Column-Based Remediation of Groundwater Nitrogen via Stimulation of Nitrification and Denitrification

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ABSTRACT: A former research laboratory facility utilized a leach field for sanitary and aqueous laboratory waste. Surrounding soils and groundwater are impacted by ammonia and pH varies between 3.6 and 5. The feasibility of employing an in-situ, two-stage, nitrification/denitrification program was evaluated via a soil column study. The objectives of the study were to a) determine the efficacy of stimulating nitrification, followed by denitrification, and b) provide a scale-up implementation design and cost for the full-scale remedy. The study consisted of monitoring the inorganic nitrogen content of leachates from 7 columns with 4 conditions over six weeks. The four conditions were 1) un-amended groundwater controls, 2) pH adjusted, o-PO4-P amended groundwater tests with daily peroxide addition, 3) condition 2 augmented with a pure nitrifying consortium and, 4) condition 3 amended with catalase enzyme. During the nitrification phase, condition 1 nitrified to a very limited degree, indicating the presence of indigenous nitrifying organisms. Condition 2 confirmed the presence of indigenous nitrifiers, although nitrification performance was incomplete and sporadic. Condition 3 significantly outperformed condition 2. No benefit was observed from catalase addition. Column exhaustion required the denitrification phase to be performed as a slurry reaction. Slurries 1 and 2 were fed nitrate, glucose, phosphate, and sulfite to scavenge oxygen. Slurry 3 was treated in the same manner with the addition of a single denitrifying bacterial strain. Slurry 1 failed to denitrify. Slurries 2 and 3 reduced 100 mg/L of nitrate at rates of 14 and 59.5 mg/L/day, respectively.

INTRODUCTION

Innovative Environmental Technologies approached Novozymes Biologicals to determine the feasibility of inoculating the vadose zone of an abandoned septic tank leach field with nitrifying and denitrifying bacteria as a means to reduce groundwater nitrogen levels.

Previous attempts to stimulate in-situ nitrogen removal involved the injection of a slow-release peroxide twelve feet up gradient of and a reducing solution seven feet down gradient of a specific monitoring well. After six months, no nitrification was observed at this well, however, some reduction in nitrate was seen at a down gradient well.

METHODS AND MATERIALS

Site soil and groundwater received in early December 2002 measured 22 mg/L tCOD, 25.4 mg/L NH_4-N and non-detect for both NO_2-N and NO_3-N

Seven soil columns were prepared in 1.75 in ϕ x 24 in (4.5 cm ϕ x 61 cm) plastic tubes to support 4 test conditions. A small funnel, lined with a fine mesh screen and filled with coated aquarium gravel was taped to the bottom of each column, and the columns were filled with 20 in (51 cm) of soil screened through ¼ in (0.6 cm) mesh screen. The columns were operated as down flow units to model the vadose zone.

Nitrogen species were monitored with a Hach DR/2000 spectrophotometer and associated methods. All are all reported as N. The Nitraver 5 method is based on a cadmium-reduction and reports the sum of NO_2- and NO_3-N; results are reported as NO_x-N. Ortho-phosphate was monitored colorimetrically with Chemetrics R-8510 CHEMets®. COD was measured with Hach low range, closed reflux COD

tubes. pH was analyzed with an Accumet AB-15 pH meter. Peroxide was dosed from a 3% solution and monitored with EM Quant® Peroxide Test strips from Merck.

Nitrification. This phase of the study compared unamended control columns (1a and 1b) that were fed as-received groundwater with three alternate conditions, each representing a different potential cost and performance level for nitrification stimulation. For each of the three alternate conditions (columns 2a, 2b, 3a, 3b, and 4), the soil was fed groundwater with the pH adjusted to 7.8 with $NaHCO_3$ and to which 5 mg/L of ortho-PO_4-P had been added; 100 mg/L of hydrogen peroxide was added separately to the groundwater feeds on a daily basis. In two of the alternate conditions (column 3a, 3b, and 4), the soil was amended once with a nitrifying consortium, and in one of these conditions (column 4), the soil was also amended once with catalase enzyme. Catalase catalyzes the formation of oxygen and water from hydrogen peroxide.

The study design and rationale for the conditions in the nitrification phase are presented in Table 1.

TABLE 1: Nitrification study design and rationale for treatments.

