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Advanced removal of organic and nitrogen from ammonium-rich landfill leachate using an anaerobic-aerobic system[☆]Hongwei Sun^{1,*}, Huanan Zhao¹, Baoxia Bai³, Yuying Chen¹, Qing Yang², Yongzhen Peng²¹ School of Environmental and Municipal Engineering, Lanzhou Jiaotong University, Lanzhou 730070, China² Key Laboratory of Beijing Water Quality Science and Water Environment Recovery Engineering, Beijing University of Technology, Beijing 100124, China³ Disease Prevention and Control Office of Urumqi Railway Bureau, Urumqi 830010, China

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ABSTRACT

A novel system coupling an up-flow anaerobic sludge blanket (UASB) and sequencing batch reactor (SBR) was introduced to achieve advanced removal of organic and nitrogen from ammonium-rich landfill leachate. UASB could remove 88.1% of the influent COD at a volumetric loading rate of $6.8 \text{ kg COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$. Nitrification-denitrification was responsible for removing 99.8% of $\text{NH}_4^+ \text{-N}$ and 25% of total nitrogen in the SBR under alternating aerobic/anoxic modes. Simultaneous denitrification and methanogenesis in the UASB enhanced COD and TN removal, and replenished alkalinity consumed in nitrification. For the activated sludge of SBR, ammonia oxidizing bacteria were preponderant in nitrifying population, indicated by fluorescence *in situ* hybridization (FISH) analysis. The Monod equation is appropriate to describe the kinetic behavior of heterotrophic denitrifying bacteria, with its kinetic parameters determined from batch experiments.

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1. Introduction

Wastewater usually contains high concentrations of organics and ammonia, so nitrogen landfill leachate without treatment will pollute the environment seriously [1]. Cost-effective and highly efficient treatments for leachate are of great interest.

In terms of cost-effectiveness and reusability, biological methods predominate compared to other treatment methods, such as ammonium tripping, ozone oxidization and reverse osmosis [2–7]. In biological processes, organic and nitrogen in the leachate can be transformed into carbon dioxide and nitrogen gas, respectively, which means real removal of organic and nitrogen without secondary pollution. The biological process with an anaerobic-aerobic system is a feasible and sustainable technology for removing organic and nitrogen from landfill leachate [8–11]. In the majority of recently published papers, organic and ammonium removal higher than 90% could be achieved, but the total nitrogen (TN) removal efficiency is not high due to the shortage of carbon source available for denitrification [12,13].

In order to improve the nitrogen removal efficiency, nitrification-denitrification theory has been proposed in recent years, involving oxidation of ammonium to nitrite and then reduction to nitrogen gas.

Compared with conventional biological processes, the nitrification-denitrification process can reduce the amount of aeration by 25% and carbon needed by 40% [14–16].

To enhance the denitrification efficiency, simultaneous denitrification and methanogenesis (SDM) was used in anaerobic reactor [17,18]. SDM has become an attractive technology for improving TN removal because it leads to better economic benefit.

In this study, a novel system coupling an up-flow anaerobic sludge bed (UASB) and sequencing batch reactor (SBR) is developed for organic and nitrogen removal from leachate. The main function of the UASB is to improve COD and TN removal through SDM. The SBR is operated under aerobic/anoxic mode to achieve ideal performance for nitrogen removal *via* nitrification-denitrification. Furthermore, batch experiments are conducted to determine the kinetic model for heterotrophic denitrification.

