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Achieving the nitrite pathway using FA inhibition and process control in UASB-SBR system removing nitrogen from landfill leachate

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An up-flow sludge blanket (UASB) and sequencing batch reactor (SBR) system was introduced to remove organics and nitrogen from landfill leachate. The synergetic effect of free ammonia (FA) inhibition and process control was used to achieve the nitrite pathway in the SBR. In previous research, inhibition of FA on nitrite oxidizing bacteria (NOB) activity has been revealed and the process control parameters (DO, ORP and pH) exactly indicate the end-point of nitritation. The method was implemented in the SBR achieving stable nitrogen removal via the nitrite pathway from landfill leachate. The degree of nitrite accumulation during the nitritation was monitored along with the simultaneous and advanced removal of organics and nitrogen in the UASB-SBR system. The nitrifying bacteria community was quantitatively analyzed by fluorescence *in situ* hybridization (FISH) techniques. Batch tests were carried out to investigate the denitritation kinetics of microbial bacteria in the SBR. Experimental results showed that the nitrite pathway could be repeatedly and reliably achieved by synergetic effect of FA inhibition and process control. FISH analysis showed the dominant nitrifying bacteria were ammonia-oxidizing *β-Proteobacteria*. Relationship between nitrite concentration and nitrite reduction rate followed the Monod-type equation. The maximum specific nitrite utilization rate (k) and half-velocity constant (K_s) were calculated as 0.44 gN gVSS^{-1} d^{-1} and 15.8 mg L^{-1}, respectively.

landfill leachate, partial nitrification, FA, FISH, process control, kinetic

1 Introduction

Landfill leachate treatment has received significant attention in recent years. However, high ammonia content and low C/N ratio present unique difficulties in treatment of landfill leachate. For removing ammonia nitrogen, biological nitrification-denitrification systems are effective and economical. It has been widely reported that partial nitritation-denitritation not only reduces the oxygen consumption in the nitrification step by 25% but also reduces the COD requirement in the denitrification step by 40% [1, 2]. For these advantages this process may be attractive as an alternative nitrogen removal technology to the conventional biological nitrification-denitrification, and has been developed for nitrogen removal from landfill leachate containing a high concentration of ammonia with low C/N ratio.

To achieve partial nitritation it is necessary to reduce the activity of nitrite oxidizing bacteria (NOB) but not the activity of ammonia oxidizing bacteria (AOB). Thus, the physiological difference between AOB and NOB has been suggested as a major factor leading to the elimination of NOB from the nitrifying system. Several factors have been identified to promote the nitrite pathway by selectively in-

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hibiting or limiting the activity of NOB over AOB. Low dissolved oxygen (DO) (AOB have a higher affinity for oxygen than NOB) [3, 4], high temperature combined with short sludge retention time(SRT) (AOB grow faster than NOB above a temperature of 25 °C, and NOB are washed out under a relatively lower sludge retention time) [5], and high concentrations of free ammonia (FA) and free nitrous acid (FNA) (AOB are more resistant to FA and FAN than NOB) [6–8] all likely contribute to the inhibition or elimination of NOB and the accumulation of nitrite. However, in most cases low DO may result in the decrease of ammonia oxidization. In addition, AOB may be eliminated under an extremely short SRT and the temperature is not susceptible to the modified and controlled. Therefore, the partial nitritation using inhibition of FA and FNA should be a very effective methodology. Moreover, many researchers have reported that nitritation was achieved through application of real-time control in SBR treating real municipal wastewater. Aeration was stopped as soon as nitritation finished as indicated by a break-points on the pH, DO and ORP profiles. However, the application of FA inhibition on NOB activity and process control to achieve nitrogen removal via nitrite from landfill leachate has not been reported.

In addition, although anarobic-aerobic process has been recommended for the treatment of landfill leachate, the UASB-SBR process has not been widely reported in previous studies [9–13].

