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Biological treatment of landfill leachate with the integration of partial nitrification, anaerobic ammonium oxidation and heterotrophic denitrification

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ABSTRACT

A biological treatment with the integration of partial nitrification, anaerobic ammonium oxidation (Anammox) and heterotrophic denitrification was successfully developed in a SBR with periodical air supply to treat landfill leachate. An operating temperature of 30 ± 1 °C and a dissolved oxygen concentration within 1.0–1.5 mg/L were maintained in the SBR. First, the mixture of Anammox biomass and aerobic activated sludge (80% w/w) were inoculated, and inorganic synthetic wastewater with progressively increased N-loading was added. The activities of maximum aerobic ammonium oxidizing and anaerobic ammonium oxidizing reached 0.79 and 0.18 (kg NH_4^+–N/kg_{dw}/day) after the inoculation lasting 86 days, respectively. Secondly, an unexpected group of heterotrophic denitrifying bacteria was inoculated into the reactor along with the feeding of raw landfill leachate, and the final maximum activities of aerobic ammonium oxidizing, anaerobic ammonium oxidizing and denitrification reached 2.83 (kg NH_4^+–N/kg_{dw}/day), 0.65 (kg NH_4^+–N/kg_{dw}/day) and 0.11 (kg NO_3^-–N/kg_{dw}/day), respectively.

1. Introduction

Landfill still remains the chief method for municipal solid waste (MSW) treatment around the world and landfill leachate is a mixture of precipitation percolating through the landfill, including organic and inorganic matters generated during decomposition of the waste in the landfill, which is characterized by high concentrations of organics, ammonium, inorganic salts, and in some cases, heavy metals. If the landfill leachate has been collected incautiously and discharged unsafely, it may become potential pollution source that threats soil, surface water and groundwater (Nehrenheim et al., 2008). Therefore, landfill leachate is recognized as an important environmental problem by modern society (Horan et al., 1997).

To minimize the risks of contamination, a suitable treatment must be given to the leachate, so as to reduce the contaminant concentrations to values lower than those required by the receiving media. Nitrogen contaminants can be removed by either physico-chemical or biological methods. The most widely used physico-chemical treatments are ammonium stripping (Cheung et al., 1997; Kurniawan et al., 2006) and chemical precipitation (Ozturk et al., 2003; Kurniawan et al., 2006), while biological nitrogen removal from landfill leachate has traditionally been carried out by means of conventional autotrophic nitrification and heterotrophic denitrification systems (Horan et al., 1997; Laitinen et al., 2006).

However, nitrogen removal from landfill leachate cannot be carried out easily by conventional biological treatments due to the high ammonium concentrations and the low biodegradable organic matter content. Taking all these constraints into account, treating this leachate through autotrophic nitrification plus heterotrophic denitrification would be expensive due to the higher costs of alkalinity and external carbon source addition. Thus, to treat high N-loading streams in a more sustainable way, new alternative systems have been developed in recent years. Some of them are combined autotrophic denitrification via nitrite with heterotrophic denitrification (Hellinga et al., 1998) or anaerobic ammonium oxidation (Anammox) (Strous et al., 1997) to form dinitrogen gas, reducing the aeration and organic carbon requirements (Ruiz et al., 2003; Van Dongen et al., 2001). In such cases, ammonium would be partly converted to nitrite (Eq. (1)) by ammonium oxidizing bacteria (AOB), and subsequently, heterotrophic denitrifying bacteria (HDB) would use nitrite as the final electron acceptor to form nitrogen gas (Eq. (1)) (Hellinga et al., 1999), or Anammox bacteria (AAOB) would convert ammonium with nitrite to nitrogen gas (Eq. (2)) (Strous et al., 1998).

$$2NO_2^- + 6H^+ + 6e^- \rightarrow N_2 + 2OH^- + 2H_2O \qquad (1)$$

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$$NH_4^+ + 1.31NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \qquad (2)$$

The idea of coupling the partial nitridation process with Anammox process has been deemed to one of the most economical process (Jetten et al., 1997; Van Dongen et al., 2001). But the Anammox process is not suitable for wastewater with the ratio of chemical oxygen demand (COD) to NH_4^+–N (C/N) above 1 where the AAOB are no longer able to defeat the HDB absolutely (Güven et al., 2005). In addition, to ensure the stabilization of the coupled system, the nitrite produced must be controlled strictly since it will completely inhibit the Anammox process at the concentrations higher than 100 mg NO_2^-–N L^{-1} (Strous et al., 1999). Thus, the coupled system may not be suitable for the treatment of landfill leachate with C/N ratio above 1. Thus, another new process was necessary to be studied. Denitrification was added into the coupled system to solve this problem. In this way, ammonium and COD can be removed in via of the partial nitrification, Anammox and denitrification simultaneously in a single reactor (Sliekers et al., 2002, 2003; Chen et al., 2009).

