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Laboratory sand column study of encapsulated buffer release for potential in situ pH control

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Abstract

Encapsulation technology is being investigated as a method for controlling pH in situ at contaminated groundwater sites where pH may limit remediation of organic contaminants. This study examined the effectiveness of using KH_2PO_4 buffer encapsulated in a pH-sensitive coating to neutralize pH in laboratory sand columns (1.5-l) under a simulated groundwater flow rate and characterized the pattern of capsule release in the flow-through system. Denitrification was used in the columns to increase the pH of the pore water. Each of three columns was equipped with three miniature mesh wells to allow contact of the buffer with column pore water, but capsules (15 g) were inserted into only one column (amended). The two other columns served as amendment (no buffer) and abiotic (no denitrification) controls. Oxidation–reduction potential, dissolved organic and inorganic carbon, NH_4^+, $NO_3^- + NO_2^-$, PO_4^{3-}, and pH were measured in the influent, two side ports, and effluent of the columns over time. Near complete conversion of 80 mg N/l of nitrate and 152 mg/l of ethanol per day resulted in a mean pH increase from 6.2 to 8.2 in the amendment control column. The amended column maintained the target pH of 7.0 ± 0.2 for 4 weeks until the capsules began to be depleted, after which time the pH slowly started to increase. The capsules exhibited pulses of buffer release, and were effectively dissolved after 7.5 weeks of operation. Base-neutralizing capacity contributed by the encapsulated buffer over the entire study period, calculated as cation equivalents, was 120 mM compared to 8 mM without buffer. This study demonstrates the potential for this technology to mediate pH changes and provides the framework for future studies in the laboratory and in the field, in which pH is controlled in order to enhance organic contaminant remediation by pH-sensitive systems.

Keywords: Encapsulation; Buffer; Remediation; pH; Denitrification

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1. Introduction

In subsurface sediment, pH changes can occur as a result of microbial processes such as mineralization and denitrification or due to the presence of chemicals such as phenols and acidic pesticides. Metabolic products such as carbon dioxide (CO_2) and organic acids can decrease the pH of groundwater (Long and Aelion, 1999). In contrast, denitrification can produce alkaline conditions in the subsurface (Kennedy and Hutchins, 1992; Hutchins and Wilson, 1994). Fluctuating pH levels could limit microbial degradation of contaminants such as chlorinated VOCs due to the sensitivity of anaerobes to pH (Borden, 1994). For example, Zhuang and Pavlostathis (1995) and Lee et al. (1997) showed that tetrachloroethylene (PCE) dechlorination by mixed microbial cultures enriched from contaminated soil was maximized at pH 7, as compared to pH levels of 4, 5, 6, 8, or 9. Even a slight increase in pH (8.30 vs. 7.48) was shown to decrease the dechlorination of PCE in landfill leachate microcosms (Leahy and Shreve, 2000).

Regulation of pH in situ usually entails the use of natural buffers present in the aquifer (Marcus and Bonds, 1999), or limestone or pyrite added to a permeable reactive barrier (PRB). An alternative method for regulating pH is the in situ triggered release of phosphate buffer encapsulated by a pH-sensitive polymer. The coatings that form the encapsulation allow the contents to be released slowly and only as they are needed. In order to maintain a neutral pH, coatings that dissolve as pH rises above 7 or drops below 7 can be used to slowly add buffer to the surrounding water. In this study, a polymer (Eudragit™ S100, Röhm America, Piscataway, NJ) that is often used to encapsulate pharmaceuticals was used to coat KH_2PO_4 buffer. The polymer dissolves at a pH greater than 7.0. The capsules are 1 mm in average diameter and are meant to be introduced into the subsurface via a PRB or screened well.

The use of capsules may be a more efficient way to add nutrients and bacteria or control environmental conditions for remediation of sources or plumes of groundwater contamination. For example, enriched bacteria encapsulated inside dialysis bags have been shown to increase the mineralization of atrazine in groundwater over natural processes (Shati et al., 1996). These bags were lowered into a screened well that was drilled into a sandy aquifer contaminated with herbicides. Sodium percarbonate has been encapsulated as a way of delivering oxygen to bacteria metabolizing propylene glycol aerobically in soils with low hydraulic conductivity (Vesper et al., 1994). Encapsulated buffer may be useful in controlling pH during nitrate-amended bioremediation of alkylbenzenes (Hutchins, 1991) and BTEX (Borden et al., 1997). Permeable reactive barrier systems that are designed to contain iron filings may be particularly conducive to loading of encapsulated buffer.

Implementation of encapsulation technology for in situ pH control requires knowledge of the specifics of the buffer release in a dynamic system. Denitrification was chosen as the pH-increasing process in the system, because it has been shown previously that denitrification coupled with the mineralization of reduced organic compounds can result in a pH increase to 8.0–8.5 in cell suspension (Vanukuru et al., 1998) and in sediment microcosms (Rust et al., 2000). The objectives of this research were to test the potential use of encapsulation for in situ pH control, and to characterize the pattern of buffer release in the flow-through system under a simulated groundwater flow rate, by

neutralizing a pH increase created by microbial denitrification in a sand column specifically designed to incorporate the capsules.

2. Methods

Three glass columns (40.5 cm in length, 8 cm I.D.) equipped with three miniature wells each (Geoprobe Systems, Salina, KS) were designed for use in the flow-through experiments (Fig. 1). All three columns were similarly constructed. The wells were 6.5 cm in length, 6 mm I.D., made from double-woven 150-μm steel mesh, and were capped with a Swagelok™ fitting (0.32 cm O.D.) on one end. These wells were designed to contain the capsules during the experiment, to allow contact of the buffer with column pore water, and to simulate screened monitoring wells that might house the capsules in the field. The wells were equipped with fittings so the spent capsules could be replaced without repacking the column. Each column included two sample ports (1.27 cm I.D.)

