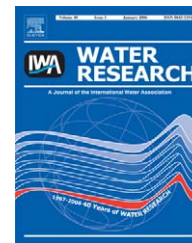


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# Chitin and corncobs as electron donor sources for the reductive dechlorination of tetrachloroethene

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## ARTICLE INFO

### Article history:

Received 4 June 2004

Received in revised form

26 February 2006

Accepted 4 April 2006

### Keywords:

Chitin

Corncobs

Tetrachloroethene

PCE

Reductive dechlorination

Fractional factorial design

## ABSTRACT

Chitin, corncobs, and a mixture of chitin and corncobs were tested as potential electron donor sources for stimulating the reductive dechlorination of tetrachloroethene (PCE). Semi-batch, sand-packed columns were used to evaluate the donors with aerobic and anaerobic groundwaters containing varying degrees of alkalinity. In all experiments, acetate and butyrate were the dominant fatty acids produced, although propionate, valerate, formate, and succinate were also detected. From a multivariable regression analysis on the data, the presence of chitin, limestone, and dechlorinating culture inoculum were determined to be the most positive predictors of dechlorination activity. Chitin fermentation products supported the degradation of PCE to trichloroethene (TCE), *cis*-1,2-dichloroethene (DCE), and vinyl chloride (VC), even in columns containing PCE DNAPL, whereas dechlorination activity was not observed in any of the columns containing corncobs alone. The longevity and efficiency of chitin as an electron donor source demonstrates its potential usefulness for passive, in situ field applications.

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## 1. Introduction

Improper handling, storage, and disposal of the toxic cleaning solvent tetrachloroethene (PCE) have contaminated sediments and groundwater supplies across the United States (National Research Council, 1994). In situ bioremediation emerged as a promising technology to remediate these contaminated aquifers more than two decades ago (Roberts et al., 1990; Semprini et al., 1992). In this technology, bacteria are stimulated to degrade PCE anaerobically by injecting electron donors and nutrients into the zone of contamination (Semprini et al., 1992). Unfortunately this injection process, which is labor and cost intensive, can result in well-clogging due to an excess of microbial growth (US Army Corps of

Engineers, 2003), and often fails to achieve the proper reducing conditions necessary for reductive dechlorination. For in situ bioremediation to gain widespread use and regulatory acceptance, a successful, low cost, and low maintenance method is likely required. This method must provide enough nutrients to maintain an electron donor and nutrient rich reducing environment favorable for reductive dechlorination, but not so much as to over-stimulate the growth of non-remediating microorganisms that can cause detrimental decreases in hydraulic conductivity.

As an alternative to liquid electron donor addition into the subsurface, numerous investigators have examined the ability of solid polymeric organic materials (POMs) to stimulate reductive processes. POM degradation proceeds through

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doi:10.1016/j.watres.2006.04.011

two main processes: hydrolysis and fermentation. The rate of initial hydrolysis is dependent on the type of polymer and the accessibility of extracellular enzymes to the polymer matrix (Cheng et al., 1987). The monomers derived from hydrolysis may affect the degree of success of remediation. Hydrolysis of cellulose and chitin, for example, results in the generation of glucose and N-acetylglucosamine monomers, respectively (Brock and Madigan, 1991). Although fermentation of both of these monomers will result in the production of fatty acids, CO<sub>2</sub>, and H<sub>2</sub> (Vera et al., 2001), the degradation of N-acetylglucosamine will also release nitrogen, a nutrient for biological growth. The optimum POM may be different for each contaminated site, and may depend on the soil/groundwater chemistry and the activity of the indigenous microbial population. For example, a site without any appreciable nitrogen source (e.g., no nitrate) might benefit from the addition of a chitinous POM, whereas a site containing a very active biomass might be better off with a slower degrading POM such as wood chips to prevent clogging. Thus, the molecular composition of the POM and the site conditions may both be important considerations in optimizing remediation.

Most research on environmental POM applications has focused on nitrate reduction to N<sub>2</sub>, although work in the area of chloroethene reduction is increasing. Volokita et al. (1996a, b) used both newspaper and cotton to treat nitrate-contaminated drinking water, and Schipper and Vojvodić-Vuković (1998) stimulated the removal of nitrate via denitrification in an aquifer by constructing a barrier wall composed of a mixture of sawdust and sand. They observed O<sub>2</sub> removal due to the degradation of sawdust, which resulted in a lower redox potential in the groundwater, and subsequently, the complete removal of nitrate. In a study by Wu et al. (1998), corn crop residue, wood chips, and newspaper were shown to support the reductive dechlorination of TCE to ethene in batch experiments. Although not a solid-phase POM, many researchers have also successfully used vegetable oils to support the reductive dechlorination of chlorinated ethenes (Hunter, 2002; Yang and McCarty, 2002). All of these studies have demonstrated the feasibility of using POM to promote reductive processes, yet very few have reported the type or concentration of fatty acids released during fermentation, or tested the POM under varying groundwater conditions. To date, there have been no published laboratory investigations on the use of chitin from crab shells for stimulating the reductive dechlorination of chlorinated ethenes.

