



Denitrification capacity of bioreactors filled with refuse at different landfill ages

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ABSTRACT

The denitrification capacity of refuse at different landfill ages in bioreactor landfill system was studied. Three reactors filled with 1-year-old refuse (R1), 6-year-old refuse (R6) and 11-year-old refuse (R11), respectively, were operated in the experiment. Nitrate solution ($1000 \text{ mg NO}_3^- \text{-NL}^{-1}$) was added into each reactor. The results showed that the reactors were all able to consume nitrate. However, 1-year-old refuse in R1 had both a higher nitrate reduction rate and concentration of N_2 . In addition, vertical differences in nitrate removal along the depth of R1 were observed. The bottom-layer refuse and the middle-layer refuse both showed higher efficiency in nitrate depletion than the top layer. Furthermore, N_2O accumulation was found in R11 with the concentration up to 19.3% of the released gas. These results suggested that 1-year-old refuse, which was partly degraded, was more suitable to use as denitrification medium.

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1. Introduction

Currently, there is increased focus on bioreactor landfills which are a relatively new trend in solid waste management [1–3]. Leachate recirculation is the main characteristic of this technology [4,5]. However, leachate, which is returned to the waste cell, may also contain higher concentration of some constituents, especially ammonia [6]. It is well known that the landfill has an anaerobic environment, and significant ammonia elimination processes do not occur under such conditions. Through leachate recirculation, ammonia may accumulate to high concentrations, which may inhibit biodegradation of refuse [7]. In addition, if leachate containing ammonia is discharged to the environment, it may trigger eutrophication of water bodies. Therefore, ammonia accumulation is a serious long-term pollution problem in landfill management. Recently, the Chinese government published a new standard for pollution control of municipal solid waste landfills, which prescribes that the emission concentration of ammonia in leachate must be lower than 25 mg L^{-1} . For these reasons, studies on the removal of ammonia nitrogen from leachate are necessary.

Ammonia removal is mostly practiced *ex situ* at present and biological nitrogen removal is the most common method. Biological nitrogen removal involves two processes, nitrification and denitrifi-

cation, both of which require large spatial and financial investment, which restricts the utilization of this treatment approach. Therefore, development of an *in situ* nitrogen removal technique would be an attractive alternative. The condition in a landfill body is predominantly oxygen-free, which is suitable for denitrification, however nitrification requires oxygen, which is unlikely to be present in landfills. Thus, nitrogen removal could involve *ex situ* nitrification of leachate which is coupled with the use of the landfill as a bioreactor for denitrification.

In situ denitrification has been reported previously. By feeding 500 and $1000 \text{ mg NO}_3^- \text{-NL}^{-1}$ into reactors filled with 1-month-old refuse, Burton and Watson-Craik [8] found that complete denitrification occurred. Price et al. [9] injected $400 \text{ mg NO}_3^- \text{-NL}^{-1}$ into a series of reactors that were actively producing methane to evaluate denitrification. The research showed that the reactors were able to convert nitrate to nitrogen, during which methane production was inhibited. These results were confirmed by El-Mahrouki and Watson-Craik [10]. Vigneron et al. [11] demonstrated that denitrification occurring during the acidogenic waste degradation phase was predominantly heterotrophic, while autotrophic reactions prevailed during the methanogenic phase. Other treatment systems consisting of *in situ* denitrification preceded by *ex situ* nitrification have been studied, and they all showed high efficiency in nitrogen removal [12–15]. These studies proved that fresh, old, aged or mature refuse could be used as denitrification media. However, there were also some studies which showed that fresh refuse in the acidogenic phase may be unsuitable for denitrification, because dissimilatory nitrate reduction to ammonium (DNRA) would occur instead of denitrification [8,11,16]. In general, these studies have

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focused primarily on either the denitrification potential of waste, effects of denitrification on methane production or development of a new combined system for nitrogen removal.

Refuse placed in landfills degrades gradually and the composition and properties of refuse vary with landfill age. Nitrate added to the refuse body will be reduced to nitrite, nitrogen gas or other nitrogenous compounds as a result of denitrification. N_2 and N_2O are two primary products during the process of denitrification. However, N_2O is a potent of greenhouse gas and it is necessary to avoid its production. It is well known that nitrate reduction rates and reaction products are related to the type and quantity of microorganisms which exist in the refuse. Thus, landfill age is a major factor which affects microbial community structure. In order to meet both economic and environmental needs, it is useful to find the landfill age which has the best characteristics for denitrification. In addition, for the purpose of better understanding nitrate conversion, different forms of nitrogenous compounds produced during the process of denitrification should be monitored.