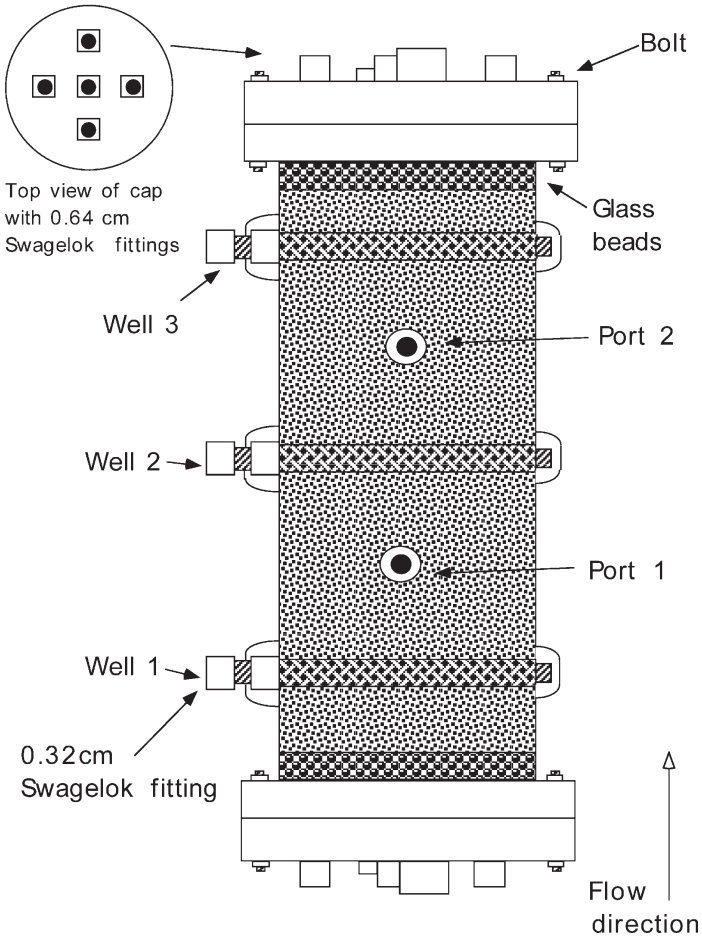

Fig. 1. Schematic of experimental flow-through sand columns showing location of sampling Ports 1 and 2 (actual size: 40.5 × 8 cm I.D.).

sealed with rubber septa and two custom-made Teflon™ caps with O-rings to seal the top and bottom. Five stainless steel Swagelok™ fittings (0.64 cm O.D.) were installed in each cap to provide sealed ports for entry and exit of liquid and for insertion of probes or syringes if necessary. In this study, only one of the ports in each cap was used for liquid flow; the other ports were plugged with M-Type septa (Alltech). The columns were operated at room temperature (23 °C) and covered with aluminum foil to shade out photosynthesizers (Nay et al., 1999). The capsules were only inserted into the wells of one column, called the amended column, in which active denitrification was taking place. The second column served as an amendment control since it underwent active denitrification but was not amended with the capsules. The third column served as an abiotic control to assure that the pH increase was due to denitrification, therefore microbial activity was inhibited using periodic 5-ml injections of 36.8% formaldehyde (final concentration 0.3%, Phelps et al., 1990) and contained no capsules. All columns were fed by timed peristaltic pumps (Manostat Varistaltic AL-Series Dual Pump for active denitrification columns, Beta Technology Speed 1000 for abiotic control column), which provided intermittent flow from one feed reservoir (5-l carboy). The cycle time was 1 min, so that the pumps were on for 6 s then off for 54 s. The columns were operated in an upflow, plug-flow manner.

The three columns were filled with a total of 75 g of 3-mm borosilicate glass beads above and beneath standard Ottawa grade sand (30–100 mesh, Thomas Scientific) that filled the column to capacity. Physical parameters of the columns (Table 1) were measured in order to set the pumps for a theoretical retention time of 24 h (Hutchins et al., 1998). Differences in the flow rate were attributed to slightly different capacities of the columns and slight variability in pump speeds. Actual residence time distribution (RTD) was measured for each column by a pulse introduction of 10 ml of a blue dye solution (1.0 g of blue food coloring/100 ml) and analysis of the outflow by spectrophotometry ($A = 400$ nm).

The columns undergoing active denitrification were inoculated by injecting suspended cells from a denitrifying batch culture grown from subsurface sediment, collected from the Savannah River Site (Aiken, SC). Five grams of KH_2PO_4 capsules were added to each of the three wells in the amended column. This was the maximum mass of capsules each well would hold. Sterile deionized water used to formulate the feed solution for the flow-through columns was sparged for 3 min/l with N_2 gas to replace the dissolved oxygen. The feed (per liter) consisted of: 400 mg ethanol, 607 mg $NaNO_3$

Table 1
Physical parameters of the sand columns

Parameter	Amended column	Amendment control column	Abiotic control column
Liquid volume (ml)	566	545	616
Sand volume (ml)	985	975	992
Volumetric flow rate (ml/day)	605	691	835
Darcy velocity (cm/day)	20.0	22.9	27.7
Retention time (h)	26.7	22.5	20.9

(100 mg N), 1 mM KH_2PO_4, 9 mg NH_4OH, 4 mg Na_2SO_4, 2 mg $MgSO_4 \cdot 7H_2O$, 1.2 mg $CaCl_2 \cdot 2H_2O$, 0.02 mg $FeCl_3 \cdot 6H_2O$, 0.8 mg $MnSO_4 \cdot H_2O$, 1.4 mg $ZnSO_4 \cdot 7H_2O$, 0.6 mg $CuSO_4 \cdot 5H_2O$, 0.4 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 0.6 mg $CoCl_2 \cdot 6H_2O$ (Vanukuru et al., 1998). Each fresh batch of nutrient solution was made daily for the duration of the flow-through experiment. To control contamination, the feed tank was autoclaved weekly and influent lines flushed periodically with a 70% ethanol solution and rinsed with sterile feed solution. Some PO_4^{3-} was added to the feed to accommodate the fact that sand was used as the medium. The encapsulated buffer was therefore evaluated assuming some background buffering capacity would be contributed by the sediment or soil medium when used in the field. High concentrations of ethanol and nitrate were added to ensure that a pH of 8.0–8.5 was achieved in the actively denitrifying columns.

For sampling, influent and effluent lines were routed through 20-ml volatile organic analysis (VOA) vials equipped with two-hole #2 rubber stoppers, similar to a configuration used by Hutchins et al. (1998). Freshly autoclaved 20-ml VOA vials were filled with the feed solution and connected to the line to replace the removed sample. Sampling from the influent and effluent was done every weekday for 52 days (7.5 weeks). For the last 5 days of the experiment, samples were taken daily from the two side ports of the column, and oxidation–reduction potential (ORP) was measured. A sterile 10-ml syringe equipped with a sterile 21G needle was used to sample 10 ml of liquid each from the two side ports of all columns. The syringes were not filled under gradient conditions.