This work presents a lab-scale UASB-SBR biological system adapting the simultaneous removal of organics and nitrogen as a feasible process for the treatment of landfill leachate. The objective of this study is to investigate the possibility of achieving nitrogen removal from landfill leachate via nitrite pathway by the synergetic effect of FA inhibition and process control. The high strength FA contained in landfill leachate is used to inhibit NOB activity, and simple process control parameters (pH, DO and ORP) are applied to exactly indicate the completion of nitritation. The biological system performance in terms of the nitrogen and organic removal efficiency and the degree of nitrite accumulation was monitored. Microbial spatial distribution of AOB and NOB in the SBR sludge was examined by fluorescent in situ hybridization (FISH).

2 Methods

2.1 Experimental setup and operational procedure

Figure 1 shows the experimental treatment system, which consists of an up-flow anaerobic bed (UASB) and a sequencing batch reactor (SBR).

The working volume of UASB was 3 L with an internal diameter of 70 mm, and a height of 1400 mm. The upper part of the reactor was designed for good separation of gas, liquid and solid. The SBR had an effective volume of 9 L, with three ports on its sidewall for insertion of a DO probe, a pH probe and a ORP probe. Dissolved oxygen was supplied through a porous diffuser installed at the bottom of the SBR. The liquor and activated sludge were completely mixed by using a mechanical stirrer at a constant speed of 40 rpm.

The UASB and SBR were operated at 30±2 °C and ambient temperature, respectively. The influent of the UASB reactor was a mixture of raw leachate and returned SBR nitrification supernatant at a desired ratio (3:1). The UASB effluent was used as the influent of the SBR. The SBR operation was as follows: transient filling → aeration (18 h) → settling (0.5 h) → supernatant recycling (0.5 h)→ anoxic mixing (7 h) → settling (0.5 h)→ discharging (0.5 h).

2.2 Characteristics of landfill leachate

The leachate used in this study was from Beijing Liulitun Municipal Solid Waste (MSW) Sanitary Landfill, and its characteristics were pH 7.8–8.9, chemical oxygen demand

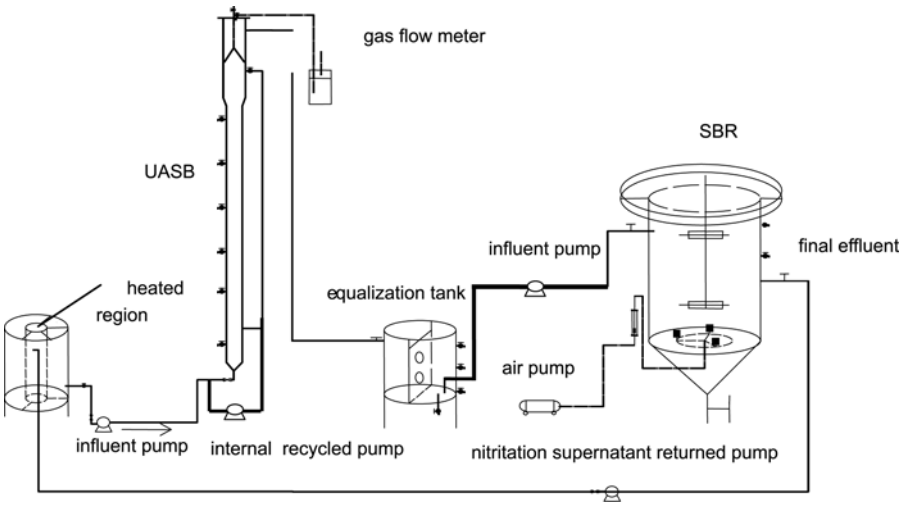

Figure 1 Schematic diagram of the UASB-SBR biological system.

(COD) 1038–8091 mg L^{-1}, total nitrogen (TN) 1005–2444 mg L^{-1}, NH_4^+ – N 890–2360 mg L^{-1}, and alkalinity (as $CaCO_3$) 5000–8500 mg L^{-1}.

2.3 Seed sludge

Granulated anaerobic sludge taken from Haerbin beer wastewater treatment plant in Heilongjiang province was used as the seed for the UASB reactor. The aerobic activated sludge withdrawn from Jiuxianqiao Municipal Wastewater Treatment Plant (WWTP) in Beijing was used as the seed for the SBR reactor.

