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Operation of partial nitrification to nitrite of landfill leachate and its performance with respect to different oxygen conditions

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ABSTRACT

The coupled system of partial nitrification and anaerobic ammonium oxidation (Anammox) is efficient in nitrogen removal from wastewater. In this study, the effect of different oxygen concentrations on partial nitrification performance with a sequencing batch reactor (SBR) was investigated. Results indicate that, partial nitrification of landfill leachate could be successfully achieved under the 1.0–2.0 mg L^{-1} dissolved oxygen (DO) condition after 118 d long-term operation, and that the effluent is suitable for an Anammox reactor. Further decreasing or increasing the DO concentration, however, would lead to a decay of nitrification performance. Additionally, the MLSS concentration in the reactor increased with increasing DO concentration. Respirometric assays suggest that low DO conditions (<2 mg L^{-1}) favor the ammonia-oxidizing bacteria (AOB) and significantly inhibit nitrite oxidizing bacteria (NOB) and aerobic heterotrophic bacteria (AHB); whereas high DO conditions (>3 mg L^{-1}) allow AHB to dominate and significantly inhibit AOB. Therefore, the optimal condition for partial nitrification of landfill leachate is 1.0–2.0 mg L^{-1} DO concentration.

1. Introduction

Leachates are defined as the aqueous effluent generated as a consequence of rainwater percolation through wastes, biochemical processes in waste's cells and the inherent water content of wastes themselves, which may contain large amounts of organics (biodegradable, but also refractory to biodegradation), ammonium, inorganic salts, and in some cases, heavy metals [1,2]. Factors that affect the quality of leachates include age, waste type, site geochemical properties, and regional climate [3]. The composition of landfill leachates, in particular, varies greatly depending on the age of the landfill. [4]. In young landfills, leachate is characterized by high concentrations of biodegradable matters, the largest group of which are volatile fatty acids (VFAs). However, much of landfill leachate is composed of recalcitrant organic molecules with increasing landfill age because VFAs are gradually converted to biogas. As a result, this type of intermediate leachate is characterized by high COD (2000–20,000 mg L^{-1}), low BOD_5 (500–1000 mg L^{-1}) and fairly high NH_3-N (1000–4000 mg L^{-1}) [5,6]. Therefore, nitrogen removal from landfill leachates by conventional biological nitrification/denitrification is difficult owing to low biodegradable organic matter content and high ammonium concentration. Recently, some new processes and operational strategies such as partial nitrification/denitrification and anaerobic ammonium oxidation (Anammox) have arisen in order to remove nitrogen compounds in wastewaters. These process is based on the facts that, since nitrite can be partially produced by ammonia-oxidizing bacteria (AOB) using ammonium, and subsequently, heterotrophic denitrifying bacteria would use nitrite as terminal electron acceptor to form nitrogen gas (Eq. (1)) [7], or Anammox bacteria (AAOB) would convert ammonium with nitrite to nitrogen gas (Eq. (2)) [8]

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

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

Partial nitrification with Anammox saves up to 40% of the oxygen supply for nitritation without the carbon requirement and with minimal sludge production [9,10]. Thus, the coupled

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system is suitable for the treatment of landfill leachate with low biodegradable organic matter and high ammonium concentration. Feeding an Anammox reactor requires an influent composed by a 1:1 ammonium-to-nitrite molar ratio. A number of strategies have been suggested to achieve partial nitrification, including reactors' selective NOB washout at elevated temperatures (30–40 °C) [11]; NOB inhibition at the free ammonia (FA) concentration of 10–150 mg L^{-1} [12]; and DO control at low concentrations favoring AOB growth [13]. Among these strategies, the easiest to manipulate is bulk DO concentration in the reactor by changing the superficial air velocity or by partial recirculation of the off-air. Manipulation of the oxygen concentration seems to be the most practical method to obtain partial nitrification [14].

The majority of studies have confirmed that low DO concentrations (<2 mg L^{-1}) are effective in achieving partial nitrification [15,16]. However, the wastewater treated in these previous investigations was predominantly synthetic or domestic-type wastewater with low organic matter content (COD = 100–200, BOD = 50–100 mg L^{-1}), such that litter competition exists between the nitrifying bacteria and the heterotrophic bacteria more akin to "pure culture. High COD concentrations in which biodegradable organic content exceeds 500 mg L^{-1} favor the development of AHB, which grow much faster than autotrophs and outcompete them for oxygen and nutrients. As a result, the autotrophic nitrifying bacteria are easily overgrown by heterotrophs, which eventually cause the nitrification efficiency to decrease [17,18]. Available literature about nitrogen removal of landfill leachate in lab-scale reactor is focused on operations at higher DO concentrations (>2 mg L^{-1}) [15,19,20]. Consequently, a question might be introduced if the partial nitrification process can conduct at a low DO (<2 mg L^{-1}) condition to treat high organic wastewater like landfill leachate. On the other hand, although many studies have successfully achieved stable partial nitrification using synthetic or domestic-type wastewater under low DO concentration (<2 mg L^{-1}), detailed investigations of nitrification with respect to further increasing the DO concentrations post-startup are largely absent from the literature. Given that the competition between the nitrifying and the heterotrophic bacteria could be enhanced under high DO conditions, particularly with high organic matter content, it needs to be investigate nitrification performance as well as organic matter content removal under higher DO concentrations.

