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CONTROL OF PH DURING DENITRIFICATION IN SUBSURFACE SEDIMENT MICROCOSMS USING ENCAPSULATED PHOSPHATE BUFFER

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Abstract—Denitrification, a process in which nitrate (NO_3^-) is used as an electron acceptor during microbial respiration, often occurs in saturated sediment where oxygen has been depleted. During denitrification, pH may increase several units as nitrate is consumed and HCO_3^- and OH^- are produced. A novel approach to control pH is the use of encapsulated buffers. Previous research has shown that KH_2PO_4 microcapsules were effective at maintaining a neutral pH during denitrification in suspended bacterial cultures. Our objective was to test the ability of encapsulated buffers to control pH in subsurface sediment microcosms amended with NO_3^-. Sediment samples from three depths (37, 40, and 43 m) in the saturated zone beneath the Savannah River Site, near Aiken, SC, were amended with 1 mg NO_3^-/g. Nitrous oxide (N_2O) production was measured by gas chromatography (GC) using the acetylene block technique. NO_3^-, ammonium (NH_4^+), total alkalinity, and sediment particle grain size also were measured as part of a nitrogen mass balance and to assess base neutralizing capacity. In two of the three samples, all of the NO_3^- added was converted to N_2O at a rate between 0.7–2.3% h^{-1}. pH levels above 8.0 were measured in vials to which no microcapsules had been added while the pH in vials containing microcapsules remained within 0.2 pH units of 7.0. The results indicated that over time periods as short as five days, denitrification increased the pH beyond the range considered to be optimal for microbial metabolism. Because these encapsulated buffers were effective at maintaining a neutral pH during denitrification in sediment microcosms, they may facilitate remediation of subsurface contaminants through the mediation of pH-limited biological or chemical processes.

Key words—pH, encapsulation, denitrification, sediment microcosms

INTRODUCTION

In situ bioremediation is often used to remove organic contaminants from saturated soils and groundwater. Usually this entails the addition of oxygen, a primary carbon source, and nutrients, but may not provide control over pH. Both increases and decreases in pH may occur as a result of specific microbial processes. For example, the production of carbonic and organic acids may decrease pH (Long and Aelion, 1999) and denitrification may increase pH (Hutchins and Wilson, 1994; Kennedy and Hutchins, 1992–1993). The high solubility of nitrate (NO_3^-) in water allows it to be very mobile in saturated sediments (Freeze and Cherry, 1979) and available for use in denitrification, one of the predominant respiratory processes that occurs in anoxic contaminated groundwater (Smith *et al.*, 1996). However, denitrification coupled with mineralization of reduced organic compounds produces hydroxyl ions and carbon dioxide, which interact to form carbonates. When the production of hydroxyl ions exceeds that of carbon dioxide, the pH of the immediate microbial environment can rise above an acceptable level of 8.3 (Drtil *et al.*, 1995). The pH preferred by most heterotrophic soil microbes is generally considered to be between 5.5 and 8.0 (Prescott *et al.*, 1990) and pH values outside of this range may have deleterious effects on metabolism and conversion of carbon compounds, e.g. contaminants.

During *in situ* bioremediation of subsurface sediments and groundwater, changes in pH could be neutralized by the environmentally-controlled release into the subsurface of phosphate buffers encapsulated in a polymer coating. The capsules are not designed or expected to move through the aquifer to specific contaminated areas. During *in situ* applications, it is anticipated that the encapsulated buffers would be added through a series of monitoring wells or drive points at specific locations. As

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groundwater flows through those points, the pH of the groundwater would be modified. This system would be analogous to that of *in situ* treatment walls in which a reactive barrier is created through which the groundwater flows. The reactive barrier is not mobile. Once the capsules have been used up, more could be added as necessary and any management of the introduction system, such as de-fouling, could be accomplished during that time.

The microcapsules are designed to release buffer (KH_2PO_4 or K_2HPO_4) into sediment pore water as a function of the polymer material used as the outer coating. Polymer coatings can be designed to dissolve at specific pH levels, releasing the buffer only when necessary and mediating not only processes that increase pH, but those that decrease pH as well. The KH_2PO_4 microcapsules designed for use in the experiments described here are 1 mm in average diameter and are coated with a polymer (Eudragit® S100, Coatings Place Inc, Verona, WI) that dissolves at pH levels above 7.0. Vanukuru *et al*. (1998) showed that the encapsulated KH_2PO_4 buffer controlled pH under denitrification conditions in suspended culture in 155-ml microcosms, which contained bacteria inoculated from activated sludge. The pH rise from 7.0 to 8.6 after 48 h of incubation was mediated to 7.0 ± 0.2 pH units in microcosms containing the encapsulated buffer.