Considering that fresh refuse had a negative effect on denitrification in some studies [8,11,16], we sampled 1-year-old refuse, 6-year-old refuse and 11-year-old refuse and placed the refuse in three reactors. Specific objectives of this work were to (1) evaluate denitrification capacity and compare nitrate reduction rates when nitrate was injected into these reactors, (2) analyze vertical differences of nitrogen conversion in the reactors, and (3) measure the concentrations of gas components produced after nitrate addition.

2. Materials and methods

2.1. Simulated landfill bioreactor construction

The experiment was conducted in three identical plexiglass reactors, Reactor 1 (R1), Reactor 6 (R6) and Reactor 11 (R11). The diameter and height of the reactors were 20 cm and 75 cm, respectively. Two ports were installed in the lid of each reactor for gas collection and leachate recirculation, while another one was installed at the bottom of the reactor for leachate drainage. Three leachate sampling ports numbered as 1#, 2# and 3# from bottom to top were equally spaced along the cylinder wall. The schematic configuration is shown in Fig. 1.

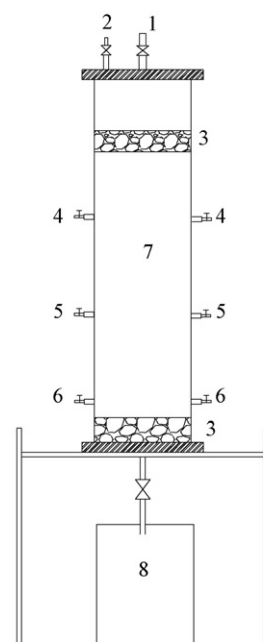
2.2. Simulated landfill bioreactor loading

Refuse was excavated and dated according to the records from the Hangzhou Tianziling Landfill in China. For each date, 3–4 excavation sites were selected at random and a total amount of 100 kg samples was collected from these sites. Before placing waste in the reactors, non-degradable matter such as plastic bags and metals was removed. Then landfill bioreactors R1, R6 and R11 were filled with 15 kg of 1-year-old refuse, 6-year-old refuse and 11-year-old refuse, respectively, at a density of 884 kg m^{-3} . Two gravel layers, both with a thickness of 5 cm were placed above and below the refuse layer in each reactor to distribute leachate and prevent clogging. After filling, reactors were sealed with silicon caulk to minimize oxygen intrusion and examined for leaks. The characteristics of refuse at different landfill ages are shown in Table 1.

Table 1

The characteristics of refuse at different landfill ages.

Landfill age (years)	pH	Water content (%)	TN (g kg^{-1})	BDM (g kg^{-1})	Humic matter (g kg^{-1})	Ammonia (g kg^{-1})	Nitrate (g kg^{-1})	Nitrite (g kg^{-1})
1	7.5	41.61	6.21	140.60	109.54	0.22	0.07	0
6	8.21	31.98	3.11	48.46	155.72	0.20	0.03	0
11	8.03	27.80	3.47	42.56	193.70	0.18	0.01	0



1. leachate recirculation adapter
2. gas collection adapter
3. gravel layer
4. 3# port
5. 2# port
6. 1# port
7. refuse
8. leachate collection container

Fig. 1. The configuration of the bioreactor used in this experiment. (1) Leachate recirculation adapter; (2) gas collection adapter; (3) gravel layer; (4) 3# port; (5) 2# port; (6) 1# port; (7) refuse; (8) leachate collection container.

2.3. Experimental design and operation

In order to reduce ammonia concentrations, 6 L distilled-water were injected three times into each reactor to wash the refuse resulting in less than 150 mg L^{-1} of ammonia-nitrogen. Then 5.5 L of KNO_3 solution ($1000 \text{ mg NO}_3^- \text{ NL}^{-1}$) were added to each reactor so that the liquid level was just above the refuse layer and allowed to drain over time. Leachate and gas samples were collected for analysis throughout the study. Ten milliliters of leachate was removed every other hour in the first 11 h, sampled every 12 h in the next 84 h, and then once every 24 h. Gas composition was analyzed daily in the first 19 days and then analyzed every 4 days. The experiment in each reactor was ended when the concentrations of nitrate was nondetectable. Throughout the experiment, all the reactors were incubated at $28 \pm 1^\circ \text{C}$.