2.4 Analytical methods

The ammonia ($NH_4^+ - N$), nitrate ($NO_3^- - N$), nitrite ($NO_2^- - N$) and chemical oxygen demand (COD) were measured according to the standard methods [14]. Total nitrogen (TN) was analyzed using TN/TOC analyzer (Multi N/C3000, AnaltikjenaAG, Germany). DO, pH and ORP were monitored using pH/Oxi 340i analyzer (WTW Company, Germany).

2.5 Fluorescence *in situ* hybridization (FISH)

Fluorescence *in situ* hybridization (FISH) was performed as described by Amann [15, 16]. Oligonucleotide probes, as shown in Table 1, were EUBmix (EUB338, EUB338II and EUB338III) for the detection of all bacteria, NSO1225 for Ammonia-oxidizing *β-Proteobacteria*, and NIT3 for *Nitrobacteria*. FISH images were collected using an OLYMPUS-BX52 fluorescence microscope. FISH quantification was performed according to Leica QWIN Software, where the relative abundance of each group was determined in triplicate as mean percentage of all bacteria.

2.6 Batch experiments

Batch experiments were performed using the sludge from the SBR. In each test, 1000 mL of sludge from SBR reactor was transferred to the batch reactor. Initial $NO_2^- - N$ concentrations of each batch reactor were individually adjusted to 5, 10, 20, 40, 60, 80 and 100 mg L^{-1}, respectively. Then external carbon source (methanol) was added to enable denitritation in the nitrite reduction period. The pH was kept approximately constant at 7.0 ± 0.1 through manually adding 0.1 mol/L HCL. Samples were taken every 30 min from the batch reactor, and the $NO_2^- - N$ reduction rate was measured at different initial $NO_2^- - N$ concentrations. The temperature was controlled at 27 ± 1 °C using an aquarium heating apparatus. The volatile suspended solid (VSS) was 1.25 ± 0.11 mg L^{-1}.

Table 1 16S rRNA targeted oligonucleotide probes used in FISH analyses

Probe	Probe sequence	Specificity	Fluorochrome labeled
EUBmix	GCTGCCTCCC GTSGGAGT	(most) bacteria	FITC
NSO1225	CGCCATTGTA TTACGTGTGA-	ammonia-oxidizing *β-proteobacteria*	Cyt3
NIT3	CCTGTGCTC CATGCTCCG	*nitrobacter*	Cyt3

3 Results and discussion

3.1 Overview of operating results of the UASB-SBR system

During the experimental periods, the average organic loading rate, COD removal rate and hydraulic retention time (HRT) of the UASB were 6.5 kg COD m^{-3} d^{-1}, 5.3 kg COD m^{-3} d^{-1} and 1 d, respectively. The removal of COD, $NH_4^+ - N$ and TN in the UASB-SBR system was monitored as shown in Table 2. The total COD removal efficiency of the system reached above 94.5%, and the contributions of UASB and SBR to the total COD removal were calculated as 81.0% and 13.5%, respectively. It can be seen that the UASB played the most important role in the COD removal, and the SBR reactor was further needed to enhance treatment efficiency and ensure the excellent effluent.

As the average feed concentrations of $NH_4^+ - N$ and TN were 2012 and 2444 mg L^{-1}, respectively, and the final effluent concentrations were less than 5 and 20 mg L^{-1}. The total removal efficiencies of $NH_4^+ - N$ and TN were consistently above 99%. The contributions of the UASB and SBR to the total $NH_4^+ - N$ removal were calculated as 76% and 24%, respectively. However, it was noted that the $NH_4^+ - N$ in the UASB effluent was significantly low compared to the

Table 2 Removal of organics and nitrogen in the UASB-SBR system

Item	COD			$NH_4^+ - N$			TN		
	influent (mg L^{-1})	effluent (mg L^{-1})	removal efficiency (%)	influent (mg L^{-1})	effluent (mg L^{-1})	removal efficiency (%)	influent (mg L^{-1})[1]	effluent (mg L^{-1}) $^{-1}$	removal efficiency (%)
UASB	6537 ± 1055	1238 ± 364	81.0	2021 ± 352	485 ± 198	76	2444 ± 362	543 ± 256	77.8
SBR	1238 ± 243	354 ± 114	71.4	485 ± 125	< 5	98.9	543 ± 156	< 30	96.3
System	–	354 ± 126	94.5	–	< 5	99.7	–	< 20	99.2

