TECHNICAL COMPLETION REPORT (TCR) FOR WRC PROJECT W-902

Bioremediation of Perchlorate in Ground Water

Abstract

A bacterial isolate, strain perc1ace, was shown to reduce the ground water contaminant, perchlorate to levels < 0.005 mg L^{-1} when grown on acetate under anaerobic conditions. The ability of perc1ace to simultaneously reduce perchlorate and another ground water pollutant, nitrate, was examined in batch studies and in columns. The strain reduced perchlorate and NO_3^- under similar growth conditions. A sand-packed column inoculated with perc1ace simulated a flow-through biotreatment system for water contaminated with a low level of perchlorate and nitrate. Celite-packed columns were also 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 92-95% of the perchlorate was removed when the flow rate was 2 ml min^{-1}. 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 rapidly 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 groundwater 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 95% of perchlorate was removed when the flow rate was 3 ml min^{-1}. Rapid removal of perchlorate by perc1ace immobilized in a bioreactor may provide an efficient, cost-effective technology for remediation of groundwater.

Introduction and Problem Statement

In the past 5 years, advances in ion chromatography have allowed the 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 9 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, Wallace et al. 1996, 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, 1-1000 mg L^{-1}, are often found near industrial facilities involved in the production and testing of rocket fuel.

The 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.

Objectives

Our research focused on:

1) isolation and identification of microorganisms capable of utilizing perchlorate as an electron acceptor in support of the anaerobic respiration of organic carbon substrates.
2) determination of the optimum conditions for perchlorate reduction.
3) design and construction of a bench-scale bioreactor for the remediation of perchlorate in contaminated ground water.
4) design of 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.

Experimental procedures

Perchlorate reducing bacterium. Enrichment of a perchlorate reducing bacterial isolate began with the transfer of 10 ml of biosolids (Water Quality Control Plant, Riverside, CA) into a 125 ml Erlenmeyer flasks containing 125 ml mineral salts medium with 1000 mg L^{-1} acetate and 500 mg L^{-1} perchlorate. The headspace of the Erlenmeyer flasks were purged with nitrogen gas and sealed air-tight with screw-cap stoppers. The mineral salts medium (FTW) contained (in g L^{-1}): K_2HPO_4, 0.225; KH_2PO_4, 0.225; $(NH_4)_2SO_4$, 0.225; $MgSO_4 \times 7H_2O$, 0.05; $CaCO_3$, 0.005; $FeCl_2.4H_2O$, 0.005, acetate, 1.0 (as sodium acetate) and 1 ml of trace elements solution (Focht, 1994) and the loss of perchlorate was detected after more than one month. The perchlorate-reducing enrichment culture was then transferred to fresh medium. The perchlorate-reducing bacterium was isolated by selective plating using agar hardened enrichment medium. A single species referred to as perc1ace was isolated (Herman and Frankenberger, 1999).

Cultivation of perc1ace. Perc1ace 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) and 1000 mg L^{-1} sodium acetate (Herman and Frankenberger, 1999). The medium (125mL) was autoclaved in 125-mL Erlenmeyer flasks, and then

inoculated with 1 mL of perc1ace cell suspension, sparged with nitrogen gas, and the flask 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 0.005 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 mM NaOH at a flow rate of 1 mL min^{-1} with conductivity detection. An ASRS-II (4 mm), operated at 300 mA with water as the regenerant (10 mL min^{-1}), 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 mM Na_2CO_3 /1.0 mM $NaHCO_3$, 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, Inc. 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}], $MgSO_4 \cdot 7H_2O$ [10 mg L^{-1}], $CaCl_2 \cdot 2H_2O$ [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, 1g L^{-1}). BMS was the preferred medium because it created less interference during ion chromatography.

Groundwater. Groundwater 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 mg L^{-1} 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.

Batch studies. Batch studies were performed using 50 ml Elernmeyer flasks sealed with rubber stoppers (size no 2). The flasks contained 50 ml of FTW with 1000 mg L^{-1} sodium acetate and the appropriate amount of perchlorate, depending on the study. The inoculum was prepared by washing perchlorate-grown perc1ace in sterile water and then adding a 1% (v/v) aliquot. The ability of the isolate to use a variety of electron acceptors other than perchlorate was examined. Optimal conditions for perchlorate reduction were assessed.