In this study, a SBR with periodical air supply was applied for the integration process. The strategy of periodical aeration made the aerobic condition and anoxic condition alternate appeared in the SBR. This is in accord with the principle that partial nitrification requires a certain aerobic condition for oxidation of ammonia, whereas denitrification (Münch et al., 1996) and Anammox occurs under anoxic condition in the presence of electron donors (Strous et al., 1997). Under oxygen limitation, ammonium is oxidized to nitrite by AOB, the nitrite in the reactor can be used by AAOB with ammonium, and finally to dinitrogen gas with small amounts of nitrate produced (Strous et al., 1999). Afterwards, COD as electron donor could deoxidize nitrate to dinitrogen gas through denitrifying process for the completely nitrogen removal performance. The integration of aerobic nitrifying, anaerobic ammonium oxidizing and anaerobic denitrifying bacteria under oxygen limitation has the potential to make an almost complete conversion of ammonium and organic carbon to dinitrogen gas and carbon dioxide.

This study focused on a SBR for the biological treatment of high ammonium content landfill leachate, with the integration of partial-nitrification process, Anammox process and heterotrophic denitrification process. First of all, the heterotrophic bacteria and nitrite oxidizing bacteria (NOB) were washed out due to the absence of organic matter when the AOB and AAOB were enriched. Secondly, a progressive adaptation of biomass from synthetic wastewater to raw leachate was carried out accompanied with an unexpected inoculation of the HDB from the leachate. And finally, the periodic variations of NH_4^+–N, NO_3^-–N, NO_2^-–N inside the reactor were analyzed during both aforesaid periods.

2. Methods

2.1. Experimental lab-scale reactor

The experimental reactor with its instrumentation and control system is schematized in Fig. 1. The working volume of the reactor was 3 L, with an internal diameter of 10 cm and a height of 40 cm, and the volume exchange ratio (VEX, the volume added to the maximum reactor volume ratio) was about 0.333. Liquid temperature inside the SBR was maintained at (30 ± 1) °C with a thermostatic water jacket. A complete mixture was achieved during the filling and reaction phases with a mechanical stirrer. Dissolved oxygen (DO) was controlled within 1.0–1.5 mg/L during all aeration reaction stages utilized an air-compressor and three micropore aerators supplied air into the rector. The pH inside the reactor was initially controlled at a maximum set-point value between 7.8 and 7.2, depending on the applied nitrogen load, adding 0.5 mol/L Na_2CO_3 or 1 mol/L HCl.

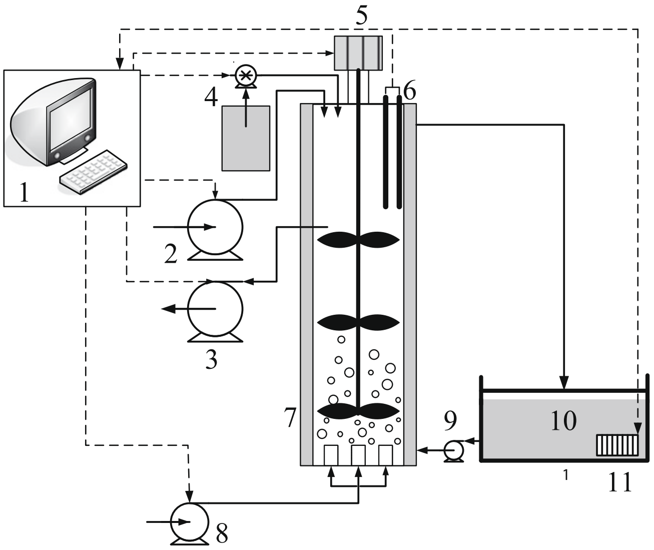

Fig. 1. Schematic representation of the 3L lab-scale SBR (1; control system, 2; influent pump, 3; effluent pump, 4; pH controller, 5; stirrer, 6; probes (pH, DO, T), 7; jacketed SBR, 8; air compressor, 9; thermostatic pump, 10; thermostatic tank and 11; heater.

An auto control system consisted of an interface card (PCL-812 PG, Advantech, USA) and two probes (one reads pH, other one reads DO and temperature) was utilized to carry out the real-time control of the thermostatic system, the aeration system and the pH system.

2.2. Inoculums

The aerobic activated sludge from the Heimifeng Landfill Leachate Treatment Plant (Changsha, China) and the biofilm from a 2 L Anammox-ASBBR-reactor in which most of the biomass consisted of planctomycete-like AAOB (Yang et al., 2007) were mixed (80% w/w). The mixed activated sludge was inoculated in the SBR with the initial concentration of biomass at 2.18 g vss L^{-1}.

2.3. Synthetic wastewater and raw leachate

The composition of the synthetic wastewater (Sliekers et al., 2002) used in this experimental was described in Table 1.

Table 1
Composition of the synthetic wastewater used in this study. Values are in mg/L except the pH.