2.4. Analytical methods

Leachate samples were analyzed for pH, chemical oxygen demand (COD), $\text{NH}_4^+ \text{-N}$, $\text{NO}_3^- \text{-N}$ and $\text{NO}_2^- \text{-N}$. All the analyses were conducted according to standard methods [17]. pH was measured using a glass electrode (DELTA 320-S) and COD was analyzed by the standard digestion method. $\text{NH}_4^+ \text{-N}$, $\text{NO}_3^- \text{-N}$ and $\text{NO}_2^- \text{-N}$ concentrations were determined by spectrophotometric method. CH_4 , N_2 and N_2O were measured using gas chromatography (GC, Agilent

7890, United States). CH_4 was analyzed on an OV-1 capillary column, coupled to a hydrogen flame ionization detector (FID). The oven temperature was 100°C and the injector and detector were maintained at 100 and 150°C , respectively. N_2O was analyzed by a GC equipped with an electron capture detector (ECD) and a HP-PLOTQ capillary column. The oven and detector temperatures were 50 and 250°C , respectively. N_2 was measured using a GC equipped with a thermal conductivity detector (TCD) and a HP-PLOTQ capillary column. The oven and detector temperatures were 35°C and 200°C , respectively.

Refuse placed in the reactors was analyzed for pH, water content, total nitrogen (TN), biological degradable matter (BDM), $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$ and humic matter. These parameters were analyzed by standard methods [18,19]. The pH of refuse was measured in a distilled-water extract of 1:5 (w/v) by a pH meter (DELTA 320-S). Water content of the refuse was determined by drying the samples in an oven at 105°C for 24 h. TN was determined by the Kjeldahl method and BDM was determined at ambient temperature by using the method for COD analysis. Refuse was extracted with 200 mL of 2 mol L^{-1} sodium chloride, then $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ were measured by the same method as leachate analysis. Humic matter was determined as recommended by the International Humic Substances Society [20].

3. Results

3.1. Variations of leachate COD and pH

COD concentration data are shown in Fig. 2. The COD concentrations for the reactors were different with the highest in R1 which was filled with partly degraded refuse. Throughout the experiment, due to refuse hydrolysis, COD concentrations of the three reactors increased slightly during the initial phase of the experiment, especially in R1. Then COD in R1 decreased over time, while it remained fairly stable in R6 and R11. Small differences in COD concentrations among different layers of R6 and R11 were observed, with the highest in the bottom layer, followed by the middle and top layers. This might be due to wash out of organic matter from top layers to the bottom. As shown in Fig. 3, pH values of R1 were in the range of 6.8–7.5 after nitrate addition and quickly initially increased then decreased over time. In contrast, pH values of R6 and R11 remained relatively constant and near neutral.

3.2. Variations of nitrate, nitrite and ammonia

Nitrate consumption data for the three reactors are presented in Fig. 4. As illustrated, nitrate removal times were different for each reactor. R1 consumed nitrate much more quickly than R6 and R11. Nitrate added in R1 was reduced completely within 287 h, while R6 and R11 required 455 h and 1127 h, respectively for complete removal. Nitrate concentrations in samples from three ports of R6 and R11 decreased gradually over time, and no apparent vertical differences were detected in either reactor. In contrast, vertical differences in nitrate reduction rates along the depth of R1 were observed. The bottom layer and the middle layer were much more effective in nitrate removal than the top layer. The bottom and middle-layers depleted the entire $1000\text{ mg NO}_3^-\text{-NL}^{-1}$ addition within 119 h. Moreover, nitrate removal efficiencies in the bottom and middle layers were as high as 66.6% and 60.0%, respectively, in the first hour.

Fig. 5 shows the nitrite concentrations in R1, R6 and R11. Nitrite accumulation was observed in R1 and R6 initially and then the concentrations decreased. However, nitrite concentrations detected in R11 were less than 2 mg L^{-1} during the entire experiment.