Column studies. We used a glass column (2.8 cm internal diameter and 14 cm length) packed with sterilized, oven dried sand (Type no. 30, Ogleby Norton Industrial Sands, San Juan Capistrano, CA, 40-70 mesh size). The pore volume within the column was 36 mL, and a 3-h retention time was maintained using 0.2 ml mim^{-1} flow rate (model P-3

peristaltic pump, Pharmacia, Piscatway, NJ). The column was saturated for 5 d from the bottom-up with a 5 mM NaCl solution, and then inoculated by loading the column with a cell suspension of perclace (Herman and Frankenberger 1999). Celite R-635 (Celite Corporation, Lompoc, CA) packed into 2-inch PVC pipe (5.2 cm internal diameter by 18 cm in length), was also used as the solid support (Giblin et al 2000). 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 days. Following saturation, the column was inoculated with a washed cell suspension of perclace. When mineral salts medium was used as the influent, nitrogen gas was continually bubbled into the medium to maintain anaerobic conditions. When groundwater 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 groundwater was filter sterilized (0.45 um 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 (4-5 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 experiments with column 1, packed with R-635 Celite, was two-fold: to determine the capacity of perclace to remove perchlorate from groundwater in a flow through system (Fig. 1a) and to determine the residence time necessary for perchlorate removal to below CDHS action levels. The concentration of perchlorate in groundwater 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 4 layers from the outlet to inlet, transferring samples of celite into test tubes containing 1 mL of 0.1 N NaOH and heating to 95°C for 10 minutes to lyse the bacteria attached to the celite. Tubes were 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, weighed and the final protein content expressed as μg 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 rapid re-circulation 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. Fig. 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 groundwater 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.

Results

The perchlorate-reducing isolate, perc1ace, is a gram-negative, curved rod, facultative anaerobe which can grow aerobically on acetate, and in the absence of oxygen, will reduce ClO_4^-, and nitrate. Perc1ace is capable of growth on ClO_4^- between 0.1 and 1000 mg L^{-1}. Identification of perc1ace was attempted using 16SrRNA gene sequence homology (MIDI labs, Newark, DE). Perc1ace had the highest sequence homology (between 90 and 92% similarity) with several members of the beta subclass of the *Proteobacteria.*

The rate of perchlorate reduction was examined at 5°C intervals between 20 and 40 °C. Perc1ace could reduce perchlorate between 20 and 40 °C, with an optimum between 20 and 30 °C. Perchlorate reduction by perc1ace was also examined between pH 5.5 and 8.5. Perchlorate reduction occurred between pH 6.5 and 8.5, with an optimum between 7.0 and 7.2. Perc1ace can grow on acetate using three different electron acceptors, namely oxygen, NO_3^- and perchlorate. The strain is capable of reducing both NO_3^- and perchlorate under similar growth conditions. A variety of carbon sources were examined to determine the range of of compounds that could be used as electron donors in the promotion of perchlorate reduction by perc1ace. Perchlorate reduction, and growth as indicated by an increase in the turbidity of the cell suspension, was supported by a variety of commercially available protein sources and by certain organic acids. Sugars and alcohols were not utilized (Herman and Frankenberger, 1999). Perc1ace reduced 580 mg L^{-1} during a 72 h period with the concomitant release of chloride, and the production of biomass, indicated by an increase in turbidity of the cell suspension. After 72 h, the amount of chloride produced represented between 97 and 100% of what had been added as perchlorate, indicating that perchlorate was completely transformed.

