Environmental Chemistry

REDUCTION OF PERCHLORATE IN RIVER SEDIMENT

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Abstract—The transformation of perchlorate was investigated in river sediment during laboratory batch and column studies to determine if reduction of perchlorate is a viable pathway in natural sediment without previous exposure to perchlorate. Perchlorate at an initial concentration of 10 μM was reduced quantitatively to chloride in 3 d after a lag phase of 2 d in sediment slurries amended with lactate. Raising the initial concentration of perchlorate to 1,000 μM increased the lag phase to 20 d before reduction occurred. At perchlorate concentrations greater than 1,000 μM, the reduction of perchlorate was not observed within 40 d. We speculate that the high concentration of perchlorate specifically was problematic to the microbes mediating the reduction of perchlorate. High levels of nitrate inhibited the reduction of perchlorate as well. In sediment slurries amended with 870 μM sodium nitrate, the reduction of perchlorate at an initial concentration of 100 μM did not occur before day 15 of the experiment, but complete removal of nitrate had occurred by day four. Sediment column studies further demonstrated the dependence of perchlorate reduction on endogenous nitrate levels.

Keywords—Perchlorate River sediment Environmental fate Biological reduction

INTRODUCTION

The ammonium salt of perchlorate is a primary ingredient in solid rocket fuel [1,2]. Because of its limited shelf life, the fuel of the military rocket inventory has to be exchanged on a regular basis, which has resulted in the accumulation of 55 million pounds of propellant for disposal as of 1997 (American Water Works Service Company, Voorhees, NJ, USA, 1998). It is projected that by 2005, the accumulated perchlorate will total 80,000 tons. Munitions facilities are thought to be the principal source of contamination found in groundwater, surface water, and drinking-water wells throughout the southwestern United States [2]. Perchlorate concentrations in Nevada, USA, groundwater have been reported in the g/L range [3]. Perchlorate has been measured in the range of 18 to 280 μg/L in California, USA, surface waters and groundwaters, impacting the water supplies of more than 12 million people [4]. Perchlorate contamination is not limited to the southwestern United States; it also has been detected in well-water samples in Indiana, Iowa, Pennsylvania, and New York, USA [2].

Other routes of potential exposure to perchlorate have been identified. Hydroponic growth studies have demonstrated that perchlorate can accumulate in tobacco plants [5]. This finding is significant, because previous studies demonstrated that perchlorate in contaminated fertilizer was taken up by roots of the tobacco plant [6]. More recently, perchlorate has been detected in supermarket milk [7] and in dairy and breast milk [8] at the low μg/L range.

Little is known about the environmental and health risks associated with exposure to low levels of perchlorate. If taken up in high concentration, perchlorate causes the discharge of stored iodide (I−) from the thyroid gland in humans and other mammals [9]. Because of this property, perchlorate is still clinically in use for the diagnosis of thyroid dysfunction [10]. Interference by perchlorate with the sodium/iodine symporter prevents the uptake of iodide by thyroid cells and, thus, disables the production of thyroid hormone [9,11]. Because of its potential health impact, perchlorate was added to the U.S. Environmental Protection Agency Contaminant Candidate List [12], and it is subject to the Unregulated Contaminant Monitoring Rule [13]. Exposure studies have demonstrated that environmentally significant concentrations of perchlorate affect thyroid function in fish (Pimephales promelas) [14] and inhibit development and metamorphosis in amphibians (Xenopus laevis) [15].

Under aerobic conditions, perchlorate is a recalcitrant chemical. Furthermore, because perchlorate is a highly soluble inorganic ion (~17 g/L for the sodium salt) that does not adsorb readily to natural surfaces, it is expected to migrate readily in aquatic ecosystems once dissolved [2,16,17]. Under anaerobic conditions, however, the potential for perchlorate reduction exists. The reduction of perchlorate to chloride proceeds through chlorate and chlorite:

\[
\text{ClO}_4^- \rightarrow \text{ClO}_3^- \rightarrow \text{ClO}_2^- \rightarrow \text{Cl}^- + \text{O}_2
\]