Therefore, the aims of our study are to (1) investigate the feasibility of achieving long-term and stable partial nitrification of landfill leachate under low DO condition, (2) test the correlation between the performances of nitrogen removal and DO dynamic characteristics, and (3) determine the kinetic parameters of the nitrifying bacteria and AHB from respirometric assays.

2. Materials and methods

2.1. Experimental lab-scale reactor

The experiments were conducted in a 3 L sequencing batch reactor (SBR) as described previously [21]. Liquid temperature inside the SBR was maintained at (28 ± 1) °C with a thermostatic water jacket. Compressed air was supplied through an air diffuser placed at the bottom of the reactor. The DO concentration in the bulk liquid was measured on-line (HI 98186, HANNA, Italy) and controlled via a Programmable Logic Controller (PLC) to remain between 0.5 and 4.0 mg L^{-1} during the aeration period using ON/OFF control. pH was measured on-line (HI 98184, HANNA, Italy) and initially controlled at a maximum set-point value between 7.2 and 7.8 by adding 0.5 M Na_2CO_3 or 1 M HCl, depending on the applied nitrogen load. The SBR was carried out on an 12 h working cycle, consisting of an aerobic reaction phase of 690 min (feeding during the first 575 min), followed by a settling phase of 30 min and, afterwards, a drawing phase which was considered to occur in an instant. The volume exchange ratio (VEX, the volume added to the maximum reactor volume ratio) of each cycle was about 0.333, resulting in a hydraulic retention time (HRT) of 1.5 days. The sludge retention time (SRT) was not a controlled parameter of the system, and was calculated considering reactor MLSS and effluent suspended solids concentrations, presented during the whole study an average value of 10 days. With an SRT of 10 days, the corresponding mixed liquor concentration was within the range of 1400–2800 mg L^{-1}.

Table 1
Composition of the synthetic wastewater used in this study. Values are in mg L^{-1} except the pH.

Component	Concentration	Component	Concentration
$(NH_4)_2SO_4$	566	$FeSO_4{\cdot}7H_2O$	10
KH_2PO_4	25	$ZnSO_4{\cdot}7H_2O$	4.4
$KHCO_3$	125	$CoCl_2{\cdot}6H_2O$	3.2
$CaCl_2{\cdot}2H_2O$	300	$MnCl_2{\cdot}4H_2O$	10.2
$MgSO_4$	200	$CuSO_4{\cdot}5H_2O$	3.2
$NiCl_2{\cdot}6H_2O$	19	EDTA	6.3
H_3BO_3	6	Glucose	625
pH	7.0–7.8		

2.2. Wastewater characteristics

The wastewater used in this study consisted of synthetic wastewater and raw leachate. The composition of the synthetic wastewater [21] was described in Table 1. The used raw leachate was collected from the Heimifeng MSW sanitation landfill site (Changsha, China) every 10 d, and stored at 4 °C. The average values of the principal chemical compounds concentration were summarized in Table 2.

2.3. Experimental procedure

The experiment was divided into two periods. In period I, the reactor was operated under low DO concentration (1.0–2.0 mg L^{-1}) condition to investigate the feasibility of achieving long-term stable partial nitrification to nitrite of landfill leachate. Mixed liquor of nitrifying sludge from our laboratory was used as inoculum for the SBR [21]. In the meantime, the leachate was diluted with synthetic wastewater (Table 1) and fed to the reactor to allow for adaptation to the real leachate over 118 days with decreasing dilution ratios.

In period II, the reactor was operated for 121 days under different concentrations of DO (0.5–1.0, 1.0–2.0, 2.0–3.0, 3.0–4.0 mg L^{-1}), during which the correlation between the performance of nitrogen removal and DO dynamic characteristics was tested. For all operations, the reactor was fed with raw leachate concentration with approximately 1300 mg $NH_4{}^{+}$-N L^{-1}. Partial nitrification was evaluated according to the ammonium oxidation rate and the concentrations of $NH_4{}^{+}$-N, $NO_2{}^{-}$-N, $NO_3{}^{-}$-N. Additionally, microbial oxygen utilization dynamics (AOB, NOB and AHB) with respect to different DO concentrations were also investigated.

Table 2
Characteristics of the raw landfill leachate from the Heimifeng municipal wastes landfill site of Changsha city. Values are in mg L^{-1} except the pH.

Compound	Average ± S.D.	Compound	Average ± S.D.
COD	3876 ± 661	$NO_2{}^{-}$-N	0
BOD_5	548 ± 236	TKN	2018 ± 512.3
$NH_4{}^{+}$-N	1312 ± 417	Alkalinity	9618 ± 3502
$NO_3{}^{-}$-N	0	pH	7.5–7.8

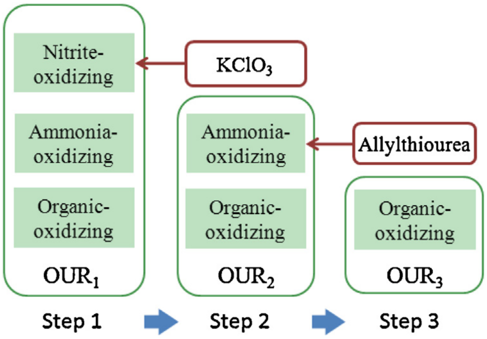

Fig. 1. The measuring processes of OUR.