In the sand-packed column, perchlorate concentrations, were reduced from 100 mg L^{-1} to <1 mg L^{-1} in the effluent. Chloride determination within the effluent revealed complete transformation of perchlorate to chloride. The influent perchlorate concentration was then reduced to 0.130 mg L^{-1} perchlorate, and at no time during the next 50 pore volumes (approximately 6 d) were effluent perchlorate concentrations > 0.005 mg L^{-1}. Starting at pore volume 51, 125 mg L^{-1} nitrate was added to the influent for the next 40 pore volumes. Following the addition of nitrate, perchlorate levels in the effluent initially increased to 0.099 mg L^{-1}. But within 5 pore volumes, perchlorate levels had declined to <0.005 mg L^{-1}, and remained below detection for the next 35 pore volumes. Effluent nitrate concentration ranged from 1.0 to 3.5 mg L^{-1} in the first 5 pore volumes (<1 d), and then decreased to < 1 mg L^{-1} (Herman and Frankenberger, 1999).

Figure 2 demonstrates the removal of perchlorate from contaminated groundwater 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 hours respectively, perchlorate was removed from 0.738 mg L^{-1} to below detectable levels by perc1ace. When the flow rate was increased to 2 ml min^{-1}, representing a residence time of 2.5 hours, 92-95 % of the perchlorate was removed. At this flow rate, perchlorate was detectable at levels in the eluent of 0.04-0.06 mg L^{-1}, levels 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 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 eluent from days 13-18, 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 μg of protein per g celite as opposed to the upper 75% of the column, which averaged about 2 μg 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 groundwater 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} whereas 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 eight days, 0.672 mg L^{-1} perchlorate was degraded to less than detectable levels, as shown in Fig. 3. On day eight the cycling pump was turned off to determine the effect. It appeared that perchlorate reduction to below 0.005 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 groundwater.

When groundwater, 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 hours) 95% of the perchlorate was removed (Figure 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 0.005 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 perc1ace has the potential to remove perchlorate to below action levels at a relatively rapid rate which should not be greatly affected by the presence of nitrate in the groundwater.

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 plus/minus 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 perc1ace 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.

Conclusion

The current study examined the reduction of perchlorate at concentrations representative of ground water contamination, and focused on demonstating the removal of perchlorate to non-detectable levels. Bacterial mediated reduction removed perchlorate to less than the State of California drinking water action level of 0.018 mg L^{-1}.

Nitrate, which can commonly occur in the ground water of agricultural regions, did have a limited effect on perchlorate reduction. In batch systems, NO_3^- was always reduced at a faster rate than perchlorate, however, perchlorate reduction did occur simultaneously with nitrate reduction, and both perchlorate and nitrate were removed within 48h.

A sand-packed column successfully demonstrated the capacity of perc1ace to remove perchlorate from a mineral salts medium (Herman and Frankenberger, 1999). Also, celite-packed columns inoculated with the perchlorate-reducing isolate, perc1ace, were capable of removing 92% to 99% of the ClO_4^- loaded into the column. 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.

Perchlorate is stable in ground water but by promoting bacterial-mediated reduction, perchlorate can be efficiently transformed to chloride. We have demonstrated a treatment system for the removal of low levels of perchlorate from contaminated water that can also be used for simultaneous removal of perchlorate and nitrate.

Published papers

Herman, D.C., and W. T. Frankenberger, Jr. 1998. Microbial-mediated reduction of perchlorate in ground water. J. Environ. Qual. 27:750-754.

Herman, D.C., and W. T. Frankenberger, Jr. 1999. Bacterial reduction of perchlorate and nitrate in water. J. Environ. Qual., 28: 1018-1024.

Frankenberger, W.T. and Herman D.C., 2000. Bacterial removal of perchlorate and nitrate. US Patent No. 6,077, 429.

Giblin, T., Herman, D., Deshusses, M.A., Frankenberger. Jr., W.T., 2000. Removal of perchlorate in ground water with a flow-through bioreactor. J. Environ. Qual. 29, 578-583.

Giblin, T; Frankenberger, WT. (2000). Rapid detection of perchlorate in ground water using capillary electrophoresis. Chromatographia, 52, 505-508.

Giblin, TL; Herman, DC; Frankenberger, WT (2000). Removal of perchlorate from ground water by hydrogen-utilizing bacteria. J. Environ. Qual. 29:1057-1062.