Encapsulation technology has been examined for *in situ* bioremediation of subsurface environments. Vesper *et al*. (1994) encapsulated sodium percarbonate (SPE) as 0.25–2.0 mm grains in order to provide a source of oxygen (from hydrogen peroxide) to enhance aerobic biodegradation of propylene glycol in soil. Encapsulated bacteria added to 0.2-μm dialysis bags and lowered into contaminated subsurface sediment have been used to enhance remediation of atrazine (Shati *et al*., 1996). Lin *et al*. (1991) co-immobilized fungal cells, cellulose co-substrate, and activated carbon in alginate beads in order to concentrate pentachlorophenol for microbial degradation. dos Santos *et al*. (1996) reported the use of co-immobilized nitrifiers and denitrifiers to remove nitrogen from wastewater systems. To our knowledge, encapsulation in environmental systems usually entails applications such as these, in which bacteria or slow release compounds are used to directly enhance biodegradation. In our approach, the environment is modified to maintain conditions conducive for denitrifying bacteria. However, encapsulated buffers could be useful not only in areas of high nitrate concentration and associated denitrifying conditions, but also in other biological or chemical remediation systems (such as iron filings used in chemical reactive barriers) where pH must be maintained in a specific range in order for a particular process to take place most efficiently. Our specific objective was to investigate the use of phosphate buffer encapsulation by a pH-sensitive polymer for maintenance of neutral pH using heterogeneous subsurface aquifer sediments and native bacteria in controlled laboratory microcosms.

MATERIALS AND METHODS

Sediment samples were taken at three different depths from a contaminated aquifer that had not previously been shown to contain active denitrifiers. Sediment particle size and total alkalinity were measured at each depth. Three sets of microcosms were allowed to incubate and were analyzed for nitrous oxide (N_2O) production over time. A subset of vials was sacrificed every 24 h and analyzed for pH, ammonium (NH_4^+), and nitrate + nitrite ($NO_3^- + NO_2^-$). In order to demonstrate the effectiveness of the microcapsules in counteracting a consistent pH increase, only denitrification and dissimilatory nitrate reduction to NH_4^+ were monitored. For simplicity, other processes that could increase or decrease pH, such as carbon mineralization and organic acid production, were not directly promoted or analyzed.

Sediment sampling

Subsurface sediment samples from 37, 40, and 43 m depths were obtained from the saturated zone on 27 February 1998 from boring hole MRS17 located in M-area at the Savannah River Site (SRS), near Aiken, SC. The water table was approximately 37 m below land surface. The sediment was contaminated with trichloroethylene (0.01–4.0 mg/l) and perchloroethylene (0.04–11.7 mg/l). The sample location was 270–360 m from a basin where chemical wastes had previously been released. The groundwater pH ranged from 4.5 to 5.5. The boring was drilled using a Rotasonic drill rig equipped with a 3″ diameter core. Three-meter cores were split and sediment was sampled from the inside of the core using an ethanol-sterilized spatula. Samples were stored in autoclaved Mason jars on ice until returned to the laboratory, where they were kept at 4°C.

Sediment characteristics

Particle size distribution and total alkalinity in sediment were analyzed from each depth. A 20-g (wet) sediment sample was disaggregated overnight in 1% sodium hexametaphosphate, and was separated into the sand fraction and the mud (silt + clay) fraction by washing with deionized water through a 63 μm sieve. Both fractions were dried in a 60°C oven and weighed. The sand fraction was then shaken for 15 min through the following series of sieves (μm): very coarse (1400–1000), coarse (710–500), medium (355–250), fine (180–125), very fine (90–63), and silt + clay (< 63). Percent recovery was recorded as the summed weight of all sieved fractions divided by the weight of the original sand fraction (g). Total alkalinity (mg/l as $CaCO_3$) of the sediment was determined by adding 10 g wet sediment to 15 ml deionized water and equilibrating overnight. Aliquots of 0.002 N H_2SO_4 were added until the pH reached 4.50 (bromcresol green endpoint). Total alkalinity in mg/l as $CaCO_3$ was then converted to mol/l of carbonate ($C_{T,CO3}$). Alkalinity production from microbial metabolism of organic material was estimated from the stoichiometry of the reaction of ethanol and nitrate to form bicarbonate ion and nitrous oxide:

$$2C_2H_6O + 2H^+ + 6NO_3^- \rightarrow 2CO_2 + 2HCO_3^- + 5H_2O + 2OH^- + 3N_2O \quad (1)$$

Ethanol was chosen as a representative carbon source because the average oxidation number of carbon ($^-2$) for this compound predicts that a pH > 8.3 will occur if denitrification is coupled with metabolism (Drtil *et al*., 1995).