2.4. Respirometry and kinetic parameters determination

The respirometer used to follow the oxygen uptake rate (OUR) dynamic of suspended biomass during batch tests was described in details by Spérandio and Paul [22]. The sludge of 10 mL was sampled from the reactor at specific times (as mentioned in the sections when DO concentration changed), then transferred to a set of 250 mL air-tight vessel. Subsequently, the leachate used in our experiment was added to the vessel with the same concentration as the reactor.

The OUR was determined by measurement of the dissolved oxygen concentration in the air-tight vessel. After bubbling the air to specific DO concentration (0, 0.5, 1, 1.5, 2, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 mg L^{-1}), the vessel was sealed with rubber stopper and the respiration rate could be directly deduced by measuring the decrease in DO

$$\mathrm{OUR} = -\frac{d[O_2]}{dt} \tag{3}$$

The steps of the measuring process of OUR (AOB, NOB and AHB) are as follows (Fig. 1):

Step 1: The OUR of the sludge was monitored without inhibitor, named OUR_1.
Step 2: The $KClO_3$ (6 mg L^{-1}) was added to inhibit the activity of NOB, then the OUR of the sludge was monitored, named OUR_2.
Step 3: Both the inhibitors of $KClO_3$ (6 mg L^{-1}) and Allylthiourea (15 mg L^{-1}) were added, thus the AOB and NOB activities were negligible. Then the corresponding OUR was measured, named OUR_3.

Therefore, the following equations were available:

$$\mathrm{OUR_{AOB}} = \mathrm{OUR_2} - \mathrm{OUR_3}; \mathrm{OUR_{NOB}} = \mathrm{OUR_1} - \mathrm{OUR_2};$$

$$\text{and}\mathrm{OUR_{AHB}} = \mathrm{OUR_3}.$$

To represent the effect of DO on oxygen utilization rate, either a Monod-type kinetic expression (Eq. (4)) or an Andrews-type expression (Eq. (5)) were used [23,24]

$$\mathrm{OUR} = \mathrm{OUR_{max}}\frac{\mathrm{DO}}{K_{O_2} + \mathrm{DO}} \tag{4}$$

$$\mathrm{OUR} = \mathrm{OUR_{max}}\frac{\mathrm{DO}}{K_{O_2} + \mathrm{DO} + \mathrm{DO}^2/K_I} \tag{5}$$

where OUR is the oxygen utilization rate, $\mathrm{OUR_{max}}$ is the maximal oxygen utilization rate, DO is the concentration of dissolved oxygen, K_{O_2} is the oxygen half saturation constants and K_I is the inhibition constant.

The maximal activity changed from one test to another, due to the change in active biomass concentration in the working period, thus it is preferred to draw $\mathrm{OUR/OUR_{max}}$ versus substrate, while the measurement of the $\mathrm{OUR_{max}}$ was according to Spanjers and Vanrolleghem [25]. The kinetic parameters in these expressions were then obtained by best-fitting Eqs. (4) or (5) to the data set of oxygen utilization rates obtained as a function of oxygen from respirometric assays using the statistical software Origin (version 8.6).

2.5. Analytical methods

All chemical analyses were performed in accordance with standard methods [26]. Ammonium nitrogen ($NH_4{}^+$-N) was measured by the Nesslerization method by reading absorbance at 425 nm, nitrite nitrogen ($NO_2{}^-$-N) was measured by a colorimetric method at 543 nm, nitrate nitrogen ($NO_3{}^-$-N) was measured by an ultraviolet spectrophotometric screening method at 220 nm. The concentrations of MLSS and BOD_5 were determined following standard methods SM 2540 D and SM 5210 B, respectively. Chemical oxygen demand (COD) was measured by dichromate oxidation, taking into account the presence of nitrite (nitrite exerts a COD of 1.1 mg O_2/mg $NO_2{}^-$-N), hydrogen peroxide was used to overcome the interference [27].

Concentration of FA and free nitrous acid (FNA) were calculated as a function of pH, temperature and total ammonium as nitrogen (TAN), for FA, or total nitrite (TNO_2), for FNA [12]:

$$\mathrm{FA(mg\ N\ L^{-1})} = \frac{\mathrm{TAN}}{1 + (10^{-\mathrm{pH}}/K_e^{\mathrm{NH}})} \tag{6}$$

$$\mathrm{FNA(mg\ N\ L^{-1})} = \frac{\mathrm{TNO_2}}{1 + (K_e^{\mathrm{NO}}/10^{-\mathrm{pH}})} \tag{7}$$

$$K_e^{\mathrm{NH}} = e^{-6344/(273+T)} \tag{8}$$

$$K_e^{\mathrm{NO}} = e^{-2300/(273+T)} \tag{9}$$

The efficiency of ammonium conversion was estimated according to Eq. (10)

$$\mathrm{Efficiency} = \left(\frac{C_{\mathrm{ini}} - C_{\mathrm{eff}}}{C_{\mathrm{ini}}}\right) \times 100\% \tag{10}$$

where C_{ini} is the initial ammonium concentration in the feeding phase; and C_{eff} is the ammonium concentration in the effluent.