The objectives of this study are to determine which fatty acids are produced from crab shell-derived chitin and corncob fermentation, and to determine if these fatty acids promote reductive dechlorination under both aerobic and anaerobic groundwater conditions, in the presence and absence of dense nonaqueous phase liquid (DNAPL) PCE, in the presence and absence of limestone (a natural buffering mineral), and in the presence and absence of a mixed dechlorinating culture. A factorial design of experiments was selected to statistically determine which of these variables are the most important for supporting reductive dechlorination. Chitin and corncobs are hypothesized to degrade at different rates, and chitin is expected to provide

more favorable conditions for reductive dechlorination because it contains nitrogen and residual calcium carbonate (CaCO<sub>3</sub>) from the shell material.

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## 2. Materials and methods

### 2.1. Chemicals

PCE, trichloroethene (TCE), cis-1,2-dichloroethene (DCE), vinyl chloride (VC), and methanol were obtained from Aldrich Chemical Co. (Milwaukee, WI). Lab grade methane and hydrogen, as well as ultra-high-purity (UHP) helium and nitrogen gasses were obtained from S. J. Smith Welding Supply (Davenport, IA). A 100-ppm hydrogen gas standard was obtained from Supelco (Bellefonte, PA). A fatty acid standard mix containing C<sub>1</sub>–C<sub>7</sub> acids at 10-mM each was obtained from Supelco (Bellefonte, PA). Limestone rocks (calcium carbonate) were donated by Prairie Central (Champaign, IL), and were broken into smaller chips using a mortar and pestle and then sieved to an 18–45 mesh (0.36–1 mm) particle size. Practical grade chitin flakes purified from crab shells were obtained from Sigma Chemical Co. (St. Louis, MO) and were found to contain 40.8% C and 5.7% N (Microanalysis Lab, University of Illinois at Urbana-Champaign (UIUC)). Corncobs (without kernels) were obtained from a farm in Champaign County, Illinois, and contained 44.0% C and 0.5% N (Microanalysis Lab, UIUC). The chitin and corncobs were pulverized using a coffee grinder, and then sieved to the particle sizes indicated below before being used in the experiments.

### 2.2. Supplementary cultures

The anaerobic pure culture, *Desulfuromonas michiganensis* strain BB1, which reductively dechlorinates PCE to DCE (Sung et al., 2003), and an anaerobic mixed culture enriched from Sangamon River sediments (Lodge Park, Piatt County, IL), also capable of reducing PCE to DCE, were added to column experiments as indicated below. (Note that subsequent enrichments of the Lodge Park culture in other work were capable of ethene production.) The cultures were grown separately in 160 ml serum bottles containing 100 ml of reduced anaerobic basal salts medium (Löffler et al., 1998) amended with Wolfe's vitamin solution (Atlas, 1997). Strain BB1 was maintained with a one-time addition of 1 mM acetate as an electron donor, whereas the mixed culture was maintained with 1 mM pyruvate. PCE was added to both cultures to give an initial aqueous concentration of 120 μM, which was replenished periodically after it was consumed. After incubation at room temperature (22 ± 1 °C) for approximately 2 months, equal volumes of the two cultures were combined in the same vessel prior to inoculation.

### 2.3. Groundwater sources

Natural groundwater from the Teays aquifer in Central Illinois was used for the semi-batch column experiments. It contained the following constituents per liter: 60.1 mg Ca<sup>2+</sup>, 38.2 mg Mg<sup>2+</sup>, 4.0 mg Fe<sup>2+</sup>, 290 mg alkalinity as CaCO<sub>3</sub>,

307 mg hardness as  $\text{CaCO}_3$ , and 3.0 mg dissolved organic carbon (DOC) (Najm et al., 1993). During these experiments, the pH of the Teays groundwater was 7.5, and the natural dissolved oxygen (DO) was 0.22 mg/l. Prior to being passed through the semi-batch columns, the groundwater treated to the desired oxygen level by either degassing it with nitrogen for at least 10 min to remove trace DO (anaerobic conditions), or by bubbling laboratory air through it for 30 min to reach a DO of 8.4 mg/l (aerobic conditions).

#### 2.4. Analytical methods

Aqueous concentrations of PCE, TCE, DCE, and VC were determined using a Tekmar Purge & Trap system (Tekmar-Dohrmann, Cincinnati, OH) connected to a Hewlett Packard 5890A gas chromatograph (GC) equipped with a photoionization detector (PID) and an electrolytic conductivity detector (ELCD). At each sampling time, 1 ml aqueous samples were preserved under 1 ml of methanol, sealed in glass vials with Teflon septa, and stored at 4 °C. At the time of analysis, 10  $\mu\text{l}$  of sample was added to 4.5 ml distilled, deionized water in a 5 ml gas-tight luer lock syringe (SGE International PTY LTD, Australia), and then transferred to the Tekmar 2016 Autosampler. Samples were purged with nitrogen gas for 11 min, and the volatilized compounds were trapped on a Tenax trap in the Tekmar 3000 Concentrator. The trap was then heated to 220 °C for 4 min, and the desorbed compounds were carried to a DB-624 column (J&W Scientific, Folsom, CA) with helium, where separation occurred isothermally at 60 °C.

