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Nitrogen management in bioreactor landfills

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Abstract

One scenario for long-term nitrogen management in landfills is ex situ nitrification followed by denitrification in the landfill. The objective of this research was to measure the denitrification potential of actively decomposing and well decomposed refuse. A series of 10-l reactors that were actively producing methane were fed 400 mg NO_3-N /l every 48 h for periods of 19–59 days. Up to 29 nitrate additions were either completely or largely depleted within 48 h of addition and the denitrification reactions did not adversely affect the leachate pH. Nitrate did inhibit methane production, but the reactors recovered their methane-producing activity with the termination of nitrate addition. In well decomposed refuse, the nitrate consumption rate was reduced but was easily stimulated by the addition of either acetate or an overlayer of fresh refuse. Addition of acetate at five times the amount required to reduce nitrate did not lead to the production of NH_4^+ by dissimilatory nitrate reduction. The most probable number of denitrifying bacteria decreased by about five orders of magnitude during refuse decomposition in a reactor that did not receive nitrate. However, rapid denitrification commenced immediately with nitrate addition. This study shows that the use of a landfill as a bioreactor for the conversion of nitrate to a harmless byproduct, nitrogen gas, is technically viable.

1. Introduction

In 2000, approximately 55% of the municipal solid waste (MSW) generated in the United States was disposed of in landfills (US EPA, 2002). While yard waste composting and recycling play prominent roles in many local waste management strategies, landfills will continue to play a significant role in MSW management in the US for the foreseeable future. Recently, there has been increased emphasis on the operation of landfills as bioreactors to enhance decomposition (Mehta et al., 2002; Pohland and Kim, 2000; Pacey et al., 1999). There are many advantages to the operation of landfills as bioreactors including: (1) settlement before placement of the final cover which decreases the risk of damage to the final cover, (2) increased effective refuse density and landfill capacity, (3) in situ leachate treatment, (4) increased rates of gas production which may make energy recovery more favorable, (5) the potential for additional revenue for commercial liquid waste disposal and (6) acceleration of refuse decomposition which may shorten the regulated post-closure monitoring period and thereby reduce the overall cost of the landfill (Reinhart et al., 2002; Barlaz et al., 1990).

Recently, landfill owners and regulators have begun to consider in more detail strategies for the long-term management of landfills after closure and one consideration is leachate quality (Barlaz et al., 2002). The biological oxygen demand (BOD) and chemical oxygen demand (COD) of landfill leachate typically decrease substantially as refuse decomposes, and ultimately the remaining organic matter in leachate from well decomposed refuse is largely humic matter (Kjeldsen et al., 2003). However, MSW has been estimated to contain about 4% protein and therefore, ammonia (NH_3–N) is produced during the decomposition of organic nitrogen (Madigan et al., 1997; Barlaz et al., 1990). Because ammonia is stable under anaerobic conditions, it typically accumulates in leachate (Burton and Watson-Craik, 1998). Thus, high concentrations of ammonia persist long after the BOD and COD have decreased to concentrations representative of well-decomposed

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refuse, and the treatment of leachate to remove ammonia is an important aspect of long-term landfill management. For example, in assessing when post-closure monitoring can be reduced or discontinued, one proposed criterion is to evaluate whether the landfill presents a risk to human health and the environment under worst-case conditions (Barlaz et al., 2002). Such conditions include the environmental impact of an ammonia discharge to surface water or groundwater.

One scenario for ammonia management is ex situ treatment. There has been considerable research on the biological treatment of ammonia-rich leachate (Carley and Mavinic, 1991; Borzacconi et al., 1999; Pelkonen et al., 1999). Ex situ treatment systems generally involve both nitrifying and denitrifying reactors. Another scenario for ammonia removal is ex situ nitrification followed by the use of the landfill as an anaerobic bioreactor for denitrification, the conversion of NO_3–N to N_2 gas, a harmless byproduct. This scenario is currently under evaluation at a field-scale bioreactor in Louisville, KY, USA.

Onay and Pohland (1998) simulated a series of landfill cells operated under methane producing, nitrifying, and denitrifying environments. Leachate concentrations of 920–1400 mg NO_3^-/l were generated in a refuse reactor that was supplied with air and the leachate was cycled to a denitrification reactor where NO_3^- removal ranged from 91 to 93%. They concluded that the use of leachate recirculation in simulated landfill bioreactors was feasible for the in situ removal of NH_3–N. Burton and Watson-Craik (1998) reported that nitrate concentrations were not detectable 6 days after adding 500 and 1000 mg NO_3–N/l to batch reactors filled with 1-month-old refuse. In further work, 1000 mg NO_3–N/l was added to methanogenic refuse and methane production was inhibited for 20 days after which it recovered.

The objective of this research was to develop an understanding of the nitrogen cycle in landfills and to further evaluate the efficacy of using landfills for denitrification. Specific objectives were to (1) evaluate whether NO_3–N could be added to refuse in various states of decomposition without adversely affecting the ability of the refuse to resume methanogenesis after nitrate depletion, (2) determine whether denitrification reactions result in an inhibitory pH increase, (3) evaluate whether nitrate addition to landfills could lead to the production of NH_3–N in place of N_2 by dissimilatory nitrate reduction to ammonium, and (4) identify the types of organic carbon required to support denitrification in landfills.

1.1. Background

Denitrification is the dissimilatory microbial process of reducing NO_3^- and NO_2^- to N_2O and N_2. It is performed by aerobic bacteria that are capable of growth with NO_3^- and NO_2^- as electron acceptors (Tiedje, 1988). Denitrification is inhibited by the presence of oxygen and is therefore limited to anoxic environments.

Nitrate reduction can occur by the following reactions:

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O \qquad \Delta G^0 = -1197.0\,\text{kJ} \tag{1}$$

$$NO_3^- + 10H^+ + 8e^- \rightarrow NH_4^+ + 3H_2O \qquad \Delta G^0 = -679.6\,\text{kJ} \tag{2}$$

Eq. (1) represents respiratory denitrification while Eq. (2) represents dissimilatory nitrate reduction to ammonium (DNRA). Nitrite is an intermediate in both reactions and N_2O, which is significant for its contribution to atmospheric climate change, is an intermediate in reaction 1. When the degradable organic carbon/nitrate level is high, microbes are electron acceptor limited and reaction 2 prevails which would be counterproductive in a landfill. In contrast, when the degradable carbon/nitrate level is low, the microbes are carbon limited and reaction 1 prevails as is desired in a landfill. DNRA has been reported to predominate in anaerobic sludge digesters, anoxic sediments, and the rumen, all of which are carbon-rich, nitrate poor environments (Tiedje, 1988). Finally, note that H^+ ions are consumed in both reactions and a pH increase during denitrification has been reported (Burton and Watson-Craik, 1999).

2. Materials and methods

2.1. Experimental design

Eleven reactors were initiated to evaluate the extent of nitrate consumption and the effect of nitrate addition on methane production, leachate pH, and N_2O production. Nine reactors (1–9) were filled with a mixture of fresh and well decomposed refuse. The decomposed refuse served as a seed to initiate methane production. Two reactors (10–11) were filled only with decomposed refuse to measure its contribution to the methane production measured in reactors 1–9. Reactors 1–9 were operated until they reached an approximate peak in methane production at which time nitrate was added to eight of the nine reactors (1–6, 8–9) while one reactor (7) served as the control. Duplicate reactors received nitrate additions for periods of 19, 35, 45, and 59 days to evaluate the effect of the duration of nitrate dose on methane production (Table 1). This first set of nitrate additions will be referred to as Phase 1.