Column	Soil	Groundwater	Rationale
1a & b	Unamended	Unamended	Control
2a & b	Unamended	pH adjusted to 7.8 ($NaHCO_3$); amended with 5 mg/L o-PO_4-P; 100 mg/L H_2O_2 added daily to groundwater feed container.	Stimulation: supportive pH range; P for cell growth, peroxide for additional oxygen.
3a & b	Amended once with 38 mg nitrifying cell mass	Same as 2a & b.	Stimulation and augmentation with nitrifying biomass.
4	Same as 2a & b with one addition of 100 CIU catalase enzyme.	Same as 2a & b.	Same as 3a & b with enzyme to increase rate of O_2 generation from H_2O_2.

Denitrification. Persistent column clogging required denitrification to be carried out as a slurry phase. The contents of columns 1a, 2a, and 3a were saturated on Feb 17 with a glucose:sulfite:nitrate solution as defined in Table 2, and each slurry received an additional 200 mg/L of glucose and 5 mg/L PO_4-P on Feb 18. Slurry 3a received a one-time inoculum of $1e^6$ CFU/mL of *Paracoccus pantotrophus*, a denitrifying bacterium. Each slurry was stirred at 20 rpm. Samples for analysis were prepared by centrifuging a small aliquot at 20,000 rpm for 20 minutes.

TABLE 2: Denitrification test amendments.

Column	Date	Additions (mg/L)				
		Glucose	Na_2SO_3	PO_4-P	NO_3-N	Cells
1a	Feb 17	100	38		100	
	Feb18	200		5		
2a	Feb 17	100	38		100	
	Feb18	200		5		
3a	Feb 17	100	38		100	$1e^6$ CFU/mL
	Feb18	200		5		

RESULTS AND DISCUSSION

The groundwater feeds for columns 2a, 2b, 3a, 3b, and 4 were initially raised by titration with sodium bicarbonate ($NaHCO_3$) to a pH of 7.8, however, the leachate pH remained in the mid-5 range during the first week of the study, which, by classic guidelines, is strongly inhibitory to nitrification. Water chemistry suggests that water saturated with $NaHCO_3$ will maintain a pH near 8.2 at room

temperature. Consequently, the groundwater feeds were saturated with $NaHCO_3$. However, the combination of soil with water chemistry resulted in a requirement for constant feed pH adjustment to offset leachate pH in excess of 9.0. Leachate pH from these columns remained in the 8-9 range throughout the remainder of the study. While this is higher than that considered optimal for nitrification, the results inform that it did not compromise the study. The zigzag pattern resulting from the groundwater pH adjustments is apparent in the pH charts for these columns.

In general, leachate flow rates declined throughout the study. Clogging was a significant issue in all but the control columns at one time or another, and ultimately resulted in performing the denitrification test in slurry phase. Significant differences in N-species measurement between the columns of any given test condition during the nitrification test are most probably explained by differential leachate rates. These differences allow major factors affecting nitrification and denitrification (oxygen and retention time) to have varying degrees of significance.

Hydrogen peroxide and ortho-phosphate were never detected in any leachate during the study.

Nitrification Phase: The nitrification phase ran for 28 days. NO_2-N and NO_3-N were non-detectable in the as-received groundwater, however, based upon the appearance of NO_2-N and NO_3-N and the loss of some NH_4-N in all of the pH-adjusted feeds, the presence of indigenous nitrifiers is suggested. In an attempt to prevent nitrification from complicating the interpretation of the appearance of oxidized nitrogen species in the leachates, the feeds for the five test columns were filtered to remove biomass during the third week. Standard glass fiber filters for total suspended solids analysis in wastewater (Whatman 934-AH) were used for this step. The effort was largely successful, although some additional increase in nitrate did occur.

Controls - Columns 1a & 1b: The total groundwater feeds through columns 1a and 1b were 3.07 and 2.16 L, respectively. Feed and leachate pHs averaged 3.1 and 4.4, respectively, with very low variability. High levels of NH_4-N were detected in the leachate throughout the trial, although NH_4-N dropped significantly from 30-35 to 20 mg/L in Column 1b in the 10 days preceding the end of the nitrification phase. Very low levels of nitrite persisted throughout the test, with a maximum of 0.12 mg/L in Column 1b. NO_x increased significantly from non-detect to near 30 mg/L in both columns, and corresponds with the increase in NOx-N seen in the 10 days preceding the end of the nitrification phase. Due to persistently low nitrite levels, the majority of NO_x-N was NO_3-N.