Fatty acid concentrations were determined using a Waters 486 (Milford, MA) high-performance liquid chromatograph (HPLC) system equipped with a HPX-87H column (Biorad, Hercules, CA). Aqueous samples were prepared for analysis by centrifuging them at 10,000 rpm for 20 min, and then filtering the supernatant through a 0.45  $\mu\text{m}$  Millex-LCR membrane (Millipore Corporation, Bedford, MA). 900  $\mu\text{l}$  of filtered supernatant was then mixed with 100  $\mu\text{l}$  of 2N  $\text{H}_2\text{SO}_4$  in a sealed 1 ml autosampler vial (Kimble Glass, Inc., Vineland, NJ) and loaded onto a Waters 712 WISP autosampler. 100  $\mu\text{l}$  of each sample was injected into the HPLC mobile phase (0.005 M  $\text{H}_2\text{SO}_4$ ) which flowed at a rate of 0.6 ml/min into the column at 60 °C.

Soluble and total chemical oxygen demand (COD) concentrations were determined using the closed reflux, colorimetric method as described in Standard Methods (Clesceri et al., 1998). Protein as bovine serum albumin (BSA) was determined using the Folin reaction as described by Hansen and Phillips (1981). DO was measured using a YSI Model 58 dissolved oxygen meter with a 5905 DO probe (Yellow Springs Instrument Co., Inc., Yellow Springs, OH). pH was measured using a Fisher Accumet pH meter (Fair Lawn, NJ) with a Corning 476306 pH electrode (Corning Incorporated, Corning, NY).

#### 2.5. Semi-batch column experiments

To uncouple the effects of electron donor source and groundwater conditions on PCE degradation using chitin, corncobs, and a chitin-corn-cob mixture, semi-batch column experi-

ments were used to test the interrelated effects of six different variables: chitin, PCE, limestone, DO, corncobs, and dechlorinating microorganisms. Chitin and corncobs were selected as independent variables, so their ability to stimulate reductive dechlorination could be determined individually and as a mixture. To test all possible combinations of these six variables at two levels (low and high),  $2^6 = 64$  experiments would have to be conducted. To reduce the number of required experiments and still statistically determine which of these variables were the most important for stimulating dechlorination activity, a  $2^{6-2}$  fractional factorial design was used (Devor et al., 1992). This method of testing allows the statistical determination of the most important variables in a reduced number of experiments ( $2^{6-2} = 16$  experiments). The experimental design matrix was developed using the generators  $I = 1235 = 2346$  to minimize the confounding of effects. The components of each of the resulting 16 experiments are listed in Table 1.

For each experiment, a stainless steel column-containing POM (i.e., POM column) was connected in series with another stainless steel column containing PCE (i.e., PCE column). The columns had inner diameters of 8.5 mm, were 25 cm long, and were connected in series by stainless steel Swagelok reducing unions and 1/16 in tubing. The POM columns were packed with a mixture of +45 mesh (<0.35 mm) silica sand (Best Sand Corporation, Chardon, OH), 0.2 g 18–45 mesh (1–0.36 mm) limestone (Prairie Central, Champaign, IL), 18–45 mesh (1–0.36 mm) chitin (Sigma), and/or  $\geq 18$  mesh ( $\leq 1$  mm) corncobs. The total mass of POM added to each column was 1.16 g, with mass ratios of chitin:sand and corncobs:sand of 1:14 and 1:10, respectively. Columns containing both chitin and corncobs were packed with 0.58 g of each so that the total mass of POM in all columns would be equivalent. The PCE columns were packed with +45 mesh silica sand, or a mixture of sand and 0.2 g 18–45 mesh limestone. After packing, all columns were purged with three pore volumes (18 ml) of groundwater to remove fines and insure proper DO levels. PCE columns were either autoclaved or inoculated with one pore volume of supplementary cultures (50.0 mg/l protein as BSA). After a 4-day equilibration period for the microorganisms, 50  $\mu\text{l}$  (1% residual) of neat PCE was injected into the influent side of the DNAPL columns (see Table 1), and sampling was initiated as described below.

For every sampling period, groundwater was collected and treated to the desired DO, as described above. The treated groundwater was then pushed through each pair of columns every 2 days using a piston pump (Eldex Laboratories, Inc.) to exchange one pore volume (6 ml). For the column sets with 24  $\mu\text{M}$  PCE, 0.4 ml of a 440  $\mu\text{M}$  PCE stock solution was added to the POM column effluents and mixed before being pushed through the PCE columns. The water collected from the end of all PCE columns was tested for pH, COD, fatty acids, and chlorinated ethenes. The columns were evaluated over a period of 1 month.