Further reading

Attaway, H., and M. Smith. 1993. Reduction of perchlorate by an anaerobic enrichment culture. J. Ind. Microbiol. 12:408-412.

Attaway, H., and M. Smith. 1994. Propellant wastewater treatment process. US patent 5, 302, 285.

Betts, K. S. 1998. Technology update: rotating ion-exchange system removes perchlorate. Env. Sci. Tech. 32: 454A-455A.

California Department of Health Services (CDHS). 1998. Perchlorate in California drinking water. www.dhs.cahwnet.gov/ps/ddwem/chemicals/perchl/perchlindex.htm.

California Department of Health Services (CDHS). 1997. Determination of Perchlorate by Ion Chromatography, Rev. No. 0, June 3, 1997. Sanitation and Radiation Laboratories Branch, Berkeley, CA.

Capen, C. C. 1994. Mechanisms of chemical injury of thyroid gland. *In* Progress in Clinical and Biological Research. Receptor mediated Biological Processes: Implications for evaluation Carcinogenesis. pp 173-191. New York, USA: Wiley-Liss.

Catts, J. G. 1998. The biochemical reduction of perchlorate at low concentrations in water-technology application for groundwater in San Gabriel Basin, California. pp. 144

– 147. *In* Proceedings of the Southwest Focused Groundwater Conference: Discussing the Issue of MTBE and Perchlorate in the Ground Water. National Ground Water Association.

Daniels, L, R. S. Hanson, and J. A. Phillips. 1994. Chemical analysis. pp. 512 – 554. *In* Gerhardt, P., R. G. E. Murray, W. A. Wood, and N. R. Krieg (Eds.), Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington, D.C.

Focht, D. D. 1994. Microbiological procedures for biodegradation research. pp. 407-426. *In* Methods of Soil Analysis, Part 2 - Microbiological and Biochemical Properties. Soil Science Society of America, Book Series 5. SSSA, Madison, WS.

Gu, B., Brown, G.M., Alexandratos, S.D., Ober, R., and V. Patel. 1999. Selective anion exchange resins for the removal of perchlorate (ClO_4^-) from groundwater. US Department of Energy, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA. ORNL/TM-13752; ORNL Envirronmental Sciences Division Publication No. 4863.

Herman, D.C., and W. T. Frankenberger, Jr. 1998. Microbial-mediated reduction of perchlorate in groundwater. J. Environ. Qual. 27:750-754.

Herman, D.C., and W. T. Frankenberger, Jr. 1999. Bacterial reduction of perchlorate and nitrate in water. J. Environ. Qual., 28: 1018-1024.

Jacob, H.-E. 1970. Redox potential. pp 91-123 *In* Method in Microbiology, vol. 2. Norris, J.R. and D.W. Ribbons (eds.), Academic Press, NY.

Korenkov, V. N., V. I. Romanenko, S. I. Kuznetsov, and J. J. V. Voronov. 1976. Process for purification of industrial waste waters from perchlorates and chlorates. US patent 3,943,055.

Lamm, S.H., Bravermman, L. E., Li, F.X., Pino, S. and G. Howearth. 1999. Thyroid health status of ammonium perchlorate workers: a cross sectional occupational health study. J. Occup. Env. Med. 41: 248-260.

Logan, B.E. 1998. A review of chlorate- and perchlorate-respiring microorganisms. Bioremediation J. 2:69-79.

Logan, B.E. and K. Kim. 1998. Microbiological treatment of perchlorate contaminated groundwater. pp. 87 – 90. *In* Proceedings of the Southwest Focused Groundwater Conference: Discussing the issue of MTBE and perchlorate in the ground water. National Ground Water Association.

Malmqvist, A., T. Welander, and L. Gunnarsson. 1991. Anaerobic growth of microorganisms with chlorate as an electron acceptor. Appl. Environ. Microbiol. 57:2229-2232.

Rikken, G.B., A. G. M. Kroon, and C. G. van Ginkel. 1996. Transformation of (per)chlorate into chloride by a newly isolated bacterium: reduction and dismutation. Appl. Microbiol. Biotechnol. 45:420-426.