Components	Concentrations
$(NH_4)_2SO_4$	132–1320
KH_2PO_4	25
$KHCO_3$	125
$CaCl_2·2H_2O$	300
$MgSO_4$	200
$NiCl_2·6H_2O$	19
H_3BO_3	6
$FeSO_4·7H_2O$	10
$ZnSO_4·7H_2O$	4.4
$CoCl_2·6H_2O$	3.2
$MnCl_2·4H_2O$	10.2
$CuSO_4·5H_2O$	3.2
EDTA	6.25
pH	7.0–7.8

The raw leachate used in this experimental was supplied from the Heimifeng MSW sanitation landfill site (Changsha, China) per 10 days, and conserved at 4 °C. The average values of the principal chemical compounds concentration were summarized in Table 2.

2.4. Experimental procedure

The experiment was divided into two periods. In Period I the reactor was fed with inorganic synthetic wastewater as described in Table 1, and the N-loading rate gradually grew following the increase of $(NH_4)_2SO_4$. During this period, the heterotrophic bacteria and NOB were washed out due to the absence of organic matter when the AOB and AAOB were enriched.

During Period II the reactor was fed with a mixture of synthetic wastewater (the concentration of $(NH_4)_2SO_4$ was always maintained at 132 mg) and landfill leachate from the Heimifeng Landfill Leachate Treatment Plant (Changsha, China). The influent ammonium concentration as well as the proportion of leachate in the feed increased during the whole period until the 100% raw leachate was reached.

The entire study was carried out on a 12 h operational cycle, consisting of four phases: (1) feeding phase, which was supposed to occur in an instant; (2) reaction phase, which contained four aeration stages and four anaerobic stages running alternately for 2 h and 1 h, respectively; (3) settling phase, which was simultaneously carried out with the last anaerobic stage of the reaction phase and (4) drawing phase, which was considered to occur in an instant like fill phase.

Moreover, in each period, concentrations of ammonium, nitrite and nitrate were synchronously measured per half hour over a 12 h cycle to study in more detail the process and the conversion of the nitrogen species. And, the maximum activity of aerobic ammonium oxidation, aerobic nitrite oxidation, maximum anaerobic ammonium oxidation and maximum denitrification were measured in each period, respectively.

2.5. Chemical analysis

Ammonium nitrogen (NH_4^+–N), nitrite nitrogen (NO_2^-–N), total Kjeldahl nitrogen (TKN), COD, mixed liquor suspended solid (MLSS), mixed liquor volatile suspended solid (MLVSS), and sludge volume index (SVI) were analyzed according to the standard methods for the examination of water and wastewater (APHA, 1995), while nitrate nitrogen (NO_3^-–N) was analyzed by using ultraviolet spectrophotometer method. BOD_5, DO and pH were measured by a BOD meter (HI99724A, Lovbond, Germany), a DO meter (HI9143, Hanna, Italy) and a pH meter (pH meter pen, Lida, China), respectively. Dry weight was measured after drying the filtered biomass in a microwave for 10 min at 300 W.

2.6. Calculations

Concentration of free ammonia (FA) was calculated as a function of pH, temperature and total ammonium as nitrogen (TAN), for FA (Anthonisen et al., 1976):

$$\mathrm{FA}\ (\mathrm{mg\ N\ L^{-1}}) = \frac{\mathrm{TAN}}{1 + (10^{-\mathrm{pH}}/K_e^{\mathrm{NH}})} \tag{3}$$

$$K_e^{NH} = e^{-6344/(273+T)} \tag{4}$$

The efficiency of ammonium conversion, total nitrogen (TN) removal, COD removal and BOD removal were all estimated according to:

$$\mathrm{Efficiency} = \left(\frac{C_{\mathrm{ini}} - C_{\mathrm{eff}}}{C_{\mathrm{ini}}}\right) \times 100\% \tag{5}$$

where C_{ini} is the ammonium (or COD, BOD, TN) concentration in the liquid after the instant fill phase; and C_{eff} is the ammonium (or COD, BOD, TN) concentration in the effluent. The summation of ammonium, nitrite and nitrate was calculated as the TN during Period I.

2.7. Activity measurements

The method of activity measurements was adapted from Third et al. (2001). The maximum aerobic ammonium oxidation activity and the maximum aerobic nitrite oxidation activity of the biomass were measured under fully aerobic conditions. A 10 mL sample of biomass was taken from the reactor and incubated in a 250 mL Erlenmeyer flask at 30 °C in a rotary shaker. Nitrite and nitrate were measured over time during 1–2 h. Maximum anaerobic ammonium oxidation activity was measured as follows. Biomass from the reactor was transferred into serum bottles with gas tight rubber stoppers. The flasks were made anaerobic using Argon. Nitrite was added to a maximum of 100 mg NO_2^-–N L^{-1}. Ammonium was already presented in the biomass suspension, due to the ammonium surplus in the reactor. Nitrite and ammonium consumption and nitrate production were tracked during a 2 h period. Maximum denitrification activity of the biomass was measured by incubating biomass with 1 g/L yeast extract, under anaerobic conditions, using bottles with rubber stoppers and a headspace of Argon.