The extent of NO_3^- conversion by day 4 (where pH 8 was first reached for two of the three sediments) was used to determine metabolic $C_{T,CO3}$.

In order to compare the base neutralizing capacity of the microcosm system (sediment and metabolically-derived alkalinity) to the additional base neutralizing capacity contributed by the microcapsules, a series of electroneutrality equations was set up to calculate the maximum base equivalents (equivalents of base able to be neutralized) generated each by $C_{T,CO3}$ and the microcapsules. First, the base equivalents of the sediment alkalinity (HCO_3^-) were calculated by setting up an electroneutrality equation at the initial pH of the sediment in the microcosm sacrificed at time 0 and solving for the base equivalents necessary to balance the HCO_3^- (Snoeyink and Jenkins, 1980). Second, the base equivalents of the system at pH 8 were similarly calculated using both sediment and metabolic HCO_3^-. Third, the base equivalents of the system at pH 8 with the microcapsules dissolved were calculated using the sediment and metabolic HCO_3^- and the $C_{T,PO4}$ (total phosphate, in mol/l). $C_{T,PO4}$ was calculated for each sediment depth based on the amount of microcapsules (mg) added to the microcosm vials. Previous studies by Vanukuru *et al.* (1998) characterized the microcapsule mass as being comprised of 30% wall material by using atomic absorption analysis of the potassium content. Therefore, a maximum of 70% of the microcapsule mass added is available as KH_2PO_4. In both cases, a pH of 8 was selected because the capsules release the buffer core continuously only at pH greater than 7. If the pH is equal to 8 and the microcapsules are not completely dissolved, then the pH will not increase further until the entire buffer microcapsule has dissolved.

Experimental setup

The experimental setup was modified from Aelion *et al.* (1997). Each treatment (capsules vs no capsules and NO_3^- vs no NO_3^-) was analyzed for N_2O and $NO_3^- + NO_2^-$ in duplicate. Approximately 2 g of wet sediment were added to 25-ml serum vials. Half of the vials received 10 (for 37-m depth), 15 (43-m), or 20 (40-m) mg of the KH_2PO_4 microcapsules. Greater amounts of capsules were used in later experiments to ensure that the microcapsules were not limiting at longer time points.

In order to fully stimulate denitrification, $NaNO_3$ was added to each treatment vial for a final concentration of 1 mg NO_3^-/g wet sediment (3 ml of liquid/vial). Live controls received sterile deionized water without NO_3^- while metabolically inhibited controls received NO_3^- and 200 μl of 7% mercuric chloride. 175 μl Oxyrase enzyme (Oxyrase, Inc, Mansfield, OH) was added to each vial to scavenge oxygen, the vials were flushed for 10 s with nitrogen gas (N_2), and were immediately sealed with a butyl rubber stopper and a crimp cap to maintain anoxic conditions. Five percent of the vial headspace was replaced with acetylene and the production of N_2O was measured over time as an indicator of denitrification using the acetylene block technique (Yoshinari *et al.*, 1977).

Headspace samples were removed using a Pressure-Lock syringe (Precision Sampling Corp., Baton Rouge, LA) and the rubber stopper was resealed using silicone rubber. Measurements of N_2O in the headspace were analyzed every 24 h for a duration of 6, 7, or 8 days on a Varian 3700 gas chromatograph (GC) fitted with a Valco 0.25-ml gas sampling loop, a Supelco Porapak Q column (1/8 in $\times$ 3 m), an electron capture detector, and an automatic data acquisition system. The carrier gas was 5% methane in argon flowing at 35 ml/min and the temperature settings were: injector at 200°C, column at 80°C (isothermal), and detector at 350°C. All vials were incubated at room temperature (23°C).

A subset of NO_3^--amended and live control vials was sacrificed at every time point, analyzed for pH, and used to measure $NO_3^- + NO_2^-$ and NH_4^+ for a nitrogen mass balance. NH_4^+ and NO_3^- concentrations were measured spectrophotometrically by the indolphenol blue method and the cadmium reduction method, respectively (Keeney and Nelson, 1982). At each time point, the sediment slurry was centrifuged (2000 rpm for 10 min) and the pH of the resulting supernatant was measured with an Orion ROSS 8104 combination electrode connected to an Orion model 420A meter. The liquid and pellet were recombined and poured back into the original vial. The centrifuge tubes were washed with 6 ml of 2 M KCl, which were returned to the original serum vial. 200 μl of 7% $HgCl_2$ were added and sediments were extracted overnight in the KCl solution. Samples were then filtered through a 0.2 μm membrane filter and stored at -20°C until analysis for the nitrogen mass balance.

