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Chapter 17

PERCHLORATE REDUCTION IN A PACKED BED BIOREACTOR USING ELEMENTAL SULFUR

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Abstract: This study investigated perchlorate reduction by sulfur utilizing perchlorate reducing bacteria (SUPeRB). SUPeRB cultures were enriched from a denitrifying wastewater inoculum in medium containing elemental sulfur (S0), crushed oyster shell, nutrients and perchlorate. Perchlorate was reduced from 5 to < 0.5 mg/L in approximately 15 days. The enrichment culture was subsequently inoculated into a continuous flow packed-bed bioreactor containing S0 and crushed oyster shell medium. High-level perchlorate concentrations (5-8 mg/L) were reduced to < 0.5 mg/L with an empty bed contact time (EBCT) of 13 hours. Low levels of perchlorate concentrations (80-120 μg/L) were treated varying two parameters, recirculation ratio and empty bed contact time (EBCT). Little or no recirculation was required to efficiently reduce perchlorate to < 4 μg/L. The system also proved somewhat independent of EBCT. Investigations of the effect of nitrate on perchlorate removal and reactor media particle size is on going and will be presented at the meeting.

1. INTRODUCTION

Perchlorate (ClO4−) contamination has primarily occurred in association with manufacturing of missiles, fireworks, and other industrial processes (Urbansky, 2000) and has been recorded in 38 US states (MADEP, 2005). Military applications have also resulted in contaminants such as nitrate and Royal Dutch Explosives (RDX) present with perchlorate as co-contaminants. (Clausen et al., 2004). Perchlorate contamination poses a significant health threat, and toxicological studies have demonstrated that it interferes with iodine uptake into the thyroid gland disrupting thyroid function (O’Connor and Coates, 2002). Although national standards have yet to be established, the Commonwealth of Massachusetts has set a maximum contaminant limit for perchlorate of 2 μg/L.

Perchlorate is highly soluble and stable in water and hence cannot be removed by conventional drinking water treatment processes such as filtration or air stripping (Tipton et al., 2003). As an alternative, biological reduction of perchlorate has been investigated by several researchers and is thought to be the most cost-effective process for perchlorate removal (Min et al., 2004). Certain bacteria have shown to metabolize perchlorate to chloride, which is harmless to the environment. Several electron donors (acetate, wastewater, hydrogen, elemental iron, thiosulfate), have been previously investigated for perchlorate reduction using pure and mixed cultures (Urbansky, 2000). Use of S0 as an electron donor for autotrophic perchlorate reduction has been previously attempted but was not successful (Bardiya and Bae, 2005).

This study investigated sulfur utilizing perchlorate reducing bacteria (SUPeRB) for perchlorate reduction. Sulfur-oxidizing bacteria have been proven to successfully convert nitrate to nitrogen (denitrification) in water and wastewater treatment applications (Sengupta et al., 2006). Since the thermodynamic values of energy gained by microorganisms from nitrate and perchlorate are close to each other (Nerenberg et al., 2002) an attempt was made to investigate perchlorate reduction using the same microbial consortium with S0 as the main electron donor. Elemental sulfur pellets have many advantages as a bioreactor packing material, namely they are inexpensive and readily available as a
waste by-product of petroleum industry. Also, since sulfur-oxidizing bacteria are autotrophs they grow slowly, producing very little sludge hence, reducing the maintenance required for backwashing.

2. RESEARCH OBJECTIVES

The overall objective of this research was to engineer a robust, reliable and inexpensive biological process for treatment of perchlorate contaminated water, using \( S^0 \) as an electron donor with SUPeRB cultures. The specific objectives were to:

1. Investigate the effects of operating parameters (perchlorate concentration, particle size, EBCT and recirculation rates) on perchlorate removal in packed bed bioreactors.
2. Test the removal efficiencies of perchlorate in the presence of nitrate as a groundwater co-contaminant.

3. MATERIALS AND METHODS

3.1 Batch Culture Enrichments

SUPeRB was enriched from mixed liquor suspended solids (MLSS) from the denitrification stage of the Berkshire mall wastewater treatment facility (Lanesboro, MA), which utilizes methanol as an electron donor. Batch cultures were set up in 1000 mL Erlenmeyer flasks containing sulfur pellets (30 g), crushed oyster shell as an alkalinity source (10 g), 250 mL MLSS, and 250 mL of synthetic perchlorate contaminated groundwater. The cultures were incubated with agitation at 150 rpm in the dark at 20°C. Groundwater (Amherst, MA) was used to prepare synthetic groundwater medium containing 5 mg/L \( \text{ClO}_4^- \), 0.5 g/L \( \text{NaHCO}_3 \), 8.5 mg/L \( \text{KH}_2\text{PO}_4 \), 21.75 mg/L \( \text{K}_2\text{HPO}_4 \), 33.4 mg/L \( \text{Na}_2\text{HPO}_4\cdot7\text{H}_2\text{O} \), 22.5 mg/L \( \text{MgSO}_4\cdot7\text{H}_2\text{O} \), 0.25 mg/L \( \text{FeCl}_3\cdot6\text{H}_2\text{O} \) and 27.5 mg/L \( \text{CaCl}_2 \). \( \text{N}_2 \) gas was periodically sparged through the cultures to maintain anaerobic conditions. The cultures were monitored for perchlorate concentration over time.