3. Results and discussion

3.1. Period I: inoculation and start-up

To start the SBR, the mixture of the aerobic activated sludge from the Heimifeng Landfill Leachate Treatment Plant (Changsha, China) and the denitrifying sludge from the First Municipal Wastewater Treatment Plant (Changsha, China) was introduced into the reactor. The aforementioned synthetic wastewater containing 56–280 mg NH_4^+–N L^{-1} was added and pH inside the reactor was maintained at 7.8.

As Fig. 2 presents, ammonium concentrations increased slightly on the first week after inoculums were introduced. And the max effluent ammonium concentration reached 63 and 60 mg NH_4^+–N L^{-1} on days 1 and 2, respectively, which were evidently higher than the influent ammonium concentration at about 56 mg NH_4^+–N L^{-1}. This phenomenon might ascribe to the changed environment of the mixed activated sludge, which resulted in the breakdown of the organic nitrogen to ammonium (Nutchanat and Suwanchai, 2007). After inoculation, the ammonium conversion efficiency gradually went up with obvious fluctuation, in spite of the stepwise increasing of N-loading. Concentration of effluent nitrate gradually increased and even reached 30.3 mg NO_3^-–N L^{-1} on day 86, since denitrifying activity was eliminated absolutely in absence of organic matter. In the first two days, TN also increased since the breakdown of the organic nitrogen. Until day 14, the removal of TN was still indiscoverable. On day 14, the removal of TN was detected in the reactor, which indicated the occurrence of an incipient Anammox activity.

Table 2
Characteristics of the raw leachate from the Heimifeng municipal wastes landfill site of Changsha city. Values are in mg/L except the pH.

Compound	Average ± S.D.	Compound	Average ± SD
COD	3876 ± 661	NO_2^-–N	0
BOD_5	548 ± 236	TKN	2018 ± 512.3
NH_4^+–N	1451 ± 417	Alkalinity	9618 ± 3502
NO_3^-–N	0	pH	7.67 ± 0.53

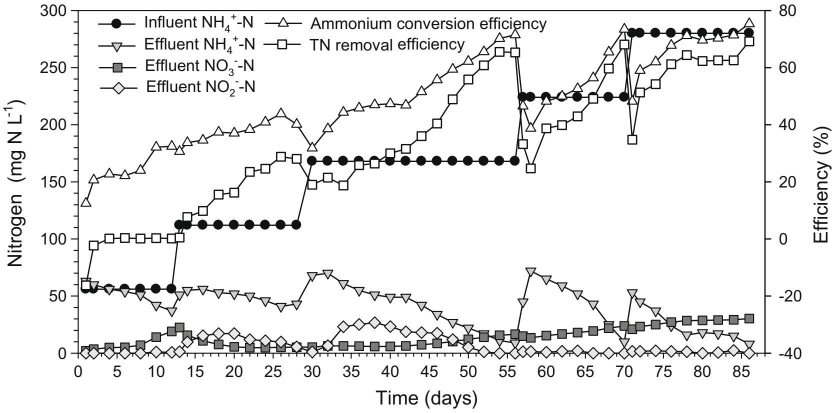

Fig. 2. Evolution of the concentration of nitrogen compounds and removal efficiency of total nitrogen in the SBR during Period I.

The molar ratio of (NH_4^+–N_{con} + NO_2^-–N_{con})/NO_3^-–N_{pro} (where con and pro is abbreviation for consume and production, respectively) was calculated according to Fig. 2. In the first month the ratio increased to 8.8, which was the theoretical ratio of (NH_4^+–N_{con} + NO_2^-–N_{con})/NO_3^-–N_{pro} for the Anammox reactor according to Eq. (2), and then the ratio maintained in values between 8.7 and 9.0. This phenomenon indicated that the AAOB had consumed almost all the available nitrite produced by the AOB. Thus, cooperation was established between the aerobic and anaerobic ammonium oxidizers.

As Table 3 shows, heterotrophic denitrifiers seem to be inactive during Period I, but an evident denitrification activity about 0.11 kg NO_3^-–N/kg_{dw}/day (where dw is abbreviation for dry weight) was detected during Period II. Then, the denitrification activity of raw leachate was measured to ascertain the origin of the heterotrophic denitrifiers. As supposed, the denitrification activity was found in raw leachate.

Moreover, the maximum ammonia oxidation activity was tested at aerobic and anaerobic conditions, respectively. As Table 3 shows, an evident ammonia oxidation activity was detected. And, nitrate was formed in the anaerobic ammonium oxidation activity measurements, but absent in the aerobic nitrite oxidation activity measurements, which indicated the disappearance of the NOB. As expected, neither nitrite nor nitrate removal was observed in the activity test for heterotrophic denitrification.

Furthermore, the concentration of FA during Period I was calculated and presented in Fig. 3 – Period I, which could explain the elimination of NOB in the reactor. As can be seen, the concentration of FA in most time of Period I was higher than 3.5 mg NH_3–N L^{-1}, an inhibitory value for NOB as reported by Anthonisen et al. (1976). In reference to the inhibition of AOB by FA, Anthonisen et al. (1976) also reported that inhibition took place in the range of 10–150 mg NH_3–N L^{-1}. This value never reached, even with the highest effluent ammonium.