Data analysis

N_2O standard gases were obtained from Scotty Specialty Gases (Plumsteadvillle, PA). N_2O production was quantified directly from the standard curve calculations and corrected by Henry's law to account for gas dissolved in the liquid phase (Stumm and Morgan, 1981). All nitrogen species concentrations were converted to μmol as N for mass balance, conversion, and conversion rate calculations. Rates were calculated for the linear portion of each curve using a linear regression (Microsoft Excel, 1997). Conversion of $NO_3^- + NO_2^-$ to N_2O was based on the amount of NO_3^- added (approximately 1 mg/g wet sediment or 18 μmol/g dry sediment); the amount added was measured as the amount extracted from the amended vials that were sacrificed at time 0. A conversion rate (following zero order kinetics) for N_2O was calculated for each vial that was repeatedly sampled over the entire linear period, which varied with depth. The reported rate is the mean ($\pm$SD) of the individual vial rates (totaling either six or eight) within the same depth and capsule treatment. The rates of $NO_3^- + NO_2^-$ disappearance were also based on the amount of NO_3^- initially added. However, since $NO_3^- + NO_2^-$ was only measured once for each vial due to destructive sampling, rates were calculated as the concentration change (over the linear period) between vials sacrificed in a time series. Conversion rates reported for $NO_3^- + NO_2^-$ disappearance are therefore the mean ($\pm$SD) of only two constructed rates, since a duplicate set of vials was sacrificed for $NO_3^- + NO_2^-$ at each time point. For pH data, the mean and one standard deviation of the mean pH of two vials receiving the same exact treatments were calculated.

All statistical analyses were performed using SAS (Statistical Analysis System, 1996). A series of one-way ANOVAs (Proc GLM) was used to compare rates of conversion to N_2O (from added NO_3^-) between vials receiving capsules and those not receiving capsules within each depth. The same type of analysis was used to detect a difference between rates of $NO_3^- + NO_2^-$ disappearance. A two-way ANOVA series compared pH levels between vials with capsules and vials without capsules over time within the same depth. Because the pH difference was not the same over all time points within the same depth, the pH data sets were split into early and late time points and analyzed separately. A one-way ANOVA was used to determine significant differences separately for the %sand and %silt + clay between depths. For all tests, an $\alpha = 0.05$ was used to determine if a statistically significant difference existed between experimental conditions.

RESULTS

Sediment and microcapsule characteristics

Sediment grain size analyses are presented in Fig.

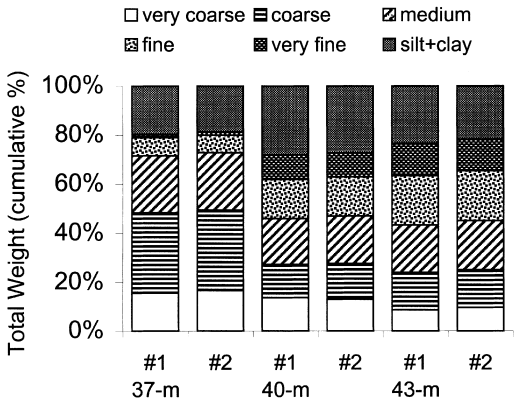

Fig. 1. Sediment grain size analyses from samples collected at three depths (in duplicate).

1. The 40-m sediment contained the most % silt + clay, followed by the 43-m, then the 37-m. The distribution of the sand fractions was different for each depth. The 37-m was predominantly coarse and medium-grain sands and contained very few fine grains. The 43-m contained more of the fine and very fine sands than the other two depths, while the 40-m was comprised of approximately equal proportions of coarse, medium, and fine-grained sands. Percent recovery was 99 ± 0 for 37-m, 98 ± 0.5 for 40-m, and 99 ± 0.5 for 43-m sediment. Statistical analysis showed that the 37-m and 43-m sediments were not significantly different from each other but were both different from the 40-m sediment in total %sand ($p = 0.0066$) and % silt + clay ($p = 0.0066$). Sediment total alkalinity (as $CaCO_3$) was measured as 53 mg/l for the 37-m, 31 mg/l for the 40-m, and 50 mg/l for the 43-m sediment. The corresponding $C_{T,CO3}$ values, $C_{T,PO4}$ values for the microcapsules added, and base equivalents for each sediment system with capsules are shown in Table 1.