perchlorate chlorate chlorite chloride

Although the reduction of perchlorate by metal cations (e.g., Ti(III) and V(II)) [16], electrochemical means [16], and zero-valent iron [18] has been observed, the abiotic reduction of perchlorate in natural systems has not been reported and is not thought to be a viable reaction pathway. The reduction of perchlorate in pure and mixed-cell cultures [19–23], wastewater [23], biotreatment systems [20,22,24,25], root zone of trees [26], and sediments and soils previously exposed to perchlorate [27], however, suggests the potential for microbial reduction to occur under anaerobic conditions. These studies have provided clear evidence that perchlorate is capable of serving as an alternative electron acceptor coupled to microbial-mediated oxidation of organic carbon. To determine if the...
reduction of perchlorate would occur under environmentally relevant conditions in sediments not previously exposed to perchlorate, laboratory batch and column experiments with natural sediment were conducted. An ion chromatographic (IC) method for the simultaneous detection and quantification of a number of anions of interest was developed. Using this IC method, perchlorate and its reduction products (chlorate, chlorite, and chloride species) as well as redox-sensitive species, including nitrate (NO$_3^-$), nitrite (NO$_2^-$), phosphate (PO$_4^{3-}$), sulfate (SO$_4^{2-}$), the electron-donor lactate, and its metabolite carbonate (CO$_3^{2-}$), were measured as a function of time and column length.

### MATERIALS AND METHODS

**Sediment**

The sediment was collected from the Oconee River (OR) (Athens, GA, USA) at a water depth of several centimeters by scraping the top 0 to 15 cm of the sediment along with the immediate overlying water. The sediment was wet-sieved (mesh size, 1 mm) while open to the air in the laboratory. Analysis of the pore water from a sediment slurry before sieving indicated that the river sediment was not reducing in its natural state, as indicated by the absence of NO$_3^-$ and SO$_4^{2-}$ and the presence of NO$_2^-$ and Mn(II) and Fe(II). Quartz, feldspar, kaolinite, and iron phases dominated the sediment mineralogy. Analysis under a petrographic microscope indicated the sediment consisted primarily of coarse quartz grains covered with a layer of very fine iron oxide (Materials Characterization Laboratory, Pennsylvania State University, University Park, PA, USA). Other sediment characteristics were as follows: Brunauer-Emmett-Teller surface area, 4.66 m$^2$/g; organic carbon, 1.79%; sand/silt/clay, 96.4%/1.0%/2.6%; cation-exchange capacity, 0.39 mEq/100 g; total iron, 1.70%; and total manganese, 0.05%.

**Batch studies**

For batch experiments, the sieved sediment was air-dried, and 10 g were added to 50 ml of prefiltered river water in 60-ml serum bottles. The bottles were spiked (both as concentrated solution or solid salt) with sodium perchlorate (10–10,000 μM), sodium lactate (0 and 100 mM), and/or sodium nitrate (0 and 100 μM). The bottles were rotated end-over-end at 25 rpm at an average temperature of 22°C. At selected intervals, 1-ml samples were taken with a gas-tight syringe and filtered via a 0.2-μm syringe filter (Acrodisc; Fisher, Atlanta, GA, USA).

**Column design and hydrodynamics**

A glass column (length, 62 cm; inner diameter, 5 cm; total volume, 1,217 cm$^3$) was equipped with 10 sampling ports consisting of 1.5-mm glass tubes with threaded bushing and Teflon® septa (Ace-Glass, Vineland, NJ, USA). The ports were located at selected distances from the inlet (0, 2, 4.5, 7, 12, 17, 22, 32, 42, 52, 60, and 62 cm). Needles (10 cm, 22 gauge) were permanently installed and kept closed with inert sample valves (Fisher). Oconee River sediment was packed in the column as an aqueous slurry. The column was oriented vertically and operated in an upward-flow mode for all experiments. Further details concerning the design and characterization of the column hydrodynamics have been described elsewhere [28]. The column hydrodynamics are summarized as follows: Dispersion coefficient, 1.79 cm$^2$/h; pore volume, 628 cm$^3$; column porosity, 0.52; pore water velocity, 2.9 cm/h; and dispersivity, 0.11 cm.

