Treatment of perchlorate- and nitrate-contaminated groundwater in an autotrophic, gas phase, packed-bed bioreactor

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Abstract

The biological degradation of perchlorate was examined using a laboratory-scale, autotrophic, packed-bed biofilm reactor. The reactor was operated in unsaturated-flow mode and continuously fed water containing perchlorate (ClO4⁻/C0) as an electron acceptor, and a gas mixture of hydrogen (5%) and carbon dioxide at a retention time of 1.5 min. In the absence of nitrate, perchlorate removal rate (rP, ppb/min) in the reactor was found to be first order with respect to perchlorate concentration (c, ppb) according to

$$r_P = 0.16 \pm 0.06c^{0.97\pm0.12} \quad (n = 11, \quad R^2 = 0.97, \quad p < 10^{-5})$$

Perchlorate removal rates in the hydrogen feed were found to be comparable to rates found by others for fixed film bioreactors using either hydrogen gas or organic electron donors such as acetate, although the rate coefficient was reduced to slightly less than unity ($r_P = 0.22 \pm 0.08c^{0.91\pm0.08}$, $n = 19$, $R^2 = 0.89$, $p < 10^{-5}$). When nitrate was present in the water, similar perchlorate removals were achieved despite nitrate concentrations three orders of magnitude higher than perchlorate concentrations. Perchlorate was removed by an average of 25±5% from a perchlorate-contaminated groundwater containing 73±2 ppb of perchlorate and 21±2 ppm of nitrate. This removal was slightly higher than the removal of 17±3% measured for a synthetic groundwater containing 79±3 ppb of perchlorate and 22±2 ppm of nitrate. In both cases, there was an average of 10% nitrate removal. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The widespread contamination of groundwater due to releases of ammonium perchlorate may affect the drinking water supplies of at least 15 million people in the US [1]. Ammonium perchlorate is used as the oxidizer in solid rocket fuel (up to 70% by weight), and occurs naturally in nitrate deposits in Chile that are used in some fertilizers [2–5]. Perchlorate (ClO4⁻) interferes with iodide, and at high doses is a human health concern because it affects regulation of hormone production and metabolism by the thyroid [6]. California has set a provisional drinking water standard for perchlorate of 18 ppb, and perchlorate has been added to the federal Contaminant Candidate List (CCL) under the Safe Drinking Water Act [6–8]. The EPA may set a higher drinking water standard of 32 ppb [6,9]. (After this paper was accepted, the State of California lowered its action level from 18 ppb to 4 ppb, and a new study by the EPA was released that could result in a perchlorate drinking water standard at 1 ppb.)

Perchlorate is highly oxidized and is not reduced by rare-earth metals or zero-valence iron [10]. It can be used by some strains of bacteria for cell respiration.
under anoxic conditions [11,12]. It is sequentially reduced via chlorate and chlorite to chloride, or as

\[ \text{ClO}_4^- \rightarrow \text{ClO}_3^- \rightarrow \text{ClO}_2^- \rightarrow \text{Cl}^- + \text{O}_2 \] [13]. Although oxygen is

produced, it is used by the perchlorate respiring bacteria and does not accumulate in solution [12,14].

The breakdown intermediates ClO_3^- and ClO_2^- do not accumulate to measurable levels [2,15,16]. Bioreactor

systems have been developed to remove perchlorate from water [12,16–18]. At first, these reactors were

designed to treat highly contaminated rocket wash waters (1000–3000 ppm) to <0.1–0.5 ppm using complex

organic feeds [17]. More recently, reactors have been designed and tested to remove perchlorate to non-
detectable levels (<4 ppb) [9,18–20].

Microbial reduction of perchlorate can be supported by hydrogen [21]. Hydrogen is only sparingly soluble in

water (0.08 mg/L at 5% H_2) [22], and the use of this inorganic energy source in a bioreactor reduces the risk of

contamination of water distributions systems by non-oxidized substrate. Perchlorate has been degraded in

saturated-flow, packed-bed reactors [23], air lift reactors [24] and unsaturated-flow, packed-bed reactors [16].

The advantage of the unsaturated-flow hydrogen reactor is that hydrogen is continuously and passively transferred

to the biofilm just as oxygen is transferred to a biofilm in a trickling filter [25,26]. It was recently shown that

perchlorate at a concentration of 740 ppb was reduced by 39% using the hydrogen gas-phase reactor with a

retention time of only slightly more than 1 min (1.1–1.3 min) [16].