Nitrous oxide

No N_2O was produced in the metabolically inhibited sediment or live controls without NO_3^- at the 37-m depth (data not shown). After a lag time of approximately 24 h, N_2O exhibited linear production in all (37-m) live NO_3^--amended vials until 72 h (Fig. 2a). N_2O conversion rates calculated from 24 to 72 h were $2.0 \pm 0.5\%$ h^{-1} for vials with microcapsules and $2.3 \pm 0.2\%$ h^{-1} for vials without microcapsules. This difference was not statistically significant ($p = 0.16$).

The N_2O production in the 40-m sediment was lower than in the 37-m sediment (Fig. 2b), and exhibited < 50% conversion of NO_3^- to N_2O in microcosms with and without capsules. A lag of approximately 24 h was again evident and approximately linear N_2O production continued until 72 h. A small amount of N_2O was produced in the 40-m live controls (0.7–1.7%), but the levels were much

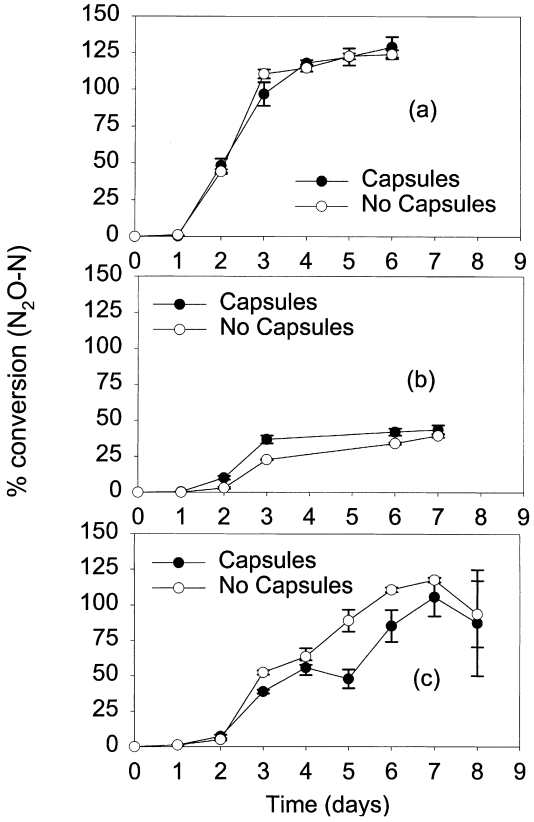

Fig. 2. Nitrous oxide (N_2O-N) production as a percentage of initial nitrate (NO_3^--N) added in sediment from (a) 37-m, (b) 40-m, and (c) 43-m depths after addition of 1 mg NO_3^-/g dry sediment ($\pm SE_x$).

lower than in the sediment amended with NO_3^-. No N_2O was detected in the metabolically inhibited vials at this depth. The rate of conversion to N_2O for sediment with capsules was $0.8 \pm 0.1\%$ h^{-1} and for vials without capsules was $0.5 \pm 0.0\%$ h^{-1}. The conversion rate was significantly higher ($p = 0.0005$) in NO_3^--amended sediment with capsules than sediment without capsules.

The N_2O production in the 43-m sediment exhibited linear production from 24 to 144 h (Fig. 2c). Complete conversion of the added NO_3^- to N_2O also occurred in this sediment, with a rate of $0.7 \pm 0.2\%$ h^{-1} in sediment with capsules and $1.0 \pm 0.1\%$ h^{-1} in sediment without capsules. Again, no N_2O was detected in vials that did not receive NO_3^- or were metabolically inhibited. N_2O rates were significantly higher ($p = 0.026$) in microcosms without capsules than those with capsules.

pH

The pH of the 37-m sediment that was amended with NO_3^-, but not microcapsules, increased over the duration of the experiment, from 5.39 ± 0.01 at 0 h up to a peak of 8.44 ± 0.08 at 120 h (Fig. 3a). The pH at the final time point (144 h) dropped to 7.97 ± 0.52, however the standard deviation was

Table 1. Total alkalinity ($C_{T,CO3}$), phosphate ($C_{T,PO4}$), and base equivalents of sediment from three depths