3. Results and discussion

3.1. Period I: inoculation and start-up

The transformations of nitrogen (ammonia nitrogen, nitrite, nitrate), and ammonium conversion efficiency during period I in SBR are shown in Fig. 2a. A stable conversion of ammonium to nitrite was first achieved on day 30 of this period, whereupon an effluent with a composition close to a molar ammonium to nitrite ratio of 1:1 was observed. During steady state of partial nitrification (t = 30–118 days), ammonia conversion was primarily to nitrite over nitrate (Fig. 2a). One important feature of period I was that a lower percentage of ammonium conversion was initially observed once the ammonium loading rate was increased. Nevertheless, after few days with stable influent ammonium concentration, the system recovered its performance. For example, on day 34 the ammonia conversion efficiency and the corresponding effluent ammonia and nitrite concentration were 35.13% and 462.86, 234.07 mg L^{-1}, whereas they were 46.92% and 387.85, 323.14 mg L^{-1} on day 40, respectively. And we assumed the reason for this was due to the sensitive of AOB to the N-loading shift.

Another feature of period I was that the concentration of FA was always higher than 3.5 mg L^{-1} (Fig. 4), an inhibitory value for NOB as reported by Anthonisen et al. [12]. In reference to the inhibition of AOB by FA, Anthonisen et al. [12] also reported that inhibition

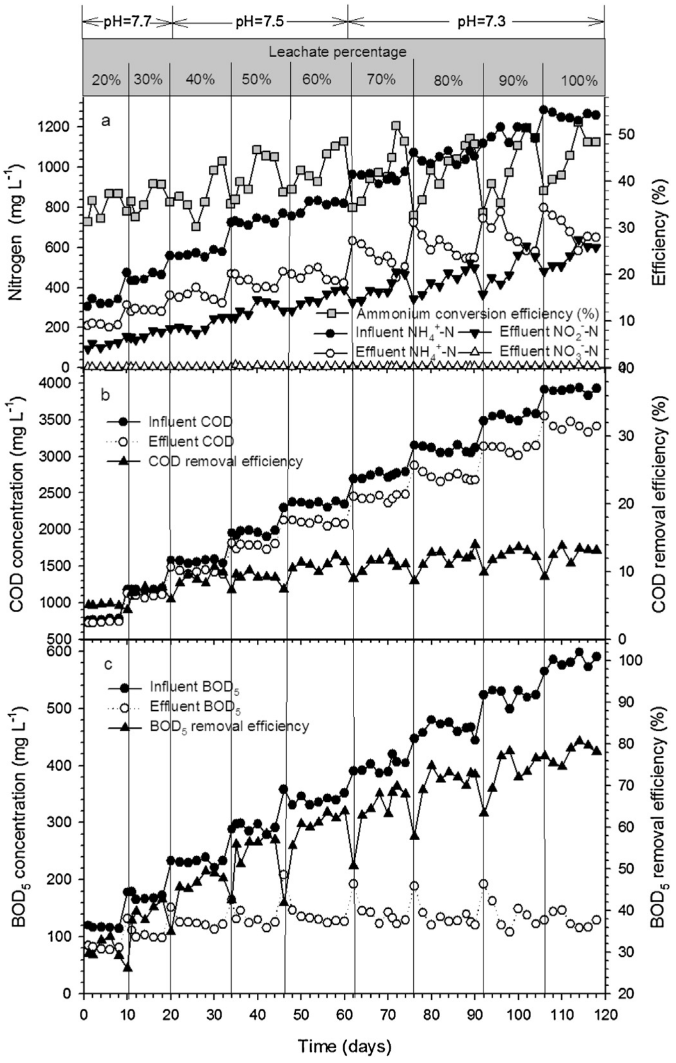

Fig. 2. Evolution of the SBR during Period I: (a) evolution of the concentration of nitrogen compounds; (b) evolution of the concentration of COD; (c) evolution of the concentration of BOD_5.

took place in the range of 10–150 mg L^{-1}. This value could not be reached except the days before pH was adjusted. Additionally, FNA has been known to inhibit NOB from the concentration between 0.06 and 0.85 mg L^{-1} [12]. In the whole period I, the average FNA was calculated to be lower than 0.06 mg L^{-1} (Fig. 4). Therefore, it can be concluded that FNA had little effect on nitrite accumulation in our study.

Except the stable ammonium conversion efficiency and high nitrogen removal efficiency during Period I, an unexpected removal of organic matter was also detected. Fig. 2b shows the variations concentration and removal efficiency of COD during Period I. As observed, the COD removal efficiency gradually increased with a small range in this period, showing the values of 5–13%. Fig. 2c presents the variations concentration and removal efficiency of BOD_5 during Period I. Also, the BOD_5 removal efficiency increased in this period. Unlike the low removal efficiency of COD, the BOD_5 exhibited much higher removal efficiency, ranging from 30% to 80%. However, by comparison of the consumption of COD and BOD_5 in the same operating cycle, it was shown that they were very close. Hence, the removed organic matters from the system were mostly the biodegradable fraction.