Table 3
Characteristics of nitrogen removal in both periods.

Parameter	Period I	Period II
1. Test day	Day 86	Day 123
2. Ammonium in (mg NH_4^+–N/L)	280	1442
3. Ammonium out (mg NH_4^+–N/L)	8	48
4. Ammonium load (kg N/m^3/day)	0.187	0.961
5. Ammonium consumption(kg N/m^3/day)	0.181	0.929
6. Nitrate production (kg NO_3^-–N/$m^3_{reactor}$/day)	0.007	0.009
7. Dry weight concentration (g/L)	1.2	1.5
8. Maximum Anammox activity (kg NH_4^+–N/kg_{dw}/day)	0.18	0.65
9. Max. aerobic NO_2^- oxidizing act. (kg NO_2^-–N/kg_{dw}/day)	0	0
10. Max. aerobic NH_4^+ ox. act. (kg NH_4^+–N/kg_{dw}/day)	0.79	2.83
11. Max. denitrification act. (kg NO_3^-–N/kg_{dw}/day)	0	0.11

3.2. Period II: acclimatising to leachate

After inoculation and start-up, the SBR was initially fed with a mixture (20% v/v) of synthetic wastewater and raw leachate. The proportion progressively was increased according to the current removal efficiency of NH_4^+–N, until 100% v/v of leachate was reached in the influent, and the influent ammonium concentration increased from 317 to 1454 mg NH_4^+–N L^{-1} simultaneously. During this acclimatization period, the pH inside the reactor was maintained at a maximum set-point value between 7.8 and 7.2, using 0.5 mol/L Na_2CO_3 or 1 mol/L HCl.

Fig. 4 presents the evolution of the SBR during Period II. Fig. 4a shows the evolution of influent ammonium concentration versus effluent ammonium, nitrite and nitrate concentrations. As observed, the percentage of leachate in effluent was progressively increased from 20% to 100% during Period II, operating for 124 days. Despite of the high influent ammonium concentration, a stable conversion of ammonium, slight nitrite and nitrate production were detected in effluent. In spite of this performance, a lower percentage of ammonium conversion was initially observed when the ammonium loading rate was increased. Nevertheless, after few days with stable influent ammonium concentration, the system recovered its performance. This effect was related to a possible slow response of AOB to the increasing N-loading rate. Therefore, after 124 days of operation, by the end of Period II, the SBR was operated with 100% of raw leachate and an influent ammonium concentration of 1450 mg N L^{-1}. Under such conditions, the reactor presented stable behavior, reached high ammonium conversion efficiency and low effluent concentrations of nitrite and nitrate.

Except the stable ammonium conversion efficiency and high nitrogen removal efficiency during Period II, unexpected removal of organic matter was detected after 5 days of feeding with mixture of synthetic wastewater and raw leachate. Fig. 4b shows the variations concentration and removal efficiency of COD during Period II. Under the specific condition of the SBR, the removal efficiency of COD was always less than 6.7%, and the average value was just 5.1%. Also, Fig. 4c presents the variations concentration and removal efficiency of BOD during Period II. Despite the low removal efficiency of COD, the removal efficiency of BOD was over 95% during majority of Period II, and the average value was 82.95%. Moreover, the consumption of COD was close to the consumption of BOD in the same operating cycle, and it could demonstrate that the removed organic matters from the system were mostly the biodegradable fraction.

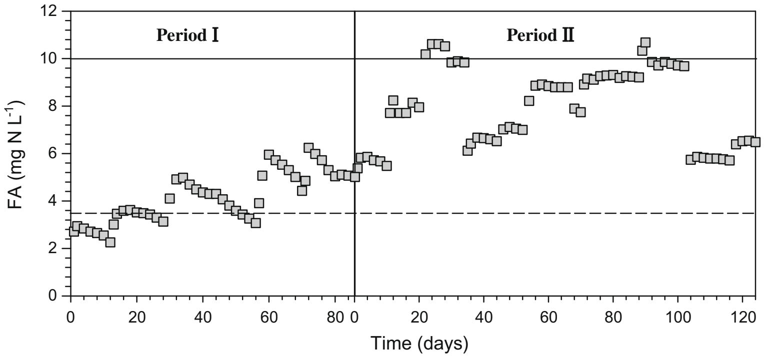

Fig. 3. Evolution of FA concentration during both Periods I and II, the dash line and the solid line indicates the inhibitory value for NOB (3.5 mg NH_3–N L^{-1}) and AOB (10 mg NH_3–N L^{-1}), respectively.

