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Removal of Perchlorate in Ground Water with a Flow-Through Bioreactor

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ABSTRACT

The bacterium, perclace, has been shown to reduce perchlorate to less than the detection limit of 0.004 mg L^{-1} when grown on acetate under anaerobic conditions. In batch studies, the presence of nitrate does not significantly hinder the reduction of perchlorate. The ability of perclace to remove nitrate and perchlorate from ground water in a flow-through system is described in this study. Celite-packed columns of 300 ml were used to demonstrate the removal of perchlorate from ground water. At a flow rate of 1 ml min^{-1}, perchlorate was removed from 0.738 mg L^{-1} to less than detectable levels and when the flow rate was 2 ml min^{-1} 92 to 95% of the perchlorate was removed. Analysis of bacterial biomass at the completion of the study revealed that most of the bacterial growth was concentrated in the inlet area of the column. A circulating pump was added, with the idea that passing the ground water multiple times through the bacterially active zone might increase the efficiency of the column. In this experiment, perchlorate in ground water was reduced from 0.550 mg L^{-1} to nondetectable levels at a flow rate of 1 ml min^{-1}. When the flow rate was increased to 2 ml min^{-1}, 98% of perchlorate was removed and when the flow rate was 3 ml min^{-1} 95% of perchlorate was removed. Rapid removal of perchlorate by perclace immobilized in a bioreactor may provide an efficient, cost-effective technology for ground water remediation.

In the past five years, advances in ion chromatography have allowed detection of the perchlorate ion (ClO_4^-) at levels as low as 0.004 mg L^{-1} [California Department of Health Services (CDHS), 1997]. Because perchlorate is known to disrupt the production of thyroid hormones (Von Burg, 1995; Capen, 1994; Lamm et al., 1999), the CDHS has advised that wells containing more than 0.018 mg L^{-1} perchlorate not be used as a source of drinking water, even though there is no federal drinking water standard for perchlorate. According to the CDHS, 144 wells in California have detectable perchlorate levels and 38 wells exceed the CDHS action level of 0.018 mg ClO_4^- L^{-1} (CDHS, 1998). In Riverside and San Bernardino, California, some drinking water wells contain up to 0.216 mg L^{-1} ClO_4^- and nine wells have been closed in this area (CDHS, 1998).

The use of microorganisms to remove perchlorate from contaminated ground water is currently an area of intense interest (Herman and Frankenberger, 1998, 1999; Logan, 1998; Urbansky, 1998). Microbially based remediation of perchlorate is attractive because it has the potential to transform perchlorate into an innocuous end product, chloride, at the expense of minimal nutrient input (Malmqvist et al., 1991; Attaway and Smith, 1993; Rikken et al., 1996).

Other proposed remediation techniques, including ion exchange resins (Betts, 1998) or reverse osmosis (Gu et al., 1999), simply concentrate the perchlorate rather than destroying it. Little research has been done regarding the capacity of microorganisms to remove low levels of perchlorate. Most microbial remediation experiments have examined the reaction when high levels of perchlorate are present. These high levels, 1 to 1000 mg L^{-1}, are often found near industrial facilities involved in production and testing of rocket fuel.

Treatment of very low concentrations of perchlorate, such as those found in southern California drinking water, has only recently become an issue because analytical methods for perchlorate detection have greatly improved. The objective of this work was to design a ground water treatment system that would remediate very low levels of perchlorate to less than the CDHS action level and preferably to less than detectable levels.

The bacterium perclace, used in this work, was described by Herman and Frankenberger (1999). Perclace displays 90% homology with members of the beta subdivision of the *Proteobacteria*, as determined by ribosomal RNA analysis. No close matches were found in the database. It is a gram-negative rod, capable of reducing perchlorate and nitrate simultaneously in batch culture, only in the absence of oxygen, with acetate as the carbon source. The optimum temperature for perchlorate reduction by perclace is between 25 and 30°C with a pH 7.0 to 7.2. Other growth characteristics of this bacterium are described in Herman and Frankenberger (1999). The ability of perclace to degrade perchlorate and nitrate simultaneously from ground water, in a flow through system, is described in this work.