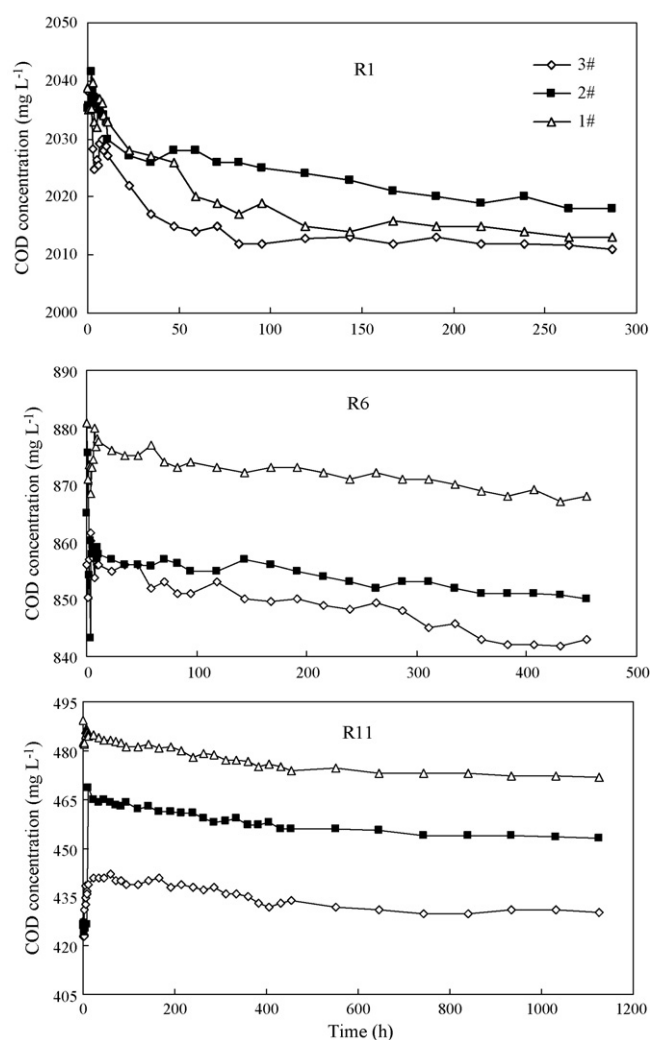


Fig. 2. The variations of COD in R1, R6 and R11 at three sampling ports. 1#: bottom port (Δ); 2#: middle port (\blacksquare); 3#: top port (\diamond).

Ammonia concentrations in R1, R6 and R11 remained relatively stable (Fig. 6). Ammonia concentrations in R1 and R6 were near 90 mg L^{-1} , while in R11 they were less than 50 mg L^{-1} .

3.3. Gas composition

Gas compositions of the reactors were measured as a percentage by volume and data are presented in Fig. 7. N_2 and N_2O concentrations from R1, R6 and R11 differed significantly. At the end of the reaction time, N_2 concentrations reached up to 82.5% and 80.2% in R1 and R6, respectively, while it reduced from initial 79.1% to 71.9% in R11. N_2O concentration in R11 followed an increasing trend and the final concentration was 19.3%. While for R1 and R6, N_2O was detected at low level. CH_4 concentrations from these three reactors were quite low (less than 0.2%), especially in R6 and R11.

4. Discussion

The nitrate consumption data suggest that the reactors all had the capacity to deplete nitrate (Fig. 4). As illustrated, R1 showed a considerably higher nitrate reduction rate than R6 and R11. This could be explained by the fact that the content of organic matter in R1 was higher than that of R6 and R11 (Fig. 2 and Table 1). It is well known that organic matter, which acts as an electron donor, plays an important role in nitrate reduction [21] and the absence

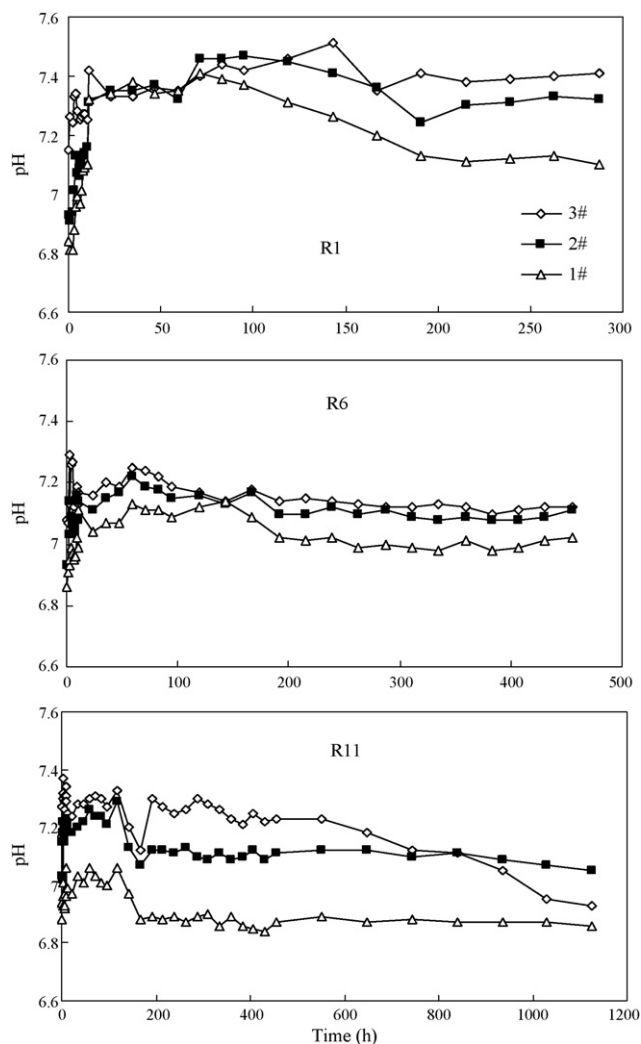
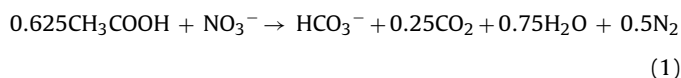
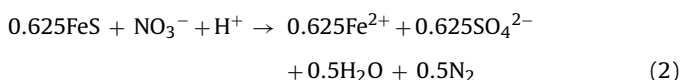