The data suggest, unexpectedly, that a degree of nitrification occurred in a pH range of 3.1 to 4.4. The gradual increase in NO_2-N and NO_3-N in the feed for these columns, followed by a significant increase in leachate NO_3-N above that present in the feed during the third week, suggests that the groundwater contained nitrifying organisms. The observation, while interesting, apparently has no practical value for remediation as the site history informs us that an unmanaged approach to will not be successful in reducing groundwater NH_4-N. Nitrification rates are highly dependent on pH, with peaks near 8.0 and, in wastewater, process failures often occur below a pH of about 6.2. The long-term exposure of the groundwater feeds to the atmosphere from the time of collection in early December ’02 through early February ’03 most likely explains the observation by permitting an extended retention time in an oxic environment.

Stimulation – Control 2a & 2b: The total groundwater feeds through columns 2a and 2b were 2.42 and 2.52 L, respectively. Figure 1 charts pH, NH_4-N, NO_2-N and NO_x-N for both columns.

Leachate NH_4-N levels varied widely throughout the groundwater feed phase, and were impacted by a column-repacking event on January 23rd. The reduction in flow rate following repacking appeared to strengthen a downward trend for the remainder of the nitrification phase. Leachate NH_4-N was 8.1 mg/L for column 2a and less than 1.0 mg/L for column 2b at the end of the nitrification phase. NO_2-N levels also varied significantly, peaking in column 2b at greater than 7.0 mg/L shortly after the