As expected, the steady-state partial nitrification to nitrite of landfill leachate was realized after 118 d operation. By the end of period I, the SBR was operated with 100% of raw leachate and an influent ammonium concentration of 1300 mg L^{-1}. Our results confirmed that low DO control strategy was feasible for the treatment high organic wastewater. Additionally, the start-up times required in our study was shorted than Ganigue et al. [28], who takes 167 d to achieve partial nitrification of landfill leachate. The reason for this was related to the inoculum of nitrifying sludge from our lab with ammonium conversion efficiency up to 80% [21,29,30], favoring the enrichments of AOB.

3.2. Period II: operation under different DO concentrations

The operational parameter and steady-state characteristics of the SBR reactor in period II are summarized in Table 3 and the results of the continuous experiment are shown in Fig. 3. As observed in Fig. 3a, on the second day (120th) after decreasing the DO to 0.5–1.0 mg L^{-1}, the operators noticed a significant reduction of the ammonium conversion rate to 33% parallel with the increase of effluent ammonia and nitrite concentrations to approximately 877.08 and 340.61 mg L^{-1}, respectively, while nitrate production was limited to a negligible amount (<2 mg L^{-1}). Additionally, within the first week of operation under 0.5–1.0 mg L^{-1} DO, the nitrification performance of the reactor continued to decrease. On the other hand, the FA value was up to 10 mg L^{-1} (Fig. 4) which achieved the range of 10–150 mg L^{-1} inhibiting both AOB and NOB activity. In this circumstance, it was unclear whether the lower DO concentration or FA inhibition or both cause the decrease of ammonium conversion. Thus, the pH value was adjusted on day 133 to eliminate the FA inhibition. Interestingly, there was a rise in ammonium conversion rate to around 36% afterwards. In contrast, the COD removal

Table 3
Operational parameter and steady-state characteristics of the SBR reactor.

Parameter	Unit	Phase				
		1 (0.5–1.0 mg L^{-1} DO)		2 (1.0–2.0 mg L^{-1} DO)	3 (2.0–3.0 mg L^{-1} DO)	4 (3.0–4.0 mg L^{-1} DO)
		FA inhibition	without FA inhibition	without FA inhibition	without FA inhibition	without FA inhibition
Influent NH_4^+-N	mg L^{-1}	1255	1293	1253	1307	1295
Ammonia convention rate	%	25.83	36.49	47.83	59.23	32.19
Effluent NH_4^+-N	mg L^{-1}	930.78	821.21	653.72	532.9	878.19
Effluent NO_2^--N	mg L^{-1}	302.82	438.24	585.23	712.12	358.61
Effluent NO_3^--N	mg L^{-1}	1.39	1.55	2.05	19.47	45.18
Influent COD	mg L^{-1}	3855	3821	3838	3836	3830
Effluent COD	mg L^{-1}	3574	3580	3328	3231	3026
COD removal efficiency	%	7.29	6.31	13.29	15.77	20.99
Influent BOD_5	mg L^{-1}	564.86	568.89	584.77	597.35	576.17
Effluent BOD_5	mg L^{-1}	354.09	353.38	162.45	41.91	3.59
BOD_5 removal efficiency	%	37.31	37.88	72.22	92.98	99.37
MLSS	mg L^{-1}	1893	1905	2110	2364	2783

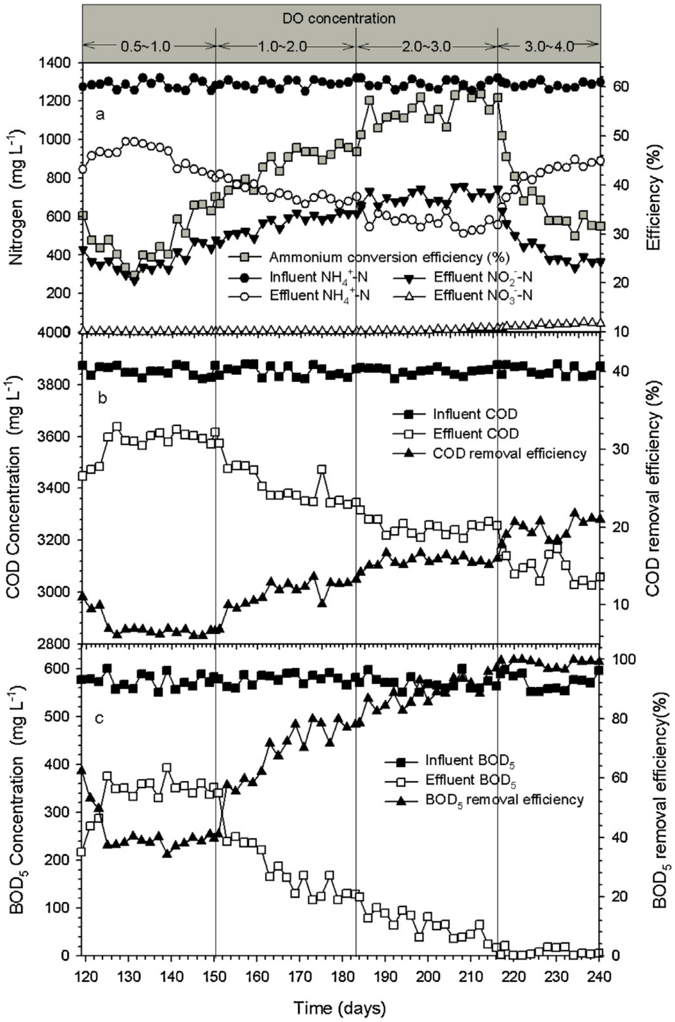

Fig. 3. Evolution of the SBR during Period II: (a) evolution of the concentration of nitrogen compounds; (b) evolution of the concentration of COD; (c) evolution of the concentration of BOD_5.