2. Materials and Methods

2.1. Reactor and operation

Fig. 1 shows the experimental system with an UASB and a SBR. The raw leachate sorted in the feed tank was used as the influent of UASB. An equalization tank was designed to meet the requirement of continuous effluent of UASB and intermittent influent of SBR, with leachate in the equalization tank utilized as the influent of SBR. The working volume of UASB and SBR was 3 L and 12 L, respectively, which is made of polymethyl methacrylate. For the SBR, dissolved oxygen (DO) and pH

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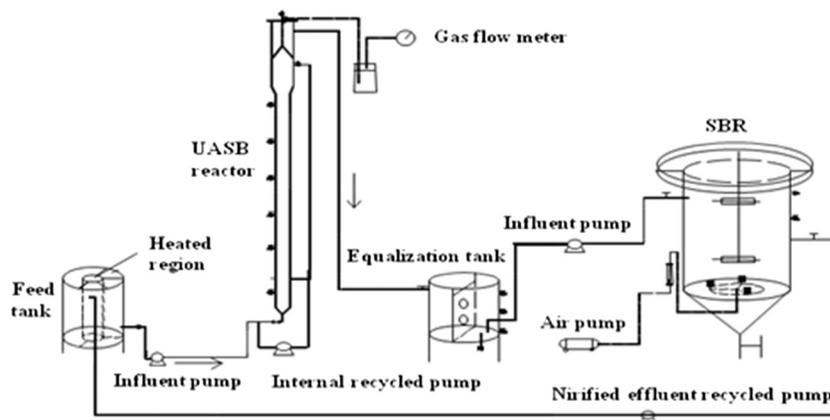


Fig. 1. Schematic diagram of the novel system coupling UASB and SBR.

meters, mechanical stirrer, and diffusers connected to an air compressor were set up. Through the temperature control apparatus, the operation temperature was controlled at $(30 \pm 2)^\circ\text{C}$ for the UASB, while the SBR reactor was operated at ambient temperature of $(20.5\text{--}31.4)^\circ\text{C}$. Nitrified supernatant in the SBR was returned to the UASB for denitrification with 300% of recirculation flow ratio.

In addition, hydraulic retention time of the UASB was 24 h. The SBR had a cycle time of 12 h, with 8 h aerobic, 0.5 h settling, 0.5 h SNS recycling, 2 h anoxic, 0.5 h settling, and 0.5 h decanting periods. The exchange volumetric rate was 50%.

2.2. Landfill leachate

Raw leachate from the Liulitun Municipal Solid Waste Sanitation Landfill Site (Beijing, China) was used as the wastewater in this experiment. The main characteristics of the leachate are shown in Table 1.

2.3. Inoculums

Granulated anaerobic sludge from a methanogenic reactor of a beer factor (Beijing, China) was inoculated in the UASB. Inoculums for SBR were aerobic activated sludge from a lab-scale oxidation ditch treating municipal wastewater, which performs nitrogen removal *via* nitrification–denitrification well. During this experiment, the mixed liquor suspended solid (MLSS) concentrations of UASB and SBR were approximately 55000 and 3500 $\text{mg}\cdot\text{L}^{-1}$, respectively.

2.4. Analytical methods

Chemical oxygen demand (COD), ammonium ($\text{NH}_4^+\text{-N}$), nitrate ($\text{NO}_3^-\text{-N}$), nitrite ($\text{NO}_2^-\text{-N}$), MLSS and volatile MLSS (MLVSS) were measured according to the standard methods [19]. TN was determined with a TN/TOC analyzer (Multi N/C 3000, AnaltikjenaAG, Germany). Temperature, DO and pH were monitored using pH/Oxi 340i analyzer (WTW Company, Germany).

Fluorescence *in situ* hybridization (FISH) was performed as specified in Amann [20]. Oligonucleotide probes used in this study were EUBmix for the detection of all bacteria, Nso1225 for ammonia-oxidizing β -Proteo bacteria, Ntspa 662 for *Nitrospira*, and Nit3 for *Nitrobaacter*. The

images of FISH samples were captured using an OLYMPUS-BX52 fluorescence microscope (Japan). The quantitative analysis of FISH images was performed using Leica QWIN software, where the relative abundance of each group was determined in triplicate as mean percentage of all bacteria.