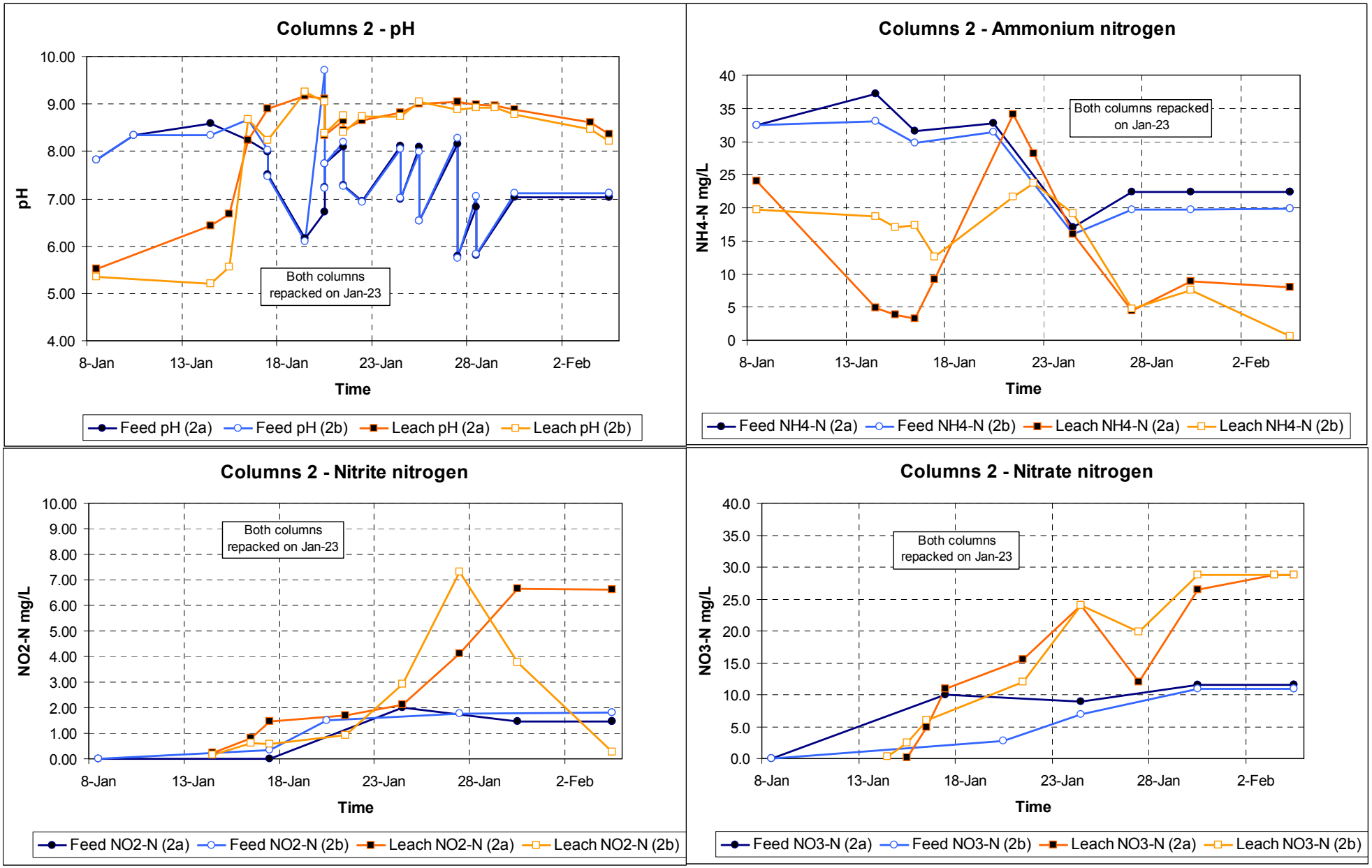

FIGURE 1. Feed and leachate pH, NH_4-N, NO_2-N, and NO_3-N for columns stimulated with pH adjusted feed, ortho-PO_4-P, and hydrogen peroxide.