Depth (m)	Initial pH	$C_{T,CO3}$ (mol/l)		$C_{T,PO4}$ (mol/l)	Base eqivalents (mol/l)	
		Sediment	Metabolic		Total alkalinity	Microcapsules
37	5.57	5.30×10^{-4}	8×10^{-3}	1.72×10^{-2}	8.24×10^{-3}	3.20×10^{-2}
40	6.33	3.07×10^{-4}	4×10^{-3}	3.43×10^{-2}	4.05×10^{-3}	6.39×10^{-2}
43	6.44	5.00×10^{-4}	4×10^{-3}	2.57×10^{-2}	4.11×10^{-3}	4.79×10^{-2}

large for this point as compared to other points. The pH in the NO_3^--amended vials containing microcapsules fluctuated slightly after 72 h, with a range of 7.09 ± 0.04 at 96 h to 6.88 ± 0.04 at 120 h. Statistical results for the NO_3^--amended vials indicated no significant difference ($p = 0.22$) in pH between microcapsule treatments at the early time points (0–72 h). However, a significant difference in pH ($p < 0.0001$) was found at the late time points (96–144 h). In the live controls (no NO_3^-), the pH increased from 5.53 ± 0.00 at 0 h to 7.33 ± 0.23 in vials without capsules and to 6.62 ± 0.11 in vials with capsules at 144 h. The pH in the metabolically inhibited vials was not measured for this depth.

The pH of the 40-m sediment did not increase substantially over time (Fig. 3b). pH values rose slightly from 6.26 ± 0.00 to 7.19 ± 0.10 in vials without microcapsules but stayed constant in vials with microcapsules. All live control vials showed no change in pH over time (data not shown). In NO_3^--amended sediments, the pH was not different between capsule treatments ($p = 0.81$) at the early time points (0–72 h), but was different at the two late time points ($p = 0.0005$).

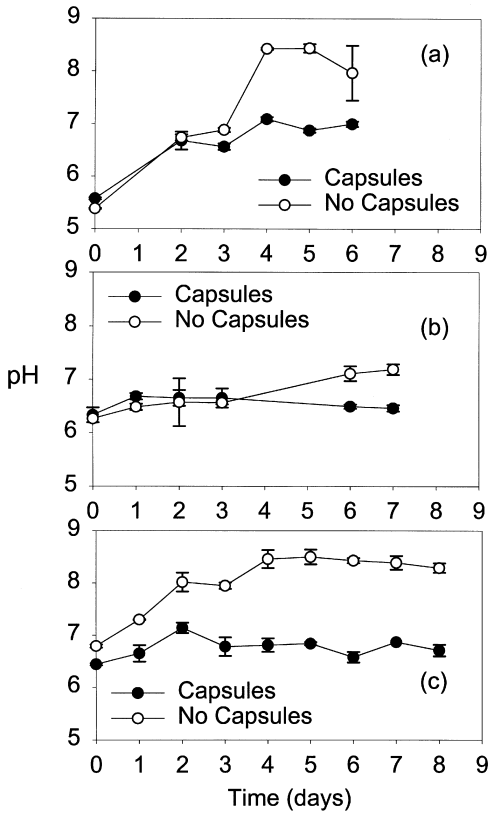

Fig. 3. Change in pH during denitrification in sediment from (a) 37-m, (b) 40-m, and (c) 43-m depths after addition of 1 mg NO_3^-/g dry sediment (±SD).

A different pattern was seen for the 43-m NO_3^--amended sediment (Fig. 3c), in which a significant difference in pH occurred at the early time points (0–72 h) between vials with and without capsules ($p < 0.0001$). During the late time points, the pH remained high for several days (8.46 ± 0.17 at 96 h to 8.29 ± 0.09 at 192 h) in vials with no capsules. The range of values for the vials containing microcapsules was 6.58 ± 0.10 to 6.87 ± 0.00 pH units for the same time period (96–192 h). With no NO_3^- addition, pH increased slightly at 48 h both in vials with and without capsules (data not shown), but returned to the 0-h readings at the next time point and remained stable. Actual pH values in the live controls were approximately 0.6 pH units higher for vials not receiving capsules than for those receiving capsules at each time point. NO_3^--amended sediment at the late time points showed a significantly higher pH ($p < 0.0001$) in vials without capsules. The metabolically inhibited microcosms at this depth had pH readings of 5.13 ± 0.01 (capsules) and 5.19 ± 0.01 (no capsules).

