor that is responsible for the maintenance of shortcut nitrification in the study. Under a special concentration range, FA inhibits NOB growth, whereas its influence on AOB was negligible. By adjusting the different effluent return ratios, the concentration of FA could be controlled appropriately.

When the ratio of feed COD to NH₄⁺-N ranged from 2 to 3, the TIN removal efficiency was 70%–80%. The biodegradable COD in raw leachate was first used as the carbon source for denitrification in UASB. Moreover, the recirculation effluent can dilute raw leachate and improve the mixing and mass transfer in the UASB.

The FISH analysis of the sludge samples from A/O reactor shows that AOB accounts for 4% of the total eubacterial population, whereas NOB accounts for less than 0.2%. Nitrobacteria constituted the major portion of NOB but no *Nitrospira* was found in NOB.

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