An Orion Model 420A pH meter equipped with a ROSS electrode was used to measure the pH in all samples immediately after removal from the column. A platinum redox probe attached to the same unit measured the ORP in samples immediately after pH was measured. Samples were then filtered through a 0.2-μm membrane filter and transferred to sterile 40-ml VOA vials for storage at 4 °C. The cadmium reduction method was used to analyze the samples for nitrate + nitrite ($NO_3^- + NO_2^-$) on a Spectronic 20D spectrophotometer as an indicator of denitrification (Keeney and Nelson, 1982). The ascorbic acid method was used to measure orthophosphate (PO_4^{3-}) (Olsen and Sommers, 1982) on a SHENA LASCA 6880 Automatic Chemistry Analyzer to determine the amount of buffer release that took place. Dissolved total carbon (DTC) and dissolved inorganic carbon (DIC) were measured using a Total Organic Carbon 5000-A analyzer (Shimadzu); the difference was recorded as dissolved organic carbon (DOC). This was measured to assure that carbon was not limiting in the system. Ammonium (NH_4^+) was measured by the indolphenol blue method (Keeney and Nelson, 1982) in some samples, to assure that NO_3^- respiration via denitrification was the dominant nitrogen-related process occurring in the columns.

The base-neutralizing capacity in the presence and absence of the encapsulated buffer was calculated by setting up two series of electroneutrality equations, one equation for each effluent sample from the actively denitrifying columns over the 52-day test period, and solving for the cation equivalents (mol/l) that would be necessary to balance the input anion concentrations. The cations consisted of K^+ and H^+ from the encapsulated buffer and others, such as Na^+, from the feed; obviously, not all cations added to the system would have an effect on pH. Using the actual measured concentrations of the

ionic compounds at each time point took into account the loss of some compounds due to biofouling and the PO_4^{3-} that was washed from the column every 24 h. Before calculating the cation equivalents produced from buffer release, two additional sets of equations were used to calculate the cation equivalents in the feed (initial conditions) and those produced from metabolism and denitrification. In order to simplify the calculations, the same initial conditions were used for each time point, which included the mean pH (6.27 ± 0.19), $C_{T,\ CO3}$ (1.25×10^{-4} M), $C_{T,\ NO3}$ (5.79×10^{-3} M), and $C_{T,\ PO4}$ (1.47×10^{-3} M) measured in the amended column influent. These values were not different in the amendment control or abiotic control column influent. An estimate for $C_{T,\ SO4}$ (4.83×10^{-5} M) was calculated from the amount added to the feed. Sulfate reduction was properly included, but its effect was negligible. The first electroneutrality equation used to set the initial conditions was as follows:

$$[M^+] + [H^+] = \beta_1 C_{T,CO3} + 2\beta_2 C_{T,CO3} + [OH^-] + \beta_1 C_{T,PO4} + 2\beta_2 C_{T,PO4} + 3\beta_3 C_{T,PO4} + C_{T,NO3} + C_{T,SO4} \quad (1)$$

where M^+ is the cation equivalents contributed by the constituents of the feed, β_1, β_2, and β_3 are ionization fractions for the multiprotic acid at the initial pH, and $C_{T,\ CO3}$, $C_{T,\ PO4}$, $C_{T,\ NO3}$, and $C_{T,\ SO4}$ are the total concentrations of the respective ions.

A second equation determined the cation equivalents in the effluent after carbon and sulfate metabolism and denitrification had occurred but before the capsules released the buffer. This calculation was performed using the mean pH (8.23 ± 0.41), $C_{T,\ CO3}$ (6.00×10^{-3} M), $C_{T,\ NO3}$ (3.14×10^{-5} M), $C_{T,\ S}$ (4.83×10^{-5} M), and $C_{T,\ PO4}$ (1.20×10^{-3} M) in the amendment control column effluent.

$$[N^+] + [M^+] + [H^+] = \beta_1 C_{T,CO3} + 2\beta_2 C_{T,CO3} + [OH^-] + \beta_1 C_{T,PO4} + 2\beta_2 C_{T,PO4} + 3\beta_3 C_{T,PO4} + C_{T,NO3} + \beta_1 C_{T,S} \quad (2)$$

where N^+ is the cation equivalents contributed by mineralization and denitrification, and $C_{T,\ S}$ is the total concentration of sulfide.

Finally, a third equation was used to solve for the cation equivalents produced at each time point from capsule release, incorporating an estimate of $C_{T,\ S}$ and the actual H, $C_{T,\ CO3}$, $C_{T,\ NO3}$, and $C_{T,\ PO4}$ values measured in each amended column effluent sample.

$$[C^+] + [N^+] + [M^+] + [H^+] = \beta_1 C_{T,CO3} + 2\beta_2 C_{T,CO3} + [OH^-] + \beta_1 C_{T,PO4} + 2\beta_2 C_{T,PO4} + 3\beta_3 C_{T,PO4} + C_{T,NO3} + \beta_1 C_{T,S} \quad (3)$$

where C^+ is the cation equivalents contributed by capsule release.

Eq. (3) was also used to calculate the daily cation equivalents in the effluent of the amendment control column for comparison purposes. The difference between these calculations and the results of Eq. (2) was that actual effluent conditions in the amendment control column at each time point were used instead of the average. A mass balance for PO_4^{3-} was determined for the capsules by subtracting the influent (feed) reading from the effluent reading at each time point, plotting these data vs. time, and calculating the approximate area under the curve to obtain a recovered amount of PO_4^{3-}.

A percent recovery was calculated by dividing the recovered amount of PO_4^{3-} by the calculated amount of PO_4^{3-} added in 15 g of capsules (30% wall material, in mol/l), then multiplying by 100. The area under the curve for the cation equivalent concentrations in the amended column effluent (including the amount in the feed) was used to calculate the mass of cation equivalents released from the capsules over the entire study period. For comparison, a similar summation was done for the cation equivalents in the amendment control column effluent for the 52-day period.

A series of one-way ANOVAs was used to evaluate the concentrations of each parameter measured (pH, $NO_3^- + NO_2^-$, ORP, DOC, DIC, PO_4^{3-}) at each sampling point separately between all three columns (SAS Institute, 1998). All analyses used a significance level of 0.05. A Pearson correlation was used to correlate pH and DIC.

