Control factors of partial nitritation for landfill leachate treatment

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Abstract

Anaerobic ammonium oxidation (ANAMMOX) technology has potential technical superiority and economical efficiency for the nitrogen removal from landfill leachate, which contains high-strength ammonium nitrogen (NH_4^+-N) and refractory organics. To complete the ANAMMOX process, a preceding partial nitritation step to produce the appropriate ratio of nitrite/ammonium is a key stage. The objective of this study was to determine the optimal conditions to acquire constant partial nitritation for landfill leachate treatment, and a bench scale fixed bed bio-film reactor was used in this study to investigate the effects of the running factors on the partial nitritation. The results showed that both the dissolved oxygen (DO) concentration and the ammonium volumetric loading rate (N_v) had effects on the partial nitritation. In the controlling conditions with a temperature of 30 ± 1°C, N_v of 0.2–1.0 kg NH_4^+-N/(m^3·d), and DO concentration of 0.8–2.3 mg/L, the steady partial nitritation was achieved as follows: more than 94% partial nitritation efficiency (nitrite as the main product), 60%–74% NH_4^+-N removal efficiency, and NO_2^--N/NH_4^+-N ratio (concentration ratio) of 1.0–1.4 in the effluent. The impact of temperature was related to N_v at certain DO concentration, and the temperature range of 25–30°C was suitable for treating high strength ammonium leachate. Ammonium-oxidizing bacteria could be acclimated to higher FA (free ammonium) in the range of 122–224 mg/L. According to the denaturing gradient gel electrophoresis analysis result of the bio-film in the reactor, there were 25 kinds of 16S rRNA gene fragments, which indicated that abundant microbial communities existed in the bio-film, although high concentrations of ammonium and FA may inhibit the growth of the nitrite-oxidizing bacteria and other microorganisms in the reactor.

Key words: landfill leachate; biological nitrogen removal; partial nitritation; anaerobic ammonium oxidation (ANAMMOX)

Introduction

Landfill lechate from an old age landfill usually contains high concentrations of ammonium nitrogen (NH_4^+-N) and refractory organics (Kjeldsen *et al.*, 2002). It is well known that ammonium nitrogen can trigger eutrophication in receiving watercourses and its removal can be completed either by physical/chemical or biological processes (Jae-Ho *et al.*, 1997). In spite of stable treatment effects, and preferable adaptability to the changes of wastewater quality and quantity, physical/chemical methods have several shortcomings: high chemical costs, high-energy consumption, and secondary pollution (Jae-Ho *et al.*, 1997). Biological technologies are more economical and effective because of lower running costs and more convenient operation when compared with the physical/chemical methods (USEPA, 1993). Traditional biological nitrogen removal processes include two steps: aerobic nitrification of ammonium to nitrate, and anoxic denitrification of nitrate to nitrogen gas using readily biodegradable organics as electron acceptor (USEPA, 1993). Therefore, some

problems always occur, such as low removal efficiency of total nitrogen (TN), high energy consumption, and unstable running when traditional biological technologies are used to treat landfill leachate with high-strength ammonium and insufficient/unavailable carbon source for denitrification (Khin and Annachhatre, 2004). Anaerobic ammonium oxidation (ANAMMOX) is a novel technology developed in the recent years (Khin *et al.*, 2004; van de Graaf *et al.*, 1995; Jetten *et al.*, 1999; Schmidt *et al.*, 2003). It does not require any organic source for nitrogen removal; therefore, it has potential technical superiority and economic benefits for landfill leachate treatment when compared with conventional biological nitrogen removal processes. In the ANAMMOX process, ammonium is converted to N_2 with nitrite (NO_2^--N) as the electron acceptor, and theoretically the concentration ratio of nitrite to ammonium is about 1.3 (Strous *et al.*, 1998; van de Graaf *et al.*, 1996). Since nitrite does not exist in the raw landfill leachate, a preceding partial nitritation step is required, which converts half of the ammonium to nitrite. However, the control of the partial nitritation is difficult. On one hand, the oxidation of nitrite to nitrate must be prevented, and on the other hand, the conversion efficiency of 57% from NH_4^+-N to NO_2^--N must be ensured on the basis of the nitrite/ammonium ratio of 1.3.