2.5. Batch tests

Batch experiments were carried out to determine the kinetics of heterotrophic denitrification. In each test, 500 ml of nitrification sludge taken from the parent SBR was transferred to batch reactor. Initial nitrite concentrations were adjusted to desired values of 5, 10, 20, 40, 60, 80 and 100 $\text{mg}\cdot\text{L}^{-1}$ by adding 10 $\text{mg}\cdot\text{L}^{-1}$ NaNO_2 solution. Ethanol was added to the sludge, resulting in an initial C/N ratio in the reactor higher than 4.0. Higher C/N ratio was used to ensure that denitrification was not limited by carbon source. The pH value was kept approximately constant to 7.0 ± 0.05 through manually adding 0.5 $\text{mol}\cdot\text{L}^{-1}$ HCl solution. Temperature was controlled at $(27 \pm 0.4)^\circ\text{C}$ using a water jacket. The MLVSS concentration was controlled at (1250 ± 110) $\text{mg}\cdot\text{L}^{-1}$. The rate of nitrite reduction was determined from the measured nitrite profile using linear regression.

3. Results and Discussion

3.1. Performance of the UASB-SBR system on landfill leachate treatment

3.1.1. Organic removal

As shown in Fig. 2, during the whole operation period lasting for 113 days, the COD in the raw leachate was (6830 ± 541) $\text{mg}\cdot\text{L}^{-1}$, corresponding to an average organic loading rate of (6.8 ± 2.3) $\text{kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. A significant decrease in UASB influent was caused by the dilution of returned nitrified supernatant. The effluent COD of UASB decreased to (802 ± 124) $\text{mg}\cdot\text{L}^{-1}$ and most of the organic matters was removed by denitrification and methanogenesis. The low influent biodegradable COD in the SBR made nitrification rapid and complete. The final effluent COD of the system was below (319 ± 82) $\text{mg}\cdot\text{L}^{-1}$ and the residual COD mainly involved refractory organic matters. The COD removal efficiency of the system was $(95.2 \pm 1.2)\%$. The contribution of the UASB and SBR to total COD removal efficiency

Table 1
Characteristics of the leachate used in this study

Items	pH	COD/ $\text{mg}\cdot\text{L}^{-1}$	TN/ $\text{mg}\cdot\text{L}^{-1}$	$\text{NH}_4^+\text{-N}/\text{mg}\cdot\text{L}^{-1}$	$\text{NO}_3^-\text{-N}/\text{mg}\cdot\text{L}^{-1}$	$\text{NO}_2^-\text{-N}/\text{mg}\cdot\text{L}^{-1}$
Range	7.8–8.9	5872–7630	1960–2444	1748–2300	0.8–3.2	0.2–1.3
Average	8.3	6830	2140	2037	1.5	0.9

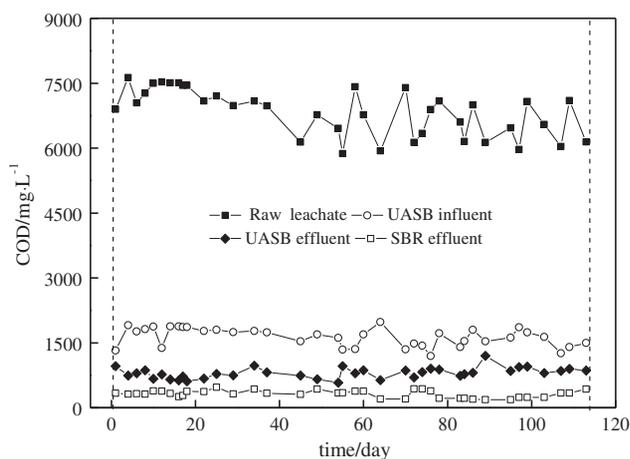


Fig. 2. COD removal performance of the UASB-SBR system.

was $(88.1 \pm 2.3)\%$ and $(7.2 \pm 2.2)\%$, respectively. Therefore, the UASB plays a major role in organic removal.