After the main predictors of dechlorination activity were determined in the first experiment, a second semi-batch column experiment was conducted to more closely examine the effects of these predictors and that of chitin loading (i.e., the mass ratio of chitin to sand) on fatty acid production and dechlorination activity. To minimize the possible confounding

**Table 1 – Fractional factorial ( $2^{6-2}$ ) design matrix for the first semi-batch column experiment**

Column I.D.	Chitin	PCE	Limestone	O <sub>2</sub>	Corncob	Cultures <sup>a</sup>
1	None	24 μM	None	Anaerobic	None	None
2	Chitin	24 μM	None	Anaerobic	Corncob	None
3	None	DNAPL	None	Anaerobic	Corncob	Cultures
4	Chitin	DNAPL	None	Anaerobic	None	Cultures
5	None	24 μM	Limestone	Anaerobic	Corncob	Cultures
6	Chitin	24 μM	Limestone	Anaerobic	None	Cultures
7	None	DNAPL	Limestone	Anaerobic	None	None
8	Chitin	DNAPL	Limestone	Anaerobic	Corncob	None
9	None	24 μM	None	Aerobic	None	Cultures
10	Chitin	24 μM	None	Aerobic	Corncob	Cultures
11	None	DNAPL	None	Aerobic	Corncob	None
12	Chitin	DNAPL	None	Aerobic	None	None
13	None	24 μM	Limestone	Aerobic	Corncob	None
14	Chitin	24 μM	Limestone	Aerobic	None	None
15	None	DNAPL	Limestone	Aerobic	None	Cultures
16	Chitin	DNAPL	Limestone	Aerobic	Corncob	Cultures

<sup>a</sup> Dechlorinating microorganisms.

**Table 2 – Full factorial ( $2^4$ ) design matrix for the second semi-batch column experiment**

Column I.D.	Chitin:sand	Limestone	Cultures <sup>a</sup>	PCE
1	1:10	None	None	15 μM
2	1:5	None	None	15 μM
3	1:10	Limestone	None	15 μM
4	1:5	Limestone	None	15 μM
5	1:10	None	Cultures	15 μM
6	1:5	None	Cultures	15 μM
7	1:10	Limestone	Cultures	15 μM
8	1:5	Limestone	Cultures	15 μM
9	1:10	None	None	DNAPL
10	1:5	None	None	DNAPL
11	1:10	Limestone	None	DNAPL
12	1:5	Limestone	None	DNAPL
13	1:10	None	Cultures	DNAPL
14	1:5	None	Cultures	DNAPL
15	1:10	Limestone	Cultures	DNAPL
16	1:5	Limestone	Cultures	DNAPL

<sup>a</sup> Dechlorinating microorganisms.

effects introduced by the fractional design of the first experiment, a full factorial design with a total of four variables was created for the second experiment. Table 2 lists this  $2^4$  full factorial design. Of the four variables, limestone, culture, and PCE addition were varied as in the first experiment, but higher chitin:sand loadings of 1:10 and 1:5 were used. The source of the chitin and the source and mesh size of sand were also modified to resemble those of a pilot field study at the Distler Brickyard Superfund site that was conducted in parallel with these laboratory experiments (Brennan, 2003).

Stainless steel columns for the second semi-batch experiment were used as described above. The POM columns were packed with a mixture of 20–40 mesh API sand (Badger

Mining), 18–45 mesh purified SC-80 grade chitin (Vanson, Inc.), and/or 18–45 mesh limestone. The rate of fatty acid release is likely affected by the surface area to volume ratio of chitin particles, so the size of chitin in the second semi-batch experiment was kept consistent with that of the first semi-batch experiment. Limestone was added to give a mass ratio of 1:5 with chitin, as indicated in Table 2. The PCE columns were packed with 20–40 mesh API sand only. After packing, all columns were treated as described before, except that both the POM and PCE columns were inoculated with supplementary cultures which included an additional mixed culture enriched from Copper Slough sediments (Champaign County, IL), capable of reducing PCE to ethene.

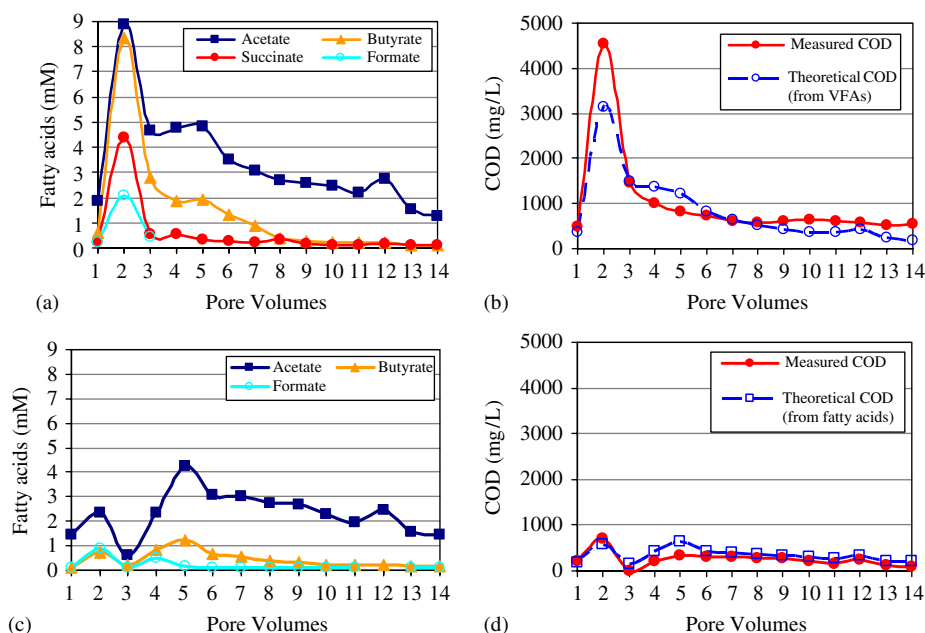


Fig. 1 – Examples of typical fatty acid and COD concentrations in the effluent of the first semi-batch column experiment. (a, b) Corncob column #5. (c, d) Chitin column #4. Column conditions are listed in Table 1.