The key of nitritation (nitrite accumulation) is to favor ammonium-oxidizing bacteria (AOB) and to inhibit nitrite-oxidizing bacteria (NOB). According to the kinetic expressions of AOB and NOB (Ruiz *et al.*, 2003; Wiesmann, 1994), the main factors affecting the nitrite accumulation include the substrate concentration (NH_4^+-N concentration), the pH value, temperature, and dissolved oxygen (DO). NH_4^+-N and the pH value are not limiting factors for AOB growth because of the high strength NH_4^+-N and the alkalinity in landfill leachate. The high free ammonium (FA) produced by high concentration ammonium and pH can inhibit the growth of NOB, but such inhibition will not be sustained because of the acclimation of NOB to FA (Abeling and Seyfried, 1992; Turk Mavinic, 1989; Villaverde *et al.*, 2000). Therefore, pH is not a controlling factor for nitritation in landfill leachate treatment. It was reported that nitrite build-up was successfully achieved at a higher temperature (35–40°C) of the SHARON (single reactor for high activity ammonia removal over nitrite) process (Hellinga *et al.*, 1998). However, the process is appropriate to high temperature wastewater for economic considerations, and higher temperature is helpful for producing more FA to inhibit NOB and AOB, which is not good for steady partial nitrification (Villaverde *et al.*, 2000). The nitrite-oxidizing bacteria have a lower affinity for oxygen than the ammonium-oxidizing bacteria, which is helpful for the inhibition to the growth of NOB at a low DO concentration (Picioreanu *et al.*, 1997). Steady nitrite accumulation can be achieved because of the different oxygen saturation coefficients of NOB and AOB (0.3 and 1.1 mg/L respectively) (Wiesmann, 1994; Garrido *et al.*, 1997).

As far as the control of the NH_4^+-N removal efficiency is concerned in partial nitritation, the chemical substances are NH_4^+-N and oxygen in this reaction. Controlling the DO concentration and the NH_4^+-N volumetric loading rate (N_v) thus becomes feasible since the manipulation of DO and N_v is effective and convenient in the actually running process. Therefore, DO and N_v were selected as the main factors to control partial nitritation in this study, and the purpose of this work is to optimize the controlling conditions to acquire steady partial nitritation for landfill leachate treatment.

1 Materials and methods

1.1 Experimental set-up

A bench scale up-flow fixed bed bio-film reactor with elastic filler was used (Fig.1). The working volume of this reactor was 11 L, and the total surface area of the filler was about 4.09 m^2. The desired DO concentration was acquired by adjusting the airflow. The temperature in the reactor was controlled at 30±1°C except in the stage of
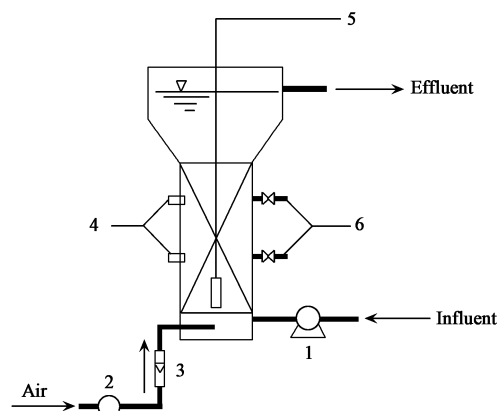

Fig. 1 Schematic diagram of the treatment process. (1) metering pump; (2) air pump; (3) flow meter; (4) DO meter; (5) heater; (6) sample point.

the testing effect on nitritation. Landfill leachate in this study was from a municipal solid wastes (MSW) sanitation landfill in Beijing of China. As shown in Table 1, the NH_4^+-N concentration of raw landfill leachate was high, in the range of 1600–3100 mg/L, and the concentration ratio of COD_{Cr} to NH_4^+-N (C/N ratio) was low (usually below 2).