Ramaswami, A., and R.G. Luthy. 1997. pp. 721 – 729. *In* Hurst, C. J., G. R. Knudsen, M. J. McInerney, L. D. Stetzenbach, M. V. Walter, (eds.), Manual of Environmental Microbiology. American Society for Microbiology, ASM Press, Washington, D.C.

Romanenko, V.I., V.N. Korenkov, and S. I. Kuznetsov. 1976. Bacterial decomposition of ammonium perchlorate. Mikrobiologiya 45:204-209.

Urbansky, E. T. 1998. Perchlorate chemistry: implications for analysis and remediation. Bioremediation J. 2:81-95.

van Ginkel, C.G., A.G. M. Kroon, G. B. Rikken, and S. W. M. Kengen. 1998. Microbial conversion of perchlorate, chlorate and chlorite. pp. 92 – 95. *In* Proceedings of the Southwest Focused Groundwater Conference: Discussing the Issue of MTBE and Perchlorate in the Ground Water. National Ground Water Association.

Von Burg, R. 1995. Toxicology Update--Perchlorates. J. App. Toxicol. 15: 237-241.

Wallace, W., S. Beschear, D. Williams, S. Hospadar, and M. Owens. 1998. Perchlorate reduction by a mixed culture in an up-flow anaerobic fixed bed reactor. J. Ind. Microbiol. Biotech. 20:126-131.

Wallace, W., T. Ward, A. Breen, and H. Attaway. 1996. Identification of an anaerobic bacterium which reduces perchlorate and chlorate as *Wolinella succinogenes*. J. Ind. Microbiol. 16:68-72.

Wirt, K., M. Laikhtman, J. Rohrer, P.E. Jackson. 1998. Low-level perchlorate analysis in drinking water and ground water by ion chromatography. American Environmental Laboratory 10(3): 4-5.

Yakovlev, S.V., J. V. Voronov, V. N. Korenkov, A. B. Nevsky, V. A. Bobrikova, T. A. Karjurkhina, I. N. Churbanova, and J. M. Laskov, J.M. 1973. Method for biochemical treatment of industrial waste water. US patent 3, 755, 156.

Table 1. Parameters for operation of celite column.

Day	Flow rate (ml min^{-1})	$[ClO_4]$ (mg L^{-1})	$[NO_3]$ (mg L^{-1})	[Acetate] (mg L^{-1})	Medium	Comments
-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[≠]	20mg L^{-1} P and 2mg 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

[+] n/a: none added

[≠]GW: groundwater

Table 2. Parameters for celite column with recycling.

Day	Flow-through rate (ml min^{-1})	[ClO_4] (mg L^{-1})	[NO_3] (mg L^{-1})	[Acetate] (mg L^{-1})	Recycle rate (ml min-1)	Medium	Comment
-6 to -4	0.25	n/a	n/a	n/a	none	H_20	Saturate column
-4 to -2	0.25	n/a	n/a	n/a	none	BMS	Load perc1ace
-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

Figure Legends

Fig. 1. Design of apparatus for experimental columns 1 and 2.

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 to 2 ml min^{-1}. Each point is the mean and standard deviation of 4 samples collected each day.

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 4 effluent samples collected daily.

Fig. 4. Perchlorate removal from contaminated groundwater 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}.