3. Results and discussion

3.1. $NO_3^- + NO_2^-$

The mean $NO_3^- + NO_2^-$ concentration for the column influent was 81 ± 16 for the amended column, 78 ± 12 for the amendment control, and 81 ± 15 mg N/l for the abiotic control column. These differences were not statistically significant ($p = 0.55$, Fig. 2). The effluent concentration of $NO_3^- + NO_2^-$ in the abiotic control column was 78 ± 12 mg N/l, indicating that little denitrification took place. The amendment control column concentration in Port 1 was 0.89 ± 0.34, in Port 2 was 1.03 ± 0.45, and in the effluent was 0.44 ± 1.2 mg N/l. The Port 1 mean $NO_3^- + NO_2^-$ concentration in the amended column was 0.89 ± 0.79 and in Port 2 was 0.36 ± 0.21 mg N/l. This indicates that most of the denitrification, and hence most of the pH increase, occurred between the influent cap and Port 1. There were two peaks in the amended column effluent samples; between Days 21–28, the effluent peaked twice at 32 and 22 mg N/l, each time dropping back to zero. The mean $NO_3^- + NO_2^-$ concentration in the amended column effluent including the peaks was 2.4 ± 7.0 and without the peaks was 0.11 ± 0.30 mg N/l. Even with the peaks included, there was no statistical difference in effluent $NO_3^- + NO_2^-$ concentration between the amendment control and amended columns ($p = 0.28$). The denitrifiers did not appear to be sensitive to pH in the range 6.23–8.5 nor were they adversely affected by the presence of the encapsulated buffer. This allowed characterization of the buffer release pattern under sustained alkaline pH conditions.

3.2. NH_4^+

The mean concentrations of NH_4^+–N in the influents were not statistically different between the amended column (1.69 ± 1.84), abiotic control (1.33 ± 1.49), and amendment control column (1.39 ± 1.58 mg NH_4^+–N/l, $p = 0.696$, data not shown). The mean effluent values were slightly lower, 0.89 ± 0.65 for the amended column, 0.26 ± 0.62 for the abiotic control, and 0.38 ± 0.29 mg NH_4^+–N/l for the amendment control. The mean effluent value for the amended column was significantly higher than that of both the

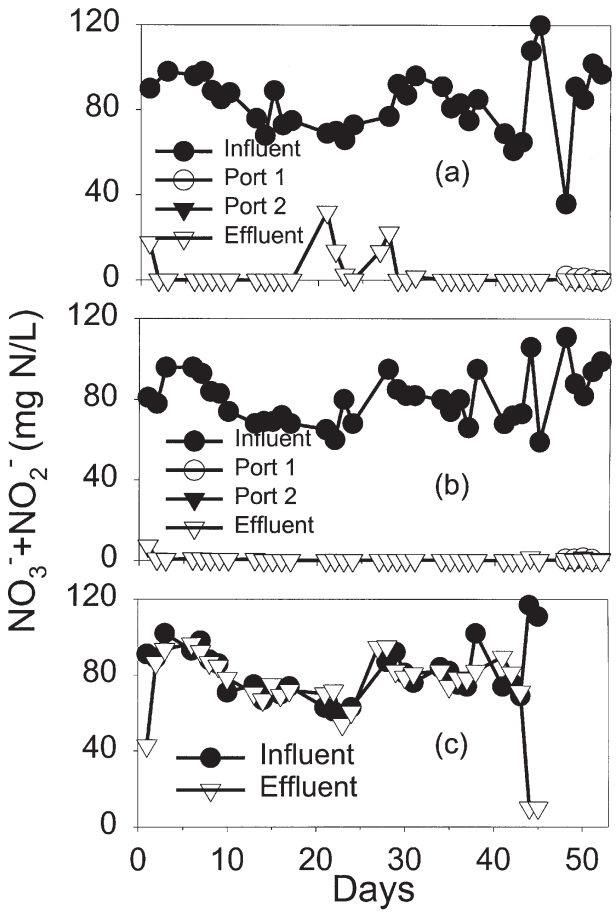

Fig. 2. Changes in $NO_3^- + NO_2^-$ over time (mg N/l) in (a) amended column, (b) amendment control column, and (c) abiotic control column.

abiotic control column ($p < 0.0001$) and the amendment control column ($p = 0.0008$), but the mean amendment control and abiotic control values were not different ($p = 0.42$). The conclusion from these results was that denitrification was the dominant nitrogen-removing process in the active denitrification columns.

3.3. DOC

Dissolved organic carbon in the samples included ethanol, added as a carbon source, dissolved polymer from the capsules, and any metabolites of ethanol. Biofouling in the influent lines and feed tank due to the large nutrient additions removed some of the ethanol before it reached the influent VOA vial. Influent VOA vials were installed in anticipation of such problems. These vials were used for sampling the actual concentrations of compounds in the influent directly before the solution entered the columns. In lieu of measuring the ethanol content the DOC was measured, since most of the influent DOC would be comprised of ethanol (no capsule polymer exposure). The mean influent DOC concentrations were 114 ± 18 mg/l C in the amended column and 106 ± 24 in the amendment control column, which were not significantly different ($p = 0.25$, Fig. 3).

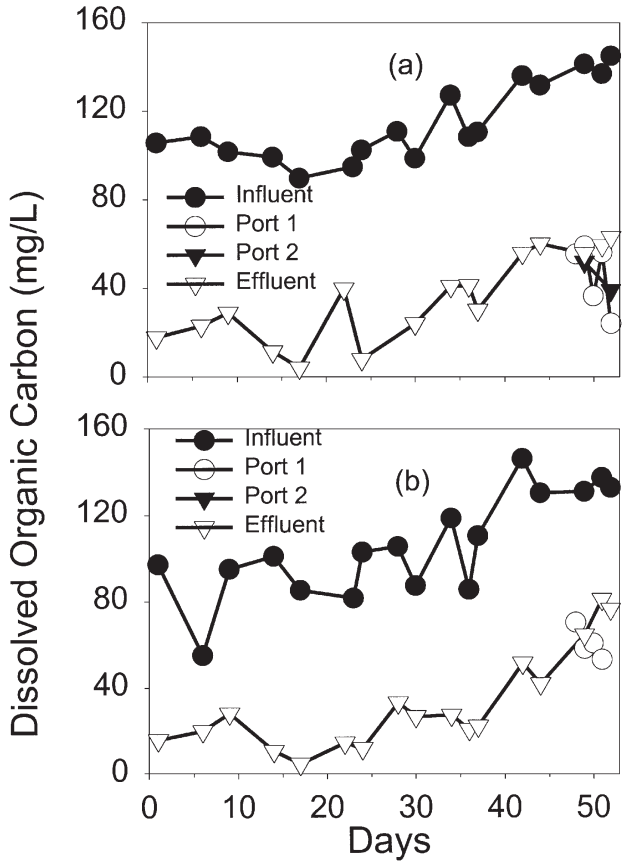

Fig. 3. Changes in dissolved organic carbon over time (mg/l) in (a) amended column and (b) amendment control column.