1.2 Start-up of reactor

From an aeration tank treating mixture of domestic sewage and leachate in an MSW transfer station, 19 g VSS sludge was inoculated in the bioreactor, and the ratio of VSS and TSS was 45%. Through acclimation and culture for about one month under the conditions listed in Table 2, the NH_4^+-N removal efficiency was more than 90%, and the partial nitritation efficiency was about 90%. Culture of partial nitritation bio-film in the bioreactor was thus considered to accomplish.

Table 2 Conditions of acclimation and culture of the bio-film in the reactor

Time	DO (mg/L)	Temp. (°C)	Raw wastewater
First week	2.0	30±1	Mixture of domestic sewage and leachate
Afterward	1.0–1.5	30±1	Leachate

1.3 Analytical methods

The concentrations of NH_4^+-N, NO_2^--N, NO_3^--N, and TN were determined according to the standard methods issued by the Environmental Protection Agency (EPA) of China.

COD_{Cr} was determined by the oxidation of potassium dichromate using a COD analyzer (CTL-12, China). The COD_{Cr} reflected value of NO_2^--N was calculated using Eq.(1), and it was deducted in this study by the difference

Table 1 Characteristics of landfill leachate

Parameter	NH_4^+-N (mg/L)	TN (mg/L)	COD_{Cr} (mg/L)	Alkalinity (Na_2CO_3) (mg/L)	pH
Range	1600–3100	1800–3300	1500–16000	8000–15000	8.0–9.0
Aver.±SD	2046.4±467.1	2241.4±494.1	2293.2±899.8	11801±2649	8.4±0.4

value between the analyzed COD_{Cr} value and the COD_{Cr} reflected value of NO_2^--N.

$$C = \frac{16}{14} C_{(NO_2^--N)_{eff}} \quad (1)$$

where, C is the COD_{Cr} reflected value of NO_2^--N; $C_{(NO_2^--N)_{eff}}$ is the concentration of NO_2^--N in the effluent.

The temperature and DO were on-line monitored using a DO meter (Oxi 330i WTW, Germany). The pH value was determined using glass electrodes connected to a pH meter (320-S, China). A scanning electron microscope (SEM) was used to observe bacteria.

TSS was analyzed by drying the sample at 105°C for at least 24 h. After burning at 600°C for 1 h, the ash was measured. The difference between TSS and ash was termed as VSS. The partial nitritation efficiency (PNE) was calculated using Eq.(2):

$$PNE = \frac{C_{(NO_2^--N)_{eff}}}{C_{(NO_2^--N)_{eff}} + C_{(NO_3^--N)_{eff}}} \times 100\% \quad (2)$$

where, $C_{(NO_2^--N)_{eff}}$ and $C_{(NO_3^--N)_{eff}}$ represent the concentrations of NO_2^--N and NO_3^--N in the effluent, respectively.

2 Results and discussion

2.1 Effects of DO and the ammonium loading rate on the partial nitritation

Limited DO concentration (less than 2 mg/L) inhibited nitrification and produced nitrite build-up (Goreau et al., 1980). DO had no effect on the nitrite accumulation at 5.7–2.7 mg/L, and both the nitrite accumulation and the

ammonium consumption decreased at a DO of 0.5 mg/L (Ruiz et al., 2003). The influence of DO on the partial nitritation at concentrations of 2.5–0.8 mg/L was thus investigated in this study. Heterotrophic bacteria oxidizing organics compete with the autotrophic AOB for oxygen, and high concentration organics make against the ammonium oxidation. The C/N ratio in the influent was often below two during the experiment. Thus, according to the two different C/N ratios (C/N<1 and 1<C/N<2), the experimental results are summarized in Figs.2 and 3, respectively.

The experimental results indicated that the partial nitritation efficiencies were more than 94% because of stable nitrite build-up resulting from steadily inhibited NOB at different conditions. DO and N_v have an interaction effect on the partial nitritation. At the same DO concentration, the NH_4^+-N removal efficiency decreased with the increase of N_v. When N_v was less than 0.5 kg/(m^3·d), almost the same partial nitritation efficiencies were achieved for two different C/N ratios at the same DO concentration. However, at N_v more than 0.5 kg NH_4^+-N/(m^3·d), the DO concentration must be increased to acquire the same efficiency for the higher C/N ratio (1<C/N<2). At the same N_v, the NH_4^+-N removal efficiency increased with the increase of DO, regardless of the C/N ratio.