In Phase 1, sufficient nitrate as KNO_3, $NaNO_3$ or $Mg(NO_3)_2$ was added to reactors 1–6, 8, and 9 every

Table 1
Schedule of Phase 1 nitrate additions

Reactor	Period of NO_3^- addition
1, 2	36–55
8, 9	36–71
3, 6	36–81
4, 5	36–95
7	59–67

other day to increase the leachate concentration by 400 mg NO_3–N/l based on the total volume of water present in each reactor. This concentration was selected to represent a hypothetical landfill in which leachate with 400 mg NH_3–N/l was nitrified and the resulting NO_3-N was returned to the landfill.

While Phase 1 experiments assessed nitrate depletion in actively decomposing refuse, the objective of Phase 2 was to measure nitrate consumption in well decomposed refuse. Nitrate (400 mg/l) was periodically added to three reactors (1, 6, 8) after their methane production rates had decreased substantially as illustrated with the results. To begin Phase 2, reactors were flushed with deionized water to remove ammonia prior to the nitrate additions. Initially, nitrate was added to the decomposed refuse to evaluate nitrate depletion. Next, acetate, which is readily degradable, was added with nitrate to evaluate whether ammonia would be produced by DNRA. In these tests, five times as much acetate as was required based on its stoichiometric oxidation under nitrate-reducing conditions was added. Once tests with acetate were complete, humic acids (Sigma Chem., St. Louis, MO) were added as a surrogate for leachate from well decomposed refuse to evaluate whether they can serve as an electron donor to support denitrification. The schedule for all nitrate, acetate, and humic acid additions is given with the results. Finally, the top 25% of the refuse in reactor 1 was removed and replaced with fresh refuse, which simulated the placement of a layer of fresh refuse over well decomposed refuse. Nitrate was added three times to evaluate whether the organic carbon from fresh refuse leachate could support denitrification.

Finally, one additional reactor (12) was initiated with fresh refuse only to study the survival of a denitrifying population. Refuse was periodically removed from reactor 12 for enumeration of the total anaerobic population and the population of denitrifying bacteria.

2.2. Materials

Fresh refuse was obtained from a vehicle that collected refuse from residential areas in Raleigh, NC. Approximately one ton of refuse was collected and shredded to less than 2×5 cm by using a slow-speed, high-torque shredder (Shredpax AZ-7H, Wood Dale, IL). The refuse was shredded twice and then sequentially quartered into about 30 piles. Piles were then randomly placed in plastic bags, transported back to the laboratory and stored at 4 °C for 1 day before use. Bags were chosen at random to fill reactors and each bag was emptied completely before a new bag was opened.

Decomposed residential refuse was used to seed reactors 1–9 to promote methane production. The seed was obtained from a 210-l reactor incubated at 37 °C in the laboratory. The refuse used to prepare the seed was obtained as described above.

2.3. Reactor construction and filling

Reactors were constructed from 10-l wide-mouth plastic containers modified for installation of leachate collection and recycling ports and a gas collection port as described previously (Rhew and Barlaz, 1995). Gas was collected in five-layer (polyester, polyvinylidene chloride, aluminum foil, polyamide, and high-density polyethylene) gas sampling bags (Calibrated Instruments, Inc., Hawthorne, NY) fitted with a straight through connection with a swage ring and a luer-fit valve (V-L/F-1). Gas samples were obtained by withdrawing a sample with a syringe through the luer valve. Leachate was collected in 2-l Viaflem bags (Baxter, Deerfield, IL) and manually recycled to the top of the reactor through tygon tubing.

To fill a reactor, fiberglass filter fabric and coarse stone were first placed over the outlet to prevent clogging. The fresh and decomposed refuse were mixed thoroughly before placement in reactors. After filling, reactors were sealed with silicone caulk and monitored for leaks. Then, deionized water was added to each reactor in a quantity sufficient to produce approximately 1 l of leachate. Reactors 1–9 each contained about 1.35 and 1.42 dry kg of fresh and decomposed refuse, respectively, and were loaded to a density of 536 kg/m^3.

2.4. Reactor operation

The reactors were operated with leachate recycle and neutralization throughout the study to enhance decomposition and to facilitate exposure of the reactor contents to nitrate. Leachate was drained from each reactor daily, neutralized to approximately pH 7 with a 1 N NaOH solution until the leachate pH remained above 6.8 and recycled. The reactors were incubated at 37 °C. These enhancement techniques minimize the acid phase and rapidly initiate methane production from fresh refuse. When chemical additions were made to a reactor (nitrate, acetate, humic acids), a concentrated solution was added to the leachate collection bag after which leachate was recirculated 3–4 times over 15–20 min to distribute the additions throughout the reactor liquid phase.

2.5. Analytical methods

Gas concentrations (CO_2, O_2, N_2, CH_4) were measured by using a GOW-MAC gas chromatograph (GC) (Bound Brook, NJ) equipped with a thermal conductivity detector and a CTR1 column (Alltech, Deerfield, IL). The injector and thermal conductivity detector temperatures were 28 and 75 °C, respectively. The carrier gas was He at 50 ml/min. Nitrous oxide (N_2O) concentrations were measured by using a Hewlett-Packard 5890 GC equipped with a ^{63}Ni electron capture detector and a GS-CarbonPLOT capillary column (J&W Scientific, Folsom, CA). The inlet and detector temperatures were 225 and 300 °C, respectively. The oven was maintained at 60 °C and the carrier gas was He at 3 ml/min. To eliminate the interference of CO_2 with the N_2O analysis, gas samples were first placed in a 5-ml vacutainer filled with 0.3-ml of 4.25 N NaOH. This sample volume was sufficient to have 3-ml available for sampling after CO_2 dissolution. N_2O concentrations were corrected back to the initial gas volume at standard temperature and pressure (STP).

To measure gas volume, gas was pumped (Welch Vacuum, Skokie, IL) from the luer valve of the gas bag to an airtight 50-l calibrated carboy with holes in the bottom. The carboy was partially submerged in acidic water (pH 2). The gas volume was determined by the volume of water displaced in the carboy. Gas volume measurements were corrected to STP.

Leachate was monitored for pH, NH_4^+, NO_3^-, SO_4^{-2}, COD, BOD, and dissolved metals. Ammonia concentrations were measured with an electrode (Orion, model 95-12) using the known addition method (American Public Health Association, 1995). Preliminary work was conducted to verify that the probe results were consistent with a wet chemistry technique and were unaffected by the presence of humic acids or metals (Price, 2001). Nitrate, phosphate, and sulfate concentrations were measured by ion chromatography (IC) with a Dionex isocratic pump and a Dionex AS4A column. A 1.8-mM carbonate/1.7-mM bicarbonate buffer served as the mobile phase. COD was measured using a Hach Kit (Hach Co. Loveland, CO). BOD was measured as described in Standard Methods (American Public Health Association, 1995). Dissolved metal concentrations in leachate were measured by inductively coupled plasma atomic emission spectroscopy (ICP–AES) using a Perkin-Elmer Optima 2000DV. The total anaerobic population and the population of denitrifying bacteria were enumerated by the most probable number (MPN) technique (Barlaz et al., 1989; Tiedje, 1994). The procedures for cellulose, hemicellulose, and lignin have been described previously (Mehta et al., 2002).