Perchlorate is typically present in groundwaters at very low concentrations (<100 ppb), although in some cases it can reach concentrations of 20 ppm or more [7,27]. Nitrate is also a common co-contaminant, and microbiological perchlorate reduction in many systems has been found to be reduced or inhibited in the presence of nitrate [11,15]. Therefore, we examined the effect of a high (ppm) concentration of nitrate on perchlorate removal using both artificial and actual groundwaters. The performance of the unsaturated-flow hydrogen gas reactor was also examined at lower (<100 ppb) perchlorate concentrations than those previously examined using this type of reactor [16].

Perchlorate removal rates obtained in the laboratory-scale reactor were analyzed to predict reactor retention times necessary to achieve complete perchlorate degradation for larger-scale systems.

2. Methods

2.1. Media and bacterial consortium

Two different reactor feeds were used: an artificial groundwater (AG); and a perchlorate-contaminated groundwater from the Texas Street pumping station in Redlands, CA. The AG contained NaClO_4 at concentrations ranging from 44 to 18,000 ppb (as ClO_4^-), and the same trace metal solution used by Miller and Logan [16] with other components at the following concentrations (ppm): K_2HPO_4 15.5; NaH_2PO_4·H_2O 8.2, NH_4H_2PO_4, 5; MgSO_4·7H_2O, 0.1, and Na_2CO_3, 1000. The groundwater sample was shipped from the Redlands site in a 55-gallon galvanized steel drum. Groundwater from the Redlands site typically contains (ppm): Ca^{2+}, 56.9; Mg^{2+}, 9.3; Na^+, 13.4; K^+, <1.0; OH^−, <1.0; CO_3^{2−}, <1.0; HCO_3^−, 181; SO_4^{2−}, 32.5; Cl^−, 5.51; Fl^−, 0.53; total alkalinity, 148 (as CaCO_3); ClO_4^−, 0.08; NO_3^−, 18.5. The sample used in this study was analyzed only for perchlorate and nitrate upon arrival at our laboratory, and was found to contain: ClO_4^−, 73±2 ppb; NO_3^−, 21±2 ppm. The following were added (ppm) to the groundwater: Na_2CO_3, 1000, and the phosphate buffer and nitrogen components used for the AG medium (ppm) (K_2HPO_4, 15.5; NaH_2PO_4·H_2O, 8.2; NH_4H_2PO_4, 10).

The reactor was inoculated with a hydrogen-oxidizing perchlorate-degrading bacterial consortium previously used [16]. Approximately 500 beads containing biofilm were removed from the reactor used by Miller and Logan [16] with a glass rod into a glass beaker, mixed with an equal number of new autoclaved 3 mm glass beads, and poured into a new reactor.

2.2. Column design and operation

The packed-bed reactor was maintained and operated in the same manner as previously reported [16] except the reactor was re-designed to contain three chambers (instead of one chamber) to better control water flow and minimize gas losses (Fig. 1). Water was pumped (Masterflex 7523-30, Cole Parmer Corp.) into the top chamber of the column where it pooled before flowing through four different holes (containing syringe needles) that distributed the flow evenly over the glass beads. The middle chamber (25 cm long, 2.5 cm inside diameter) contained the glass beads (3 mm diameter; 1200 m^2/m^3 calculated surface area) supported by a stainless-steel mesh. Gas entered as before (7.5 mL/min CO_2; 35 mL/min 5% v/v H_2 in N_2) through a side port at the top of the second chamber. The bottom chamber (an open tube below the mesh holding the beads) was designed to allow the water to pool in the bottom to minimize gas losses; the gas exited from the bottom in the water effluent. No effort was made to optimize the gas composition or flow rate other than to maintain an effluent pH of ~7. The reactor was operated at room temperature (~23°C).

The reactor was initially operated in a recycle mode for 3 days to allow the bacteria to colonize the new glass beads. Following this acclimation period, the column was switched over into a once through mode (no recycle) and fed AG containing 18 ppm of perchlorate for 19
days. The perchlorate concentrations in the AG feed were varied to determine the effects of perchlorate concentration on perchlorate removal rates. Nitrate (22 ± 2 ppm) was added as noted in some tests. Redlands water was used as received, except for indicated nutrient additions. The hydraulic retention time was measured as previously described [16] and found to be 1.5 min at a constant feed rate of 2.3 mL/min.