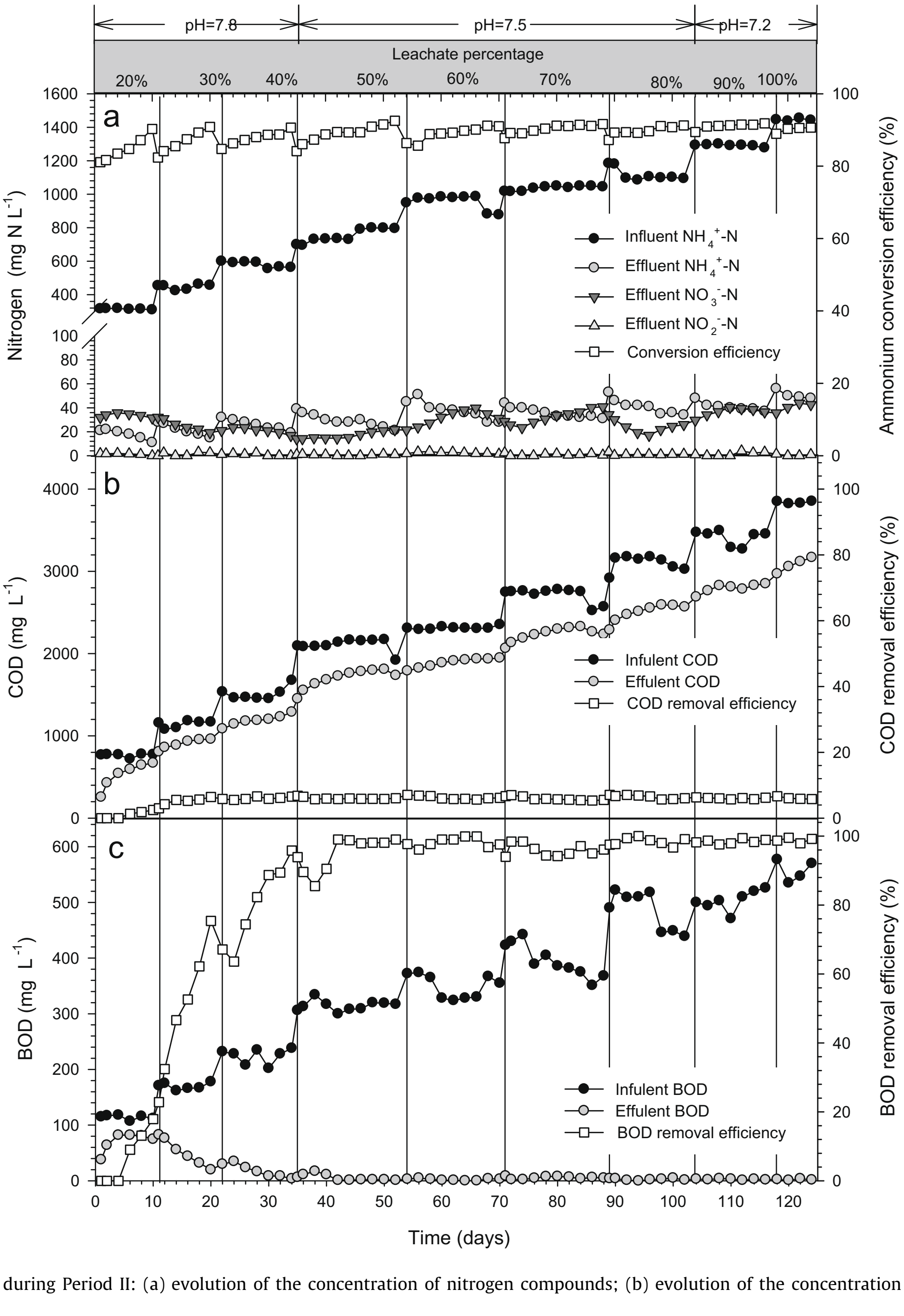

Fig. 4. Evolution of the SBR during Period II: (a) evolution of the concentration of nitrogen compounds; (b) evolution of the concentration of COD; (c) evolution of the concentration of BOD.

The removal of BOD (or COD) implied that the heterotrophic bacteria (i.e. heterotrophic denitrifiers) appeared in the reactor again, which had been washed out by the specifically operational condition of Period I. The activity test for heterotrophic denitrification during Period I (on day 86) and Period II (on day 123) confirmed the disappearance and reappearance of heterotrophic denitrifiers, respectively. As Table 3 shows, heterotrophic denitrifiers seem to be inactive during Period I, but an evident denitrification activity (0.11 kg NO_3^-–N/kg$_{dw}$/day) was detected during Period II. Then, the denitrification activity of raw leachate was measured to ascertain the origin of the heterotrophic denitrifiers, as supposed, the denitrification activity was found in raw leachate.

During Period II, the concentration of FA also was calculated and presented in Fig. 3, the concentration of FA was between 3.5 and 10 mg NH_3–N L^{-1} during nearly entire Period II except the days before pH was adjusted. The value was suitable for the AOB, but affected the NOB with opposite result. And, the appropriate concentration of FA ensured the absolute superiority of the AOB, which oxidized ammonium to nitrite at aerobic stages in every operational cycle.