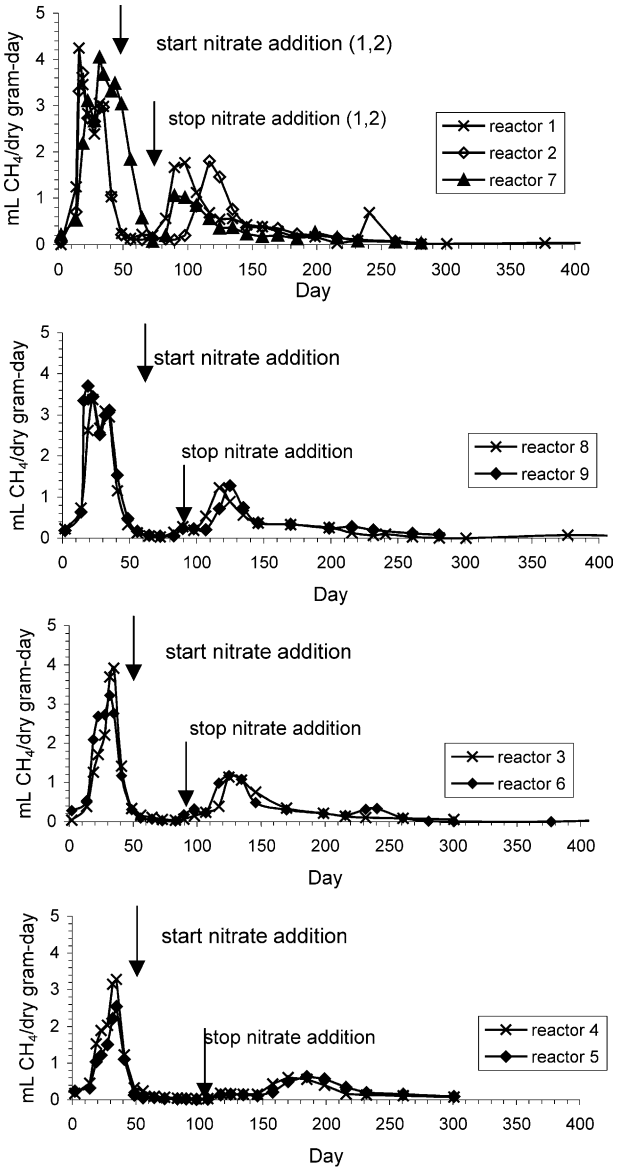

Fig. 1. Effect of nitrate addition on methane production. Nitrate was mistakenly added to the control reactor (7) on days 59–67. Gas production rates are per gram of dry fresh refuse.

3. Results and discussion

The results of nitrate addition during active methane production are presented first, followed by presentation of the results of nitrate addition to well-decomposed refuse. Finally, the development and survival of the total anaerobic population and the population of denitrifying bacteria during decomposition is discussed.

3.1. Phase 1: Nitrate addition during active methane production

3.1.1. Methane production

Methane production rate data for reactors 1–9 are presented in Fig. 1, and cumulative methane yields are presented in Table 2. The methane production rate

Table 2
Cumulative methane yields and methane production recovery times

Reactor[a]	Cumulative yield through Phase 1[b,c] (l CH_4/dry kg)	Cumulative yield through Phases 1 and 2[b] (l CH_4/dry kg)	Methane production recovery time (days)
1	148.3	160.3[d]	28
2	131.4	138.1	52
8	117.5	130.2[d]	36
9	120.8	133.8	46
3	102.1	110.8	44
6	110.2	127.3[d]	36
4	83.0	94.1	63
5	61.7	78.8	63
7	165.5	171.6	23

[a] Reactors are grouped based on the nitrate addition schedule.
[b] Gas production rates are per kg of dry fresh refuse and are corrected for methane production attributable to the seed.
[c] Phase 1 is through day 199.
[d] Yield includes 9.6, 10.8, and 3.8 l/kg of measured methane in reactors 1, 6, and 8, respectively, that can be attributed to the methane potential of the acetate added in excess of the stoichiometric amount of NO_3.

decreased sharply in all reactors shortly after the nitrate addition regiment began on day 36. A more gradual decrease in methane production was observed in reactor 7, which did not receive nitrate until erroneous additions between days 59 and 67. The inhibition of methane in the presence of nitrate was expected and indicates that the bulk of the refuse was exposed to nitrate. In every case, methane production rates recovered once nitrate addition was terminated (Table 2), suggesting that the methanogen population was inhibited but not killed by the high nitrate addition.

3.1.2. Nitrate conversion

Nitrate depletion data for each reactor are presented in Fig. 2 and overall nitrate conversion data are presented in Table 3. As illustrated, the added nitrate was typically consumed within 48 h. However, after 14–26 nitrate additions, some reactors lost their ability to deplete the entire 400 mg NO_3–N/l addition within two days. As discussed below, reduced nitrate depletion corresponded to a reduction in leachate COD. Nitrate was always added to increase the leachate concentration by 400 mg/l, so at times the refuse was exposed to concentrations greater than 400 mg/l.

The rate of nitrogen production mirrored that of methane production and data for selected reactors are presented in Fig. 3a. N_2 production rates increased with the addition of nitrate and then decreased with the termination of nitrate addition. A similar trend was observed for N_2O, which was barely detected prior to the nitrate addition (Fig. 3b). The fraction of the added nitrate converted to N_2 ranged from 89.5 to 101.2% while recovery of nitrate as N_2 plus N_2O ranged from 95.2 to 106.2% (Table 3). The nitrate mass balance is the result of multiple measurements of gas concentration and volume and the range of mass balance recoveries, some slightly above and some slightly below 100% can be attributed to experimental error. NO, which was not measured, is a minor product of denitrification (Tiedje, 1988).

3.1.3. pH and COD

pH data for selected reactors are presented in Fig. 3c. The leachate pH was externally neutralized for the first 14–28 days of the study. The pH of the reactors that started receiving nitrate on day 36 was generally higher than the pH of the control reactor (7). The pH of all reactors decreased shortly after their final nitrate addition and then increased once methane production recovered (Price, 2001). In no case did the reactor pH exceed 8.

Table 3
Cumulative nitrogen addition and recovery during Phase 1 and 2 nitrate additions[a]

Reactor	NO_3–N added (mol)	Nitrate recovered as		Total recovery of added nitrate (%)
		N_2 (%)	N_2O (%)	
Phase 1				
1	1.67	100.1	6.1	106.2
2	1.66	99.7	4.3	104.1
8	2.95	89.5	5.7	95.2
9	2.92	100.4	5.2	105.6
3	3.74	91.8	5.9	97.7
6	3.67	98.9	3.3	102.2
4	5.00	94.1	2.7	96.8
5	4.98	101.2	1.7	102.9
7[b]	0.66	98.9	3.5	102.4
Phase 2				
1	1.21	82.4	1.5	83.9
6	2.23	91.3	2.5	93.8
8	2.10	89.4	0.8	90.2

[a] Reactors are grouped based on the nitrate addition schedule.
[b] Nitrate was mistakenly added to reactor 7 on days 59–67.

Leachate COD data for selected reactors are presented in Fig. 3d. COD concentrations consistently decreased during active methane production and throughout the nitrate additions. The brief increase in leachate COD concentrations after the final nitrate addition is discussed in the following section. This trend was observed in all reactors except 4 and 5 for which insufficient data were collected (Price, 2001).