Fig. 3. The variations of pH in R1, R6 and R11 at three sampling ports. 1#: bottom port (Δ); 2#: middle port (\blacksquare); 3#: top port (\diamond).

of easily biodegradable organic carbon could delay denitrification. The experiment performed by Vigneron et al. [11] indicated that the reactors with high concentration of easily biodegradable carbon reduced nitrate more quickly than those with low organic matter content. It was also observed that leachate COD concentrations declined in R1 but not significantly, which implies that heterotrophic denitrification and autotrophic denitrification both occurred in R1. Heterotrophic denitrification will occur when an organic carbon source is available, for example, acetate used as an electron donor follows Reaction 1 [11,22].



In contrast, autotrophic denitrification will occur in environments with low organic carbon source [11,23]. Reaction 2 represents an example of autotrophic denitrification [22].



Furthermore, COD in R6 and R11 remained relatively constant, suggesting autotrophic denitrification occurred as the main pathway for nitrate consumption. Reaction 2 shows that sulphate is released when nitrate is reduced. Research by Giannis et al. showed that a decrease in the nitrate concentration was coupled with an

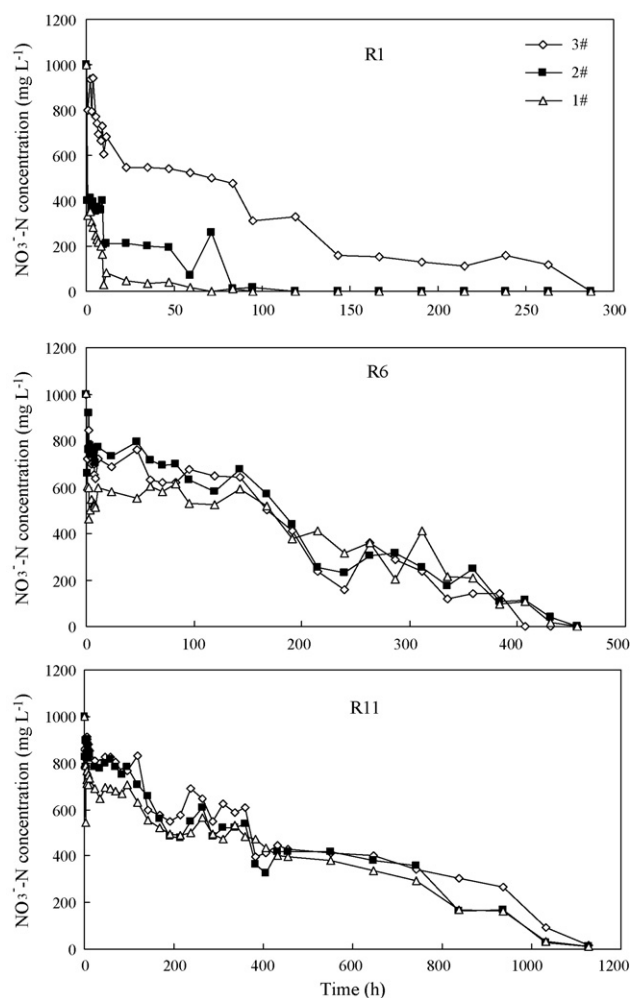


Fig. 4. The variations of NO₃⁻-N in R1, R6 and R11 at three sampling ports. 1#: bottom port (Δ); 2#: middle port (\blacksquare); 3#: top port (\diamond).

increase in the sulphate concentration in the experiment, suggesting autotrophic denitrification [24]. Unfortunately, we did not measure sulphate concentration.