### 3. Results

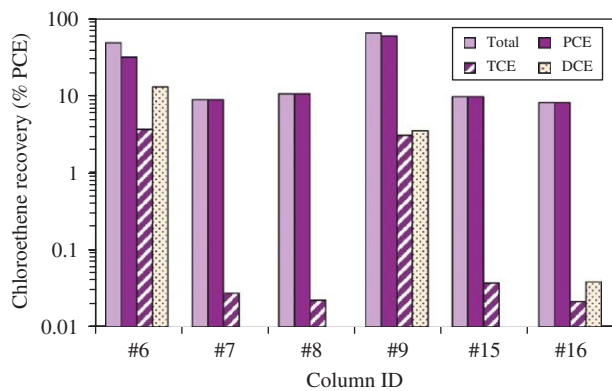
To determine which experimental variables were the most important for supporting dechlorination activity in a POM-augmented remediation system, statistically designed semi-batch column experiments were performed. Natural groundwater was exchanged in the first set of semi-batch columns for a total of 14 pore volumes. By the second pore volume, fatty acid concentrations were observed to increase in all columns. Examples of typical fatty acid and COD concentrations for a column containing corncobs and a column containing chitin are shown in Fig. 1.

All columns exhibited similar COD and fatty acid profiles, but with different magnitudes depending on the POM. In general, the columns showed maximum peaks of fatty acids within the first four pore volumes, which then gradually decreased to a concentration between 1 and 2 mM by the end of the experiment, regardless of the POM. Table 3 lists the maximum fatty acid and COD concentrations observed in each of the different types of POM columns. The high, initial spikes in the corncob columns are probably due to the presence of easily degraded fines (the corncobs used for the experiment were greater than 18 mesh ( $\leq 1$  mm)), whereas the chitin was 18–45 mesh (1–0.36 mm)). Sugars produced from the hydrolysis of corncob fines could also explain why the measured COD is above the theoretical COD (from fatty acid concentrations) in the second pore volume (Fig. 1). After the second pore volume, when the fines had been flushed through, agreement between the measured and theoretical COD in the corncob columns was very good. Measured and theoretical COD in columns containing chitin generally concurred throughout the experiment.

Table 3 – Maximum fatty acid and COD concentrations observed in the effluent of columns containing chitin, corncob, and a chitin–corncob mixture in the first semi-batch column experiment

	Chitin	Corncob	Chitin–corncob mix
Fatty acids (mM)	2–4	8–12	5–8
COD (mM)	22	140	63

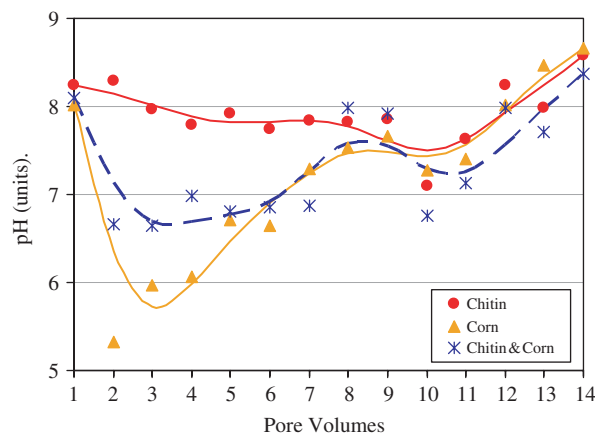
For all columns, acetate was the dominant fatty acid, generally being sustained at concentrations between 1 and 5 mM. In addition to acetate, butyrate was produced and was especially elevated at early times in the columns containing corncobs. Formate, succinate, propionate, and valerate were also detected, but at lower levels ( $< 1$  mM). The type of POM seemed to determine the types and concentrations of fatty acids produced. For example, succinate and formate were significant at early times in columns containing corncobs, but not in columns containing chitin. Although acetate was detected in all columns, its concentration decreased steadily over time in columns containing only corncobs, and was maintained at slightly higher concentrations at late times in columns containing chitin. Mass balance calculations on the cumulative fatty acids released during the experiment revealed that an average of 4.23, 1.92, and 3.18% of the POMs as carbon had been degraded into fatty acids in the columns containing corncobs, chitin, and the chitin–corncob mixture, respectively. The average of the corncob and chitin values, 3.07%, is almost equal to the value obtained for the corncob–chitin mixture, indicating that the mixture behaves roughly according to the relative contribution of its two components. Hydrogen was not measured in these experiments, despite its



**Fig. 2 – Cumulative PCE dechlorination products recovered in the effluent of the first semi-batch column experiment after 14 pore volumes. Recovery from columns containing DNAPL (columns #7, #8, #15, and #16) was primarily due to dissolution and was only a fraction of the total PCE injected into the columns. Column conditions are listed in Table 1.**

importance as a substrate for many dechlorinators. Other studies performed in our laboratory have shown that measured hydrogen represents a small fraction (generally <0.01%) of the total electron donating capacity released during chitin fermentation, whereas the majority is acetate (Brennan, 2003). Furthermore, measured values of hydrogen do not necessarily reflect hydrogen consumption rates (Löffler et al., 1999), so the intrinsic value of taking hydrogen measurements may not be that significant in fermentative systems where hydrogen is certainly produced at greater quantities than it is measured, such as the one tested here.