3.1.4. Discussion of denitrification during active methane production (Phase 1)

The inhibition of methane production in reactors that received nitrate was expected. While initial reports attributed inhibition to the rise in redox potential caused by the addition of nitrate (Bollag and Czlonkowski, 1973), more recent reports attribute inhibition to toxic denitrification intermediates (NO_2^-, NO, N_2O)

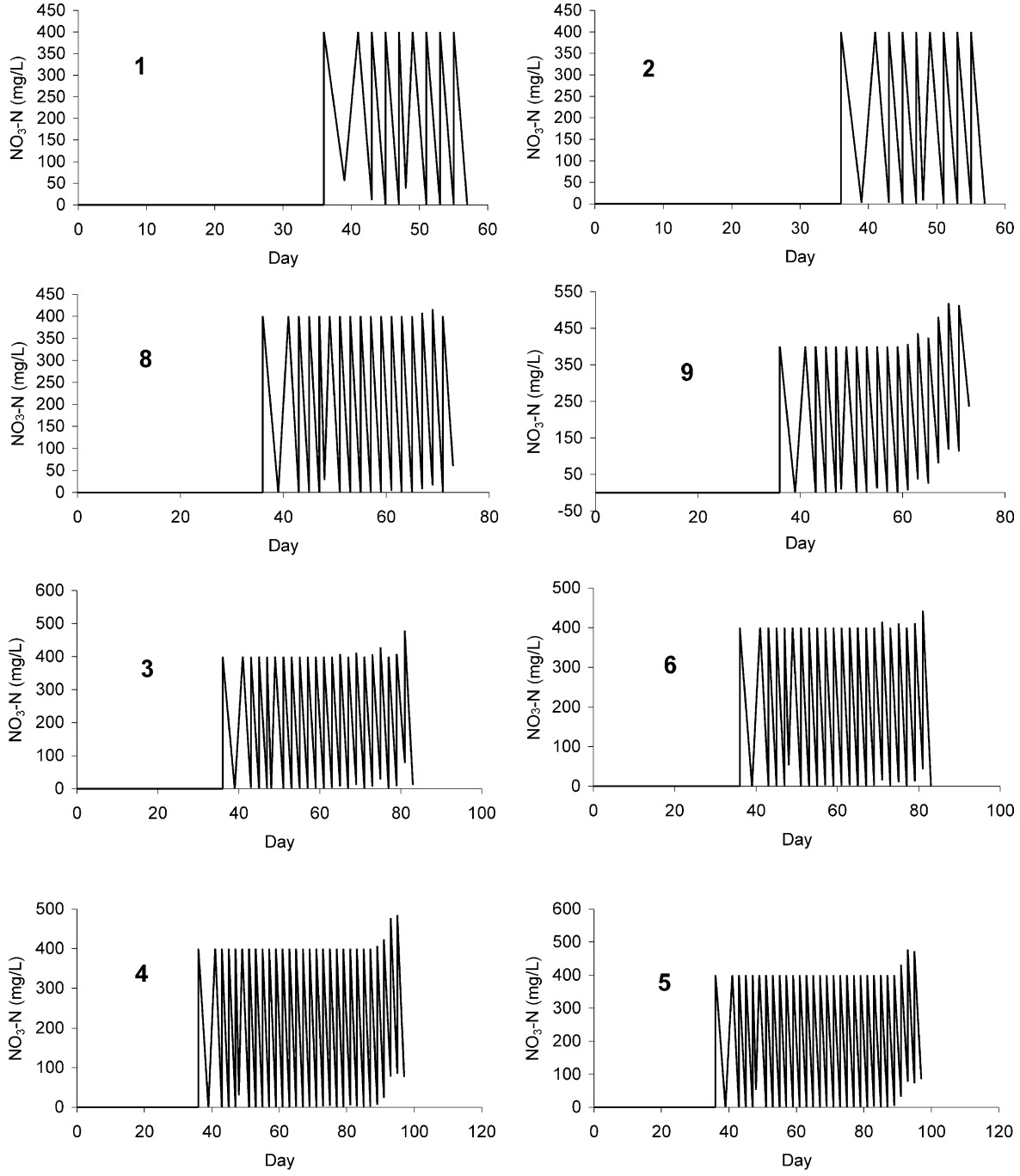

Fig. 2. Nitrate concentrations during Phase 1 (note variation in *x*-axis). The 400 mg NO_3–N/l concentrations were calculated based on the volume of water in each reactor and the mass of nitrate added. Plots are ordered sequentially by the duration of the nitrate addition.

(Roy and Conrad, 1999). The methane production recovery times suggest that methanogens were inhibited but not killed by the high nitrate additions (Table 2). The Phase 1 methane yields were inversely proportional to the mass of nitrate added, which is consistent with the diversion of electrons from CO_2 reduction to NO_3 reduction.

The nitrate depletion data suggest that the reactors had the capacity to deplete nitrate (Fig. 2). However, nitrate consumption rates did decrease such that not all nitrate was consumed in 48 h. As illustrated in Fig. 2, nitrate depletion slowed in reactors 3, 4, 5, 6, 8, and 9 on days 79, 91, 91, 79, 71, and 65, respectively. In each case, this day corresponds to the lowest measured COD concentration in the reactor (Fig. 3d, Price, 2001). The decrease in available organic carbon would explain why the nitrate depletion rate was reduced. However, there is no apparent explanation for the variation in the time of the lowest leachate COD concentration. Similarly, there was not a correlation between the mass of nitrate added and the methane production recovery time (Tables 2 and 3). One explanation for this is that the volume of methane production, which was used to calculate the methane production rate, was measured as gas bags approached capacity. The time interval between volume measurements varied from 2 to 10 days. As such, the magnitude of changes in methane production rates may have been dampened by the gas volume measurement frequency.

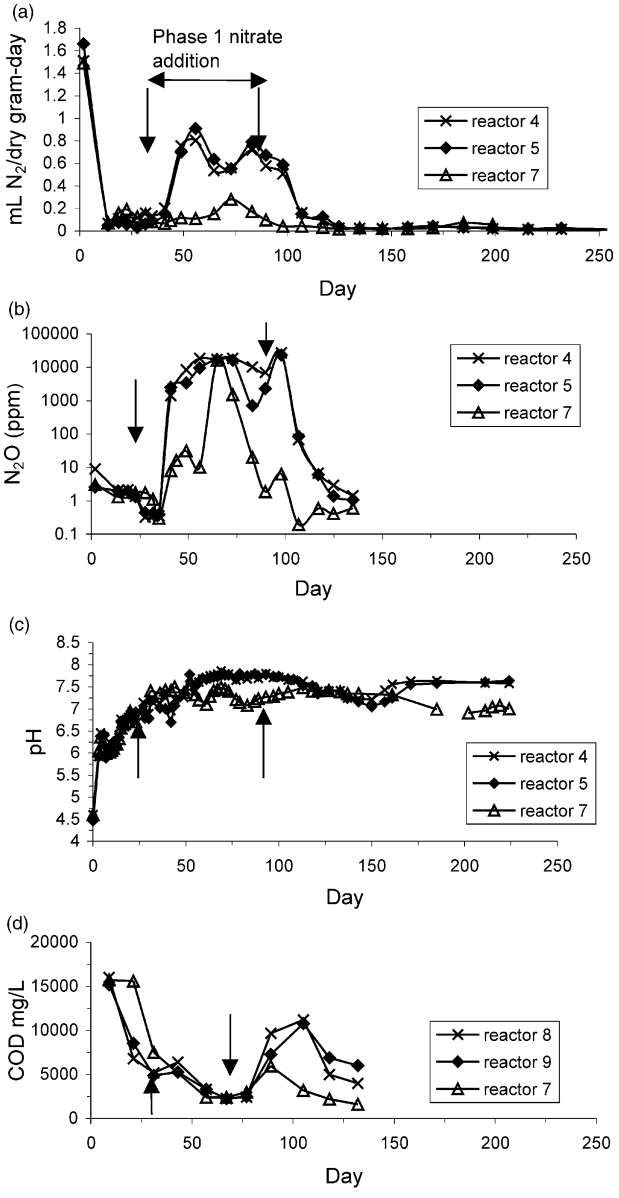

Fig. 3. Performance in Reactors: (a) Nitrogen production rates, (b) N_2O concentrations, (c) pH and (d) COD. Arrows represent the initial and final Phase 1 nitrate addition. Nitrate was mistakenly added to reactor 7 on days 59–67.