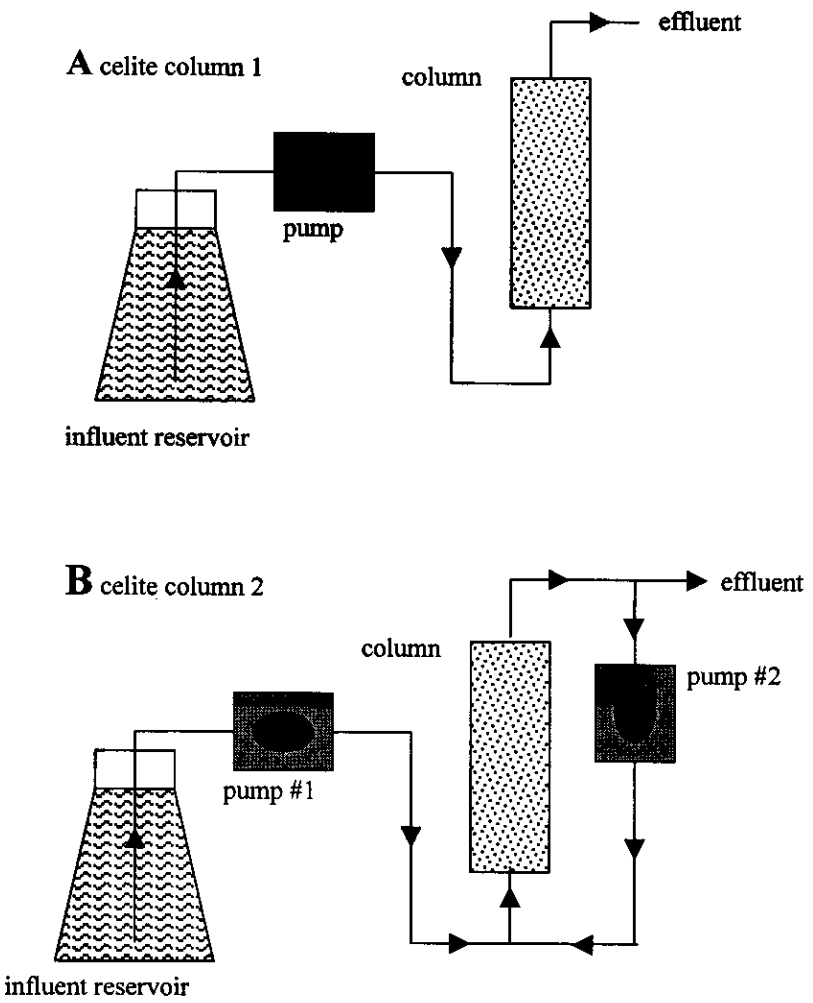
A celite column 1
effluent
column
pump
influent reservoir
B celite column 2
effluent
column
pump #2
pump #1
influent reservoir

Fig. 1

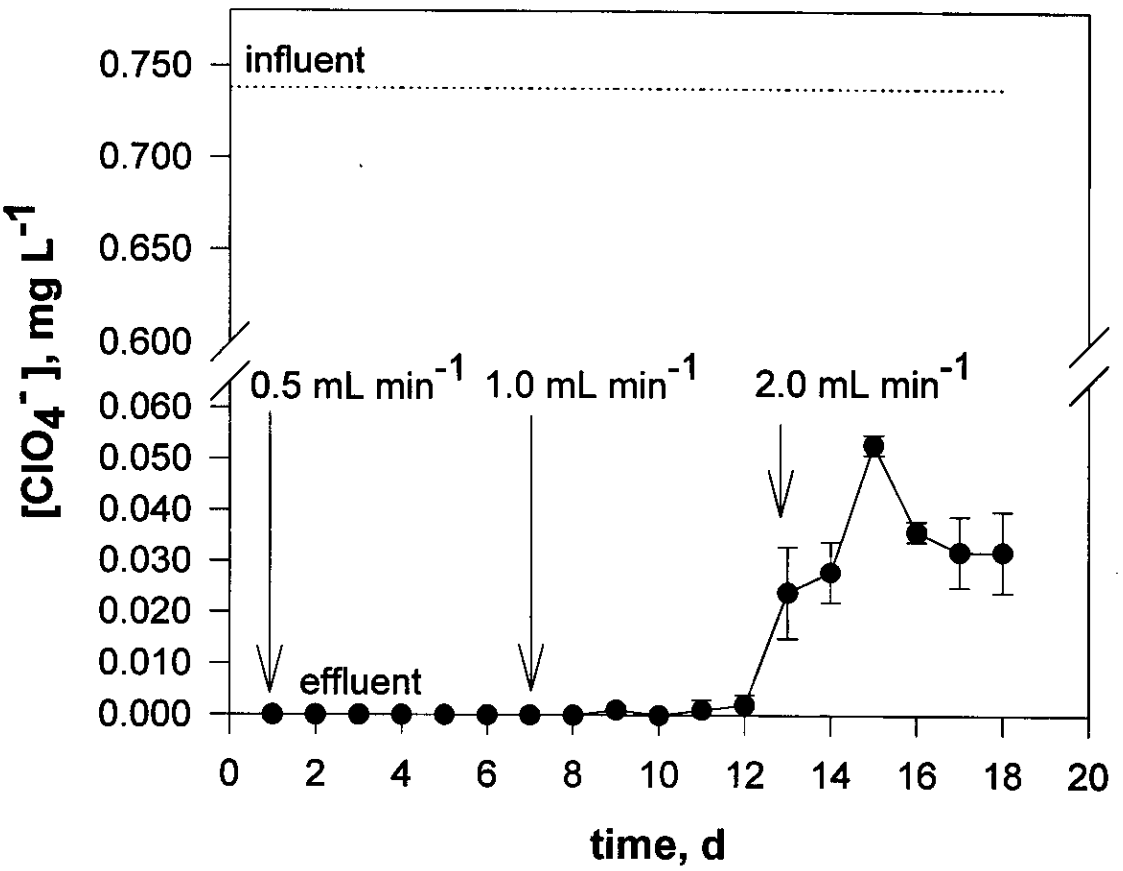
influent
0.750
0.700
0.650
0.600
0.060
0.050
0.040
0.030
0.020
0.010
0.000
0.5 mL min-1
1.0 mL min-1
2.0 mL min-1
effluent
[ClO4-], mg L-1
0
2
4
6
8
10
12
14
16
18
20
time, d