MATERIALS AND METHODS

Cultivation of Perclace

Perclace was maintained in culture by monthly transfers into a mineral salts medium (FTW) with 500 mg L^{-1} of ClO_4^- (added as sodium perchlorate, Aldrich, Milwaukee, WI)

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Abbreviation: CDHS, California Department of Health Services.

and 1000 mg L^{-1} sodium acetate (Herman and Frankenberger, 1999). The medium (125 mL) was autoclaved in 125-mL Erlenmeyer flasks, inoculated with 1 mL of perclace cell suspension, and sparged with nitrogen gas. The flasks were sealed with a screw cap. Although the redox potential necessary for perchlorate reduction was not measured, it was estimated to be below −110 mV. This is known to be the E_h at which the redox indicator, resazurin, is reduced from pink to clear (Jacob, 1970), a visible change that was always necessary for perchlorate reduction to occur. This phenomenon has been described previously for another perchlorate-reducing culture (Attaway and Smith, 1993).

Perchlorate, Acetate, Nitrate, and Chloride Analysis

A perchlorate specific electrode (model 93-81, Orion Research, Boston, MA) was used to detect perchlorate concentrations between 1 and 1000 mg L^{-1}. Detection of perchlorate to 0.004 mg L^{-1} was performed by ion chromatography using an IonPac AS11 column (Dionex, Sunnyvale, CA) and following the procedure described by Wirt et al. (1998). The eluent was 100 m*M* NaOH at a flow rate of 1 mL min^{-1} with conductivity detection. An ASRS-II (4 mm, Dionex, Sunnyvale, CA) operated at 300 mA with water as the regenerant (10 mL min^{-1}), which suppressed the eluent. The sampling loop was 0.740 mL.

An IonPac AS14 column was used for analysis of nitrate and acetate with 3.5 m*M* Na_2CO_3 /1.0 m*M* $NaHCO_3$ at a flow rate of 1.5 mL min^{-1} as the eluent. The ASRS-II was operated at 50 mA. Chloride was determined using a HBI digital chloridometer (Haake Buchler Instruments, Saddle Brook, NJ).

Medium

Medium was either FTW (Herman and Frankenberger, 1999) or BMS [KH_2PO_4 (20 mg L^{-1}), Na_2HPO_4 (75 mg L^{-1}), NH_4Cl (60 mg L^{-1}), $MgSO_4 7H_2O$ (10 mg L^{-1}), $CaCl_2 2H_2O$ (10 mg L^{-1}), $FeCl_2 4H_2O$ (2 mg L^{-1}), plus a mixture of trace metals described by Focht (1994)]. The carbon source was acetate (as sodium acetate, 1 g L^{-1}). The BMS was the preferred medium because it created less interference during ion chromatography.

Ground Water

Ground water was pumped from perchlorate-contaminated wells in the San Gabriel Valley, CA. The initial sample of water contained 0.200 mg L^{-1} perchlorate while the second contained 0.550 mg L^{-1} perchlorate. Both samples had a pH of 7.5 and 26 mg L^{-1} NO_3^-. Two milligrams per liter phosphorus (as KH_2PO_4) and 20 mg L^{-1} nitrogen (as NH_4Cl) were added as well as 250 mg L^{-1} sodium acetate as the carbon source.

Column Studies

Column studies used Celite R-635 (Celite Corporation, Lompoc, CA) as the solid support packed into 2-inch PVC pipe (5.2-cm internal diameter by 18 cm in length). Celite R-635 is a 7- by 12-mm pellet with a mean pore size of 20 mm made from diatomaceous earth. The void volume of this column was approximately 300 ml. Side-ports were evenly spaced along the column for sampling purposes.