Dechlorination activity was observed in 6 of the 16 semi-batch columns (Fig. 2), and followed the typical, step-wise, reductive pathway known to occur under anaerobic conditions in the presence of more traditional electron donors. The most activity was observed in columns containing chitin, limestone, and supplementary cultures. Although column #9 (no chitin) also had dechlorination products, they were all observed at a single pore volume, indicating that activity was not sustained. Columns #6 (chitin) and #16 (chitin-corn cob mixture), however, exhibited increasing DCE concentrations at later pore volumes (i.e., at later times) indicating that dechlorination activity increased with time (data not shown). The importance of chitin as a POM is apparent when comparing columns #5 (corn cob) and #6 (chitin) which are otherwise identical: column #6 showed dechlorination activity, whereas #5 did not. Indeed, dechlorination activity was not observed in any of the columns containing corn cobs as the sole POM. This could be due to the unstable pH conditions observed in columns containing corn cobs, relative to those containing chitin. Effluent pH values in columns without any limestone for enhanced buffering capacity are shown in Fig. 3. Similar profiles were observed for columns containing limestone, but were raised by an average of 0.16 and 0.14 pH units, in the corn cob and chitin columns, respectively. Columns containing corn cobs displayed pH changes of as much as 3 units, even in columns containing limestone, dropping below pH = 6 in three of the four columns. By comparison, columns

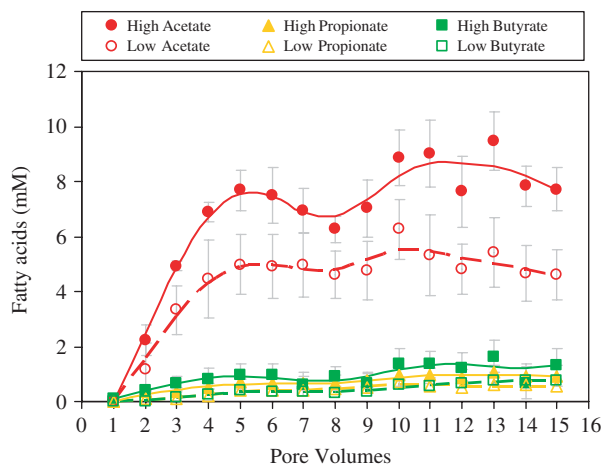


**Fig. 3 – Effluent pH values in semi-batch columns containing chitin, corn cobs, and a mixture of chitin and corn cobs, all without limestone buffer. Data points are duplicate averages; lines are the moving averages of the data points.**

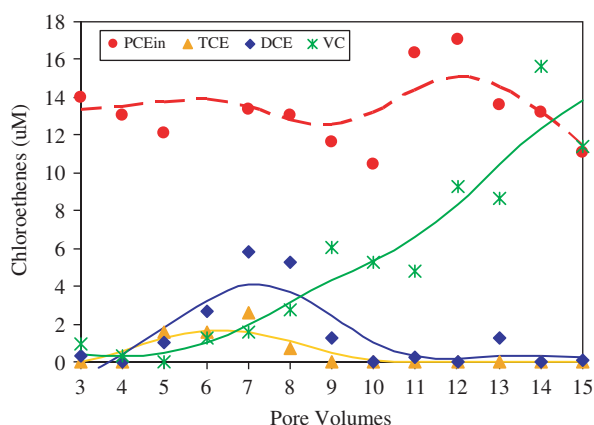
containing chitin alone generally fluctuated within 1 unit and remained above pH = 7, with and without limestone present. Of the six columns that exhibited dechlorination, half of them contained chitin or a chitin mixture, and the other half contained no POM at all. Those columns without POM presumably obtained their carbon and electron donor source from the groundwater, or in the case of column #15, from the inoculum as well. With a DOC = 3 mg/l, the groundwater had the capacity to donate enough electrons to support the dechlorination of 24  $\mu$ M PCE to DCE assuming a carbon oxidation state of -3 (the most liberal estimate). If the protein in the inoculum (50.0 mg/L protein as BSA) was used entirely as an electron donor source, it could support the dechlorination of 34  $\mu$ M PCE to DCE, assuming the formula for protein is  $C_{16}H_{24}O_5N_4$  (Sawyer et al., 1994). Either of these scenarios could account for the degradation products seen in these columns.

The most common predictor of dechlorination activity in this experiment was the presence of limestone: five of the six columns that displayed dechlorination activity contained limestone. Since limestone did not significantly affect pH (as described above), it may have instead benefited dechlorinating bacteria by serving as a source of  $CO_2$  or  $HCO_3^-$  for cell metabolism and growth (Samuelov et al., 1991). Regardless of what the beneficial mechanism behind limestone addition is, it appears to benefit the reductive dechlorination process.