3.2 Bioreactor

A bench scale bioreactor (working volume one-liter) was constructed from acrylic glass tubing with an inner diameter of 6.1 cm and a 34 cm in height. Four sample ports, evenly distributed along the height of the reactor, were sealed with septum ports for obtaining profiles of perchlorate vs. depth. Recirculation from the effluent to the influent was provided using a variable speed peristaltic pump. The reactor was packed with 4 mm sulfur pellets (Georgia Gulf Sulfur Corp., Valdosta, GA) as the electron donor and crushed oyster shell as an alkalinity source (3:1 by volume). To test the effect of small sulfur size particles, elemental sulfur and oyster shell were crushed and sieved to 0.85 mm (ASTM date???) and were used as a packing material in one of the bioreactors.

3.3 Experimental Program

Four packed bed bioreactors were packed with sulfur/oyster shell media, inoculated with SUPeRB enriched from batch cultures and operated in an upflow mode. Table 1 shows the experimental program used to investigate the following bioreactor operating parameters: perchlorate concentration, recirculation rate, EBCT, particle size and the presence of nitrate as a co-contaminant. During the Phase I experiments, the synthetic groundwater used to feed the bioreactor was the same as was used in the enrichment studies (above). During Phase II, the synthetic groundwater was diluted with additional groundwater to achieve the target concentration of 0.08-0.12 mg/L.

Table 1. Experimental program for operation of packed bed reactors
### 3.4 Perchlorate Analysis

Samples were prepared for perchlorate analysis by filtering through 47 mm Millipore glass fiber filter. Perchlorate was analyzed using USEPA Method 314.0 (USEPA, 1999). For Phase I experiments, high level perchlorate concentrations were measured using a DX-500 Ion Chromatograph (IC) system (Dionex, Sunnyville, CA) equipped with an Ionpac AS16 column, an AG16A guard column, and a CD20 conductivity detector. The eluent used was 35 mM NaOH at 1 mL/min. The detection limit was 0.5 mg/L. For Phase II experiments, low level perchlorate concentrations (0.5-50 μg/L) were measured using the same IC with a 1000 μL injection loop. Samples were manually filtered through onguard silver (Ag) and barium (Ba) cartridges to remove chloride and sulfate. The reporting limit was 4 μg/L. Nitrate was measured using the same IC but with Ionpac AS14 column, AG14A guard column. The eluent was 8.0 mM Na₂CO₃/1.0 mM NaHCO₃ at 1 mL/min. The lowest reporting limit was 0.01 mg/L NO₃--N. The pH-values were measured using an Orion 720A pH meter.

### 4. RESULTS AND DISCUSSION

#### 4.1 Batch Cultures

The reduction of perchlorate by SUPeRB using S₀ in batch culture from an initial concentration of 4.5 mg/L a final concentration of 0.5 mg/L was achieved within the first 15 days (Figure 1). The flask was then spiked with perchlorate to the original concentration and sparged with N₂ to maintain anaerobic conditions. This procedure was repeated each time perchlorate concentration was reduced to below 0.5 mg/L. The data indicate that perchlorate can be biologically reduced by denitrifying cultures using S₀ as an electron donor. These results are comparable to those observed for nitrate reduction (Lopez-Luna et al., 2005).
4.1.1 Phase I: Bioreactor Performance at High Perchlorate Concentrations

The packed bed bioreactor was inoculated with SUPeRB from the batch cultures and initially operated with a 100 hour EBCT and an influent perchlorate concentration of 5 mg/L (high perchlorate). An acclimation period of approximately 26 days was observed after which a steady effluent perchlorate concentration was observed. Average removal efficiencies for the high perchlorate concentration experiments at varying EBCTs with and without recirculation are given in Table 2. Intermittent recirculation was employed on selected days to promote mass transfer of perchlorate to the biofilm; however, the effect of recirculation on perchlorate removal during this phase was inconclusive. On day 259, the influent perchlorate concentration was raised to ~8 mg/L to challenge the system with higher perchlorate concentrations. The spike had no significant effect on effluent concentration, which resulted in a 96% perchlorate removal efficiency.