3.1.2. $\text{NH}_4^+\text{-N}$ removal

Fig. 3 shows the ammonium removal in this system. $\text{NH}_4^+\text{-N}$ in the raw leachate ranged from 1748 to 2040 $\text{mg}\cdot\text{L}^{-1}$, with the average value of $(2037 \pm 157) \text{mg}\cdot\text{L}^{-1}$, while effluent $\text{NH}_4^+\text{-N}$ of the system was in the range of $(0.1\text{--}23.8) \text{mg}\cdot\text{L}^{-1}$, with the average value of $(4.4 \pm 4.2) \text{mg}\cdot\text{L}^{-1}$, giving $\text{NH}_4^+\text{-N}$ removal efficiency of $(99.8 \pm 0.1)\%$. The ammonium removal is excellent. According to the pathway of ammonium removal, the operation period of SBR is divided to two stages (stage I and stage II). In stage I, days 0 to 37, ammonium was oxidized to nitrate and nitrite during the aerobic periods of SBR cycles. In other words, nitrate and nitrite co-existed in the reactor. As experiment proceeded, nitrate concentration decreased gradually, while nitrite concentration increased obviously, which means that nitrite began to accumulate in this period. As depicted in Fig. 3, the level of nitrite accumulation in the SBR, measured as the amount of $\text{NO}_2^- \text{-N}$ produced per $\text{NO}_x^- \text{-N}$, reached 94.2% on day 37. This suggests that nitrite pathway is achieved in a short period. In stage II, from days 38 to 113, nitrite was the primary product of nitrification during the aerobic period, which accumulated to about $(98.6 \pm 11.4) \text{mg}\cdot\text{L}^{-1}$, while the nitrate concentration was always below $(4.9 \pm 2.6) \text{mg}\cdot\text{L}^{-1}$. A higher level of nitrite accumulation of $(95.2 \pm 2.3)\%$ was maintained until the end of stage II, suggesting that the nitrification performance of the SBR is good.

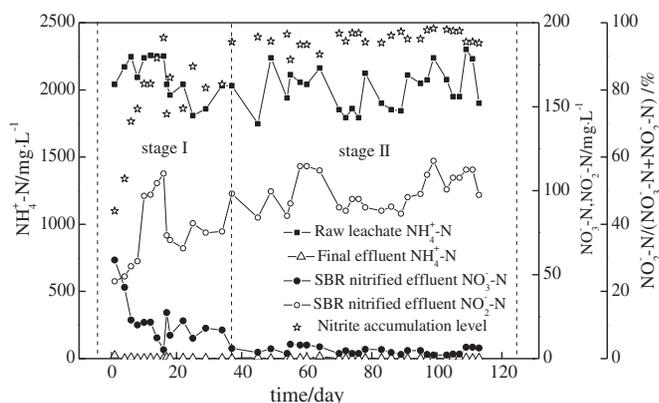


Fig. 3. Ammonium removal performance of the UASB-SBR system.

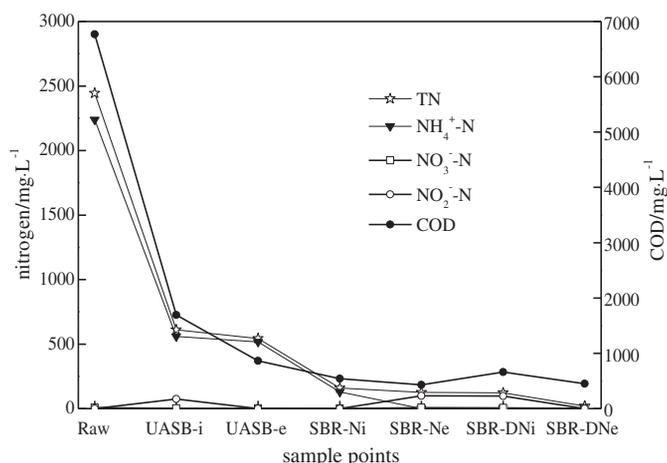


Fig. 4. Variations of nitrogen and organic in the UASB-SBR system. Raw: raw leachate; UASB-i: UASB influent; UASB-e: UASB effluent; SBR-Ni: SBR nitrification influent; SBR-Ne: SBR nitrification effluent; SBR-DNi: SBR denitrification influent; SBR-DNe: SBR denitrification effluent.