Sodium perchlorate salt was added to the mobile phase (prefiltered OR water) to achieve a concentration of 80 μM perchlorate anion. The perchlorate-amended mobile phase was air-saturated before entering the column by passing it through an open gas-exchange chamber. The flow rate was 0.5 ml/min. Sodium lactate solution was introduced to the mobile phase by a syringe pump to achieve a final concentration of 100 μM after passing through the gas-exchange chamber. Pore water was sampled over the length of the sediment column with a 2.0-ml, gas-tight syringe (Hamilton, Reno, NV, USA) through the inert sample valves at each of the sampling ports. Aliquots of 2 ml were removed over a 4-min period. Sampling was initiated at the top of the column and progressed sequentially up-gradient. The aliquots were then filtered (0.2-μm syringe filter; Whatman Anotop, Florham Park, NJ, USA) and subdivided as described below for analysis.

**Pore-water analysis**

Aliquots (100 ml) of pore water were analyzed by IC (DX500; 4-mm AS11 column; Dionex, Atlanta, GA, USA). A sodium hydroxide gradient was employed at a flow rate of 2 ml/min (0.1 M initially, 0.002 M at $t = 0$ min, 0.002 M at $t = 4$ min [start of data collection and gradient], 0.005 M at $t = 10$ min, 0.018 M at $t = 15$ min, 0.1 M at $t = 16$ min, and 0.1 M at $t = 24$ min). Detection occurred by measurement of the effluent’s conductivity (CD20; Dionex) and absorbance (AD20; Dionex). A second 50-ml aliquot was analyzed for Fe(III), Fe(II), and Mn(II) by IC (CS5A; Dionex) and absorbance detection at 530 nm after postcolumn reaction with 4-(2-pyridyldiaz)resorcinol (PC10; Dionex). Details for the analysis of the metal cations by IC have been described previously [29,30].

### RESULTS AND DISCUSSION

#### Analysis of perchlorate and redox indicators

Excellent resolution of all redox-sensitive anions was achieved with IC using a gradient method and detection by ultraviolet/visible light and conductivity (Fig. 1). Conductivity detection gave up to four orders of magnitude of linear response for perchlorate. The highest concentrations of linear range for the anions besides perchlorate, especially for the close eluting nitrate and chloride, were limited by mutual over-
could not be detected at any time during the course of the reaction. The intermediate chlorate reached a concentration of 50 μM at day 6 and was complete by day 21. The loss of perchlorate was concomitant with a significant increase in chloride concentration. The microbial-mediated reduction of perchlorate in OR sediment slurry most likely can be attributed to high cellular levels of the toxic intermediate(s) chloride and/or hypochlorite [22].

Batch kinetic studies

Analysis of the aqueous phase from a freshly collected sediment slurry after sieving (mesh size, 1 mm) indicated that the river sediment was not reducing in its natural state, as indicated by the presence of NO₃⁻ (90 μM) and SO₄²⁻ (80 μM) and the absence of Mn(II) and Fe(II) (Fig. 2). The use of such sediment (i.e., not strongly reducing) provided the opportunity to investigate the effect of nitrate-reducing conditions on perchlorate reduction. No loss of perchlorate from the OR sediment slurry could be observed under these redox conditions, lactate, which is a source of readily biodegradable organic carbon, was added to the sediment slurry at an initial concentration of 100 μM. Anion concentration is plotted on the x-axis, and chloride mass balance is plotted on the y-axis.

Influence of nitrate on perchlorate reduction kinetics

The microbial-mediated reduction of perchlorate in OR sediment amended with lactate most likely can be attributed to nitrate-reducing bacteria. Several studies have provided evidence that both dissimilatory and assimilatory nitrate-reducing bacteria are capable of using perchlorate as an alternative electron acceptor [19,25,31]. Because nitrate levels occur at concentrations typically 100- to 1,000-fold greater than perchlorate in groundwater, determining the effect of nitrate concentration on perchlorate reduction kinetics is essential for the development of predictive fate models. Accordingly, we studied perchlorate reduction kinetics using batch systems in which the initial nitrate concentration was varied from 0 to 870 μM.