Note: The values were the average±standard error during the 113 days experiment.

influent $NH_4^+ - N$ largely due to the dilution by returned SBR nitritation supernatant rather than any reaction. Thus, in this system, the SBR acted as the true undertaker for the ammonia nitrogen removal through aerobic nitritation. The results reported above clearly show that simultaneous organic and nitrogen removal from landfill leachate was successfully achieved in the UASB-SBR system.

3.2 Achievement of partial nitritation in the SBR

To determine the performance of nitrite accumulation in the SBR, nitrite accumulation rate (NAR) was calculated using the following equation (1).

$$\mathrm{NAR} = \frac{\left[\mathrm{NO_2^- - N}\right]}{\mathrm{NO_2^- - N + NO_3^- - N}} \times 100\% \quad (1)$$

where $NO_2^- - N$ and $NO_3^- - N$ are the concentrations of $NO_2^- - N$ and $NO_3^- - N$ in the nitritation effluent of the SBR, respectively.

Figure 2 presents the achievement of partial nitritation in the SBR along with the ammonia concentration in the influent and effluent of the system throughout stages I and II.

During stage I (day 0–42), the application of FA inhibition and process control of each aeration period resulted in a gradual accumulation of nitrite. From day 0 to 16, the NAR rapidly increased from 43.9% to 95.5% along with increase of $NO_2^- - N$ and decrease of $NO_3^- - N$ in SBR nitrification effluent. However, from day 17 to 36, the short-term fluctuation of NAR was observed likely due to the unsteadiness of the SBR. The nitrite accumulation was quickly recovered from day 21 to 42, and NAR reached 96.5% at the end of stage I. This high level of nitrite accumulation was maintained steadily throughout stage II (day 43–113). During state II, the $NH_4^+ - N$ and $NO_3^- - N$ concentration in the SBR nitritation effluent was less than 5 mg L^{-1}, and $NO_2^- - N$ concentration was about 100 mg L^{-1}, indicating complete ammonia oxidization via nitrite pathway was achieved. Therefore, the FA inhibition and process control were successful in achieving the stable nitritation in the SBR.

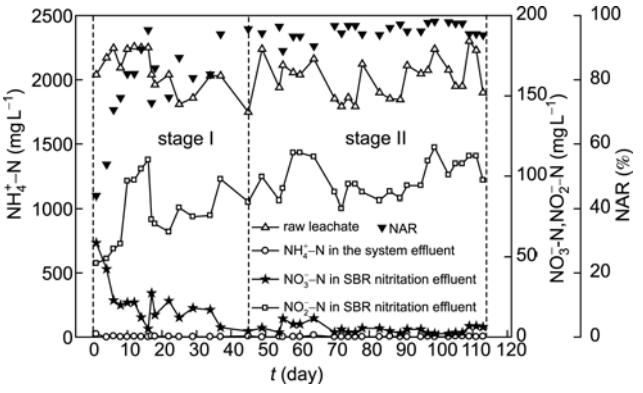

Figure 2 Variations of $NH_4^+ - N$ in the influent and effluent in the system and $NO_3^- - N$, $NO_2^- - N$ and NAR in SBR nitritation effluent.

3.3 Conversion of organics and nitrogen in the UASB-SBR system

Figure 3 clearly shows the typical variations of COD, TN, $NH_4^+ - N$, $NO_3^- - N$ and $NO_2^- - N$ in the system during nitritation period. The influent of the UASB was a mixture of raw leachate and the returned SBR nitritation supernatant, which resulted in the significant decrease of COD, TN and $NH_4^+ - N$ in the UASB influent compared to raw leachate. The organics in the UASB influent were superfluous as carbon source for denitritation of the returned SBR nitritation supernatant. The $NO_2^- - N$ in the SBR returned supernatant was about 100 mg L^{-1}, but the $NO_2^- - N$ in the UASB effluent was less than 1.6 mg L^{-1}. The above results indicate that simultaneous denitritation and methanogenesis were achieved in the UASB. In other words, organics contained in raw leachate were removed by denitritation and methanogenesis in the UASB.