3.2. SDM in the UASB and nitrification–denitrification in the SBR

In order to demonstrate the conversions of nitrogen and organic in the system, typical variations of TN, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^- \text{-N}$, $\text{NO}_3^- \text{-N}$ and COD during complete nitrification on day 102 are depicted in Fig. 4. Diluted by the returned nitrified supernatant, the influent concentrations of TN, $\text{NH}_4^+\text{-N}$ and COD in the UASB decreased sharply, while influent $\text{NO}_2^- \text{-N}$ concentration increased obviously. In the UASB, the organic matters were superfluous as carbon source for denitrification of returned nitrified supernatant. $\text{NO}_2^- \text{-N}$ in the returned nitrified supernatant was about $99.5 \text{mg}\cdot\text{L}^{-1}$, while the effluent $\text{NO}_2^- \text{-N}$ was less than $0.5 \text{mg}\cdot\text{L}^{-1}$. Therefore, simultaneous denitrification and methanogenesis appeared in the UASB. Because of further dilution by the remaining sludge, the initial TN, $\text{NH}_4^+\text{-N}$ and COD concentrations in the SBR were 158.6, 129.8 and $865 \text{mg}\cdot\text{L}^{-1}$, respectively. In the aerobic period, more than 99% of ammonium was oxidized to nitrite, while about $34 \text{mg}\cdot\text{L}^{-1}$ TN was removed, likely caused by simultaneous nitrification and denitrification. In the following anoxic period, nitrite was reduced to N_2 , decreasing TN and COD concentrations simultaneously. The effluent TN, $\text{NH}_4^+\text{-N}$ and COD values of the system were 18.6, 0.2 and $451.4 \text{mg}\cdot\text{L}^{-1}$, respectively, with corresponding removal efficiency of 99.2%, 99.9% and 93.3%. This demonstrates that advanced removal of organic and nitrogen is achieved in the system.

3.3. Replenishment of alkalinity consumed in nitrification by denitrification in the UASB and SBR

It is well-known that 7.14g alkalinity was consumed per gram of ammonium oxidized in nitrification reaction, while one equivalent of alkalinity is produced per equivalent of nitrite reduced, which equals 3.57g of alkalinity production per gram of nitrite reduced in denitrification, so that by denitrification about one-half of the amount destroyed by nitrification can be recovered [21].

Fig. 5 indicates the variations of pH and alkalinity in the same period as that in Fig. 4. With the returned nitrified supernatant, influent pH and alkalinity of the UASB decreased compared with those in the raw leachate. The denitrification of nitrite increased both pH and alkalinity in the UASB. The ratio between alkalinity produced and nitrite reduced was $3.32 \text{g CaCO}_3 \cdot (\text{g NO}_2^- \text{-N})^{-1}$, which is slightly lower than the theoretical value of $3.57 \text{g CaCO}_3 \cdot (\text{g NO}_2^- \text{-N})^{-1}$. During the nitrification, the decrease in pH and alkalinity was caused by alkalinity reduction and acid production. At the end of nitrification, pH decreased to the lowest point of 7.8, which is known as “ammonia valley”, and alkalinity also decreased to

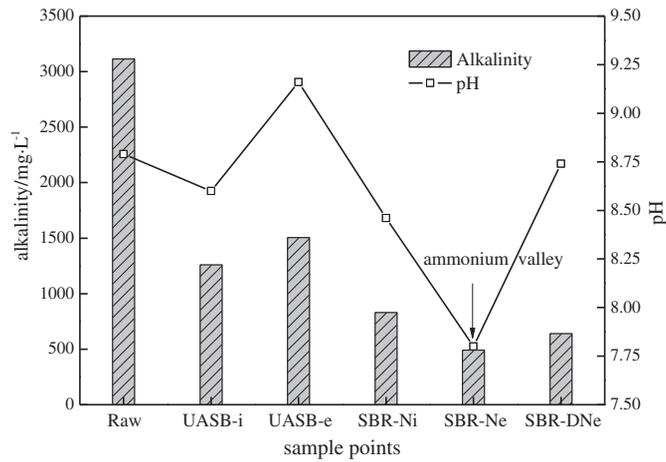


Fig. 5. Variations of pH and alkalinity in UASB-SBR system.