Nitrate

Measurements of NO_3^- disappearance showed a complete conversion of added NO_3^- to N_2O by 96 h in the 37-m sediment (Fig. 4a) and 168 h in the 43-m sediment (Fig. 4c). The 40-m sediment (Fig. 4b) showed some conversion (25%) in sediment with capsules and more (50%) in sediment without capsules. The conversion of NO_3^- was much faster in the 37-m sediment ($1.3 \pm 0.08\%$ h^{-1} for vials with and without capsules) than in the 43-m sediment, (0.32 ± 0.01 for vials with capsules and $0.73 \pm 0.14\%$ h^{-1} for vials without capsules). The rates of disappearance in the 40-m sediment were 0.23 ± 0.25 and 0.4% h^{-1}, for sediment with and without capsules, respectively. Statistical analysis revealed no difference in NO_3^- loss rates between capsule treatments for either the 37-m ($p = 0.77$), 40-m ($p = 0.68$), or the 43-m ($p = 0.055$) sediment. The background NO_3^- and NH_4^+ concentrations in these sediments were used in the mass balance calculations as those measured in the live control vials at 0 h. The background NO_3^- levels varied with depth

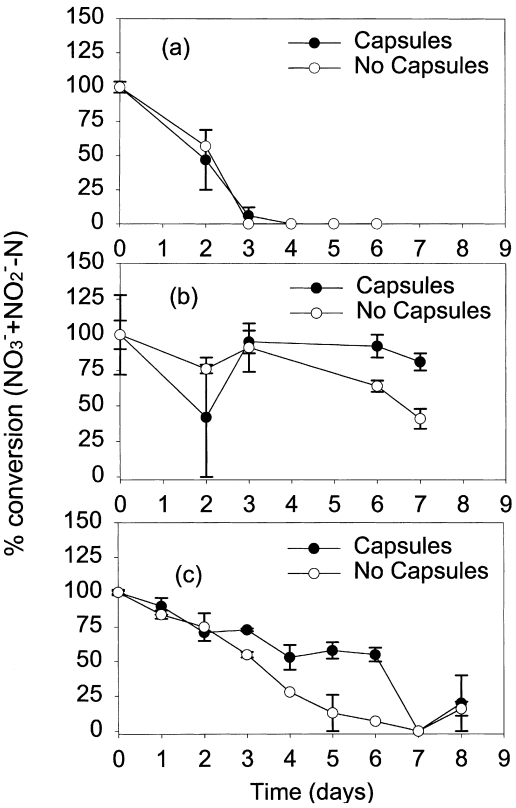

Fig. 4. Nitrate (NO_3^--N) consumption as a percentage of the initial nitrate (NO_3^--N) added in sediment from (a) 37-m, (b) 40-m, and (c) 43-m depths ($\pm SE_x$).

from 0.06–0.26 μmol NO_3^--N/g dry sediment. Results of nitrogen mass balance analyses showed nitrogen recoveries of 70–118% for the 37-m depth, 81–135% for the 40-m depth, and 72–110% for the 43-m depth in sediments to which NO_3^- had been added.

Ammonium

Background NH_4^+ concentrations ranged from 0.34–0.55 μmol NH_4^+-N/g dry sediment with depth. The final NH_4^+ concentrations over all treatments ranged from 0.16 ± 0.02 to 1.85 ± 0.82 μmol NH_4^+-N/g dry sediment for the 37-m, 0.12 ± 0.01 to 1.09 ± 0.17 for the 40-m, and 0.18 ± 0.03 to 1.15 ± 0.03 for the 43-m sediment. NH_4^+ levels fluctuated with time in all experiments and showed no pattern, except for possibly a gradual increase in the 43-m NO_3^--amended sediment. NH_4^+ concentrations in the metabolically inhibited vials were 0.33 ± 0.04 μmol NH_4^+-N/g dry sediment (capsules and no capsules) for the 37-m sediment, 0.26 ± 0 (capsules) and 0.25 ± 0 (no capsules) for the 40-m sediment, and 0.28 ± 0 (capsules) and 0.6 ± 0 (no capsules) for the 43-m sediment.

DISCUSSION

Few studies have been reported on the effect of high pH (>8.0) in the environment on microbial activity. It has been reported that pH levels of 4.0 (Bradley *et al.*, 1992) and 4.6 (Koskinen and Keeney, 1982) will slow denitrification processes in subsurface sediments. Previous studies have reported that pH > 8.3 will also reduce denitrification activity (Bogaert *et al.*, 1997; Drtil *et al.*, 1995). High pH also may affect contaminant degradation and mineralization processes by increasing organic contaminant solubility and mobility (LaFrance *et al.*, 1994; Stapleton *et al.*, 1994). For example, deBeer *et al.* (1992) found that the high local pH value within methanogenic aggregates in municipal wastewater had a greater influence on decreasing microbial conversion of acetate to carbonic acid than did the acetate concentration.