Perchlorate removal rates were calculated based on the log-mean perchlorate concentrations in the reactor, $C_{lm}$, calculated [20] as

$$C_{lm} = \frac{C_{in} - C_{out}}{\ln(C_{in}/C_{out})}$$

(1)

where $C_{in}$ and $C_{out}$ are the influent and effluent perchlorate concentrations, respectively. The perchlorate removal rate $r_p$ was calculated as

$$r_p = \frac{(C_{in} - C_{out})}{\theta},$$

(2)

where $\theta$ is the hydraulic retention time in the reactor. Linear regressions were calculated using a Microsoft Excel 2000 spreadsheet.

2.3. Analytical procedures

Perchlorate was measured using standard procedures [28] on a Dionex DX 500 ion chromatography system (Dionex, Sunnyvale, CA), equipped with an IonPAC AS 11 column and guard column. The perchlorate detection limit was 4 ppb with all calibration curves having $R^2 > 0.997$ based on standards of typically 5, 10, 20 or 25, 50 and 95 or 100 ppb. Samples above this range were diluted with deionized water. Nitrate concentrations were measured on a Technicon Autoanalyzer II (Bran and Luebbe, Buffalo Grove, IL) as combined nitrite and nitrate. Nitrate concentrations were calculated by difference by re-analysis of samples for nitrite. The minimum detection limit for nitrate was 0.04 ppm. Perchlorate was purchased from GFS Chemicals (Powell, OH) and gases from MG Industries (Allentown, PA). All other chemicals were purchased from Sigma (St. Louis, MO).

3. Results

3.1. Perchlorate removal rates

Perchlorate removals were measured in the unsaturated-flow reactor over a period that spanned 127 days. The percent of perchlorate removed in AG at the retention time of 1.5 min was the same at the two lower influent perchlorate concentrations, with 30 ± 7% and 29 ± 3% perchlorate removed at an influent concentrations of 1200 and 79 ppb, respectively (Fig. 2). At the highest influent perchlorate concentrations (18,000 ppb), the percent removal was lower (8.2 ± 2.9%).

Perchlorate removal rates demonstrated overall first-order kinetics with influent perchlorate concentration over most of the perchlorate concentration range of 44–18,000 ppb (Fig. 3). Influent perchlorate concentrations in AG were successively decreased from 866 to 44 ppb over 17 days. Average removal rates for these experiments are shown in Fig. 3 along with the average removal rates for the three experiments presented in Fig. 2. A regression of all data indicated that the perchlorate removal rate significantly increased in proportion to the log-mean concentration in the reactor, with a slope of $0.97 \pm 0.12$ ($R^2 = 0.87$, $p < 10^{-5}$). Omitting the lowest and highest removal rates from this regression improved the $R^2$ (0.95) but still produced a correlation with a slope not significantly different from unity (slope = $0.96 \pm 0.08$, $p < 10^{-7}$). This second regression analysis suggests indicates that low removal (8.2%) for the very highest perchlorate concentration shown in Fig. 2 was different from that observed at the other perchlorate concentrations.

3.2. Effect of nitrate on perchlorate removal rates

Perchlorate degradation occurred simultaneously with nitrate degradation in the autotrophic reactor despite substantially larger concentrations of nitrate than
perchlorate. Perchlorate removal initially averaged 29 ± 3% over a 5-day period at an average influent perchlorate concentration of 79 ± 3 ppb in the absence of nitrate (Fig. 4). On day 109, the reactor was switched to an identical feed of artificial groundwater, except this feed contained 22 ± 2 ppm of NO$_3$-N. Nitrate removals were variable but averaged 10 ± 5%. Perchlorate removals decreased to an average of 17 ± 3% over the seven-day period with nitrate in the feed (Fig. 4). This decrease was not due to changes in the influent perchlorate concentration as the perchlorate concentration in the feed remained constant at 78 ± 3 ppb.

3.3. Perchlorate degradation with a groundwater sample

After 118 days of operation the feed of the reactor was switched to a groundwater sample containing 73 ± 2 ppb of perchlorate and 21 ± 2 ppm of nitrate. Perchlorate was successfully degraded in this water sample despite the presence of substantially higher concentrations of nitrate than perchlorate. Perchlorate removals for this groundwater varied over a relatively large range of 16–34% (Fig. 5). In contrast to the results obtained with the artificial groundwater, there was no apparent change in perchlorate removals over time. Overall, the average perchlorate removal of 25 ± 5% was slightly higher than the removal measured for the artificial groundwater in the presence of nitrate (17 ± 3%) at similar nitrate concentrations.