The ratios of NO_2^--N to NH_4^+-N in the effluent were increased correspondingly with the enhancement of the NH_4^+-N removal efficiencies. Notably, the ratios were also related to the TN removal efficiencies, which resulted from the simultaneous nitritation and denitrification (SND) via nitrite at limited DO concentration (Christine and Sabine, 1998). Higher removal efficiency of TN will decrease the
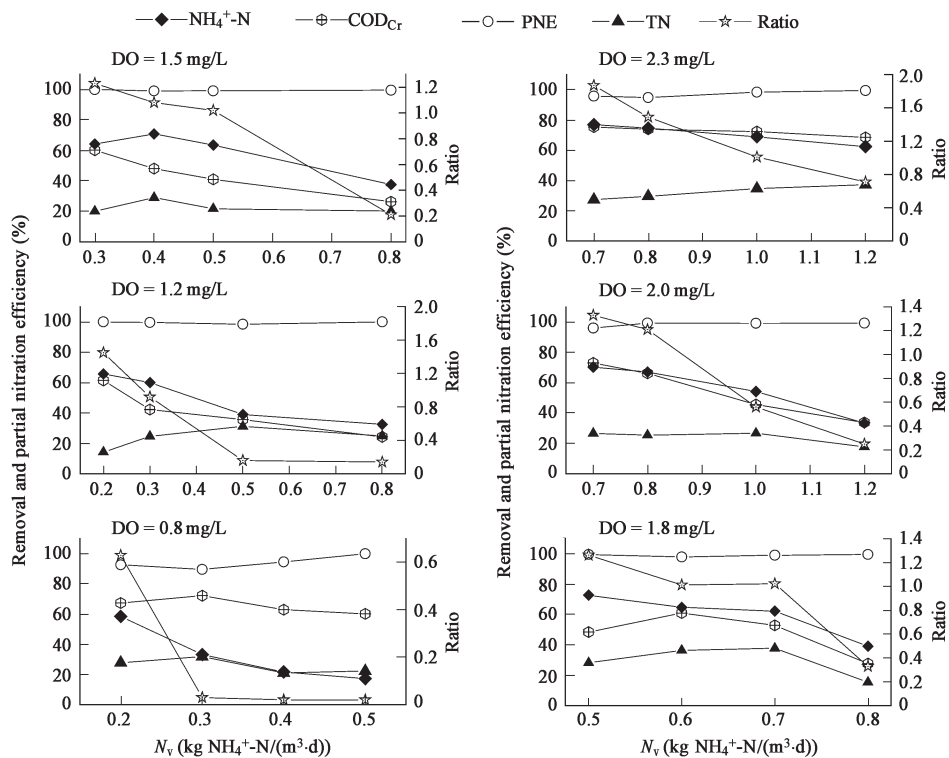