In order to compare the denitrification capacity in each reactor more clearly, nitrate reduction rates were calculated assuming zero-order removal. Rates were determined by linear regression of nitrate concentration as a function of time, from time 0 to the point at which nitrate concentration was nondetectable. Since regression coefficients exceeded 95%, the assumption of zero-order was confirmed. Denitrification rates in R1, R6 and R11 were 6.80 mg NO₃⁻-N kg-TS_{waste}⁻¹ h⁻¹, 3.00 mg NO₃⁻-N kg-TS_{waste}⁻¹ h⁻¹ and 1.10 mg NO₃⁻-N kg-TS_{waste}⁻¹ h⁻¹, respectively. Obviously, R1 had the highest nitrate reduction rates which consumed nitrate more quickly than R6 and R11. Vertical differences in nitrate concentrations detected in R1 might be explained by leachate shortcircuiting. During sampling R1, biogas was sometimes released from the top port prior to leachate flow. This suggested that biogas was accumulating in the top layer and preventing liquid from contacting the refuse in this layer, which resulted in different nitrate reduction rates among refuse layers in R1.

Nitrite is an intermediate product of denitrification. Unlike R11, nitrite accumulated in R1 and R6 (Fig. 5). Betlach and Tiedje [25] stated that substrate concentration was the most important factor influencing the nitrite reduction rate. Nitrite reductase enzyme activity is not stimulated until nitrite is produced; therefore the

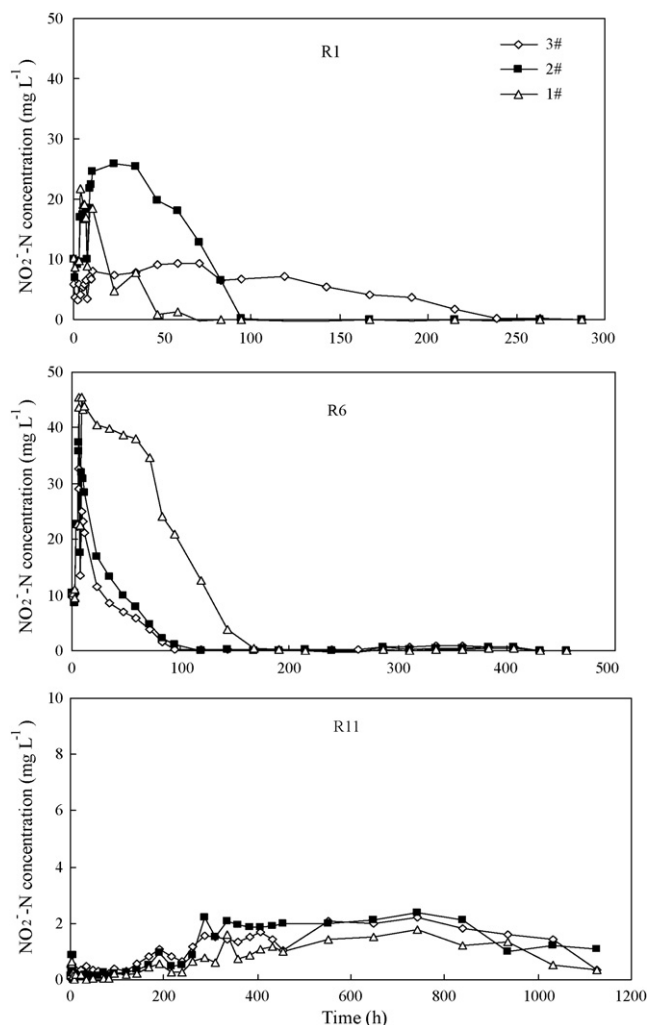


Fig. 5. The variations of NO_2^- -N in R1, R6 and R11 at three sampling ports. 1#: bottom port (Δ); 2#: middle port (\blacksquare); 3#: top port (\diamond).

activity was low at the beginning of the study leading to nitrite accumulation in R1. Additionally, nitrite accumulation has been reported during refuse hydrolysis [11]. Refuse placed in R1 was only partly degraded and hydrolysis might still dominate in the reactor. As nitrite concentration increased in R1, nitrite reductase enzyme activity was enhanced; therefore, nitrite was detected at low levels in R1 ultimately. As shown in Fig. 4, the curves of nitrate concentrations in R11 changed gradually, which would likely result in balanced nitrogenous compound conversion, and nitrite did not accumulate. However, there is no reasonable explanation for the nitrite changes over time in R6 which were similar to R1.