These mean results corresponded to 219 mg/l ethanol in the amended column and 203 mg/l in the amendment control column. Influent samples obtained from the abiotic control column were similar to these results (data not shown).

The Port 1 samples showed a decrease in DOC concentration to 46 ± 15 in the amended column and 61 ± 7.2 mg/l C in the amendment control column, indicating that significant metabolism occurred between the influent cap and Port 1 of both active denitrification columns. The mean effluent concentrations of DOC were 32 ± 23 in the amendment control and 35 ± 20 mg/l C in the amended column, which were not significantly different ($p = 0.71$). These DOC values suggested that microbial metabolism was not carbon-limited. Beginning at Day 42, the values of both the influent and the effluent DOC in the active denitrification columns increased approximately 20 mg/l C, which accounts for the high standard deviation of the reported values for the entire study period. This was attributed to better control of the biofouling in the influent lines toward the end of the study. The change in DOC between influent and effluent remained at approximately 80 mg/l C for both columns, therefore, the increase in DOC in the influent did not appear to increase uptake. The mean DOC consumed every sampling period was 6.63 ± 0.78 mmol C in the amended column and 6.14 ± 1.33 mmol C in the amendment control column. This assumes that the DOC contributed by the

polymer portion of the capsules was minimal. It was determined that each gram of capsules could contribute 195 mg TOC to the system (Baker, 2000). Assuming this was all dissolved, the total amount of DOC contributed over the entire study period was 2300 mg C. Since the DOC concentration in the effluent of the amended column was similar to levels seen in the amendment control column, then the DOC contributed by the capsules must have been rapidly consumed in the column regardless of concentration and did not inhibit uptake of ethanol. DOC and DIC data for the effluent of the abiotic control column were not comparable to the other columns because formaldehyde was used to inhibit the column, resulting in large concentrations (>2000 mg/l) of carbon. DOC and DIC from the other carbon sources could not be distinguished.

3.4. DIC

The DIC in the influent samples was 1.8 ± 0.95 in the amendment control column and 1.5 ± 0.47 mg/l C in the amended column ($p = 0.16$, Fig. 4). At Day 48, Port 1 values were 63 ± 4.3 in the amendment control column and 70 ± 2.7 in the amended column, further indicating that significant mineralization took place between the influent

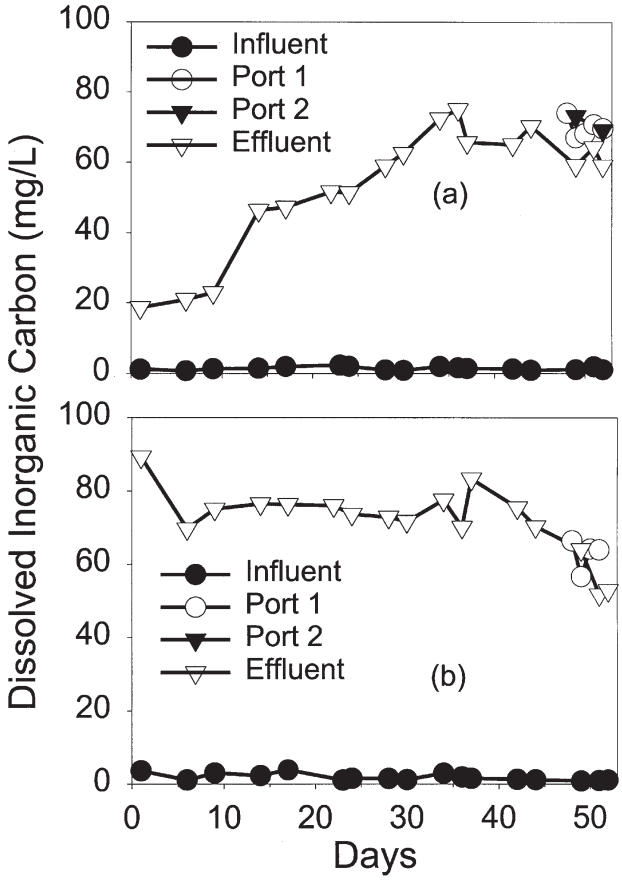

Fig. 4. Changes in dissolved inorganic carbon over time (mg/l) in (a) amended column and (b) amendment control column.

cap of both active denitrification columns and Port 1 (zone of denitrification). The DIC measured in the amendment control column effluent had a mean of 72 ± 9.3 mg/l C and remained relatively constant. The DIC in the effluent of the amended column, however, took longer to reach values around 70 mg/l C (36 days). The DIC data trends were different between the active denitrification columns, and each positively correlated with the pH data trends for that column ($r = 0.76$ and $p < 0.0001$). The DIC produced was 4.35 ± 1.42 mmol C in the amended column and 5.86 ± 0.71 mmol C in the control column, which was similar to the DOC consumed. The difference between the mean DIC and DOC in the effluent and in the mean DIC between columns may reflect loss of carbon dioxide through volatilization or incorporation of DOC into cellular material.