efficiency was marginal during this phase (119–133 days) (Fig. 3c), with values ranging from 6% to 9%. A same phenomenon was also observed in BOD_5 removal efficiency (37–40%). It was thus concluded that both lower DO concentration and FA inhibition led in the decrease of AOB activity, but FA inhibition was responsible for this depletion, while COD and BOD_5 removal only related to DO concentration.

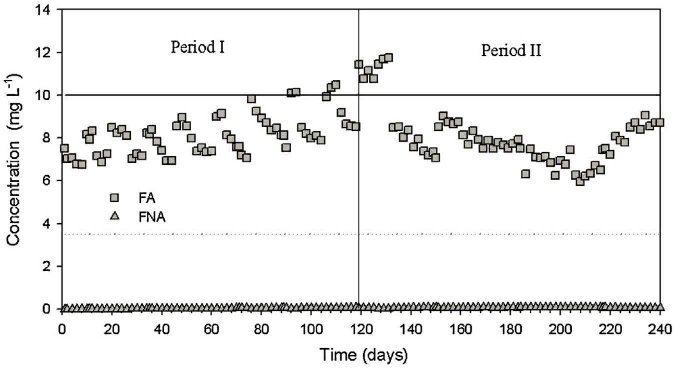

Fig. 4. Evolution of FA and FNA concentration during both Periods I and II, the dash line and the solid line indicates the inhibitory value for NOB (3.5 mg NH_3-N L^{-1}) and AOB (10 mg NH_3-N L^{-1}), respectively.

Starting from the 134 day, DO concentration was increased to 1.0–2.0 mg L^{-1}. After one week, the effluent ammonium to nitrite ratio closed to 1:1 again, suggesting that nitrification performance had been recovered (Fig. 3a). It has been reported that a dissolved oxygen concentration below 2.0 mg L^{-1} adversely affect the growth of nitrite oxidizers more so than ammonium oxidizers, which favors the dominance of latter community [31–33]. This was also confirmed in our study, as evidenced by the effluent nitrate values never exceed to 2 mg L^{-1} under both 0.5–1.0 and 1.0–1.5 mg L^{-1} DO conditions. However, when comparing the results of the above two conditions, it appeared that the ammonium conversion rate was lower under 0.5–1.0 mg L^{-1} DO condition, resulting in the effluent ammonium to nitrite ratio higher than 1.8:1, which was unsuitable to feed an Anammox reactor. It was in contrast with Wyffels et al. [34], who observed that partial nitrification with 50% ammonium conversion was attained at oxygen concentrations below 0.2 mg L^{-1} in a biofilm system. This is possibly related to the different wastewater fed in the two systems. As known, landfill leachate is a complex wastewater, the specific toxic substances such as PAHs – polyaromatic hydrocarbons, AOXs – adsorbable organic halogens, PCBs – polychlorinated biphenyls, and heavy metals could specially inhibit the sludge activity [1,2].

After recovering the nitrification performance, the concentration of DO was further increase to 2.0–3.0 mg L^{-1} (Fig. 3a). In the first weeks of this phase, the concentration of ammonia decreased, increasing the ammonium conversion rate, whereas an effluent of nitrate production (higher than 10 mg L^{-1}) was witnessed. After a month (with a DO of 2.0–3.0 mg L^{-1}), effluent nitrate concentrations increased to about 10 times greater than DO limits conditions (<2.0 mg L^{-1}), suggesting that partly recover of NOB activities. In the meantime, the COD and BOD_5 removal efficiency was also increased to about 16% and 95%, respectively, in the later part of this phase (Fig. 3b and c). Please note that, the FA value during this phase was still in the range of NOB inhibition but not AOB (Fig. 4). Two possible reasons can explain this observation: (1) NOB would assimilate to FA after long-term operation, resulting in less sensitive to FA inhibition [35,36]; or fast-growing heterotrophs in the reactor that weaken the inhibition of FA.

In the final phase, the oxygen concentration was controlled at high level of 3.0–4.0 mg L^{-1}. As expected, effluent COD and BOD_5 concentrations in the reactor decreased dramatically (Fig. 3b and c). This indicated that, the experimental high DO concentrations improve the fast-growing of AHB that favoring the removal of organic matter. Nevertheless, a significant increase of the effluent ammonia was observed, indicating significant inhibition of the ammonium conversion by high DO concentrations. Interestingly, there was a sharp continuous increase in effluent nitrate concentrations. Additionally, stable operation of the very similar FA evolution has also been shown at high DO concentrations. Ciudad et al. [37] reports that the supervisory pH control can remarkably act as an enhancement factor to reach a stable partial nitrification even under oxygen concentration at 5 mg L^{-1}. Similarly, Guo et al. [16] who observe that nitrite accumulation ratio of above 95% by using real-time aeration duration control in a SBR system at higher DO concentration (DO > 3 mg L^{-1}). However, the present research suggested that in a SBR reactor treated landfill leachate, it is impossible to achieve partial nitrification under higher DO condition (DO > 3 mg L^{-1}). The direct cause of the reduction of ammonia convention has not yet been identified, but it is speculated that a competition for oxygen and space between AOB and AHB is intense under high DO concentration.