In all cases, the support was washed in water, autoclaved, oven dried, and then packed into the columns. The dry weight of the support in each column was determined. The columns were saturated from the bottom up at a flow rate of 0.25 mL min^{-1} for 2 to 3 d. Following saturation, the column was inoculated with a washed cell suspension of perclace. Five-hundred milliliters of perclace culture, which was more than 8 d old, was centrifuged and the cell pellet suspended in approximately 1 L of mineral salts medium (FTW). The turbidity was adjusted by dilution to a reading of 0.05 at 600 nm, which represents approximately 9×10^5 cells per ml.

When mineral salts medium was used as the influent, nitrogen gas was continually bubbled into the medium to maintain anaerobic conditions. When ground water was the influent, nitrogen gas was used to sparge only the headspace of the reservoir to limit undesirable pH changes in the water. The medium was autoclaved prior to passing through the column, while ground water was filter sterilized (0.45 μm nylon filter, Gelman Scientific, Ann Arbor, MI). Perchlorate in the column eluent was monitored with the Orion electrode. Samples were taken daily from each column eluent (four to five samples per day), filtered and frozen prior to ion chromatographic analysis. Tables 1 and 2 describe each column in detail. In every case, Day 1 is the beginning of column operation while the time used for loading and conditioning the column is indicated as a negative.

Column 1 (Celite)

The purpose of the experiments with Column 1, packed with R-635 Celite, was to determine the capacity of perclace to remove perchlorate from ground water in a flow through system (Fig. 1a) and determine the residence time necessary for perchlorate removal to below CDHS action levels. The concentration of perchlorate in ground water was raised from 0.200 mg L^{-1}, the level of natural contamination, to 0.738 mg L^{-1} using sodium perchlorate. Eluent samples were taken four times per day. Following completion of the flow-through experiment, the distribution of biomass within the column was determined using a modified Lowry method for protein analysis. The celite was removed in four layers from the outlet to inlet, transferring samples of celite into test tubes containing 1 mL of 0.1 *M* NaOH and heating to 95°C for 10 min to lyse the bacteria attached to the celite. Tubes were

Table 1. Parameters for operation of celite column.

Day	Flow rate	[ClO_4]	[NO_3]	[Acetate]	Medium	Comments
	ml min^{-1}	mg L^{-1}				
−11 to −9	0.25	n/a†	n/a	n/a	H_2O	Column saturation
−8 to −7	0.25	n/a	n/a	n/a	FTW	Load bacterium
−6 to 0	0.25–3.0	100	n/a	1000	FTW	Establish biomass
1–6	0.5	0.738	26	250	GW‡	20 mg L^{-1} P and 2 mg L^{-1} N added
7–12	1	0.738	26	250	GW	none
13–18	2	0.738	26	250	GW	none
19–21	2	0.040	n/a	n/a	GW	Pass GW eluent through 2nd time
23–25	2	0.674	n/a	250	BMS	Sideport samples taken

† None added.
‡ Ground water.

Table 2. Parameters for celite column with recycling.

Day	Flow rate	$[ClO_4]$	$[NO_3]$	[Acetate]	Recycle	Medium	Comments
	ml min^{-1}	mg L^{-1}			ml min^{-1}		
−6 to −4	0.25	n/a†	n/a	n/a	none	H_2O	Saturate column
−4 to −2	0.25	n/a	n/a	n/a	none	BMS	Load perclace
−1 to 0	0.25–3.0	100	n/a	1000	100	BMS	Recycling pump on
1–7	3	0.672	n/a	150	100	BMS	Day 4 + 7 sideport samples
8–10	3	0.672	n/a	150	none	BMS	No recycling
11–12	3	0.672	n/a	150	100	BMS	Recycling resumes Day 11
13–15	3	0.550	26	150	100	GW‡	none
16–18	2	0.550	26	150	100	GW	none
19	1	0.550	26	150	100	GW	none

† None added.
‡ Ground water.

vortexed and an aliquot of the supernatant, which contained the bacterial lysate, was analyzed for protein content using the Lowry method (Daniels et al., 1994). The celite in each test tube, which was unmodified by the protein analysis experiment, was then oven-dried and weighed. The final protein content was expressed as mg protein per g celite (dry weight). The details of column operation are listed in Table 1.