The $NH_4^+ - N$ in the UASB effluent was oxidized to $NO_2^- - N$ during the SBR aerobic period (Figure 4). The

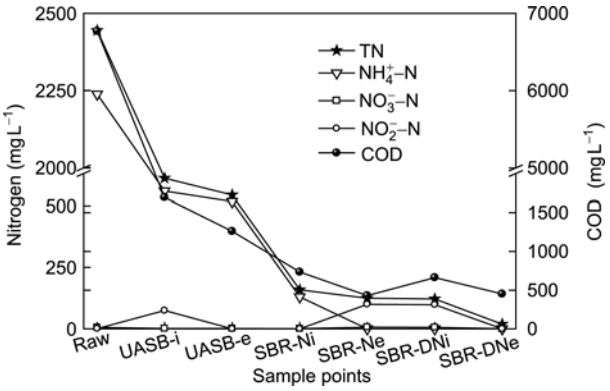

Figure 3 Variations of the nitrogen and COD in the UASB-SBR system.

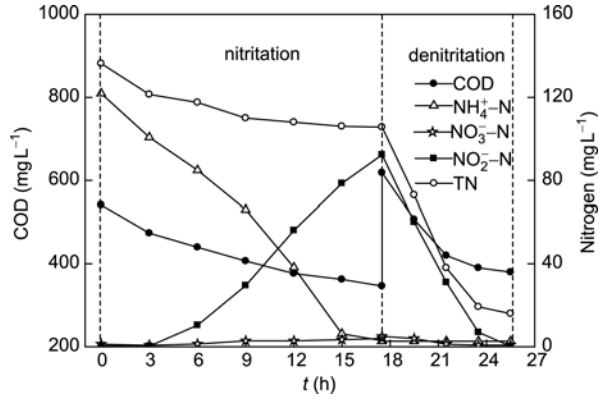

Figure 4 Variations of nitrogen and organic concentrations during nitritation and denitritation in the SBR.

$NO_2^- - N$ produced was reduced to N_2 in both the SBR anoxic period and the UASB. The denitritation efficiencies of $NO_2^- - N$ in both the reactors were over 99%. During the whole experiment period, the final effluent TN concentration was below 20 mg L^{-1}, indicating that advanced nitrogen removal from landfill leachate via nitrite pathway was achieved in UASB-SBR system.

3.4 Mechanism of partial nitritation achievement in SBR

The free ammonia (FA) concentration was calculated by equilibrium as follows [6],

$$FA = \frac{17}{14} \times \frac{\left[NH_4^+ - N\right] \times 10^{pH}}{\left(K_b / K_w\right) + 10^{pH}} \quad (2)$$

where $NH_4^+ - N$ is the $NH_4^+ - N$ concentration in the SBR, pH is the pH in the SBR, K_b is the ionization constant of the ammonia equilibrium equation, and K_w is the ionization constant of water and $K_b/K_w = e^{(6344/273 + °C)}$.

The inhibition of FA on AOB and NOB activities is well known, and NOB are more sensitive to FA in the range of 0.1–1.0 mg L^{-1} while AOB are inhibited in the range of 10–150 mg L^{-1} [6]. Kim *et al*. demonstrated that activity of NOB decreased by approximately 50% when FA concentration was 0.7 mg L^{-1} [17]. During the SBR nitritation process, a clear decreasing trend in FA concentration was observed with decreased $NH_4^+ - N$ concentration and pH (Figure 5). The FA concentration was reduced to 0.21 mg L^{-1} when nitritation was almost completed. This suggests that inhibitory effect of FA on NOB activity should gradually weaken with decreased FA concentration during the SBR nitritation process. Further, FA may lose its inhibitory effect once aeration was not stopped immediately when nitritation finished.