In addition to the transformation of nitrogen, COD and BOD_5, the evolution of MLSS concentration was also investigated in period II (Table 3). As observed, the MLSS concentrations were found to be much higher under high DO condition compared to low DO

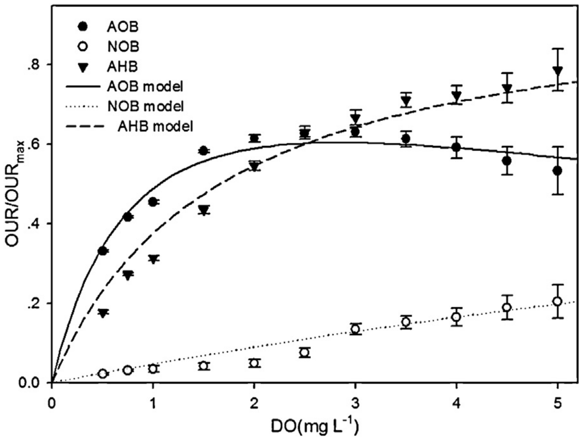

Fig. 5. Evolution of OUR/OUR$_{max}$ as a function of DO for AOB, NOB and AHB.

condition. Additionally, lowest MLSS concentration was monitored at 0.5–1.0 mg L^{-1} DO condition.

Since the volatile fatty acids (VFAs) represent the largest group of biodegradable matters in leachate [5]. In addition, acetic acid ($C_2H_4O_2$) is at a higher concentration among VFAs [38,39]. Hence, acetic acid is used to represent the leachate. The stoichiometry of catabolism and anabolism for AOB, NOB and AHB is reported as follows (Eqs. (11)–(13)) [24]:

$$\begin{aligned}\text{AOB}: &\ 55NH_4{}^{+} + 76O_2 + 109HCO_3{}^{-} \rightarrow C_5H_7O_2N\\ &+ 54NO_2{}^{-} + 57H_2O + 104H_2CO_3\end{aligned} \tag{11}$$

$$\begin{aligned}\text{NOB}: &\ 400NO_2{}^{-} + NH_4{}^{+} + 4H_2CO_3 + HCO_3{}^{-} + 195O_2\\ &\rightarrow C_5H_7O_2N + 3H_2O + 400NO_3{}^{-}\end{aligned} \tag{12}$$

$$\text{AHB}: 6C_2H_4O_2 + 7O_2 + NH_3 \rightarrow C_5H_7O_2N + 7CO_2 + 10H_2O \tag{13}$$

where $C_5H_7O_2N$ is presented the composition of the bacteria.

According to Eqs. (11)–(13), the real yield coefficients for AOB, NOB and AHB are obtained:

$$Y^{o}_{B/O_2}(\text{AOB}) = \frac{113}{32 \times 76} = 0.0047\ \text{g} \cdot \text{odm} \cdot \text{per} \cdot \text{g} \cdot O_2 \tag{14}$$

$$Y^{o}_{B/O_2}(\text{NOB}) = \frac{113}{32 \times 195} = 0.018\ \text{g} \cdot \text{odm} \cdot \text{per} \cdot \text{g} \cdot O_2 \tag{15}$$

$$Y^{o}_{B/O_2}(\text{AHB}) = \frac{113}{32 \times 7} = 0.504\ \text{g} \cdot \text{odm} \cdot \text{per} \cdot \text{g} \cdot O_2 \tag{16}$$

where odm is the organic dry matter.

The yield coefficients indicated that consumption of 1 kg O_2 would result in 4.7 g biomass of AOB, 18 g biomass of NOB and 504 biomass of AHB. Thus, it seems that the typical characteristic of nitrification is high oxygen consumption and a low biomass production. Nevertheless, high DO concentrations favor the growth of AHB. This was in line with biomass measurements in our study, and supported the notion that the SBR was dominated by AOB in low DO condition, whereas AHB enriched in high DO condition. Furthermore, the comparable sludge loss might thus explain the ammonia conversion reduction under 0.5–1.0 mg L^{-1} DO condition.