Column 2 (Celite with Recycling)

The objective of this series of experiments was to determine whether recirculation of the treated liquid would affect the removal of perchlorate. The experimental apparatus was designed just as in the previous experiment, as shown in Fig. 1a. Figure 1b shows the addition of a second pump to the design. The second pump can produce flow rates of 100 ml min^{-1} to rapidly recycle the liquid through the column while maintaining an overall flow rate through the column of 1 to 3 ml min^{-1}. In doing this, a differential reactor with an ideally mixed behavior was established and external mass transfer resistance was neglected.

For this experiment, acetate was added directly into the influent line rather than in the medium reservoir and ground water was obtained with a higher level of contamination (0.550 mg L^{-1} ClO_4^-), so it did not require perchlorate amendment. Samples were taken four times per day. Table 2 details the running parameters of this column.

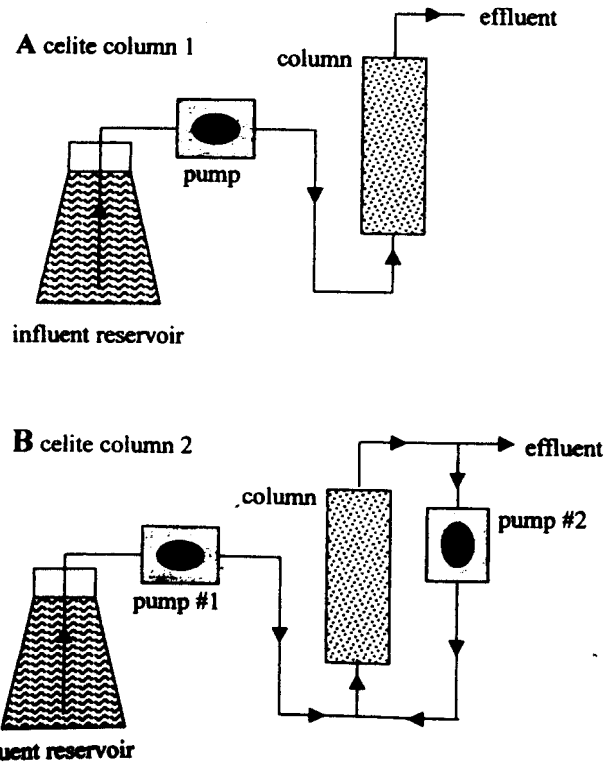

Fig. 1. Design of apparatus for Experimental Columns 1 and 2.

RESULTS

Figure 2 demonstrates the removal of perchlorate from contaminated ground water by the first celite column. Perchlorate was added to this ground water to raise the concentration from 0.200 to 0.738 mg ClO_4^- L^{-1}. At flow rates of 0.5 and 1.0 ml min^{-1}, representing residence times of 10 and 5 h respectively, perchlorate was removed from 0.738 mg L^{-1} to below detectable levels by perclace. When the flow rate was increased to 2 ml min^{-1}, representing a residence time of 2.5 h, 92 to 95% of the perchlorate was removed. At this flow rate, perchlorate was detectable at levels in the eluent of 0.04 to 0.06 mg L^{-1}, which are just slightly above the CDHS action level of 0.018 mg L^{-1}.

Because it was possible to completely remove perchlorate from mineral salts medium at high flow rates (during the conditioning period, Day −6 to Day 0, data not shown), a concern arose that a component of the ground water was inhibiting complete perchlorate removal. To address this question, a portion of the column