3.5. ORP

The mean ORP of the influent was 182 ± 48 mV in the amended column, 183 ± 34 in the amendment control, and 181 ± 41 mV in the abiotic control column ($p = 0.99$, Fig. 5). The mean ORP of the Port 1 samples was -40 ± 54 mV for the amended column and -32 ± 33 mV for the amendment control ($p = 0.76$). The mean ORP of the

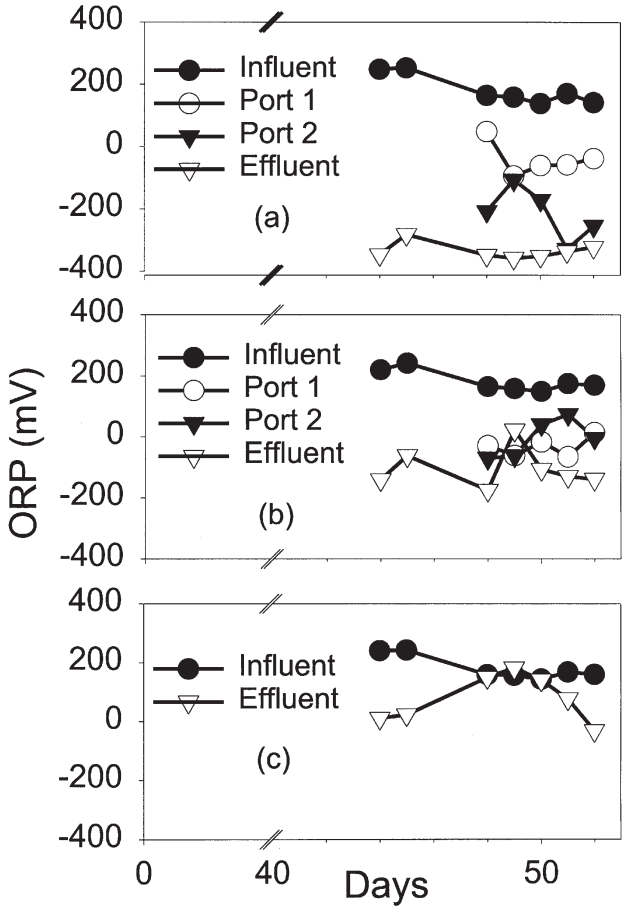

Fig. 5. Changes in oxidation–reduction potential (ORP, mV) over time in (a) amended column, (b) amendment control column, and (c) abiotic control column.

Port 2 samples was -214 ± 84 mV in the amended column and -4.6 ± 62 mV in the amendment control ($p = 0.0021$). No port samples from the abiotic control column were analyzed for ORP. Effluent samples were even more reduced in the active denitrification columns, -335 ± 26 mV in the amended column and -104 ± 65 mV in the amendment control. The abiotic control column had a mean of 79 ± 81 mV in the effluent, indicating no discernible microbial activity. The mean ORP of the effluent was statistically different for each column ($p < 0.0001$). The Port 2 and effluent samples were not as reduced in the amendment control column as in the amended column.

As evidenced by the decrease in ORP between the influent cap and Port 1 in the amended column, other anaerobic processes such as sulfate reduction and methanogenesis may have been occurring in the portion of the amended column between Well 3 and the effluent cap. Sulfate reduction can result in an increase in pH, while methanogenesis can decrease pH by a net production of CO_2. These reactions were considered to be less important as mechanisms influencing pH than denitrification because of the large concentrations of nitrate added to the columns. Since the interpretations of the encapsulated buffer release pattern are more closely tied to the pH maintained throughout the study and the ion concentrations in the feed solution, the fact that metabolic reactions other than denitrification may have occurred in the columns does not interfere with analysis of the buffer release pattern. The ORP did not decrease as much in the amendment control column, therefore it was possible that the pH control in the amended column created more favorable conditions for growth of sulfate-reducers and methanogens.

3.6. pH

The mean pH values ($\pm$ SD) for the column influent, 6.27 ± 0.19 in the amended column, 6.22 ± 0.20 in the amendment control, and 6.30 ± 0.24 in the abiotic control, were not statistically different ($p = 0.25$, Fig. 6). The mean pH inside the amendment control column was 8.38 ± 0.12 in Port 1 and 8.47 ± 0.17 in Port 2, which were similar to the mean effluent pH of 8.23 ± 0.41. This two-pH unit increase was attributed to denitrification activity. In the abiotic control column the pH of the effluent showed periodic decreases and the mean was 5.69 ± 0.89. This was attributed to the formaldehyde plug that moved through the column to inhibit microbial growth. The pH of the amended column effluent was 8.71 on Day 0 when the capsules were added. The pH of the effluent quickly dropped to 6.8 after the addition of the capsules. The pH remained at 7.0 ± 0.2 for 4 weeks after the addition of the capsules. There appeared to be a linear increase in effluent pH throughout the study period. In the last 1.5 weeks the pH reached a maximum of 7.8. Despite this slow increase, the mean pH of the effluent in the amended column (7.33 ± 0.39) was statistically lower than in the amendment control column ($p < 0.0001$). By Day 48, when samples were taken from Ports 1 and 2 in the amended column, the pH was 8.51 ± 0.19 in Port 1 and 8.32 ± 0.13 in Port 2. Because of the location of the ports, these results indicated that the capsules in Wells 1 and 2 had dissolved by the time port samples were taken.

At Day 44, the pH in the amendment control column effluent began to decrease from 8.4 to 7.5 at Day 52, while the pH of the Port 2 samples remained high. The pH of the

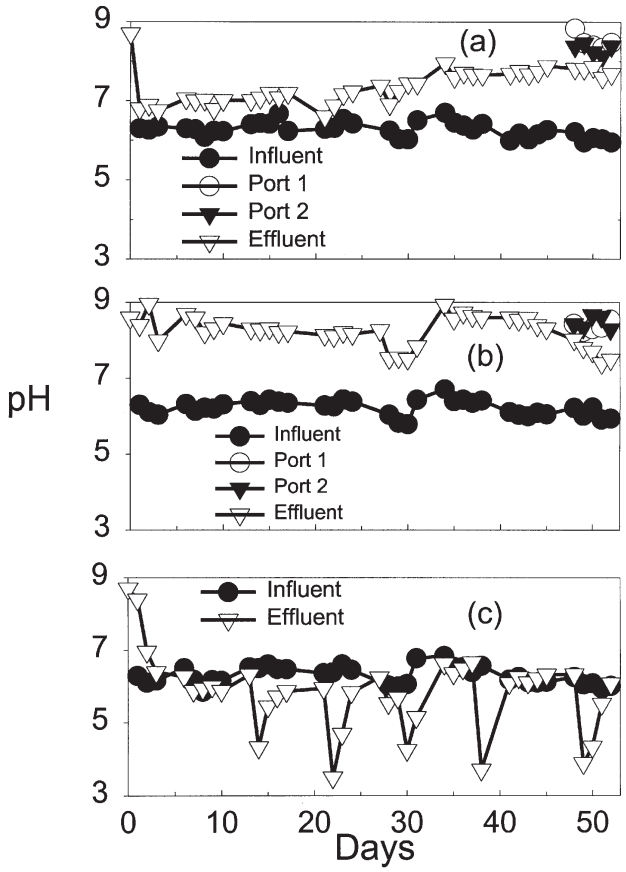

Fig. 6. pH change over time in (a) amended column, (b) amendment control column, and (c) abiotic control column.

last five amended column effluent samples also was lower (7.82–7.68) than the Port 2 samples (8.32 ± 0.13). Fermentative bacteria could have been responsible for the decrease in effluent pH in the active denitrification columns at the end of the study by producing organic acids. Methanogenic activity also could have decreased the effluent pH from CO_2 production. DOC increased in the effluent during this time period, which would be expected if metabolism had shifted from CO_2 production to fermentation. Nitrate uptake remained nearly 100% during this period, therefore the decrease in pH was not from a reduction in denitrification.