490.8 mg·L⁻¹. After nitrification completion, the pH value and alkalinity continuously increased with denitrification due to alkalinity production. Moreover, the ratio of alkalinity produced in denitrification to alkalinity consumed in nitrification was calculated as 0.44 g CaCO₃·(g NO₂⁻-N)⁻¹, which is lower than the theoretical value of 0.5 g CaCO₃·(g NO₂⁻-N)⁻¹. Thus, alkalinity destroyed by nitrification in the SBR can be recovered effectively by denitrification in the UASB and the anoxic phase of SBR.

3.4. Denitrifying activity of heterotrophic bacteria

The sludge samples from SBR reactor were analyzed by FISH in order to examine the change of bacterium community, especially, the population of ammonia oxidizing bacteria (AOB) and *Nitrobacter* (NOB). FISH analysis (Fig. 6) shows that AOB accounts for 4.5% of eubacterium, while NOB accounts for less than 0.1%. *Nitrospira* is not detected, and the remaining 95.4% is considered as heterotrophic bacteria. This result strongly suggests that AOB is the preponderant nitrifying bacteria in the sludge.

It should be noted that heterotrophic bacteria were dominant in the sludge system according to the FISH analysis. As a result, batch tests were carried out to determine the denitrifying activity of heterotrophic bacteria at different nitrite concentrations. Fig. 7 shows the nitrite concentration profile with time. Under pH 7.0 and initial nitrite concentration of 60 mg·L⁻¹, nitrite concentration decreased gradually, implying that denitrification occurred. The rate of this process was determined as 0.29 g N·(g VSS)⁻¹·d⁻¹ through linear regression. The

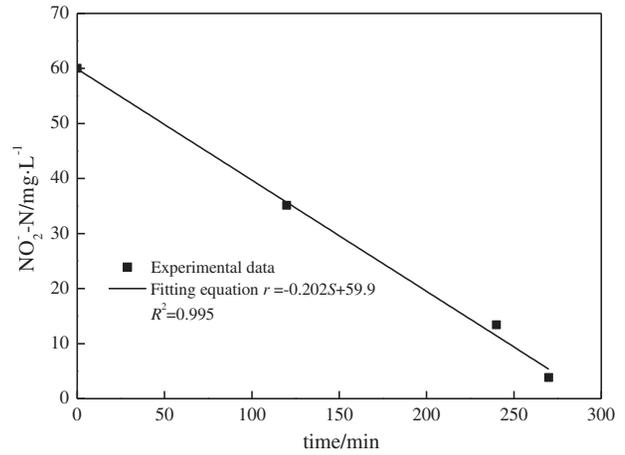


Fig. 7. Nitrite concentration profile measured under pH 7.0 and initial nitrite concentration of 60 mg·L⁻¹.

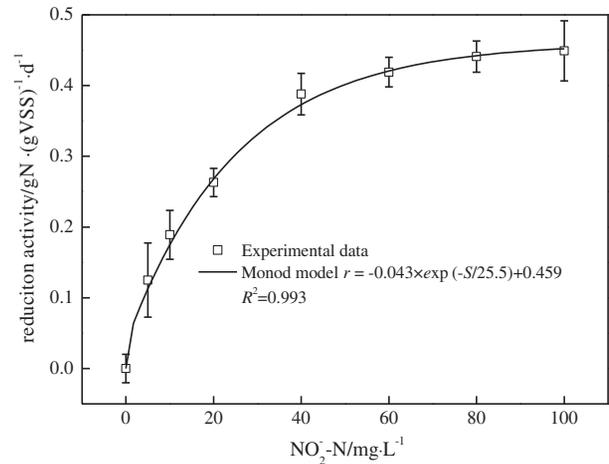


Fig. 8. Effect of nitrite concentration on denitrifying activity of heterotrophic bacteria in the SBR.

denitrification rates in other batch experiments were determined in a similar way.