3.3. Evolution of the nitrogen over a 12h SBR cycle

In order to study in more detail the process and the conversion of the nitrogen species, a 12 h cycle profile was analyzed on day 86 of Period I (Fig. 5a) and day 123 of Period II (Fig. 5b), respectively. In this experiment, ammonium, nitrite and nitrate concentrations were synchronously measured per half hour over a 12 h cycle.

Fig. 5a presents the evolution of ammonium, nitrite and nitrate inside the reactor on day 86 of Period I. According to the profile, the ammonium concentration gradually decreased from 103.3 mg/L (the mixed concentration of influent and residue of previous cycle) to 8.0 mg/L (the concentration of effluent) after an operation cycle, the conversion rate of ammonium in anaerobic stages was slightly higher than that in aeration stages. Along with the conversion of ammonium at each aeration stage, nitrite was gradually accumulated, and a transient peak could be observed at the end of each aeration stage. But, the nitrite produced at the aeration stage was consumed at the sequent anaerobic stage soon. As expected, nitrate was only produced at anaerobic stage, which was considered to be the outgrowth of the Anammox process.

The evolution of ammonium, nitrite and nitrate concentrations over a 12 h SBR cycle were shown in the Fig. 5b. As shown, although the ammonium load of Period II was higher than Period I, the ammonium and nitrite profiles of them were quite similar. At aeration stages, ammonium was converted to nitrite at a stable rate, and at anaerobic stages, ammonium was converted to nitrogen gas with the nitrite previous produced at aeration stages. But it could be observed via the slope of nitrate curve that the accumulate rate of nitrate during Period II was evidently lower than that during Period I.

To study the results in more depth and quantify the nitrogen conversion process, experimental data were analyzed and the consumption or production of ammonium, nitrite and nitrate at each reaction stage were calculated, as shown in Table 4, a part of ammonium was converted to nitrite at aeration stages and sequentially converted to nitrogen gas together with other ammonium at anaerobic stages in both Periods I and II.

According to Table 4, a stable nitrogen loss was detected at each aeration stage during both periods, but the loss quantity of Period II was obviously higher than that of Period I. It was supposed that such nitrogen loss would be caused by three processes. First,

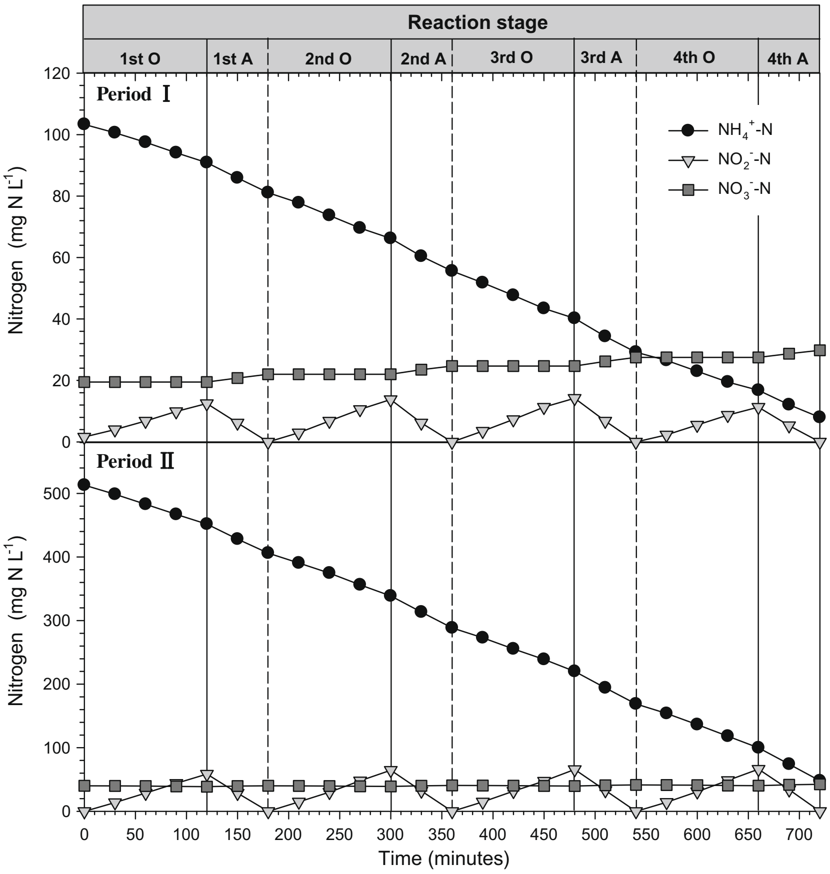

Fig. 5. Evolution of nitrogen species over a 12 h SBR cycle: (a) Period I; (b) Period II. Aeration and anaerobic were abbreviate to O and A in this figure, respectively.

Table 4
Consumption (been described as positive numbers) and production (been described as negative numbers) of nitrogen species and the molar ratio between NO_2^-–N (or NO_3^-–N) with NH_4^+–N in each reaction stage.