To more closely examine the effects of the most important variables on dechlorination, a second semi-batch column experiment was performed. Natural groundwater was exchanged in the second set of semi-batch columns for a total of 15 pore volumes. As in the first experiment, fatty acid concentrations were observed to increase in all columns by the second pore volume. Fig. 4 illustrates the fatty acid profiles from the effluent of semi-batch columns containing chitin at low (1:10) and high (1:5) ratios with sand and no added cultures. For all columns, acetate was the dominant fatty acid produced, with concentrations generally sustained between 2–6 mM and 6–10 mM for chitin:sand ratios of 1:10,



**Fig. 4 – Electron donor production observed in the effluent of semi-batch columns containing chitin at low (1:10) and high (1:5) ratios with sand and no added dechlorinating cultures. Data points are quadruplicate averages with 90% confidence intervals; lines represent the moving averages of the data points.**



**Fig. 5 – Influent PCE concentrations and effluent dechlorination products observed in a semi-batch column loaded with chitin at a ratio of 1:10 with sand. Lines are the moving averages of the data points.**

and 1:5, respectively. In addition to acetate, the fatty acids butyrate, propionate, isobutyrate, isovalerate, and formate were also detected, but at lower concentrations (<2 mM).

In the second set of semi-batch column experiments, chitin fermentation products supported dechlorination activity in all of the columns containing supplementary cultures, even in those containing PCE DNAPL. PCE was reduced to TCE, DCE, and vinyl chloride (VC) in these experiments. The final product of dechlorination, ethene, was not observed, which may have been due to the short retention time in the columns (2 days). The most dechlorination activity was observed in columns containing low loadings of chitin (1:10), low concentrations of PCE (15 µM), and limestone. Fig. 5 illustrates the typical dechlorination products observed in the effluent of a semi-batch column loaded with chitin at a ratio of 1:10 with sand and fed 15 µM PCE. Higher VC concentrations at later

pore volumes (i.e., at later times) indicate that dechlorination activity, or at least the extent of dechlorination, increased in the column with time.

To determine which were the most significant variables affecting dechlorination activity in the semi-batch column experiments, underdetermined, multivariable regression analyses were performed on the results (Devor et al., 1992). Here, dechlorination activity was defined as the cumulative mass of TCE, DCE, and VC (PCE dechlorination products) observed for each column. The statistical distributions of the resulting regression coefficients were examined for each dechlorination product using normal probability plots (not shown). Effects were considered significant based on their coefficient's distance from the mean. Variables with significant positive or negative effects on dechlorination in the first and second semi-batch column experiments are noted in Tables 4 and 5, respectively.

In the first set of semi-batch column experiments, positive predictors (variables with the most positive effects) of dechlorination activity are the presence of DNAPL PCE, limestone, cultures, chitin+limestone, DNAPL+limestone, and chitin+limestone+cultures. Overall, chitin and limestone are the most positive predictors of dechlorination activity, whereas the presence of corncobs is the most negative predictor. The presence of cultures had a significant positive effect on the generation of DCE, but not TCE. Oxygen seems to have neither a positive nor a negative effect on dechlorination activity. It is likely, therefore, that oxygen was entirely consumed in the POM columns before entering the PCE columns, where it could have had an inhibitory effect on dechlorinating microorganisms. This indicates a very positive advantage for using a fermentable substrate like chitin as an electron donor source: it has the potential to be successfully used for chloroethene remediation in either aerobic or anaerobic aquifers.

The most positive predictor of dechlorination activity in the second semi-batch column experiment was the presence of dechlorinating culture inoculum. This is consistent with the observation that all columns inoculated with dechlorinating cultures displayed dechlorination activity. The presence of DNAPL PCE had a slightly positive effect on TCE generation, but a negative effect on DCE generation. Higher chitin:sand ratios had negative effects on both DCE and VC generation, presumably due to the low pH conditions caused by the production of high concentrations of fatty acids during fermentation.

#### 4. Discussion

In these experiments, acetate and butyrate were the dominant fatty acids produced, which supported the dechlorination of PCE to VC. Acetate is a good electron donor for reductive dechlorination, and its oxidation has been shown to support the complete dechlorination of PCE to ethene in the presence of syntrophic microorganisms (He et al., 2002). Butyrate is also a good electron donor for chloroethene remediation because it is fermented further to acetate and hydrogen; the latter is a principle electron donor for reductive dechlorination (Fennell et al., 1997). Results from He et al.

**Table 4 – Regression analysis results for determining the most significant variables on dechlorination activity in the first semi-batch column experiment**

Possible variable combinations	TCE		DCE	
	Coeff.	Effect	Coeff.	Effect
Mean	5.756		5.675	
Chitin	-1.544		3.313	
PCE	2.944	+	-1.775	
Limestone	4.156	++	3.913	
O <sub>2</sub>	0.644		-0.613	
Chitin+PCE, or limestone+corncobs	-1.156		-0.613	
Chitin+limestone or PCE+corncobs	0.056		5.075	+
Chitin+O <sub>2</sub> , or corncobs+cultures	-1.706		-1.775	
PCE+limestone	4.544	+++	-0.013	
PCE+O <sub>2</sub> or limestone+cultures	0.256		3.313	
Limestone+O <sub>2</sub> or PCE+cultures	-0.956		-2.375	-
Corncobs	-2.756	-	-2.375	-
Chitin+limestone+cultures	1.106		5.675	+++
Error	-0.106		-0.013	
Cultures	1.856		5.075	+
O <sub>2</sub> +corncobs or chitin+cultures	-0.494		3.913	

Positive and negative effects on generation of TCE and DCE are noted with a "+" and "-", respectively. The greater the dechlorination activity, the more +'s indicated. Effects considered significant if:  $-2 > TCE_{coeff} > 3$  and  $-2 > DCE_{coeff} > 5$ .