The observed pH trend in R1 (initial increase, then decrease) might be attributed to denitrification occurring in the reactor, which consumed H^+ and led to an increase in pH. The rapid initial increase of pH corresponded with a high nitrate reduction rate. With the depletion of nitrate, nitrate reduction would decline, possibly resulting in the accumulation of carboxylic acids prior to re-initiation of methanogenesis, and a slight decrease in pH of R1 [9,13]. In contrast, the slow nitrate reduction rates could be the primary cause of the stability of pH in R6 and R11. This might also indicate that the refuse in these two reactors had a greater pH buffering capacity.

In the experiment, the behavior of N_2 in R1 and R6 and the presence of N_2O in R11 supported the fact that denitrification occurred. Both gas composition and volume were measured during the study.

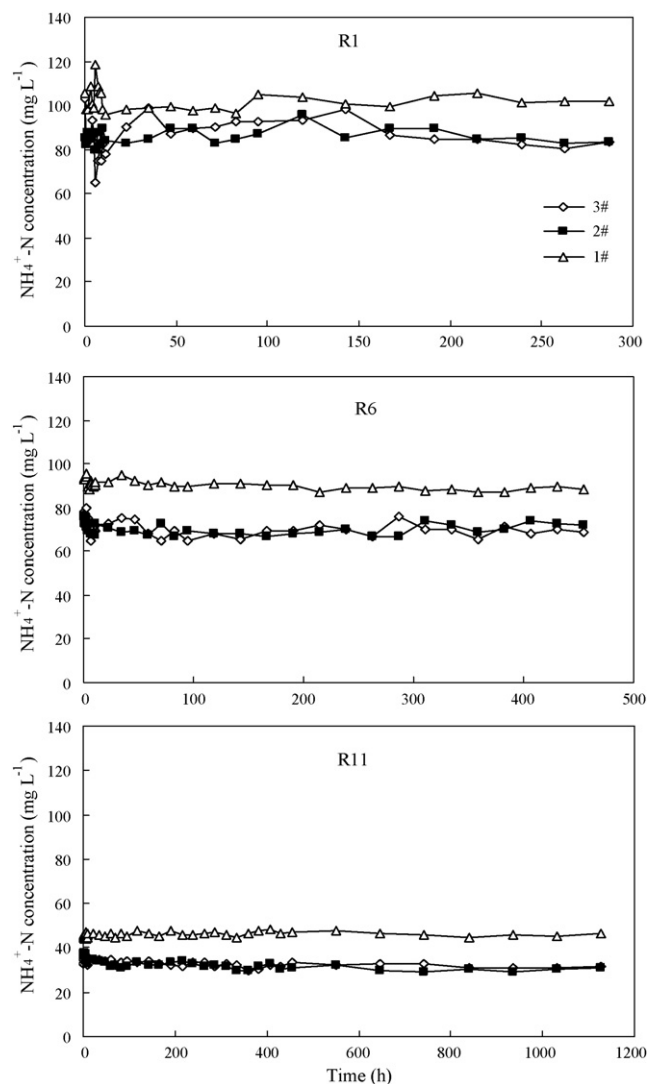


Fig. 6. The variations of NH_4^+ -N in R1, R6 and R11 at three sampling ports. 1#: bottom port (Δ); 2#: middle port (\blacksquare); 3#: top port (\diamond).

Gas bags were used to collect the released gas and the volume was measured by a water displacement technique. Unfortunately, due to operation error, gas volume data were lost. However, visual observation suggested that gas volumes of the three reactors were approximately the same, so it is reasonable to compare reactor behavior by the gas constituent concentrations. Generally speaking, N_2 is a final product during the process of denitrification, however, N_2O sometimes accumulated, as observed in R11. Hong et al. [26] reported that a low C/N ratio could result in N_2O production, therefore it might be inferred that the ratio of C/N in R11 promoted N_2O production. Price et al. [9] also found N_2O , although the amount was small. N_2O is a potent greenhouse gas with high ozone depletion potential, therefore it is important to minimize its emission. Given the accumulation of N_2O in R11, it can be concluded that use of aged refuse as denitrification medium is not suitable. N_2 concentration in R6 was lower than that of R1. This might be explained by the fact that other gases such as NO, which was not measured, accumulated in R6 due to chemical denitrification [11,22,27,28].