Fig. 2

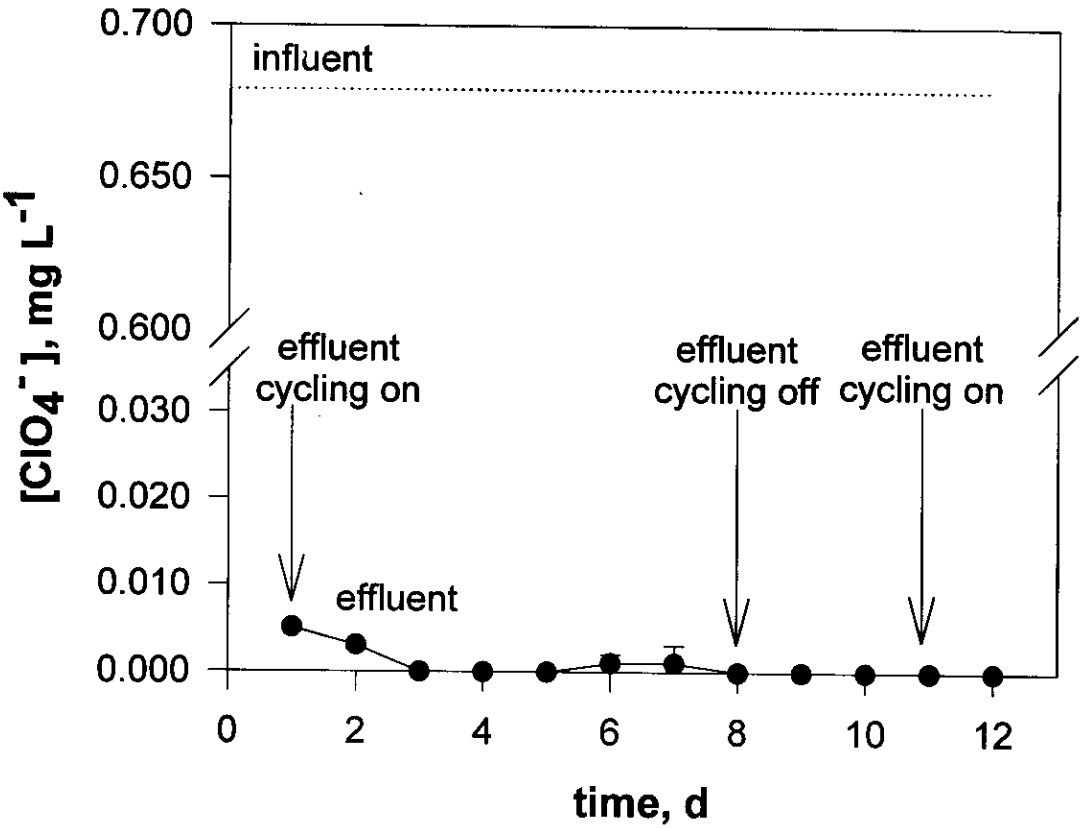
0.700
influent
0.650
0.600
effluent
cycling on
effluent
cycling off
effluent
cycling on
0.030
0.020
0.010
effluent
0.000
0
2
4
6
8
10
12
time, d
[ClO4-], mg L-1