Periods	Nitrogen species	1st O	1st A	2nd O	2nd A	3rd O	3rd A	4th O	4th A
I	NH_4^+–N (mg N L^{-1})	−12.4	−9.8	−14.9	−10.6	−15.4	−11.1	−12.3	−8.8
	NO_2^-–N (mg N L^{-1})	10.9	−12.5	13.8	−13.8	14.2	−14.2	11.3	−11.3
	NO_3^-–N(mg N L^{-1})	0	2.5	0	2.7	0	2.9	0	2.3
	Nitrogen loss(mg N L^{-1})	−1.5	–	−1.1	–	−1.2	–	−1	–
	NO_2^-–N/NH_4^+–N	–	1.28	–	1.30	–	1.28	–	1.28
	NO_3^-–N/NH_4^+–N	–	0.26	–	0.25	–	0.26	–	0.26
II	NH_4^+–N (mg N L^{-1})	−61.4	−45.5	−67.4	−50.2	−68.5	−51.2	−69.6	−51.2
	NO_2^-–N (mg N L^{-1})	58.5	−58.5	64.1	−64.1	65.8	−65.8	64.9	−64.9
	NO_3^-–N (mg N L^{-1})	−1.3	1.4	−1.1	1.7	−1.1	1.9	−1.2	2.2
	Nitrogen loss(mg N L^{-1})	−4.2	–	−4.4	–	−3.8	–	−5.9	–
	NO_2^-–N/NH_4^+–N	–	1.29	–	1.28	–	1.29	–	1.27
	NO_3^-–N/NH_4^+–N	–	0.03	–	0.03	–	0.04	–	0.04

continuous aeration resulted in an effect of air ammonium stripping. Second, anaerobic micro-ecological environment possibly exists where Anammox process might be carried out with the simultaneous consumption of ammonium and nitrite, whereas the Anammox process would be inhibited by very low concentration of dissolved oxygen. Third, cells were synthesized with the requisite of nitrogen species, while a variety of metabolize process was taking place. From Table 4 it also can be seen that when the SBR presented a stable ammonium conversion ratio to nitrite at all aeration stages, the nitrate concentration was constantly remained during Period I and even decreased during Period II, which further confirmed the elimination of NOB activity. But as abovementioned, the nitrogen loss at aeration stages was possibly ascribed to the Anammox process with the nitrate as inevitable outgrowth. This contradiction implies an absolute inactivation of Anammox activity during aeration stages.

Moreover, the value of molar ratios between NO_2^-–N_{con} and NH_4^+–N_{con} at anaerobic stages were calculated and listed in Table 4. As shown, the molar ratios of NO_2^-–N_{con}/NH_4^+–N_{con} always maintained in values that between 1.28 and 1.30, which was close to the theoretical value of 1.31 according to Eq. (5). In reference to Anammox process, Trigo et al. (2006) reported simultaneously removal of ammonium and nitrite in anaerobic condition was an essential characteristic of the Anammox process, additional, the ratio of NO_2^-–N_{con}/NH_4^+–N_{con} was regarded as the important criterion to confirm the extent of the Anammox process. The perfect ratios demonstrated that the AAOB gained an immense ascendancy at anaerobic stages, in spite of a reversible inactivation caused by the presence of dissolved oxygen during the aeration stages of the operational cycle.

Furthermore, from the absolute molar values of ratios between NO_3^-–N_{pro} and NH_4^+–N_{con} at anaerobic stages, it can be seen that the vast difference between Periods I and II. As Table 4 shows, a consumption of 1 mol NH_4^+–N along with a production of 0.25–0.26 mol NO_3^-–N during Period I. This also was as expected for the Anammox process (Strous et al., 1998). But during Period II, nitrate was detected with a lower concentration, since the heterotrophic denitrifiers quickly consumed the nitrate produced by AAOB.

4. Conclusions

It should be considered that an integration of partial-nitrification process, Anammox process and heterotrophic denitrification process may be applied to the SBR system to treat the urban landfill leachate. First of all, the operating temperature and the pH value were controlled in the optimal range to maintain the concentration of FA between 3.5 and 10 mg NH_3-N L^{-1}, which made sure the nitrification process stopped at the ammonium oxidation step, while the DO concentration were maintained within 1.0–1.5 mg/L. Afterwards, inoculating the mixture of Anammox biomass and aerobic activated sludge into a single reactor and feeding with inorganic synthetic wastewater allowed a combined process of partial nitrification and Anammox. The maximum activities of aerobic ammonium oxidizing and Anammox achieved 0.79 and 0.18 (kg NH_4^+–N/kg$_{dw}$/day), respectively, without significant heterotrophic denitrification being observed. Finally, a progressive adaptation of biomass from synthetic wastewater to raw leachate was carried out via increasing the percentage of leachate in effluent from 20% to 100%, and an unexpected inoculation of the HDB from the leachate was achieved. The final maximum activities of aerobic ammonium oxidizing, Anammox and denitrification reached 2.83 (kg NH_4^+–N/kg$_{dw}$/day), 0.65 (kg NH_4^+–N/kg$_{dw}$/day) and 0.11 (kg NO_3^-–N/kg$_{dw}$/day), respectively.

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