Fig. 2 Influence of DO and N_v on the partial nitritation. C/N<1; T=30±1°C; ratio represents the ratio of NO_2^--N to NH_4^+-N in the effluent; PNE: partial nitritation efficiency.
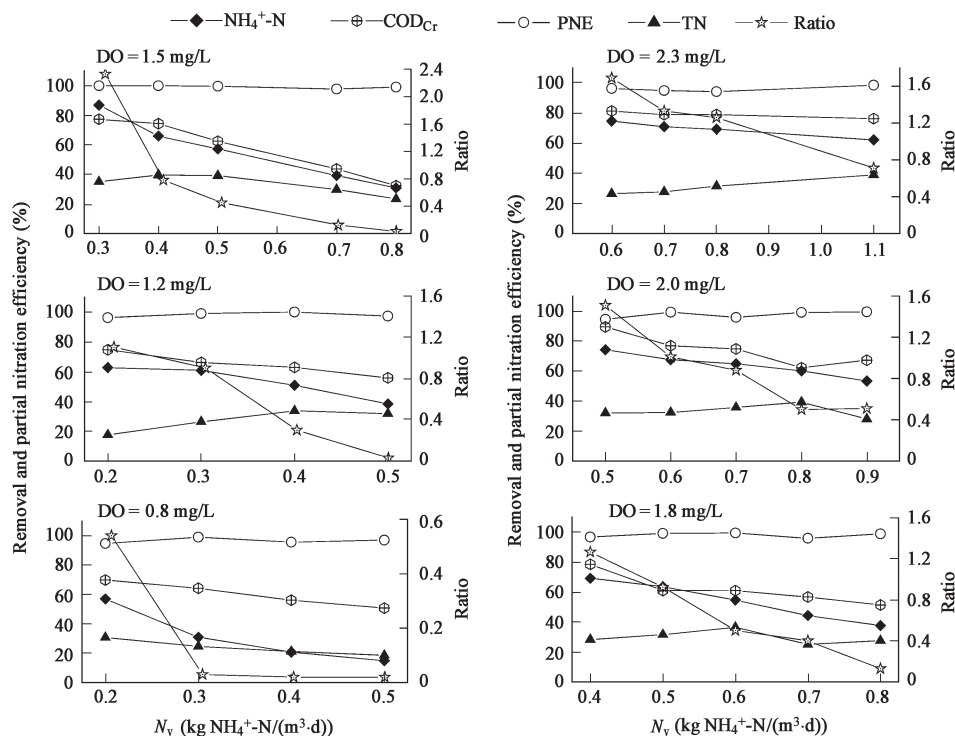
Fig. 3 Influence of DO and N_v on the partial nitritation. 1<C/N<2; T=30±1°C; and ratio represents the ratio of NO_2^--N to NH_4^+-N in the effluent. PNE: partial nitration efficiency.

ratios of NO_2^--N to NH_4^+-N because SND consumes part of the nitrite. Therefore, the actual NH_4^+-N removal efficiency (from NH_4^+-N to NO_2^--N) must be more than its theoretical value (57%) to acquire the appropriate ratio of the nitrite/ammonium mixture that will be fed into the following ANAMMOX reactor. Increasing the DO concentration will enhance the NH_4^+-N removal efficiency (from NH_4^+-N to NO_2^--N), but will result in decreased TN removal efficiency because high DO concentration is not good for SND, especially for treating landfill leachate with low C/N ratio.

As shown in Figs.2 and 3, the removal efficiencies of organics and TN changed with the variation of DO and N_v. At the same DO, the increased TN removal and the decreased COD_{Cr} removal occurred correspondingly with the increase of N_v. However, the TN removal started to decrease at the time of high N_v because of less NO_2^--N caused by the sharp decrease of the NH_4^+-N removal. Parts of biodegradable organic matters were removed by the biological oxidation of heterotrophic bacteria, and other parts were used in denitrification via nitrite. Higher COD_{Cr} concentration can provide more organics for denitrification in SND, and then result in the increase of TN removal at high C/N ratio. In this study, the average removal efficiencies of COD_{Cr} and TN were 63% and 29%, respectively.

According to the experimental results, the controlling conditions to acquire partial nitritation are summarized as follows: more than 94% PNE, 60%–74% NH_4^+-N removal efficiency, and NO_2^--N/NH_4^+-N ratio of 1.0–1.4 in the effluent (Table 3). For treating raw landfill leachate with high C/N ratio (C/N>2), the DO concentration must be increased to meet the requirements of ammonium oxidation

Table 3 Controlling conditions to acquire partial nitritation in landfill leachate treatment at temperature 30±1°C

| COD_{Cr}/NH_4^+-N<1		1<COD_{Cr}/NH_4^+-N<2	
DO (mg/L)	N_v (kg NH_4^+-N/ $(m^3 \cdot d)$)	DO (mg/L)	N_v (kg NH_4^+-N/ $(m^3 \cdot d)$)
0.8–1.2	0.2–0.3	0.8–1.2	0.2–0.3
1.2–1.5	0.3–0.5	1.2–1.5	0.3–0.4
1.5–1.8	0.5–0.7	1.5–1.8	0.4–0.5
1.8–2.0	0.7–0.8	1.8–2.0	0.5–0.6
2.0–2.3	0.8–1.0	2.0–2.3	0.6–0.8

Temperature 30±1°C.

and organics oxidation.