It is acknowledged that the presence of lag for FA is because this level is calculated according to eq. (2). Thus, how to establish a simple and reliable methodology to overcome the above problems was very critical.

Figure 5 also presents the DO, pH and ORP profiles during an SBR cycle.

The simultaneous appearance of ammonia valley, DO break-point and ORP break-point on the pH, DO and ORP curves, respectively, was observed at the end of nitritation. These characteristic points not only represented the completion of nitritation exactly, but also maintained the inhibition of FA on NOB activity by avoiding excessive aeration. The process control was reliable and easy in detecting the end of nitritation as indicated by the dot lines in Figure 5. In this study, we applied the synergetic effect of FA inhibition and process control to achieve stable nitrogen removal via nitrite pathway in the SBR. The success of this methodology was further confirmed by the good long-term performances of the SBR presented previously.

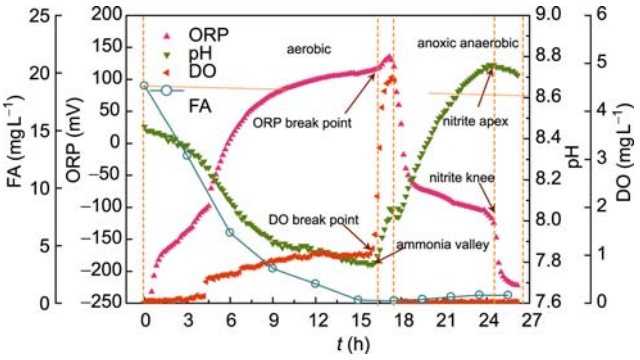

Figure 5 Variations of FA concentration and DO, ORP and pH in the SBR reactor.

3.5 Microbial community analysis and denitritation kinetics

To obtain a biological understanding of AOB with high nitritation ability, the microbial community in the SBR was analyzed by FISH. Figure 6 shows four FISH images of all bacteria (a, c), AOB (b) and NOB (d), which were entrapped in the sludge sample from the SBR on day 105. Presence of AOB was confirmed, in particular, ammonia-oxidizing *β-Proteobacteria* seem to be dominant. To quantify the ammonia-oxidizing *β-Proteobacteria* and *Nitrobacter*, the populations of both groups of bacteria were analyzed by direct cell count. The relative percentage of NSO1225-positive cells to the EUBmix-positive cells was above 4.3%, but the relative percentage of NIT3-positive cells to the EUBmix-positive cells was less than 0.3%. Therefore, the dominant nitrifying bacteria were ammonia-oxidizing *β-Proteobacteria*. This results show that the growth of NOB was likely to cease and FA might inhibit anabolism of NOB during SBR nitritation period. Hence, NOB was washed out from the system, following which stable partial nitritation could occur.

Moreover, to clarify the denitritation capability of microbial community, the relationship between initial $NO_2^- - N$ concentration and $NO_2^- - N$ reduction rate was investigated through batch tests. As a result, the relationship between initial $NO_2^- - N$ concentration and $NO_2^- - N$ reduction rate followed the Monod equation (Figure 7). The half-saturation constant (K_s) and the maximum specific utilization rate (k) of nitrite were calculated as 15.8 mg L^{-1} and 0.44 $gNO_2 - N$ $gVSS^{-1}$ d^{-1}, respectively. In reported literature, no studies have been reported on denitritation kinetics of microbial bacteria contained in an SBR for the treatment of landfill leachate. Therefore, this study was the first report on values of K_s and k which have significance to guide the operation of landfill leachate treatment plants.