**Table 5 – Regression analysis results for determining the most significant variables on dechlorination activity in the second semi-batch column experiment**

Possible variable combinations	TCE		DCE		VC	
	Coeff.	Effect	Coeff.	Effect	Coeff.	Effect
Mean	20.321		155.280		6.405	
Chitin:sand ratio	-2.989		-21.435	-	-1.148	-
Limestone	-0.806		-3.075		0.450	
Cultures	17.021	+++	144.825	+++	4.770	+++
PCE	15.619	+	-22.050	-	0.405	
Chitin+limestone	8.764		-4.200		-0.758	
Chitin+cultures	-4.219		-12.360		-1.058	-
Chitin+PCE	-3.701		5.340		0.818	
Limestone+cultures	-2.921		6.900		0.255	
Limestone+PCE	-2.929		-16.860		-0.540	
Cultures+PCE	12.319	+	-32.085	-	-0.750	
Chitin+limestone+cultures	8.209		-13.035		-0.368	
Chitin+limestone+PCE	6.971		11.790		0.428	
Chitin+cultures+PCE	-4.931	-	14.655		0.938	+
Limestone+cultures+PCE	-5.044	-	-7.275		-0.765	
Chitin+limestone+cultures+PCE	6.416		2.745		0.698	

Positive and negative effects on generation of TCE, DCE, and VC are noted with a "+" and "-", respectively. The greater the dechlorination activity, the more +'s indicated. Effects considered significant if:  $-5 > TCE_{coeff} > 16$ ,  $-25 > DCE_{coeff} > 20$ , and  $-1 > VC_{coeff} > 1$ .

(2002) indicate that methods that increase the flux of both acetate and hydrogen may be the most promising to support complete dechlorination at certain field sites. The fermentation of chitin is an excellent approach to produce both of these electron donors.

Greater dechlorination activity was observed in columns containing chitin (which were maintained near pH = 7.9) than in those containing corncobs (which fluctuated between pH 5.3 and 8.6). Residual calcium carbonate present in the

chitin shell material may have helped to maintain a more neutral pH and promote dechlorination activity. These results are consistent with the observation that PCE dechlorination is most favorable near neutral conditions (Gerritse et al., 1999). The lower pH observed with corncobs may have been due to the inclusion of corncob fines ( $\geq 18$  mesh,  $\leq 1$  mm) in the columns, which fermented rapidly to fatty acids within the first few pore volumes of the first experiment.

In addition to the electron donors, nutrients, and buffering capacity produced by chitin, it also has considerable longevity. Chitin degrades slowly, and once installed can last for considerable time ( $\geq 14$  pore volumes) before needing replacement. Under the conditions tested in this study, chitin maintained reducing conditions and continued to produce fatty acids throughout the experiment (one month). On-going field studies have shown chitin to produce fatty acids for as long as 18 months after emplacement (work in progress). This could imply significant cost savings for passive treatment systems in which chitin is installed as an in situ electron donor source. Since chitin is an abundant waste product of the crab fishing industry, its availability is not limited and costs are low. Chitin itself is relatively inexpensive, and retails for between \$1 and \$6 per pound depending on purity (JRW Bioremediation, LLC).

## 5. Conclusions

Results from these column studies indicate that using chitin from crab shells as an electron donor source may have several advantages over conventional, liquid electron donor injection for the in situ bioremediation of chlorinated ethenes:

- The diversity of chitin derivatives simultaneously provides numerous electron donors and nutrients, potentially allowing a general treatment for a variety of contaminants.
- The presence of chitin was shown to have a beneficial effect on dechlorination, and this may be due in part to buffering provided by residual calcium carbonate in the shell material.
- Chitin degrades slowly, and once installed can last for considerable time ( $\geq 14$  pore volumes) before needing replacement.

To our knowledge, this is the first laboratory study to show that chitin fermentation products support the reductive dechlorination of chlorinated ethenes. This work represents a necessary first step to assess whether chitin stimulates reductive dechlorination using field relevant materials. Additional work under continuous-flow conditions and in the field is necessary to determine how our results scale. The ability of chitin to support reductive dechlorination in a continuous-flow column system is demonstrated in a companion paper (Brennan et al., 2006).

## Acknowledgements

This research was performed with the support of a National Science Foundation Small Business Innovative Research (SBIR) Grant (Award no. DMI-0109868). The support of an NSF Graduate Research Fellowship and an Environmental Protection Agency STAR Fellowship for R.A. Brennan at the University of Illinois at Urbana-Champaign are also gratefully acknowledged. Frank Löffler of Georgia Institute of Technology is thanked for his donation of *Desulfuromonas michiganensis* strain BB1, and Kent Sorenson, Jr, now of Camp Dresser & McKee, is thanked for his many helpful discussions.

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