3.7. PO_4^{3-}

The mean concentration of PO_4^{3-} in the influent was 140 ± 33 in the amended column and 133 ± 41 mg PO_4^{3-}/l in the amendment control column. There was no statistical difference between these concentrations ($p = 0.42$, Fig. 7). The amended column had concentrations of 113 ± 0.76 mg PO_4^{3-}/l in Port 1 and 116 ± 3.8 mg PO_4^{3-}/l in Port 2. In the amendment control column, the Port 1 reading was 109 ± 7.9 mg PO_4^{3-}/l and the Port 2 reading was 110 ± 4.2 mg PO_4^{3-}/l. The mean PO_4^{3-} concentration in the amendment control column effluent was 114 ± 21 mg PO_4^{3-}/l. Fig.

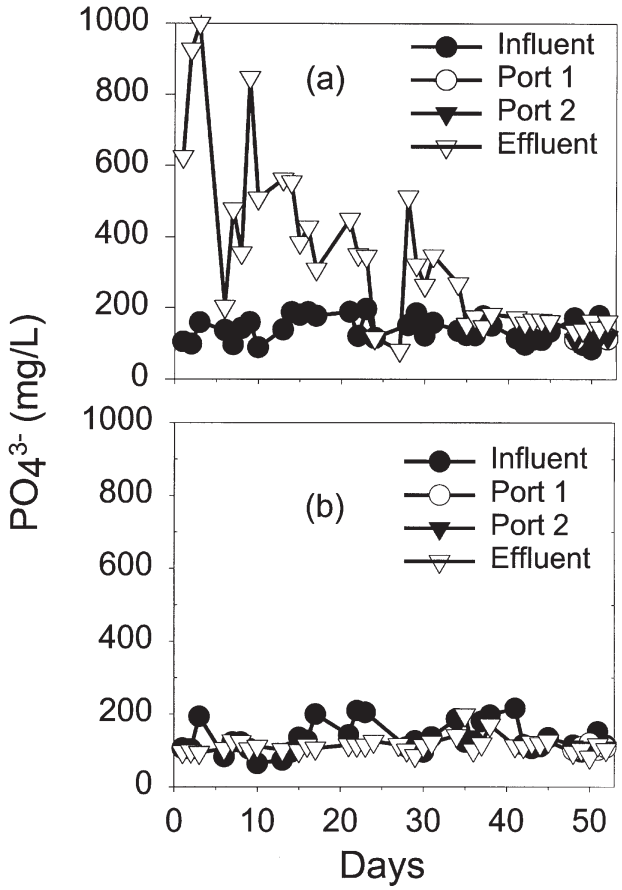

Fig. 7. Release of orthophosphate over time (mg/l) in (a) amended column containing 15 g of KH_2PO_4 buffer capsules, and (b) amendment control column without capsules.

7a shows the pattern of PO_4^{3-} release due to capsule rupture, measured as the PO_4^{3-} concentration in the effluent of the amended column. The difference in PO_4^{3-} between the amended and amendment control columns was attributed only to capsule release. The first and highest peak reached 1000 mg PO_4^{3-}/l on Day 2. The last peak appeared on Day 38 at a concentration of 182 mg PO_4^{3-}/l. The peaks became smaller over time until finally the PO_4^{3-} concentration leveled off around Day 36. The absence of peaks after Day 36 indicated that the capsules were near depletion around this time point. However, the mean PO_4^{3-} concentration in the amended column effluent between Days 38 and 52 was 159 ± 14 mg/l, which was higher than the concentration in the amendment control column for this same period, 114 ± 22 mg PO_4^{3-}/l. Of the 140 mM PO_4^{3-} that was inserted into the wells, 110 mM was recovered (79.3%).

The PO_4^{3-} data indicated that the buffer capsules released their cores in pulses of decreasing concentration. Spikes of release occurred because high-pH water dissolved several of the capsules at once; dissolution then stopped briefly due to neutralization of the pore water until it was pumped from the immediate proximity of the well and ultimately out of the column. Dissolution resumed once fresh feed entered from the influent end of the column, denitrification activity increased the pH, and the water

reached the capsules in the well. The first high-concentration PO_4^{3-} peak at Day 2 occurred because capsules from all three wells released at once. Capsules from all three wells responded because they were added when there was already a high pH throughout the entire column. However, after this time, water that reached Wells 2 and 3 should have been partially neutralized by buffer released from Well 1. A second peak at Day 9 may indicate that Well 1 was depleted at this time, forcing a large amount of the capsules in Well 2 to release in response to a plug of high-pH water that was not buffered by capsules in Well 1. A more continuous release of buffer took place after the second peak. A third peak at Day 28 may indicate depletion of the capsules in Well 2, with a subsequent release of capsules from Well 3, which decreased until the PO_4^{3-} concentration finally leveled off starting at Day 38. It is important to note that this pattern may have been different if the capsules had been introduced into water that was already at a neutral pH, or if the system recirculated the effluent water (batch mode).

Because the PO_4^{3-} in the effluent of the amended column was still higher than that in the control column after the concentration evened out at Day 38, there might have been a few capsules still in the wells releasing residual PO_4^{3-}. The Port 1 and Port 2 PO_4^{3-} readings were similar between columns and were not different from the influent, so if capsules remained in the amended column, they might have been limited to Well 3. Further evidence that only the capsules in Well 3 were still present was the pH readings from Port 2, which were higher than the effluent samples in the amended column at the end of the study. Conversely, the port readings for both pH and PO_4^{3-} could merely indicate that the area of influence of the released buffer may not have included the area from where the ports samples were drawn. In any case, the capsules were effectively dissolved during the last 5 days of the study period, since the target pH was no longer being maintained. At the high rate of PO_4^{3-} applied in the sand column, microbial uptake was insignificant in changing the concentration and therefore did not affect the release pattern.