The SRS sediment bacteria produced N_2O in microcosms amended with NO_3^-, indicating that these sediments contained active denitrifiers. Though some trichloroethylene and perchloroethylene were present in the sediment, it was not evident that these chemicals had any toxic effect on the denitrifying bacteria. The rates for NO_3^- disappearance were faster than for N_2O production, as would be expected from the production of nitrite, which is used as an electron acceptor to produce N_2O. The complete loss of NO_3^- in the 37-m and 43-m sediments indicated that the production of N_2O could be attributed almost completely to the conversion of the supplied NO_3^-. There was no clear enhancement or adverse effect of the microcapsules on denitrification activity. The N_2O data for vials with capsules were indistinguishable from the data for vials with no capsules in the 37-m sediment, were higher than vials without capsules in the 40-m sediment, but were lower in the 43-m sediment. The 37-m and 43-m sediments were shown to be very sandy while the 40-m sediment was very clayey. Konopka and Turco (1991) and Aelion (1996) suggested that sandy subsurface sediments tend to exhibit higher microbial activity than clayey sediments, possibly due to moisture- and nutrient-limiting conditions in the micropores of the impermeable clay layers. This may explain why there was more activity, as far as N_2O production, in the two sandy sediments than in the clayey sediment.

Bacterial denitrification processes occurring in the 37-m and 43-m sediments caused pH levels to rise above 8.0, generally considered to be the highest pH optimal for heterotrophic sediment microbial activity. The microcapsules maintained a pH of 7.0 ± 0.2 units over all time points in the 37-m and 43-m sediments. This was most clearly demonstrated in the longest experiment, carried out on the 43-m sediment, where pH > 8.0 was prolonged for several days in microcosms without capsules. The observation of a minimal pH increase (as compared to the other two depths) in the 40-m sediment, from which < 50% NO_3^- conversion to N_2O was measured, also implied that there was a strong link between denitrification and increasing solution pH.

The results of the NH_4^+ analysis showed that little of the pH change over time was consistent with the change in NH_4^+ concentration for any of the sediments.

The pH increase that occurred during the first 24 – 48 h (N_2O lag time) in the 37-m and 43-m sediment may be due to the release of natural alkalinity present in the sediment or due to microbial HCO_3^- production. The calculations for base neutralizing capacity support the theory that the maximum base equivalents attributable to the phosphate in the microcapsules might have a greater impact on controlling the pH increase in the microcosms than the natural sediment alkalinity and/or microbial bicarbonate production. The base equivalent values for $C_{T,PO4}$ were an order of magnitude higher than the values for the sediment and metabolic $C_{T,CO3}$ in all of the sediments.

This study demonstrated that the encapsulated buffer was effective at maintaining a pH at the set point chosen for polymer rupture in saturated sediment microcosms. The data showed that when a bacterial process such as denitrification is induced, naturally occurring buffers might be insufficient to counteract any subsequent pH increase. Three different depths were chosen and a consistent response was observed from the encapsulated buffer under varying sediment particle characteristics, inherent alkalinity, and denitrification activity. In sediment such as the 40-m, which remained at neutral pH over the time points observed, the polymer did not release the buffer. However, it is possible that the 40-m sediment would have showed a $> 50\%$ NO_3^- conversion in a longer experiment, in which case the pH would increase more slowly at this depth than at the others. The microcapsule buffer would still be available at this later time point. Once the buffer is in place, the pH may no longer be a limiting factor for contaminant degradation, even in sediment slow to produce an inhospitable environment for microbes. The results from the microcosm study indicate that examining pH control to enhance biodegradation of a contaminant in more complex flow-through systems and contaminated aquifers is warranted.

CONCLUSIONS

The results of the sediment microcosm experiments indicated that in the presence of NO_3^-, denitrification in microcosms by subsurface aquifer microbes increased pH. Furthermore, phosphate buffer microcapsules coated with a pH-sensitive polymer neutralized this pH increase in the sediment that was amended only with 1 mg NO_3^-/g wet sediment. A pH of 7.0 ± 0.2 pH units was maintained in sediment containing microcapsules at all of the three depths tested. Two of the depths produced twice as much N_2O as the third, which may have been impacted by sediment particle characteristics as evidenced by the higher N_2O production in sandy sediment as compared to clayey sediment. NH_4^+ was produced in all sediments but did not appear to influence pH changes or bacterial activity over time. Base neutralizing capacity was limited in the system, as evidenced by prolonged high pH associated with N_2O production. Therefore, the ruptured microcapsules and not the total alkalinity achieved most neutralizing of base. The study indicates that microcapsules have the potential to be used *in situ* in other biological or chemical remediation systems where pH must be maintained at a set point in order for a particular process to take place most efficiently.

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