The low methane concentrations in all reactors suggest that methanogenesis was significantly inhibited by the high nitrate addition. Previous studies have proved the inhibitory effect of nitrate on methanogenesis because of intermediates such as NO and N_2O produced during denitrification [8,9,13,29,30]. However,

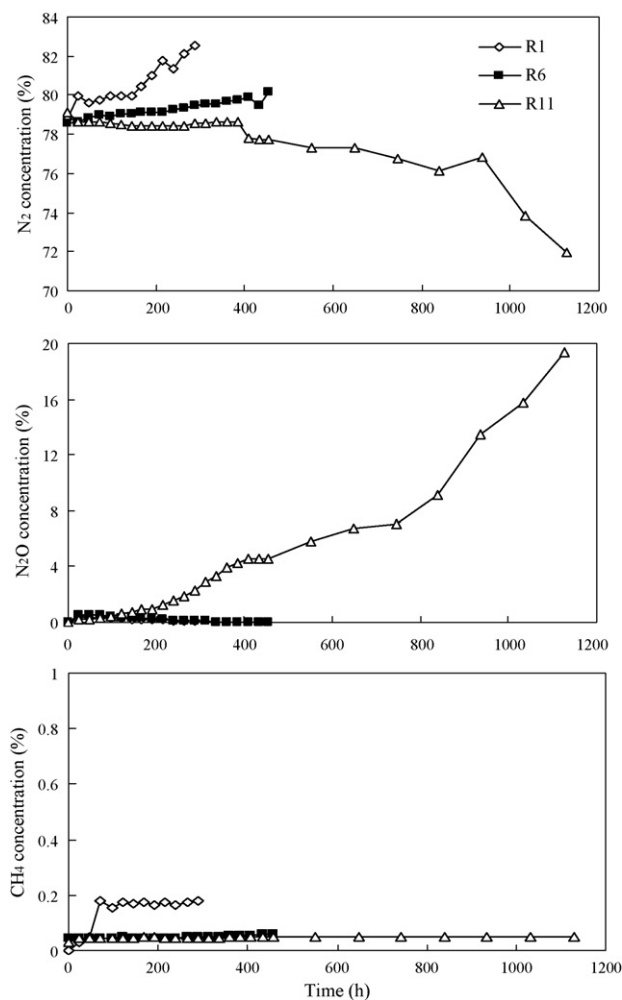


Fig. 7. The variations of N₂, N₂O, CH₄ in R1, R6 and R11.

methanogens are typically inhibited but not killed and methanogenesis will recover after nitrate depletion [9]. One exception was detected in the experiment made by Jokela et al. [14] where denitrification did not delay methanogenesis and methane was produced simultaneously with denitrification. They hypothesized that the added initial nitrate load was lower than in other studies. Nevertheless, the inhibitory effect of nitrate on methanogenesis was considerably lower even after nitrate and nitrite were depleted. Moreover, CH₄ production has also been related to refuse characteristics and COD concentration [15]. As shown in Table 1, 6-year-old refuse and 11-year-old refuse had low BDM contents. The BDM is widely used to analyze the content of biologically degradable matter in the refuse and it decreases rapidly with the decomposition of organic matter. So the BDM data in Table 1 suggested that refuse placed in R6 and R11 was well decomposed. In addition, the COD concentrations in both reactors were low compared to R1 (Fig. 2) leading to low CH₄ yield in R6 and R11.

As illustrated in Fig. 6, ammonia removal was not observed in the reactors. Therefore, it could be deduced that adsorption capacity of the refuse was satisfied [23,31]. Previous studies have reported anaerobic ammonia oxidation (Anammox) in oxygen-limited systems, where ammonium is converted to dinitrogen gas with nitrite as the electron acceptor [32]. However, during the denitrification process, ammonia concentrations measured in the leachate did not decrease significantly, so it appears that Anammox did not occur in these reactors. Due to low oxygen concentration, nitrification, the

main path for ammonia removal, will not occur. The reactors all had relatively low ammonia concentrations, which was attributed to refuse washing. Nevertheless, R11 had the lowest ammonia concentration, which may be mainly related to the flushing over time in the landfill. In addition, refuse in R11 was mature, and it probably has a strong capacity for ammonia adsorption because it contains a lot of humic matter (Table 1) [15]. This might be another reason that R11 possessed a lower ammonia concentration.

5. Conclusions

The study suggested that refuse at all landfill ages have a large denitrification capacity. R1 possessed a higher reaction rate for conversion of nitrate to gas than R6 and R11. Nitrate added in R1 was completely consumed within 287 h. Moreover, vertical differences in nitrate reduction rates were observed only in R1. The bottom and middle-layers depleted nitrate much more quickly. It was found that N₂O accumulated in R11, presumably due to the low ratio of refuse C/N, while R1 produced a higher concentration of N₂. Therefore, 1-year-old refuse, which represented partly degraded refuse, was more suitable to use as denitrification medium. Field-scale studies should be conducted in the future to confirm these results.

Acknowledgements

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