Nitrate removal in the groundwater sample averaged 10% during the first nine days. This percent removal was similar to removals measured in experiments with the artificial groundwater. The last few nitrate measurements (days 125 and 127) were significantly larger than those during the first few days (119–122), suggesting that the nitrate removal was increasing, but this effect was not noticed until the water samples were analyzed a few days after the reactor operation had been discontinued.
Thus, it was not possible to determine if perchlorate removal by the reactor would have eventually changed had the reactor continued to be operated with the groundwater sample.

4. Discussion

Perchlorate was reduced in the unsaturated-flow reactor by $30 \pm 7\%$ and $29 \pm 3\%$ at influent concentrations of 1200 and 79 ppb, respectively, at a retention time of 1.5 min. These removals are only slightly less than the 39% removal found by Miller and Logan [16] using a similar reactor. The incomplete removal of perchlorate under hydrogen-oxidizing conditions is a result of the short retention time. Giblin et al. [23] found that they could achieve complete perchlorate removal at an influent perchlorate concentration of 700 ppb at a retention time of 40 min. The only way to achieve longer retention times under unsaturated-flow conditions would be to use a longer reactor. The time necessary for complete perchlorate removal under unsaturated-flow conditions can be estimated by an analysis of existing perchlorate removal rate data.

Overall perchlorate removal rates were found to be first-order with respect to the log-mean perchlorate concentration (Fig. 3). These removal rates appear to be consistent with other studies on perchlorate removal rates in biological reactors. When the data from the current study is combined with previously published perchlorate data [20], most of the data appear to fall on the same regression line (Fig. 6). The slope of this line is somewhat less than unity due to the data at the very highest and lowest perchlorate concentrations. Perchlorate removal rates appeared to decrease at perchlorate concentrations above 10 ppm. This may be due to a
transition from first-order to zero-order kinetics as half-saturation constants have been found to be 15 ppm for a mixed culture with hydrogen as and electron donor [16], and 12 ppm (Dechlorisomomas sp. PDX) and 33 ppm (Dechloromomas sp. KJ) with lactate as an organic electron donor [29]. Two data points at the lowest perchlorate concentrations fall well below the regression line suggesting at these low-perchlorate concentrations overall removal rates in the reactor are significantly decreased.

Using the regression equation shown in Fig. 6, we estimated the reactor retention time for a given influent perchlorate concentration, and we assumed a non-detectable (4 ppb) effluent perchlorate concentration. For a typical influent perchlorate concentration of 80 ppb, the log-mean perchlorate concentration is 25.4 ppb, producing a rate from Fig. 6 of 4.1 ppb/min (1.8–9.2 ppb/min; 95% CI). This translates to a retention time of 18.5 min (8–42 min; 95% CI) and reactor length of 1.5 m (0.65–3.4 m). However, it should be noted that this value of \( C_{lim} \) falls in the range outside that considered valid for the correlation given in Fig. 6. Because the correlation overestimates rates as a function of concentration below 55 ppb, the longer reactor length of 3.4 m is likely a more reasonable length than the length basis on the correlation average. A more accurate estimate of the size of a full-scale system will require pilot-scale testing with a specific medium and groundwater.

The reactor performance could also depend on the concentration of alternate electron acceptors present in the groundwater such as sulfate and nitrate. Sulfate, however, has not been found to be removed in this unsaturated-flow reactor [30], and was not an electron acceptor for a perchlorate reducing microorganism obtained from this hydrogen-oxidizing consortium [16]. Many perchlorate respiring bacteria can use nitrate as an electron acceptor [11] [31,29], but some strains do not [32]. Nitrate has been previously reported to completely inhibit perchlorate degradation under acetate-oxidizing conditions [15]. However, nitrate did not affect perchlorate removal rates in a hydrogen-oxidizing packed-bed reactor [23]. Similarly, it was found here that despite much larger concentrations of nitrate than perchlorate, there was no measurable change in perchlorate removal rates in tests using a contaminated groundwater from Redlands, CA. It appeared that nitrate removals increased during tests using the groundwater, suggesting that the reactor may have required more time to adapt to the elevated nitrate concentrations. In tests with the artificial groundwater, perchlorate removals decreased from 29% to 17% in the presence of nitrate. Taken together, these data suggest that the effect of nitrate on perchlorate removal rates will be dependent on the water source and extent of nitrate exposure time.

5. Conclusions

Perchlorate removal rates were found to be a function of the log-mean perchlorate concentration in the reactor over a perchlorate concentration range of 44–18,000 ppb. Nitrate will likely decrease overall perchlorate removal rates, although this effect appears to be a function of the electron donor and water source. Pilot-scale systems will be necessary to more accurately assess the effects of these different parameters on perchlorate degradation in bioreactors used for drinking water treatment.

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References