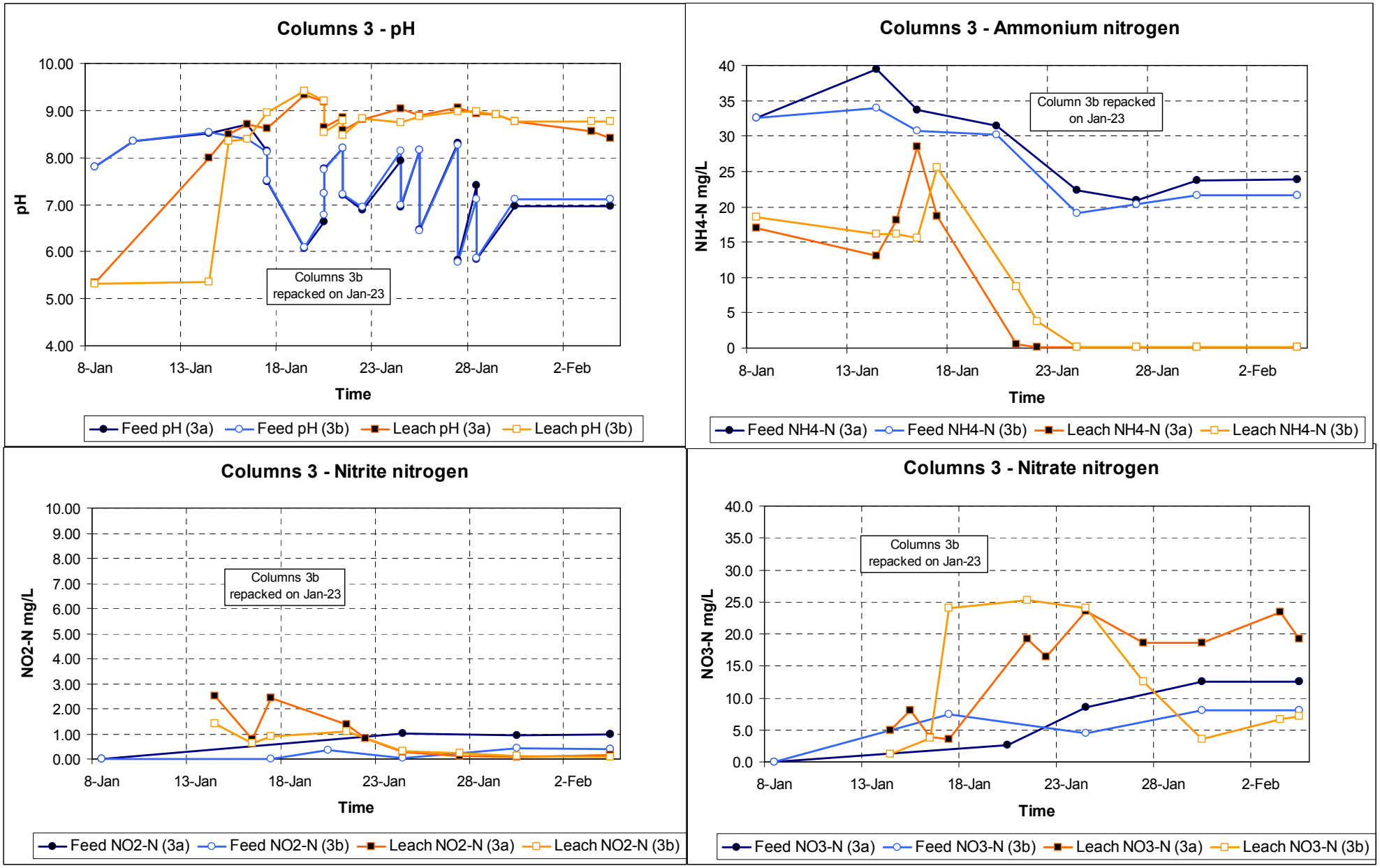

FIGURE 2. Feed and leachate pH, NH_4-N, NO_2-N, and NO_3-N for columns stimulated with pH adjusted feed, ortho-PO_4-P, and hydrogen peroxide and augmented with nitrifying cells.

repacking event, followed by a decline to near zero. Column 2a NO_2-N increased steadily to greater than 6.0 mg/L and remained at this level until the nitrification phase ended. NO_x-N levels generally increased in both columns throughout the nitrification phase. Due to the significant levels of nitrite, leachate NO_x-N contains significant contributions from both NO_2-N and NO_3-N.

Nitrification occurred due to the presence of indigenous organisms, and was much stronger than in columns 1a and 1b, however, the variability and persistence of NH_4-N and NO_2-N leads to concerns of persistence of these species as groundwater passes from a zone influenced by nitrifiers.

Augmentation - Columns 3a & 3b: The total groundwater feeds through columns 3a and 3b were 3.19 and 2.31 L, respectively. Figure 2 charts pH, NH_4-N, NO_2-N and NO_x-N for both columns.

Leachate ammonium levels initially increased from 15-20 mg/L to 25-30 mg/L, and then rapidly decreased to non-detectable levels for the remainder of the nitrification phase. Leachate nitrite levels initially increased to 1.5 – 3.0 mg/L, and then declined to less than 0.30 mg/L for the remainder of the study. NO_x levels increased strongly early in the nitrification phase, and remained elevated in column 3a leachate. Following the January 23rd repacking of Column 3b, a decline in leachate nitrate for that column occurred with no increase in leachate ammonia, suggesting that partial denitrification occurred within the column.

Complete nitrification was established. Nitrification occurred more strongly and consistently earlier in the study and at slightly higher average leachate rates than in columns 2a and 2b. While leachate NH_4-N declined sharply in columns 3a and 3b between January 16 and January 22, ammonium levels in both columns 2a and 2b were increasing at leachate rates that averaged 10% less. Following the repacking of column 3b, ammonium remained non-detectable throughout the remainder of the nitrification phase of the study, and nitrite gradually declined from 0.3 to 0.09 mg/L. There was no nitrite spike.

Augmentation plus catalase - Column 4: The total groundwater feed through column 4 was 2.41 L. Complete nitrification was also established in column 4, however, the data reveal no benefit to catalase addition. The lack of benefit may be due to rapid reaction of the added hydrogen peroxide with the upper soil layer or to degradation of the protein-based enzyme by indigenous microflora, or a combination of these factors.

Denitrification Phase: Column clogging forced this phase to be conducted with slurries prepared from the contents of Column 1a, 2a, and 3a. Amendments to the slurries are quantified under Materials and Methods. Glucose provided an electron donor; sodium sulfite (Na_2SO_3) scavenged dissolved oxygen; noting that ortho-PO_4-P was never detected in any leachate, an excess of ortho-PO_4-P was added to remove P-limited growth as a potential limiting factor. Nitrate removal data are presented in Table 3.

TABLE 3: Denitrification performance – all concentration data in mg/L.