Effect of perchlorate concentration on reduction kinetics

To determine the capacity of the OR sediment for reducing perchlorate, the reduction kinetics for perchlorate were measured over an initial perchlorate concentration range of 10 to 10,000 μM (data not shown for 10,000 μM ClO₄⁻) (Fig. 3). At the lower end of this concentration range (10, 60, and 100 μM ClO₄⁻), the lag phase for perchlorate reduction increased (from 2 to 7 and 20 d, respectively) in lactate-amended OR sediment slurries. Although the increase in perchlorate concentration resulted in longer lag phases for perchlorate reduction, the rate of perchlorate reduction was not significantly affected. At much higher initial aqueous concentrations of perchlorate (i.e., 1,000 and 10,000 μM), however, reduction was not observed over a 40-d period, but the microbial activity, as indicated by production of carbonate in the sediment slurries treated at lower concentrations of perchlorate, did not change significantly. We speculate that the higher perchlorate concentrations inhibit the perchlorate-reducing bacteria, which might be attributed to high cellular levels of the toxic intermediate(s) chloride and/or hypochlorite [22].
for perchlorate reduction doubled to approximately 2 d in the sediment slurry treated with perchlorate at an endogenous [NO$_3$]$_0$ of 100 μM (Fig. 4B). Complete reduction of nitrate was observed in the first 24 h of the experiment. In the nitrate-amended sediment slurry at an [NO$_3$]$_0$ of 850 μM, the lag phase for reduction of perchlorate increased from 2 to 15 d, but nitrate had been reduced completely after 4 d (Fig. 4C). For chloride, the initial background concentration of approximately 180 μM rose to more than 300 μM. In each of these experiments, excellent mass balance (>90%) for chloride was observed. The inhibition of perchlorate reduction by nitrate appears to be a general phenomenon [32]. In a study of sediments and soils previously exposed to perchlorate, the reduction rates of perchlorate were not affected by nitrate; however, nitrate did increase the lag phase for perchlorate reduction [27]. Rates for the plant-mediated reduction of perchlorate were significantly slower at high concentrations (>100 to 400 mg/L) of nitrate [26]. This result was attributed to competing reactions in which both perchlorate and nitrate were used as electron acceptors. A bacterial isolate also has been found to reduce perchlorate faster in the absence of nitrate than in its presence; however, perchlorate and nitrate reduction occurred simultaneously in batch studies and column studies [25].

**Column studies**

The observation in the batch systems that nitrate had to be removed before perchlorate reduction occurred was confirmed by data from column experiments: Within the first 5 cm of the column, facile reduction of endogenous nitrate occurred; the initial concentration of nitrate decreased from 53 to less than 5 μM (Fig. 5A). Nitrate reduction was followed by the reduction of perchlorate, which was concomitant with reductive dissolution of manganese, indicating that these processes may be competitive electron-accepting processes (Fig. 5A). The initial concentration of perchlorate decreased from 73 to 8 μM over the first 20 cm of the column. Three days after the initial treatment with perchlorate, the column influent reservoir was refilled with perchlorate solution ([ perchlorate]$_0$ = 80 μM). During this run, perchlorate reduction was observed before the detection of dissolved manganese (Fig. 5B). The initial concentration of perchlorate decreased from 80 to less than 3 μM within the first 2 cm of the column, suggesting that adaptation of the microbial population to perchlorate had occurred. The
observed adaptation for reduction and the lack of evidence for a chemical reductant (i.e., Mn(II) or Fe(II)) indicate that biologically mediated reduction serves as the primary process for the observed attenuation of perchlorate.

CONCLUSION

The present study demonstrates that perchlorate can be reduced in river sediment by naturally occurring microbes that have not been exposed previously to perchlorate. Reduction of perchlorate results in the formation of chloride, which is further reduced to chloride anion. By comparison, perchlorate is highly recalcitrant under aerobic conditions and moves without noticeable retardation through the mineral matrix of aquifers [17]. Although the identification of the microbes mediating electron transfer was not an objective of the present study, nitrate-reducing bacteria are thought, based on previous studies, to be responsible for this reaction process. The observation that nitrate had to be reduced before reduction of perchlorate suggests that nitrate is the preferred electron acceptor for the nitrate-reducing bacteria; however, in the absence of nitrate, perchlorate will serve as a viable electron acceptor for these bacteria. Consequently, the levels of nitrate may serve as an indicator for the viability of perchlorate reduction in anaerobic sediments. The necessary addition of an electron donor to promote reducing conditions by stimulating microbial activity could be a low-cost way to remediate contaminated sites, thus avoiding the use of expensive excavation or pump-and-treat techniques.

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REFERENCES