Fig. 8 shows that nitrite concentration has significant effect on the denitrifying activity of heterotrophic bacteria. The denitrifying activity increases sharply with nitrite concentration in the low concentration range of 0–40 mg·L⁻¹, but the effect of concentration is less at nitrite

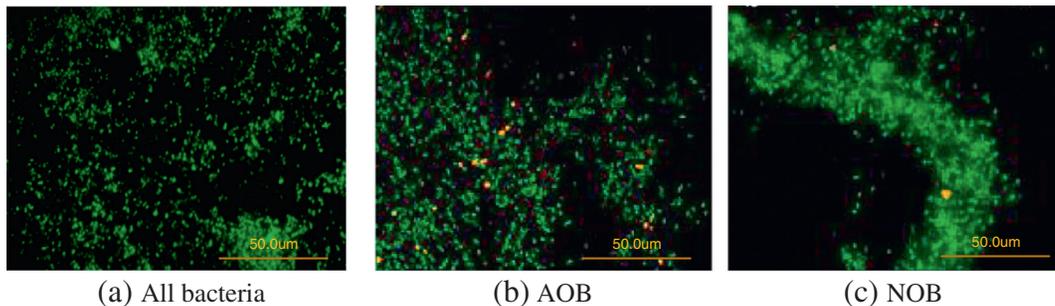


Fig. 6. FISH results for AOB and NOB in the SBR reactor on day 102. (a) EUBmix target for all bacteria; (b) NSO1225 target for AOB; (c) NIT3 target for *Nitrobacter*, Ntspsa662 target for *Nitrospira*.

concentrations greater than $60 \text{ mg}\cdot\text{L}^{-1}$. The data suggest that the Monod kinetic model may be applicable [22]:

$$r = r_{\max} \frac{S}{K_s + S} \quad (1)$$

where r and r_{\max} are the denitrification rate and its maximum value, respectively, $\text{g N}\cdot(\text{g VSS})^{-1}\cdot\text{d}^{-1}$; S is the nitrite concentration, $\text{mg}\cdot\text{L}^{-1}$; and K_s is the half-saturation constant, $\text{mg}\cdot\text{L}^{-1}$. The equation is expressed as $r = -0.043\exp(-S/25.5) + 0.459$. The value of K_s is $15.8 \text{ mg}\cdot\text{L}^{-1}$ and r_{\max} is $0.435 \text{ g N}\cdot(\text{g VSS})^{-1}\cdot\text{d}^{-1}$ for the heterotrophic bacteria.

4. Conclusions

The main conclusions of this study are as follows.

1. UASB-SBR system is suitable for advanced treatment of ammonium-rich landfill leachate, achieving COD, TN and $\text{NH}_4^+\text{-N}$ removal efficiency higher than 95.2%, 99.2% and 99.8%, respectively, with the effluent COD, TN and $\text{NH}_4^+\text{-N}$ less than 450, 0.5 and $20 \text{ mg}\cdot\text{L}^{-1}$, respectively.
2. Simultaneous denitrification and methanogenesis could be achieved in the UASB, which enhances COD and TN removal and replenishes alkalinity consumed during nitrification period of SBR. Nitrification-denitrification could be achieved in the SBR, improving nitrogen removal from leachate.
3. The effect of nitrite concentration on the denitrifying activity of heterotrophic bacteria is described by the Monod model. The kinetic parameter values of the half-saturation constant and maximum specific denitrification rate are $15.8 \text{ mg}\cdot\text{L}^{-1}$ and $0.435 \text{ g N}\cdot(\text{g VSS})^{-1}\cdot\text{d}^{-1}$, respectively.

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