The N_2 production rate increased with the addition of nitrate and decreased with the termination of nitrate addition. In addition, essentially all of the added nitrate was recovered as N_2. These results are consistent with denitrification as represented by Eq. (1) and suggest that denitrification was the means by which nitrate was consumed.

The pH increased in the reactors during nitrate additions. These results are consistent with the stoichiometry of Eq. (1). While the pH increased above levels reported as optimal, significant methane production has been reported in laboratory simulations of refuse decomposition at pH 8 (Eleazer et al., 1997; Barlaz et al., 1990). Therefore, it does not appear that the pH increased to inhibitory levels. Interestingly, there was a pH decrease and COD increase in each reactor leachate a few days after their final nitrate addition. Just after the end of the nitrate addition phase, both methane-producing and nitrate-reducing activity could have been depressed as the methanogens were recovering from nitrate inhibition and the denitrifers were deprived of nitrate. This would likely result in the accumulation of carboxylic acids due to fermentation, which explains the pH decrease and COD increase. Once methane production recovered, the pH increased and COD concentrations again decreased.

3.2. Phase 2: Nitrate addition to well-decomposed refuse

The objective of Phase 2 was to evaluate the potential for denitrification in well-decomposed refuse. Reactors 1, 6, and 8 were selected for testing based on their methane production rate curves, which showed that they contained well-decomposed refuse on day 203 when Phase 2 nitrate additions began (Fig. 1).

Phase 2 nitrate depletion data for reactors 1, 6, and 8 are presented in Fig. 4. Nitrate depletion rates were slower than the rates measured in Phase 1. The addition of acetate at a concentration equal to five times the amount required to convert the added nitrate to N_2 stimulated nitrate depletion. For example, after acetate

addition, nitrate was depleted in 2 days in reactors 1, 6, and 8, while nitrate depletion required 5–15 days before acetate addition. As illustrated, nitrate was depleted rapidly several times after an acetate addition. This is likely because acetate was added in excess of the amount required for depletion of 400 mg NO_3–N/l.

Humic acids are likely a major contributor to the COD of leachate from well decomposed refuse. However, the addition of 400 mg humic acid/l to reactors 1 and 6 on days 262 and 329, respectively, did not stimulate nitrate depletion (Fig. 4). The failure of the humic acid to stimulate nitrate depletion is consistent with its measured BOD of 5 mg/l for an 800 mg/l humic acid solution. Once acetate was added to reactor 1 on day 282, the remaining nitrate was depleted within 48 h (Fig. 4). On day 440, the top 25% of the refuse in reactor 1 was removed and replaced with fresh refuse to simulate the burial of a lift of fresh refuse over well-decomposed refuse. Nitrate was then rapidly depleted within 24 h on three occasions (Fig. 5).

3.2.1. BOD and COD concentrations

BOD and COD data for reactors 1, 6, and 8 are presented in Fig. 6. During the first three Phase 2 nitrate additions, BODs ranged from 58 to 82, 316 to 362 mg/l, and 130 to 161 mg/l in reactors 1, 6, and 8, respectively. The corresponding CODs were 225–300, 675–750, and 625–800 mg/l, respectively. The addition of acetate resulted in significant increases in BOD and COD. Once the excess acetate was consumed, BOD and COD concentrations decreased to levels similar to those prior to acetate addition.

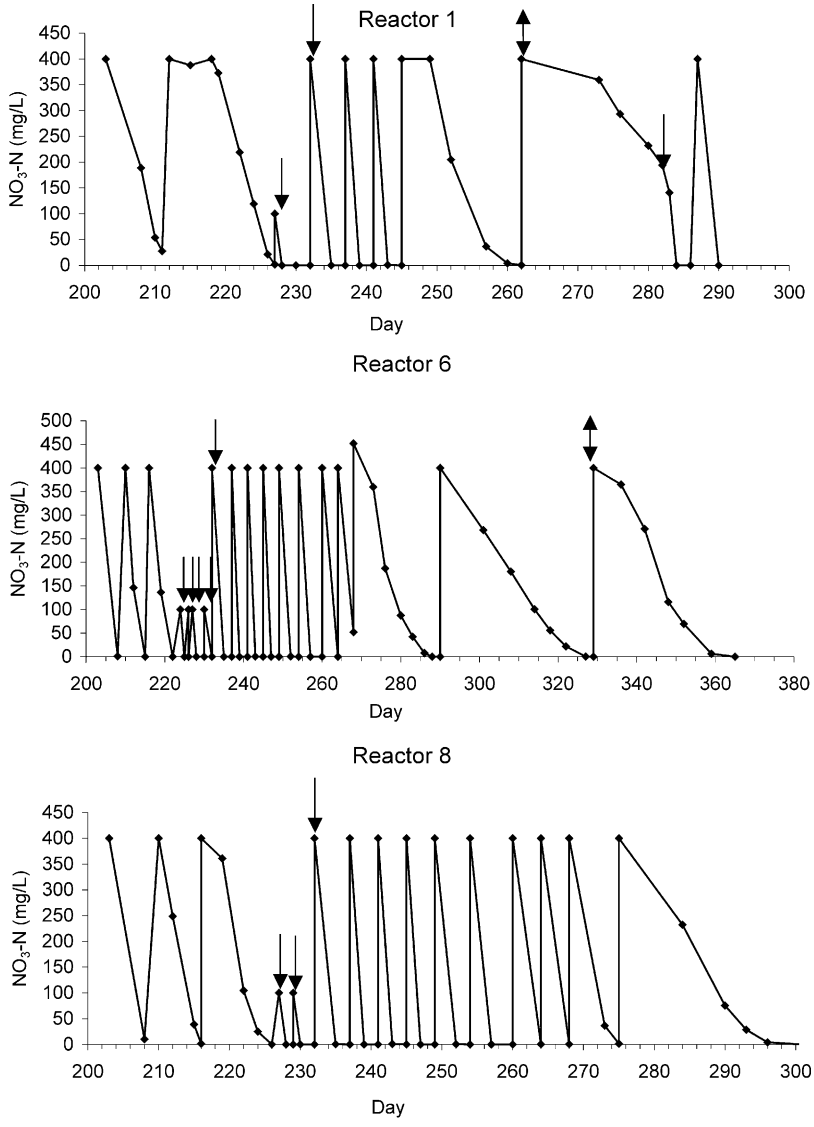

Fig. 4. Nitrate depletion during Phase 2 (Note variation in *x*-axis). The 400 mg NO_3–N/l concentrations were calculated based on the volume of water in each reactor and the mass of nitrate added. ↓ represents acetate addition and ↕ represents 400 mg/l humic acid addition. Each step increase in nitrate concentration represents a nitrate addition.

3.2.2. Methane and nitrogen production

Methane production rates decreased with the first three nitrate additions and subsequently increased with the addition of excess acetate (Fig. 7a). Reactor 1 had the most significant increase in methane production while reactor 8 did not show a significant increase in methane production after the acetate addition. The corresponding N_2 production rates are illustrated in Fig. 7b. These data show increases in N_2 production with the addition of nitrate. The failure of reactor 8 to show an increase in methane production is surprising. However, the increase in N_2 production (Fig. 7b) eliminates gas leakage as an explanation.

Cumulative N_2 and N_2O production data for Phase 2 are presented in Table 3. The fraction of added nitrate recovered as N_2 and N_2O ranged from 82.4 to 91.3 and 0.8 to 2.5%, respectively.