3.3. Kinetic comparison of AOB, NOB and AHB in the SBR reactor

The evolution of the OUR/OUR$_{max}$ ratio function of DO concentration is shown in Fig. 5. The low DO (<2 mg L^{-1}) was favorable for AOB growth, resulting in high OUR/OUR$_{max}$ ratio of AOB compared to AHB and NOB (Fig. 5). According to Hawkins et al. [40] and Anthonisen et al. [12], it is assumed that FA inhibition combined with higher affinity of AOB to oxygen is responsible for suppressing NOB activity. Wiesmann [24] has showed that an oxygen half-saturation constant of 0.08 mg L^{-1} O_2 for AHB, thus it is expected to be dominance community at low DO condition. However, unpublished results in our lab showed that the oxygen utilization rate of AHB was lower than AOB in a typical low DO range (<2 mg L^{-1}). The possible of the interpretation for this is that long-term operation (start-up period) results in a strong population of AHB washed out. Nevertheless, a comparable oxygen utilization rate between AOB and AHB was observed at 2.5 mg L^{-1} DO (Fig. 5). Thereafter, AHB surpassed AOB when DO was higher than 3 mg L^{-1} (Fig. 5). This could best be explained by the fact that competition between AHB and AOB for substrates (oxygen and carbon) resulted in the fast-growing heterotrophic bacteria dominating the reactor [41]. At low organic loadings, the heterotrophs and autotrophs can coexist. However, at high organic carbon loadings nitrifiers are non-uniformly distributed in the reactor, with the excessive growth of heterotrophs [42]. Kindaichi et al. also showed that the carbon metabolism in the reactor must be efficient to support heterotrophic bacteria. And the FISH analysis showed that the reactor was composed of 50% nitrifying bacteria (AOB and NOB) and 50% heterotrophic bacteria [43]. Fatihah pointed out that a low percentage of AOB was obtained when the oxygen concentration was high, whilst very high percentage of heterotrophic bacteria was found in the reactor [41].

Interestingly, during the same period for NOB, a sharp rise in OUR/OUR$_{max}$ ratio was also discovered. Hence, we assumed that the fast growth of heterotrophs may propose some “shielding effect” of FA inhibition, leading to acclimatization of NOB to FA, and transiently to favorable growing conditions for NOB, with $NO_3{}^{-}$-N production detected at 3 mg L^{-1} DO condition. The acclimatization phenomenon was previously reported [35,36] but has been poorly quantified until now. Thus, our experiment is difficult to clearly distinguish this adaptation which caused by a change in the resistance of some species or a switch from living environment to another. This aspect should be investigated in depth in further studies, especially in relation with the identification of the bacteria species.

Fig. 5 demonstrates that the Monod kinetics equation, fitted by non-linear least sum of squares regression, fits AHB distributions quite well (R^2 = 0.9271), whereas the Andrews-type kinetic model is better at representing the kinetic behavior of AOB (R^2 = 0.9861). For NOB, although not perfect (R^2 = 0.8743), the Monod kinetics equation also used to estimate NOB activity for utilizing of oxygen. Based on the data shown in Fig. 5, the estimated kinetic parameters for the heterotrophic bacteria and NOB were $K_{O_2} = 1.66$ and 20.25, respectively, while for AOB were $K_{O_2} = 0.93$, $K_I = 8.69$. Specifically, the value of kinetic parameters obtained in our study was completely different from other literatures [23,32,40]. The reason for this is possibly related to the different operation modes.

These results do indeed confirm the results that under low DO conditions (<2 mg L^{-1}), the AOB enriched but NOB and AHB were significantly inhibited, and led to the partial nitrification; for high-DO conditions (>3 mg L^{-1}), AHB became the dominate community whereas AOB were significantly inhibited and exhibited a poor ammonia convention. Since AOB had shown a higher value of OUR/OUR$_{max}$ ratio under 1.0–2.0 mg L^{-1} DO compared to 0.5–1.0 mg L^{-1} DO condition, and NOB activity partly recovered under 2.0–2.5 mg L^{-1} DO condition, it was proved that optimal condition for operation of partial nitrification to nitrite of landfill leachate was at 1.0–2.0 mg L^{-1} DO concentration.

4. Conclusions

The feasibility of low DO (1.0–2.0 mg L^{-1}) control strategy for the treatment of landfill leachate to obtain a suitable influent for an

Anammox reactor has been demonstrated in our research. Further decreasing the DO concentration to 0.5–1.0 mg L^{-1}, however, cause a decrease of ammonia convention rate, resulting in the effluent ammonium to nitrite ratio higher than 1.8:1. Although the ammonium conversion rate increased in 2.0–3.0 mg L^{-1} DO condition, although FA value is in the range of NOB inhibition, a rise in nitrate production occurs (higher than 10 mg L^{-1}). The reason for this may be related to the assimilation of NOB to FA or fast-growing heterotrophs that weaken the inhibition effect of FA. At high level of DO concentration phase (3.0–4.0 mg L^{-1}), a significantly decrease of ammonia conversion combined with a dramatically increase of COD removal efficiency is observed. Although biomass measurements (MLSS) in our study suggest that AHB dominate in high DO condition. The extent of comparable nitrogen and organic matter (COD and BOD_5) removal in the reactor configuration needs further microbiological evidence. The kinetic comparisons confirm that low DO conditions (<2 mg L^{-1}) favor the AOB but inhibit NOB and AHB, thus lead to the partial nitrification; while AHB became the dominate community whereas AOB were significantly inhibited under high-DO conditions (>3 mg L^{-1}), thereby resulting in a poor ammonia convention. Based on these results, the long-term operation of partial nitrification of landfill leachate is optimal at a DO concentration of 1.0–2.0 mg L^{-1}.

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