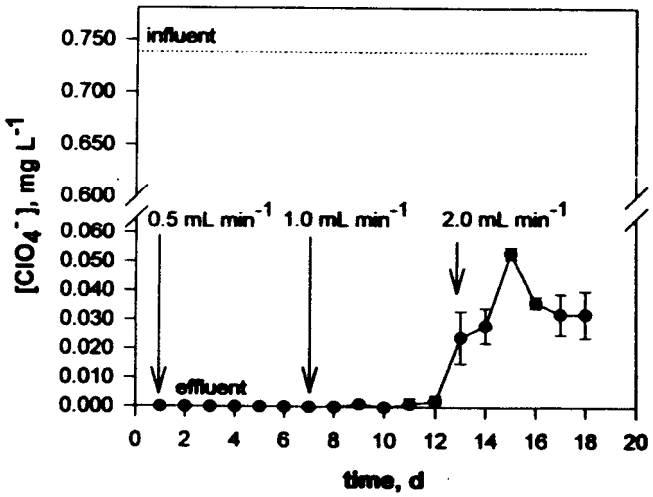

Fig. 2. Perchlorate ion breakthrough during treatment of contaminated ground water in the first celite column. The arrows indicate increases in flow rates from 0.5 to 1.0 to 2.0 ml min^{-1}. Each point is the mean and standard deviation of four samples collected each day.

eluent from Days 13 to 18, containing low levels of perchlorate breakthrough, was filter-sterilized and again passed through the column. This second passage resulted in perchlorate removal to below detectable levels (not shown), indicating that no components of the ground water were inhibiting microbial perchlorate reduction.

After completion of the flow through experiment, the column was disassembled and the biomass distribution examined. Based on protein concentration, it appeared that the majority of the microorganisms were colonized in the first quarter of the column, near the inlet. This region contained 60 mg of protein per g celite as opposed to the upper 75% of the column, which averaged about 2 mg protein per g celite. Analyzing the side-port eluent samples supported this finding. The majority of perchlorate was removed from the eluent before it reached the first sampling port, indicating that the bacterially active zone of the column was the initial portion of the column.

The second column was constructed with a primary goal of passing the contaminated ground water multiple times through the bacterially active zone near the inlet and testing potential external mass transfer limitations. A second pump was included to rapidly move the eluent through the column multiple times. Pump #2 had a flow rate of 100 ml min^{-1} while the pump maintaining flow into and out of the column (Pump #1) had a flow rate never exceeding 3 ml min^{-1}. The column was conditioned with BMS medium containing perchlorate at 3 ml min^{-1}. For the first 8 d, 0.672 mg L^{-1} perchlorate was degraded to less than detectable levels, as shown in Fig. 3. On Day 8 the cycling pump was turned off to determine the effect. It appeared that perchlorate reduction to below 0.004 mg L^{-1} was maintained in the absence of recycling. On Day 11, Pump #2 was again turned on to 100 ml min^{-1} and remained on for studies using ground water.

When ground water, contaminated with 0.550 mg L^{-1} ClO_4^-, was loaded into the column at 3 ml min^{-1} (a residence time of 1.25 h), 95% of the perchlorate was removed (Fig. 4). When the flow was reduced to 2 ml min^{-1}, 98% of the perchlorate was removed so that the breakthrough concentration was 0.010 mg L^{-1}, which is below the CDHS action level. Only at 1 ml min^{-1} was perchlorate removed to less than detectable levels of 0.004 mg L^{-1}. Analysis of nitrate concentrations in the influent and eluent show that at all flow rates, nitrate was removed from 26 mg L^{-1} to below detectable levels. Sideport sampling from this column once again indicated that the microbially active zone was in the first 25% of the column. These results indicate that perclace has the potential to remove perchlorate to below action levels at a relatively rapid rate that should not be greatly affected by the presence of nitrate in the ground water.

Acetate was measured in the effluent of both columns. The concentration of acetate added to the influent was in excess of the amount needed to carry out perchlorate reduction. In Column 1, the influent acetate concentration (mean ± standard deviation) was 215.2 ± 12.8 mg L^{-1} and the effluent acetate concentration was 168.2 ± 8.5 mg L^{-1}. This indicates that approximately 47 mg L^{-1} of acetate were consumed by metabolism within column. To reduce 0.738 mg L^{-1} perchlorate to chloride, only 0.45 mg L^{-1} acetate would be required, if the reaction proceeded stoichiometrically. Because perclace also reduces nitrate, which is present at levels of 26 mg L^{-1}, approximately 18 mg L^{-1} acetate should be used for this reaction.