During 166 d running of partial nitritation combined with ANAMMOX, the partial nitritation reactor was controlled according to these conditions to obtain stable partial nitritation. At the given conditions, i.e. 0.27–1.2 kg NH_4^+-N/$(m^3 \cdot d)$ of N_v, 0.8–2.3 mg/L of DO, and 1400–2800 mg NH_4^+-N/L in the influent (C/N<1), the NO_2^--N/NH_4^+-N ratios in the effluent were stable at about 1.0–1.3, the NH_4^+-N removal efficiencies were about 60%–70%, the PNE was more than 94%, and the NO_3^--N concentrations in the effluent were less than 43 mg/L (Liang *et al.*, 2007).

2.2 Influence of temperature on partial nitritation

The oxygen transfer rate must be equal to the oxygen requirement rate (r_{O_2}) in the nitritation process (Eq.(4)) (Garrido *et al.*, 1997). The r_{O_2} is related to the ammonium oxidation rate $(r_{NH_4^+-N})$ according to the ammonium oxidation process (Eqs.(3), (5), and (6)).

$$NH_4^+ + 1.5O_2 \longrightarrow NO_2^- + H^+ + H_2O \tag{3}$$
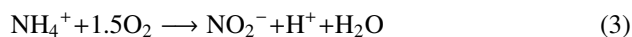
$$\frac{dC}{dt} = K_{La}(\beta C_S - C) = r_{O_2} \tag{4}$$

$$r_{O_2} = 48 r_{NH_4^+-N}/18 \tag{5}$$

$$r_{NH_4^+-N} = R N_v \tag{6}$$

By combining these equations, the following equation is obtained:

$$r_{NH_4^+-N} = 18 K_{La}(\beta C_S - C)/48 = R N_v \tag{7}$$

where, K_{La} is the overall oxygen transfer coefficient in wastewater; βC_S (mg/L) is the equilibrium oxygen concentration in wastewater with relation to temperature; C (mg/L) represents the actual oxygen concentration; R (%) is the removal efficiency of NH_4^+-N, and N_v (kg/(m^3·d)) is the NH_4^+-N volumetric loading rate. Thus, the relationship of $r_{NH_4^+-N}$ with R and N_v at a certain temperature can be known via Eq.(7).

At three different DO concentrations (1.0, 1.5, 2.0 mg/L) and different N_v, the impact of temperature on the partial nitration was investigated (Figs.4a, 4b, and 4c).

The results showed that at certain temperature, the conversion efficiency of NH_4^+-N to NO_2^--N decreased with increasing N_v. In a word, the lower the temperature, the further the NH_4^+-N removal efficiency decreased. At certain N_v, the NH_4^+-N removal efficiency increased with the increase of temperature (20–30°C), but started to decrease at a temperature of 35°C (except at low N_v of 0.4 kg NH_4^+-N/(m^3·d)). Such phenomenon mainly resulted from obvious inhibition on AOB because of the increased FA at high temperature, and it was confirmed by the decrease of $r_{NH_4^+-N}$ at 35°C. The $r_{NH_4^+-N}$ generally increased with the increase of N_v, temperature, and DO. However, the $r_{NN_4^+-N}$ began to fall at N_v above 0.8 kg NH_4^+-N/(m^3·d) and below 25°C temperature, which indicated that the high N_v and the low temperature had combined inhibition on the growth of AOB.