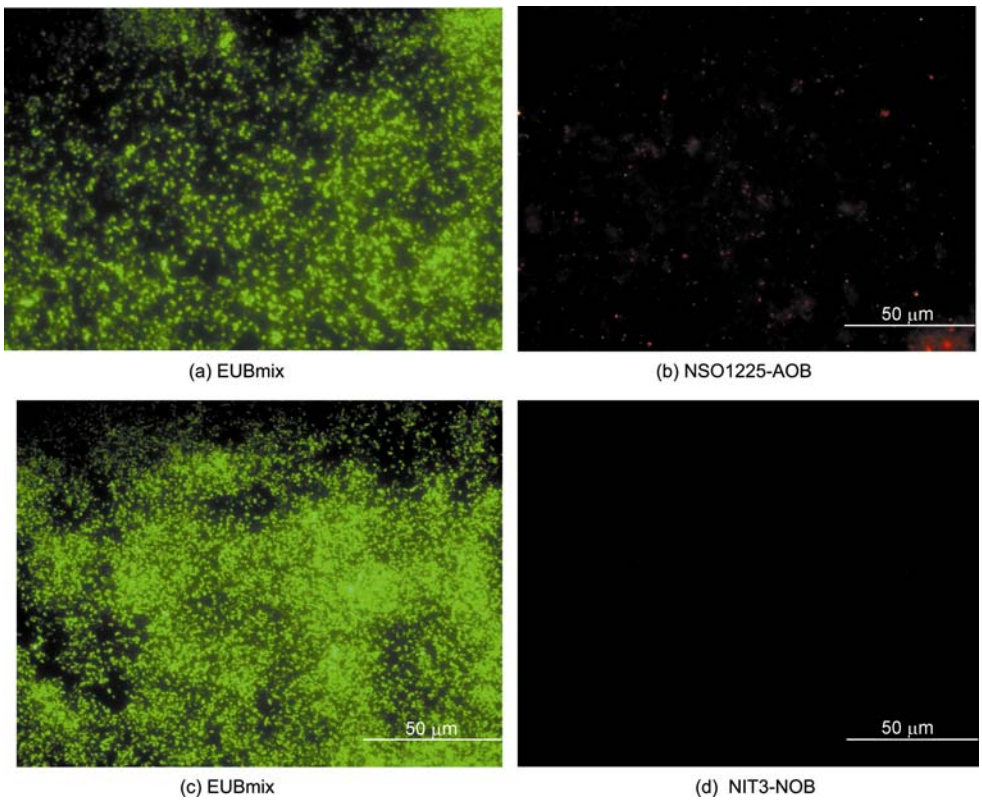

Figure 6 FISH analysis of the nitrifying bacteria in the SBR reactor on day 105.

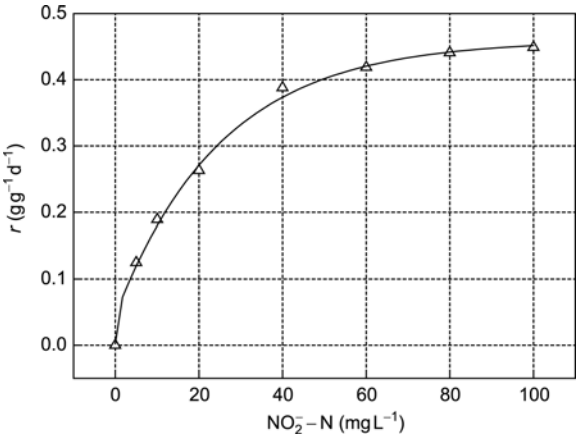

Figure 7 Monod equation using nitrite as an electronic acceptor.

4 Conclusions

In this study, the UASB-SBR system was found to be a feasible process for simultaneous and advanced removal of organics and nitrogen from landfill leachate. Average raw influent concentrations of COD, NH_4^+ – N and TN were 6537, 2021 and 2444 mg L^{-1}, respectively, and the final effluent could reach below 354, 5 and 20 mg L^{-1}. The corresponding removal efficiencies were 94.5%, 99.7% and 99.2%.

Nitrogen removal via the nitrite pathway could be achieved in SBR through the synergetic effects of FA inhibition and process control. This novel methodology could fully utilize the inhibition of FA on NOB activity by using process control parameters as the indication point of nitritation. FISH revealed that the dominant nitrifying bacteria were ammonia-oxidizing *β-Proteobacteria*. The Monod constants (K_s and k) of nitrite reduction were obtained as 15.8 mg L^{-1} and 0.44 gNO_2^-–N gVSS^{-1} d^{-1}, respectively.

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