Condition	Column	Starting NO_3-N	Date	Results	
				NO_3-N	pH
Control	1a	100	Feb 17	72	4.23
			Feb18	81	4.22
			Feb 19	78	4.22
Stimulation	2a	100	Feb 17	102	7.97
			Feb18	98	7.94
			Feb 19	52	7.89
			Feb 24	4	
Augmentation	3a	100	Feb 17	123	8.00
			Feb18	93	7.88
			Feb 19	4	7.22
			Feb 24	ND	

Slurry 1a failed to denitrify. Slurry 2a reduced NO_3-N by 98 mg/L over 7 days for a rate of 14 mg/L/day. Slurry 3a reduced NO_3-N by 119 mg/L over 2 days for a rate of 59.5 mg/L/day. The augmented column denitrified at a significantly higher rate than the stimulated column.

FULL SCALE

A full-scale application has not occurred. The site plan involves the injection of 1000 gallons/day (3785 L/day) of pH-adjusted well water amended with 2.5 mg/L of o-PO_4-P to an impacted soil volume of approximately 420,000 ft^3 (11,890 m^3), for an exchange of 0.24% per day on a soil volume basis. Figure 3 presents a leachate pH titration curve using $NaHCO_3$ to use as a guideline, however, monitoring of the injection areas is recommended to assess the impact of soil chemistry on the equilibrium pH.

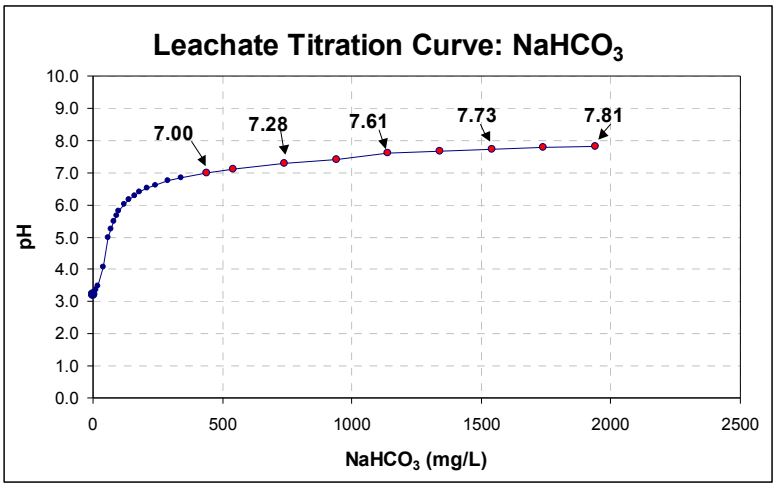

FIGURE 3. Leachate titration curve with $NaHCO_3$.

The augmented columns received 38 mg of cell mass in 0.97 L of soil, and resulted in confirmed nitrification in 10 – 14 days, providing considerable room for scale-down to reach cost-effectiveness.

CONCLUSIONS

The observation of a slight degree of nitrification in the control columns was unexpected. Variances in the column environment from that of the collection site for the groundwater include continuous exposure of the groundwater to the atmosphere during the column test and a column environment that approximated a shallow vadose zone. The site history informs us that an unmanaged approach to remediation will not be successful in reducing groundwater ammonium.

The evidence for indigenous nitrifiers was confirmed by observation of columns 2a and 2b, although nitrification in these columns was sporadic and incomplete for the majority of the nitrification phase of the study. The degree to which stimulation alone, as practiced in this study, can result in reduced groundwater ammonium, presuming adequate distribution of amendments, will depend on groundwater

flow rates and the position of compliance points relative to the zone influenced by the amendments, and the potential tolerance for nitrite at these points.

The augmented columns (3a, 3b, and 4) significantly outperformed the stimulated columns. Nitrification was established more strongly and consistently earlier in the study at slightly higher leachate rates and nitrite spikes did not appear. The significance of the increased robustness of the augmented process to a site application will be related to the increase in flow rates through the vadose and saturated zones caused by the distribution of amendments, resulting in lower water retention times, and the confidence that elevated populations of nitrifying populations have been placed in the water flow. No measurable benefit from catalase addition was observed in Column 4.

Denitrification was observed in slurry-phase reactions of the contents of both the stimulated and augmented columns. The data imply that a barrier saturated with the addition of a simple organic compound, glucose in this case, in the absence of oxygen, will be adequate to denitrify the groundwater. The increased rate of denitrification seen with the introduction of *Paracoccus pantotrophus* to the augmented column provides a tool for reaction to incomplete denitrification at the application site.