3.8. Base neutralizing capacity.

The mean value for cation equivalents of the feed (initial conditions) was calculated from Eq. (1) to be 7.50 mM. The mean amount of cation equivalents produced by metabolism and denitrification was calculated from Eq. (2) as 8.25 mM. These equivalents were continually pumped into or produced within both actively denitrifying columns, so these numbers can be considered a daily input into the flow-through system and are not totals for the entire study period. The concentrations of cation equivalents produced over time were calculated for the effluents of the actively denitrifying columns and are compared in Fig. 8. The equivalents released from the capsules obviously followed a similar pattern as the PO_4^{3-} release and are assumed to consist mostly of H^+. The cation equivalents reached a maximum of 9.53 mM on Days 2 and 3 in the amended column, then decreased until Day 36 to nearly the same level as in the amendment control column effluent. Following the large initial spike was a decline to zero, indicating that a large capsule release took place initially in response to being introduced into alkaline pH conditions throughout the column. Once this initial spike had neutralized the pH within the entire column, a more gradual release of PO_4^{3-} occurred

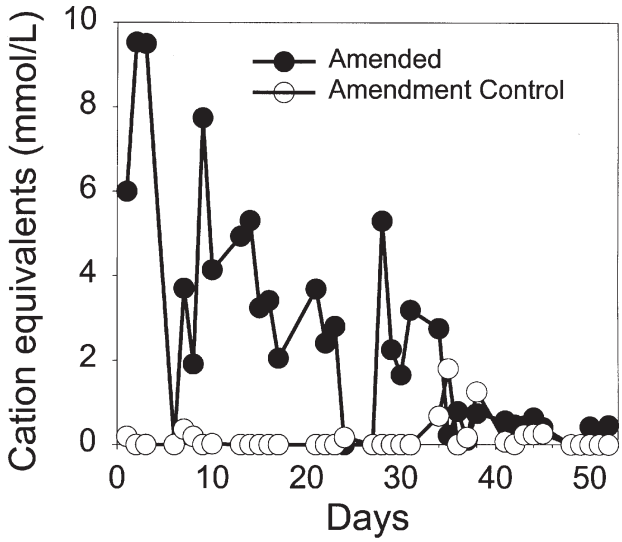

Fig. 8. Calculated cation equivalents in pore water over time (mmol/l) in amended and amendment control columns.

as high-pH water entered from the influent end of the column. The period between Days 6 and 24 showed continual capsule release with a majority of the daily cation equivalent concentrations remaining between 2 and 4 mM. When compared to the pH data during this same time period, it appears that the capsule release was in an equilibrium with base production, since the target pH was being maintained, and there were not as many drastic changes in cation equivalent concentrations. However, at Days 24–27 there was another decrease to zero, indicating that some change had rapidly occurred in the capsule concentration, such as depletion of at least one well. A smaller period of release occurred until Day 35 but the target pH was no longer being maintained, which may indicate that a fast depletion of the remaining capsules took place during this 11-day period. Between Days 35 and 52, the cation equivalents stayed more stable than in previous time points and approached the concentration in the amendment control column effluent. The pH continued to slowly increase in the amended column during this period of time.

The cation equivalents in the amendment control column effluent reflect base-neutralizing capacity in the absence of the encapsulated buffer. These values were zero for a majority of the study with some values of 0.19 at Day 1 and 0.17 mM at Day 24. Since no encapsulated buffer was added, the values for cation equivalents in the effluent of the amendment control column reflected slight variations in the influent PO_4^{3-} concentration and in the metabolism of ionic components. Between Days 34 and 38, there were readings of 0.67, 1.80, and 1.25 mM. Prior to this, during Days 28–31 there was a drop in the pH to 7.5 with a sudden rebound to 8.5, which may have indicated a loss of metabolic activity and a slight increase in ionic concentrations. The amount of cation equivalents released from the capsules over the entire 52-day study period was calculated to be 120 mM, compared to 8 mM that was available in the amendment control column effluent. The result of 120 mM was slightly higher than the concen-

tration of PO_4^{3-} (110 mM) that was reported because the influent values were not subtracted from the cation equivalents as was done for PO_4^{3-}.

4. Conclusions

Microbial denitrification proved to be a successful tool to increase pH to 8.0–8.5 in the sand columns so that the pattern of encapsulated buffer release could be elucidated. The pH in the amendment control column quickly increased two units over that in the influent due to denitrification. The same extent of denitrification and metabolism took place in the amended column as in the amendment control column, as evidenced by similar $NO_3^- + NO_2^-$ (80 mg N/l) and DOC (80 mg C/l) removal. However, DIC data were difficult to compare between the actively denitrifying columns since DIC was correlated with pH. It was assumed that the same pH increase seen in the amendment control column would have occurred in the amended column if not for the triggered release of encapsulated PO_4^{3-} buffer.

In this particular sand column configuration, considering column size, flow rate, initial pH, and extent of nitrate conversion, approximately 15 g of capsules maintained a neutral pH for 4 weeks. A general linear increase in pH was seen over the 52-day study period as the buffer was released and washed out of the sand column. The capsules contributed significant base-neutralizing capacity to the solution in the amended column, calculated to be an additional 112 mM of cation equivalents over the amount in the amendment control column. The pattern of release was pulses of decreasing PO_4^{3-} concentration and the approximate time to effective depletion was 7.5 weeks. At this time, the target pH was no longer being maintained even though some PO_4^{3-} was still measured in the effluent.

In a field situation, PO_4^{3-} would be released inside the PRB or screened well and would move through the aquifer with the groundwater. The released PO_4^{3-} could continue to act as a buffer unless it bound to the sediment. One disadvantage of using PO_4^{3-} is that in strongly acid soils insoluble precipitates can form. Because it is a nutrient, microbial uptake could also remove it from the groundwater. These issues must be addressed in future studies. The sensitivity of the encapsulated buffer to pH conditions in the flow-through system attests to the potential for in situ pH control using encapsulation technology. This study provides the framework for modeling and further investigations in the laboratory of using pH control, to enhance remediation of organic contaminants by pH-sensitive systems.

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