3.2.3. Ammonia production

To evaluate the potential for DNRA, six reactors (1, 2, 6, 7, 8, 9) were flushed repeatedly with DI water to remove dissolved NH_3–N. Nitrate and acetate were then added to three of the reactors (1, 6, 8) to simulate a high organic carbon/electron acceptor ratio, while the remaining three reactors (2, 7, 9) did not receive nitrate or acetate and served as controls. The mass of ammonia measured in all 6 reactors steadily increased with time and selected data are presented in Fig. 8. The total mass of ammonia in a reactor was calculated from the leachate NH_3–N concentration and the volume of water in each reactor. The theoretical increase in the mass of NH_3–N, based on the conversion of the added NO_3–N to NH_3–N, was 2.5–4.0 g NH_3–N in reactors 1, 6, and 8. The measured NH_3–N mass increases were only 0–0.3 g. Thus, DNRA did not occur to an extent that it had a noticeable effect on NH_3–N concentrations in the reactors. As no soil was added to the reactors, it seems unlikely that there would have been sufficient ammonia associated with the solid phase to alter this conclusion although solid phase ammonia was not measured. In addition to DNRA, two additional mechanisms for an increasing mass of ammonia include (1) the release of ammonia from protein hydrolysis and fermentation and (2) the release of ammonia that was attached to the refuse solid phase by ion exchange.

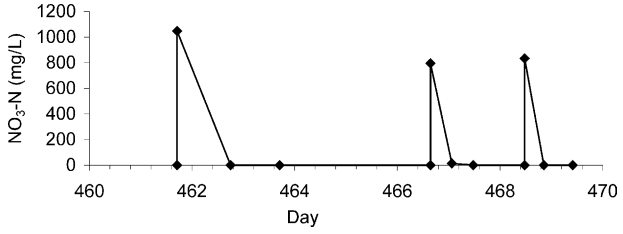

Fig. 5. Nitrate depletion after addition of fresh refuse to top 25% of reactor 1.

3.2.4. Discussion of denitrification in well-decomposed refuse (Phase 2)

In refuse with relatively low methane production rates, the depletion of nitrate prior to acetate addition required 5–15 days and this period of slow nitrate depletion corresponded with lower BOD and COD concentrations (Figs. 4 and 6). The addition of excess

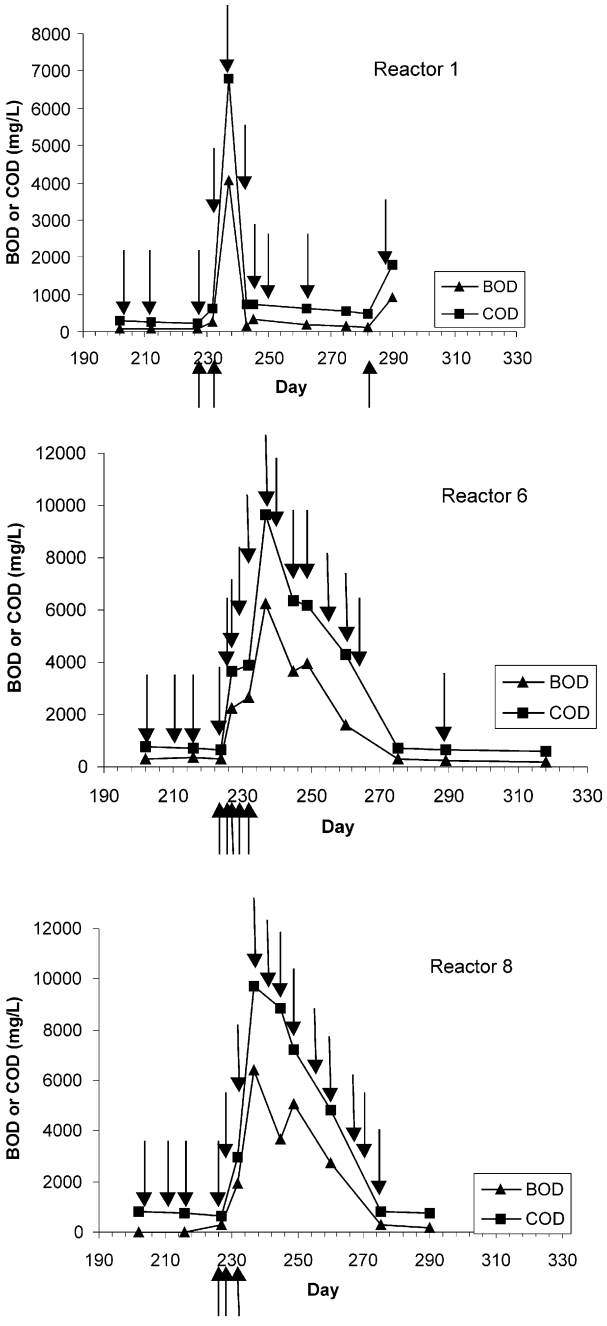

Fig. 6. BOD and COD concentrations during Phase 2 nitrate addition. The arrows pointing up and down represent acetate and nitrate additions, respectively.

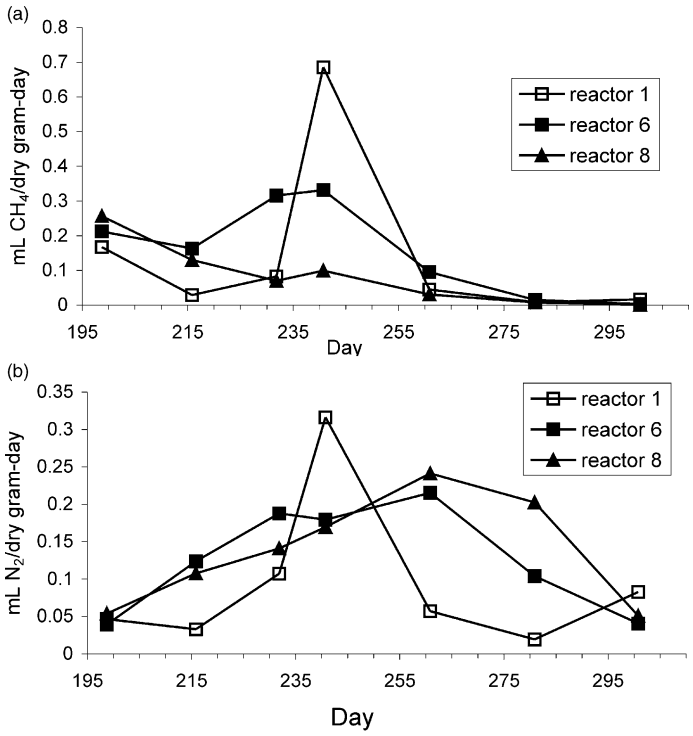

Fig. 7. Methane (a) and nitrogen (b) production during Phase 2 nitrate addition.

acetate stimulated nitrate depletion and resulted in a significant increase in BOD and COD. Even after acetate additions ceased, nitrate was rapidly consumed until the excess acetate had been depleted, which corresponded with a decrease in BOD and COD. Nitrate depletion was also stimulated in reactor 1 by removing the top 25% of the well-decomposed refuse and replacing it with fresh refuse. This simulated the addition of fresh refuse to the top layer of a landfill. Thus, the results of the acetate and fresh refuse additions are consistent in showing that degradable organic carbon limits the rate of nitrate depletion in decomposed refuse.

The amount of added acetate that was converted to methane was higher in reactor 1 than reactors 6 and 8 (Fig. 7a). A potential explanation is that the methanogens were less inhibited in reactor 1. Reactor 1 received only two Phase 2 nitrate additions before the acetate addition while reactors 6 and 8 both received three nitrate additions prior to the acetate addition.