Fig. 3

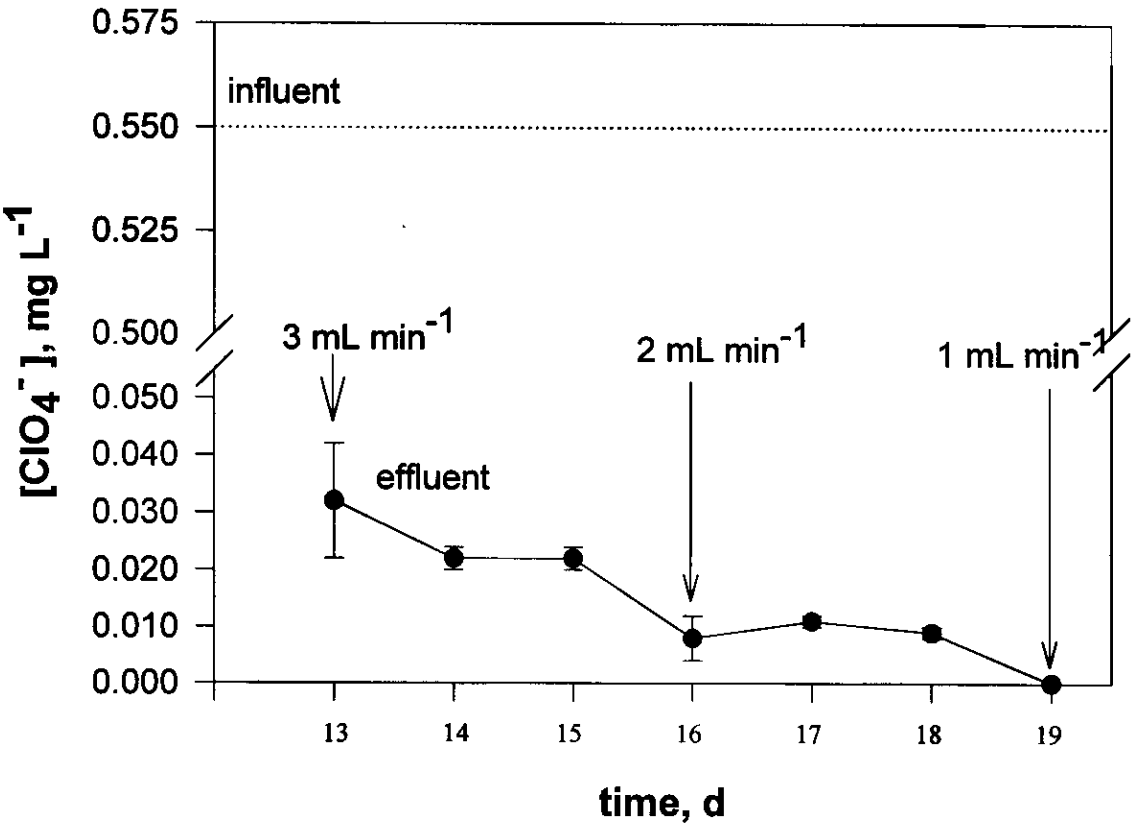
0.575
0.550
0.525
0.500
0.050
0.040
0.030
0.020
0.010
0.000
influent
3 mL min-1
2 mL min-1
1 mL min-1
effluent
[ClO4-], mg L-1
13
14
15
16
17
18
19
time, d

Fig. 4