2.3 Free ammonia (FA) and free nitrous acid (FNA)

FA and FNA rather than ammonium and nitrite inhibit nitrifying bacteria (Anthoniesm *et al.*, 1976). The toxicities of FA and FNA depend on the environmental pH at certain temperature. In this study, the average alkalinity (Na$_2$CO$_3$) in the influent was 11801 mg/L, and all pH values in the effluent were above 8.5 (average value was 8.6). According to the average pH value, the values of FA and FNA listed in Table 4 were calculated using the following equations (Anthoniesm *et al.*, 1976):

$$FA = \frac{C_{NH_4^+-N} \times 10^{pH}}{K_b/K_w + 10^{pH}} \tag{8}$$

$$FNA = \frac{C_{NO_2^--N}}{K_a \times 10^{pH}} \tag{9}$$

$$K_b/K_w = \exp(6344/(273 + t)) \tag{10}$$

$$K_a = \exp(-2300/(273 + t)) \tag{11}$$

FA at above 10–150 mg/L will inhibit ammonia oxidation and cause failure of nitrite build-up (Anthoniesm *et al.*, 1976). However, the long-term stable partial nitration was achieved in this study at FA concentration of 122–224 mg/L, which showed that the oxidation of nitrite was inhibited by FA, whereas the oxidation of ammonium was not inhibited by FA. AOB can be acclimated to high FA, i.e., it was reported that nitrifier strains adapted to high ammonium concentration (up to 3000 mg/L) at a pH of 8.2 (Princic and Mahne, 1998). In addition, the inhibitory effect of FA was related to the amount of attached biomass (Polanco *et al.*, 1994). Therefore, the inhibitory effect of FA in the bio-film reactor can be mitigated because of a large amount of attached biomass in the reactor. FNA over 0.04 mg/L will completely produce inhibition on NOB and AOB (Anthoniesm *et al.*, 1976). In this study, owing to the high pH, the FNA concentration was extremely low (at 0.01–0.025 mg/L) and hence its inhibition was ignored.
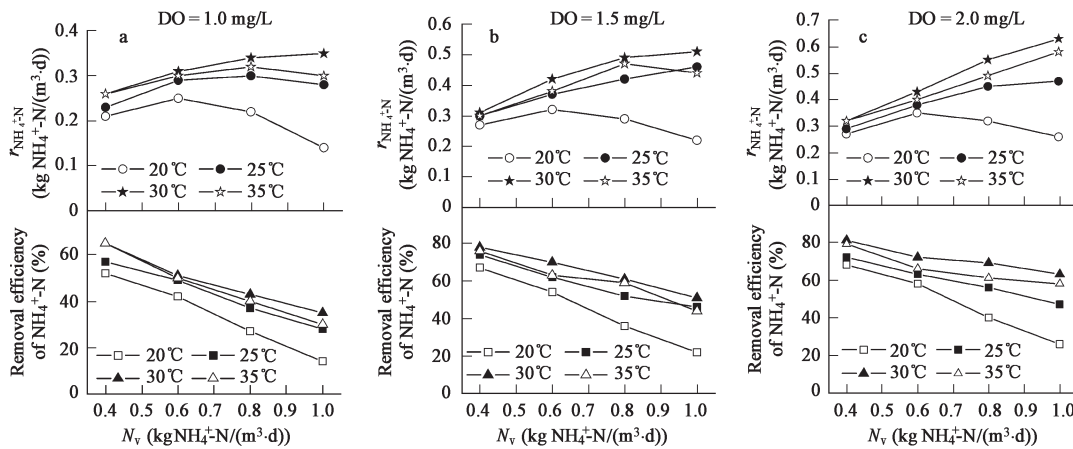

Fig. 4 Influence of temperature on partial nitration at different DO and N_v.

Table 4 FA and FNA in the reactor during the landfill leachate treatment at temperature 30±1°C