The addition of a high ratio of degradable organic carbon to nitrate did not lead to a detectable increase in ammonia production that could be attributed to DNRA. A possible explanation is that a significant portion of the added carbon was consumed by methanogens. Methane production was inhibited but did not cease when nitrate was added (Fig. 7a). Therefore, it is possible that the nitrate reducing bacteria were exposed to a lower electron donor/electron acceptor ratio than that which was added to the reactors.

Tiedje (1988) reported that DNRA microbes need a tenfold greater population than denitrifiers to reduce 50% of the nitrate. Prior to the additions of carbon and nitrate in a ratio that would favor DNRA, the reactors had received 2–3 nitrate additions under carbon limited conditions that favor respiratory denitrification. Therefore, at the time that the organisms were exposed to conditions that might have supported DNRA, there had been selective pressure towards a population that would denitrify to N_2. This may have reduced the potential for DNRA in reactors 1, 6, and 8. In contrast to this study, Burton and Watson-Craik (1999) reported $^{15}NH_3$ production from $^{15}NO_3$ in batch cultures with methanogenic refuse though the mechanism was not elucidated.

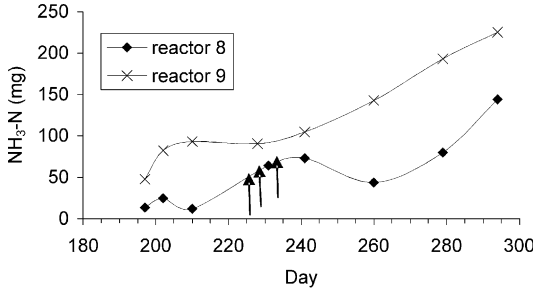

Fig. 8. Ammonia mass in Reactors 8 and 9 during Phase 2 nitrate addition. Arrows represent acetate additions to Reactor 8. Reactor 9 did not receive acetate or nitrate.

Table 4
The effect of sulfide oxidation on dissolved metal concentrations in leachate (mg/l)[a]

Day	SO_4^{-2}	Cd	Cr	Fe	Mn
Reactor 2					
315	0.90	<0.01	<0.01	1.39	0.07
318	0.46	<0.01	<0.01	1.42	0.08
322	0.47	0.01	<0.01	1.38	0.07
331	674	0.01	<0.01	0.67	0.08
336	642	0.02	<0.01	0.63	0.09
342	1310	0.02	<0.01	0.18	0.1
Reactor 7					
315	0.36	<0.01	<0.01	1.42	0.07
318	0.27	<0.01	<0.01	1.52	0.07
322	0.38	0.01	<0.01	1.57	0.07
331	272	<0.01	<0.01	0.76	0.06
338	502	0.02	<0.01	0.36	0.05
342	597	0.01	<0.01	1.01	0.06
Reactor 9					
315	0.43	<0.01	<0.01	1.01	0.03
318	0.26	<0.01	<0.01	1.04	0.03
322	0.30	<0.01	<0.01	0.89	0.02
331	361	<0.01	<0.01	0.92	0.02
338	505	0.01	<0.01	0.49	0.02
342	816	<0.01	<0.01	0.65	0.03

[a] Nitrate addition commenced on day 329.

4. Dissolved metals

While measuring nitrate concentrations in the leachate, large increases in sulfate concentrations were observed. The increase in sulfate implies that sulfide (S^{-2}) was oxidized by nitrate. This is consistent with the following stoichiometry and is spontaneous at standard conditions.

$$8NO_3^- + 5S^{-2} + 8H^+ \rightarrow 4N_2 + 5SO_4^{-2} + 4H_2O \ (E^o = 1.09) \quad (3)$$

In previous work with compost that was used to simulate well decomposed refuse, Onay and Pohland (2001) suggested that this reaction could have occurred as a result of autotrophic denitrification although it is unclear whether Eq. (3) was driven by a biological or abiotic mechanism in this study.

It is well established that metals concentrations in landfill leachate are low and one explanation is that metals form sparingly soluble metal sulfide (MeS) precipitates (Barlaz et al., 2002; Kjeldsen et al., 2003). Since the solubilities of metal sulfate complexes are much greater than those of metal sulfides, dissolved metal concentrations in leachate could be expected to increase when sulfides are oxidized to sulfates. To evaluate this, dissolved metal concentrations in leachate with low sulfate (pre-nitrate addition) and high sulfate (post-nitrate addition) concentrations were measured. Sulfate and metal concentrations for reactors 2, 7 and 9 are presented in Table 4. Although not reported in Table 4, Cu, Ni and Pb concentrations were always below 0.01, 0.05, and 0.1 mg/l, respectively. The increase in sulfate concentrations did not lead to an increase in metal concentrations. In addition to sulfide precipitation, metal mobility may be limited by precipitation as hydroxides and carbonates, by ion exchange and by sorption (Gould et al., 1990; Christensen et al. 2001). Given the absence of an increase in metal concentrations, it is likely that one or more of these other mechanisms limited the increase in metals concentrations in leachate.

5. MPN tests for total anaerobic and denitrifier populations

MPN tests were performed to measure the survival of the denitrifier population relative to the total anaerobic population during refuse decomposition. MPN results along with the corresponding state of methane production at the time of sampling are presented in Table 5 for reactor 12. The total anaerobes decreased by an order of magnitude from day 0 to day 121, which corresponds to the acid phase of refuse decomposition. Once the refuse was actively producing methane, total anaerobes increased by at least two orders of magnitude and remained at approximately 10^9 cells/dry g through the decomposition cycle. The denitrifying bacteria population decreased by about five orders of magnitude during refuse decomposition. This is likely due to the fact that no nitrate was added to the reactor.

To address whether the decrease in the denitrifier population had an effect on denitrification activity, 400 mg NO_3–N/l and acetate were added to reactor 12 on

Table 5
Methane production and microbial populations in reactor 12

Day	Methane production status	CH_4 production rate (ml CH_4/(dry g-day)	Total anaerobes (cells/dry g)	Denitrifiers (cells/dry g)
0	Fresh refuse	0	6.2×10^8	2.7×10^8
121	Prior to onset of methane production	0	1.8×10^7	8.7×10^3
252	38 days past peak methane production	1.1	$>6.2\times10^9$	1.6×10^3
364	150 days past peak methane production	0.05	4.8×10^9	7.5×10^3

days 373 and 376. Both nitrate additions were depleted within 24 h, suggesting that denitrification activity was not affected by the decrease in denitrifier population.

6. Effect of nitrate on methane yield and solids decomposition

The added nitrate served as an alternate electron acceptor for refuse decomposition that normally occurs under methanogenic conditions. As expected, the methane yields decreased as the mass of added nitrate increased. The correlation coefficient (r^2) for the relationship between methane yield and molar nitrate addition is 0.62 based on the data in Tables 2 and 3.