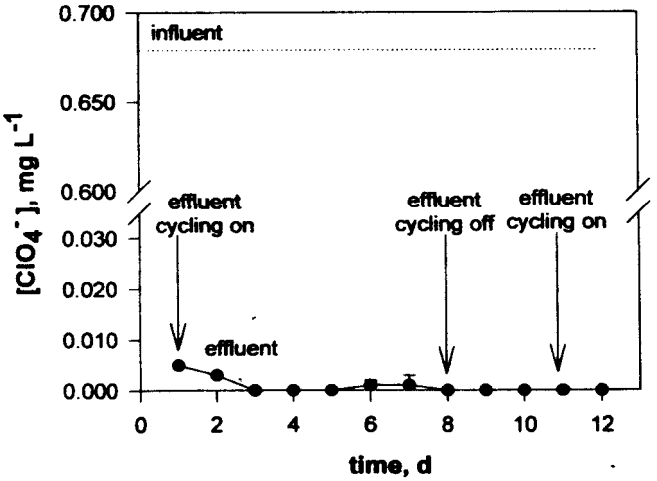

Fig. 3. Perchlorate ion breakthrough during treatment of defined mineral salts medium at a flow rate of 3 ml min^{-1} in the second celite column. The arrows indicate the point at which effluent cycling was turned off (Day 8) and on again (Day 11). Each point represents the mean and standard deviation of four effluent samples collected daily.

DISCUSSION

As described by Herman and Frankenberger (1999), perclace reduces ClO_4^- and produces Cl^-. That work also demonstrated the capacity of perclace to reduce both perchlorate and nitrate in batch culture. Nitrate is a common co-contaminant in ground water and it is crucial that the presence of nitrate at 100- to 1000-fold levels greater than perchlorate will not inhibit perchlorate reduction. Preliminary experiments indicated that

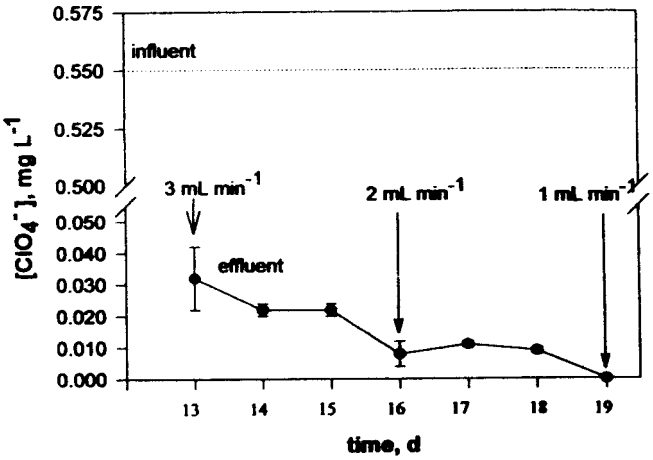

Fig. 4. Perchlorate removal from contaminated ground water using the second celite column. The arrows indicate the point at which the overall flow rate through the column was decreased from 3 to 2 to 1 ml min^{-1}.

the simultaneous reduction of perchlorate and nitrate also would occur in a flow-through system (Herman and Frankenberger, 1999). A sand-packed column successfully demonstrated the capacity of perclace to remove perchlorate from a mineral salts medium. Our current work describes two column studies that addressed the rate of perchlorate removal from contaminated ground water obtained from the San Gabriel Valley, CA.

Successful remediation of perchlorate from wastewater using immobilized microorganisms in a bioreactor was first demonstrated 25 years ago (Yakovlev et al., 1973; Korenkov et al., 1976; Attaway and Smith, 1994). This system utilized *Vibrio dechloraticans* Cuznesove B-1168, which will not reduce perchlorate in the presence of nitrate (Korenkov et al., 1976; Romanenko et al., 1976). More recently, Wallace et al. (1998) described the use of an up-flow anaerobic fixed-bed reactor containing a consortium of facultative anaerobic microorganisms including *Wolinella succinogenes* HAP-1. This bacterium was effective at reducing perchlorate levels from 1500 mg ClO_4^- L^{-1} to less than 100 mg L^{-1} at a rate of 1 g ClO_4^- L^{-1} in the bioreactor.