$C_{(NH_4^+-N)_{eff}}$ (mg/L)	Removal efficiency (%)	$C_{(NH_4^+-N)_{eff}}$ (mg/L)	$C_{(NH_4^+-N)_{eff}}$ (mg/L)	FA (mg/L)	FNA (mg/L)
1600–3100	60–65	600–1100	620–1200	122–224	0.01–0.025
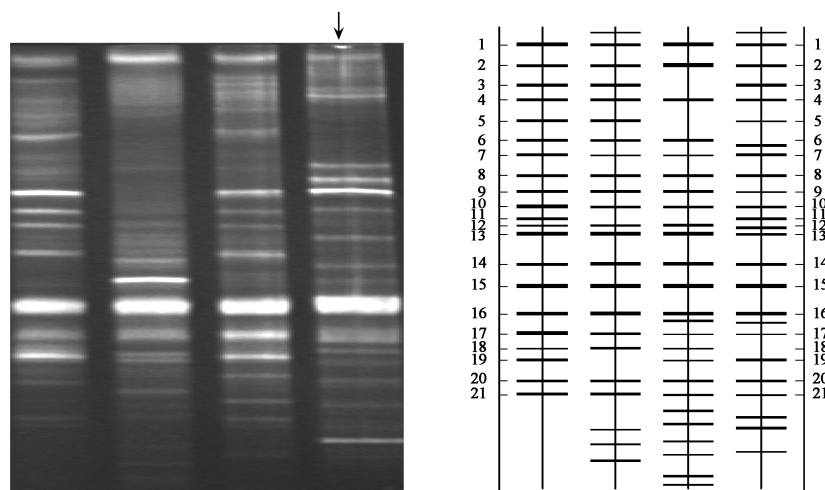
Fig. 5 DGGE analysis of the bio-film.

(a) *Nitrosomonas europaea*
(20000×)

(b) *Nitrosococcus nitrosus*
(15000×)

(c) *Nitrobacteria winogradskyi*
(10000×)
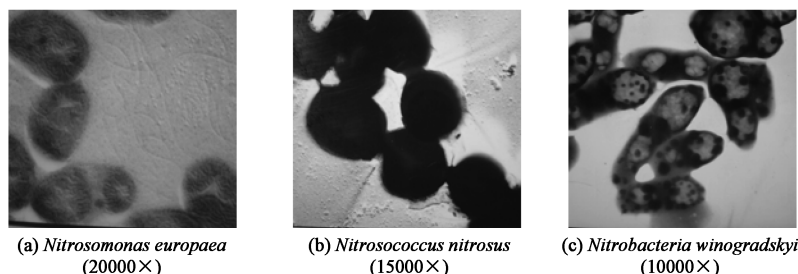
Fig. 6 Nitrobacteria in the bio-film of the Partial Nitritation Reactor.

2.4 DGGE analysis and identification of microorganism

According to the denaturing gradient gel electrophoresis (DGGE) analysis result of the bio-film in the reactor (Fig.5), there were 25 kinds of 16S rRNA gene fragments in the bio-film. These results showed that abundant microbial communities existed in the bio-film, which was confirmed by the microbial identification. The results of microorganism identification showed that two kinds of AOB, *Nitrosomonas europaea*, and *Nitrosococcus nitrosus*, and one NOB, *Nitrobacteria winogradskyi*, were present in the bio-film (Fig.6), in which *Nitrosomonas europaea* turned the most amount of germ.

The results of the DGGE analysis and the microbial identification proved that the nitrifier population adapted to high-strength ammonium and FA ensured the achievement of long-term steady partial nitritation in this study.

3 Conclusions

DO and N_v can be used to control the partial nitritation for the landfill leachate treatment. In the controlling conditions with a temperature of 30±1°C, N_v of 0.2–1.0 kg NH_4^+-N/(m^3·d), and DO concentration of 0.8–2.3 mg/L, steady partial nitritation (over 1.0 of the NO_2^--N/NH_4^+-N ratio in the effluent) was achieved.

During the course of partial nitritation, the average removal efficiencies of organics and TN were 63% and 29%, respectively.

The impact of temperature on the partial nitritation

was related to N_v and DO. High N_v (above 0.8 kg NH_4^+-N/(m^3·d)) and low temperature (below 25°C) had interactive inhibition on the ammonium oxidizing bacteria. The temperature range of 25–30°C was suitable for treating high strength ammonium leachate.

Ammonium oxidizing bacteria in this study could be acclimated to high FA at 122–224 mg/L. The results of the DGGE analysis and the microbial identification proved that abundant microbial communities containing nitrifying bacteria existed in the biofilm of the reactor.

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