The stoichiometry for the conversion of cellulose to CO_2 with nitrate as the electron acceptor is given in Eq. (4). Based on this stoichiometry, up to 15.3% of the initial mass of cellulose plus hemicellulose present in a reactor was diverted from methane to CO_2 production. This calculation was based on the maximum nitrate addition to a reactor (5 mol—Table 3), the average mass of refuse in a reactor, and the corresponding quantities of cellulose and hemicellulose. The initial cellulose, hemicellulose and lignin concentrations in fresh refuse were 43.9, 21.1, and 25.1% (dry wt.), respectively. The corresponding concentrations in the decomposed refuse used as a seed were 10, 5.2, and 20.4%, respectively.

$$5C_6H_{10}O_5 + 24NO_3^- + 24\ H^+ \rightarrow\ 30CO_2 + 12N_2 + 37\ H_2O \qquad (4)$$

While the impact of nitrate addition on methane yield in the laboratory system was as high as 15%, the potential effects at field-scale are considerably lower. Two calculations were performed to estimate the potential effect of nitrate on methane yields. First, consider waste with 50% cellulose plus hemicellulose. Based on a leachate concentration of 400 mg-NO_3/l, 0.495 m^3 of leachate per metric ton of refuse (137 gal per ton) would be required to convert the added cellulose plus hemicellulose to CO_2 with NO_3^- as the electron acceptor. This is equivalent to increasing the refuse moisture content from 20 to 47.4% in 1 day. For a second estimate of the impact of nitrate on methane yields, consider a 1 ha landfill cell with a 25 m depth of refuse with 50% cellulose plus hemicellulose. Assuming a refuse density of 891 kg/m^3 (1500 lb/$yard^3$) and leachate containing 400 mg-NO_3–N/l, the amount of cellulose converted to CO_2 by nitrate reduction would be 0.0082% of the cellulose buried per day based on a leachate addition of 9353 l/ha-day (1000 gal/acre-day). These estimates indicate that nitrate will not have a significant effect on methane yields in full-scale landfills and that the recirculation of nitrate-rich leachate is primarily a means of nitrogen management.

Microorganisms obtain more energy for growth under nitrate-reducing conditions relative to methane-producing conditions. To evaluate whether this increased energy translated into increased rates of cellulose decomposition, the total rate of cellulose decomposition from nitrate-reduction and methanogenesis was calculated using the stoichiometric relationships given in Eqs. (4) and (5) and the measured rates of N_2 and CH_4 production.

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow\ 3nCO_2 + 3nCH_4 \qquad (5)$$

The cellulose decomposition rate for two sets of reactors is presented in Fig. 9. The reactors presented received the largest quantities of nitrate so any effect should be most apparent. As illustrated, the rate of cellulose decomposition decreased with nitrate addition. A parallel decrease was observed in the control reactor (7) which only received nitrate on days 59–67. This decrease in cellulose decomposition is likely a reflection of the decreasing biodegradability of cellulosic substrates as the more easily degradable materials are consumed. However, the cellulose decomposition rate in all reactors actually increased after the termination of nitrate addition. Thus, there is no evidence to suggest that

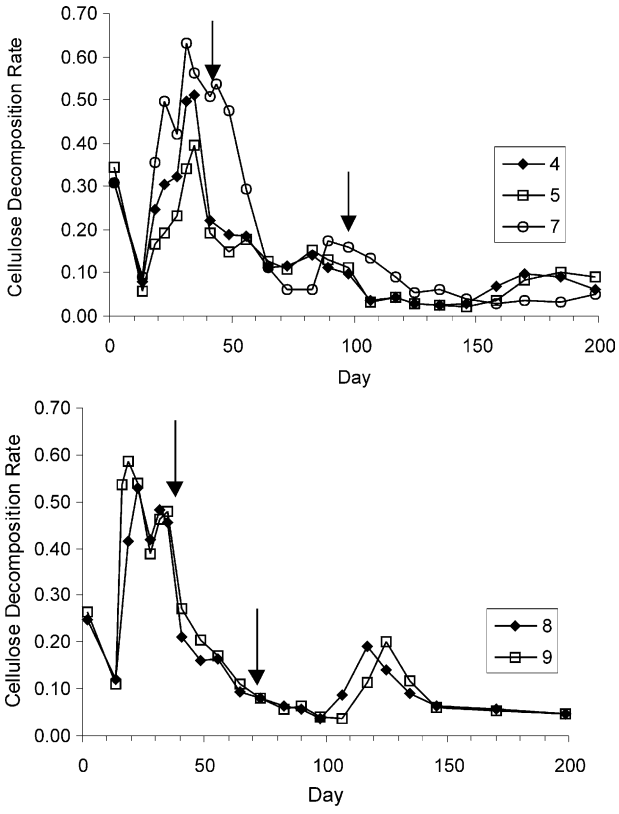

Fig. 9. Cellulose decomposition attributable to nitrate reduction plus methanogenesis. The combined cellulose decomposition rate was calculated by multiplying the N_2 production rate by 5/12 and the CH_4 production rate by 1/3 per Eqs. (4) and (5). The units are moles cellulose/(dry gram-day) times 10,000. Arrows represent the initial and final Phase 1 nitrate additions for reactors 4, 5, 8, and 9. Nitrate was mistakenly added to reactor 7 on days 59–67.

nitrate addition stimulated the cellulose decomposition rate relative to the rate measured under methane-producing conditions.

7. Summary

The refuse had a large capacity to consume nitrate and the bacteria responsible for denitrification maintained their activity even after a year without the presence of nitrate. Nitrate reduction was significantly faster in actively decomposing refuse, where nitrate was depleted in <48 h, relative to the 5–15 days required for nitrate depletion in well decomposed refuse.

Two lines of evidence suggest that the reduced rate of nitrate depletion in well decomposed refuse can be attributed to the reduced availability of organic carbon to serve as an electron donor. First, the COD in actively decomposing refuse (Fig. 3d: 2430–16,000 mg/l) was much higher than that in well decomposed refuse (Fig. 6: 300–800 mg/l). Second, when acetate or fresh refuse was added to well decomposed refuse, nitrate depletion rates increased (Figs. 4 and 5). The reduced capacity for nitrate reduction in well decomposed refuse is consistent with the overall reduction in cellulose decomposition which can be represented by the decreasing methane production rate with time in all reactors. This trend in methane production and cellulose turnover is typical of refuse decomposition and is consistent with the depletion of cellulose and hemicellulose, and enrichment of the remaining solids in lignin which is recalcitrant.

Even during the most active period of nitrate reduction, the pH did not increase to levels that would cause a concern in a full-scale system. In addition, once nitrate addition stopped, methane production resumed after some lag time. Although some N_2O was measured, the amount is likely above the upper limit of what would occur in a full-scale landfill. This is because in a full-scale landfill, the retention time of the gas would be longer, allowing more time for the complete reduction of N_2O to N_2.

The anammox process [Eq. (6)] represents a potential sink for ammonia in landfills (Jetten et al., 2001):

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (6)$$

However, the loss of ammonia by this process requires approximately equimolar concentrations of ammonia and nitrite. One ecological niche for this process is at the interface of an aerobic/anaerobic ecosystem, in which oxygen is depleted during the conversion of ammonia to nitrite (Schmidt et al., 2002). In this niche, there is the potential for nitrite and ammonia to accumulate in concentrations that would allow Eq. (6) to occur. However, the basis for this study was the conversion of ammonia to nitrate in an ex situ aerobic reactor. Thus, the presence of equimolar concentrations of nitrite and ammonia in recirculated leachate would not occur. Further clarification of the significance of the anammox process in refuse will require studies with ^{15}N to carefully account for all endproducts.

Although the consumption of organic carbon limited nitrate reduction rates, this could easily be managed in a full-scale landfill. The simplest way to enrich the leachate from a particular landfill cell in organic carbon would be to add fresh refuse to the top of the cell. If a landfill cell was no longer receiving fresh refuse, then leachate from another section of the landfill that contains a higher BOD, or a liquid waste with degradable organics could be added to provide sufficient carbon to drive denitrification. Thus, the results of this research suggest that landfills have significant capacity to convert nitrate to nitrogen gas that can be safely released to the atmosphere, thus providing a viable alternative for the long-term management of nitrogen in landfills. Finally, given the high capacity for refuse to reduce nitrate, any added nitrate is likely to be consumed close to the point of addition. Thus, another potential benefit of nitrate addition may be to reduce methane production near the landfill surface, where methane collection is most difficult.

Acknowledgements

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