Previous bioreactor systems were designed for the removal of perchlorate in the 1 to 1000 mg L^{-1} range, which is representative of levels found near industrial sites. Recent work has described ground water treatment systems that treat lower levels of perchlorate contamination and commercial operations are already being tested (Giblin and Frankenberger, unpublished; Catts, 1998; van Ginkel et al., 1998; Logan and Kim, 1998). These systems utilize autotrophic (hydrogen utilizing and heterotrophic (acetate oxidizing) processes to reduce perchlorate to below detectable levels.

The objective of our treatment system is the removal of ClO_4^- to less than the California State action level (0.018 mg L^{-1}) and preferably to less than detectable levels (approximately 0.004 mg L^{-1}). The current study showed that celite-packed columns inoculated with the perchlorate-reducing isolate, perclace, were capable of removing 92% to 99% of the ClO_4^- loaded into the column. Removal of ClO_4^- to less than 0.004 mg L^{-1} from ground water only occurred at flow rates of 1 ml min^{-1} or less (a residence time in the column of 5 h). Increasing the flow rate above 1 mL min^{-1} resulted in perchlorate breakthrough to levels greater than 0.018 mg L^{-1}. Logan and Kim (1998) also reported an increase in perchlorate breakthrough with an increase in loading rates using a sand-packed column inoculated with a mixed consortium of bacteria.

The data presented here indicate that perchlorate breakthrough is flow-rate dependent and that biological activity within the column is primarily associated with the column inlet. In the first celite column experiment, cycling of the effluent through the column a second time increased the total amount of perchlorate removal, indicating that the components of the ground water do not prevent complete removal of perchlorate.

In the second celite column, eluent was passed repeatedly over the column inlet where bacterial biomass was concentrated. When this reactor design was tested using perchlorate in a defined mineral salts medium, ClO_4^- removal to <0.005 mg L^{-1} was achieved at a flow-through rate of 3.0 mL min^{-1}. In contrast, when ground water was the influent, removal of ClO_4^- to <0.004 mg L^{-1} could not be maintained at a flow rate of 3 mL min^{-1} and required a reduction in flow rate to 1.0 mL min^{-1}. Therefore, the concept of cycling the effluent repeatedly through the bacteriologically active zone at the column inlet did not result in efficient removal of perchlorate from ground water. Our work indicated that external mass transfer was not the limiting factor in the first column. It suggests that an increase in cell density might be an important optimization measure. It appears that perchlorate removal is more efficient when perchlorate is present in a defined mineral salts medium as opposed to ground water.

The interaction of perchlorate with components present in ground water may explain the presence of residual ClO_4^- in the column effluent at the higher flow rates. There is currently little information concerning the interaction of ClO_4^- with humic materials or other components within ground water and these possible complexation reactions may effect bioavailability (Urbansky, 1998). Additionally, processes such as sorption and pore-size exclusion can limit availability and slow the rate of biodegradation. This phenomenon has been well established for organic pollutants in soil (Ramaswami and Luthy, 1997).

CONCLUSION

In this study we describe the use of celite-packed columns inoculated with the perchlorate-reducing strain, perclace, which was previously shown to reduce ClO_4^- to <0.004 mg L^{-1} (Herman and Frankenberger, 1999). The removal of up to 0.738 mg L^{-1} ClO_4^- to <0.004 mg L^{-1} was demonstrated, but was dependent on the flow rate of ground water through the column. We also found that the removal of perchlorate was more easily accomplished when ClO_4^- was present in a defined mineral salts medium as compared with ClO_4^- present in ground water. Future studies will be aimed at increasing the biomass throughout the column. Finally, optimization of the process will require further investigation to determine whether physicochemical factors effect the availability of very low perchlorate concentrations in ground water.

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