### Table 2. Packed bed reactor performance at high perchlorate concentrations

<table>
<thead>
<tr>
<th>Days of Operation</th>
<th>Empty bed contact time in hrs</th>
<th>Recirculation velocity at 9.7 cm/min</th>
<th>Average removal efficiency</th>
</tr>
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<tbody>
<tr>
<td>1-30</td>
<td>100</td>
<td>No</td>
<td>66 %</td>
</tr>
<tr>
<td>31-43</td>
<td>100</td>
<td>Yes</td>
<td>61 %</td>
</tr>
<tr>
<td>44-57</td>
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<td>No</td>
<td>90 %</td>
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<tr>
<td>58-62</td>
<td>61</td>
<td>Yes</td>
<td>99 %</td>
</tr>
<tr>
<td>63-68</td>
<td>61</td>
<td>No</td>
<td>58 %</td>
</tr>
<tr>
<td>69-129</td>
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<td>96 %</td>
</tr>
<tr>
<td>130-132</td>
<td>36</td>
<td>Yes</td>
<td>99 %</td>
</tr>
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<td>133-175</td>
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<td>199-210</td>
<td>13</td>
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<tr>
<td>211-258</td>
<td>13</td>
<td>No</td>
<td>81 %</td>
</tr>
<tr>
<td>259-268*</td>
<td>13</td>
<td>No</td>
<td>90 %</td>
</tr>
<tr>
<td>269-280*</td>
<td>13</td>
<td>Yes</td>
<td>90 %</td>
</tr>
</tbody>
</table>

*indicates higher influent concentration of perchlorate (~ 8 mg/L)

4.1.2 Phase II: Bioreactor performance at low concentration

After conducting the high perchlorate concentration experiments, the contents of the packed bed reactor, including biomass, were divided and mixed with fresh sulfur/oyster shell media to construct two new packed bed reactors (Reactor 1 and Reactor 2). Both reactors were operated at low perchlorate concentrations (80-120 μg/L), more typical of contaminant groundwater levels. Reactor 1
was operated at a constant EBCT of 30 hrs and varying recirculation ratios while Reactor 2 was operated with no recirculation and varying EBCT. Figure 2 shows the effect of recirculation ratio on treatment of low perchlorate concentrations at an EBCT of 30 hrs. Decreased removal efficiencies were observed at increased recirculation ratios. Perchlorate reducing bacteria are slow growing autotrophs and the loss of biofilm from the sulfur pellets under turbulent conditions may have resulted in lower perchlorate removal efficiencies at higher recirculation ratios. The highest removal efficiency (92%) was observed at the lowest recirculation ratio (Qr/Q=52), however due to pump limitations further reductions in recirculation ratios were not investigated.

![Graph showing the effect of recirculation velocity on low level perchlorate removal. Qr is the recirculation flow rate and Q is the influent flow rate.](image)

Reactor 2 was used to investigate low level perchlorate removal at varying EBCT without recirculation (Figure 3). After observation of steady perchlorate removal efficiency at an EBCT of 30 hours, the EBCT was reduced in steps to a final value 8 hours. Average perchlorate removal efficiencies at EBCTs of 30, 15, 12 and 8 hours were 75%, 90%, 87% and 96%, respectively, showing that removal efficiency was independent of EBCT within this range but in general showed steady improvement over time of operation of the reactor. By the end of 130 days, consistent effluent perchlorate concentrations below a MDL of 4 μg/L were achieved at influent perchlorate concentrations of 80-120 μg/L and an EBCT of 8 hours. The bioreactor was then operated with 100 μg/L perchlorate and 10 mg/L NO₃-N. The experiments are on going and will be presented at the meeting.

Reactor 3 was started with SUPeRB from enriched batch cultures, 0.85 mm sulfur and oyster shell packing and an initial EBCT of 22 hours. An influent perchlorate concentration of 0.08-0.1 mg/L was maintained. An average perchlorate removal of 63% was observed in this reactor over an operating period of 54 days. Stepwise reduction of EBCT is presently underway and the results will be presented at the meeting.
Figure 3. Performance of packed bed reactor with no recirculation and varying EBCTs (EBCT values shown above the arrows)

Perchlorate concentration profiles over the length of the column are shown for Reactors 2 at two EBCTs in Figure 4. Active perchlorate degradation was observed in the first 10 cm of Reactor 2, closest to the inlet, suggesting that most bacteria resided and formed biofilms where the electron acceptor was readily available and that there was little change in the concentration profile when the EBCT was decreased. The column profile for a 22 hr EBCT in Reactor 3 (Figure 4) shows that perchlorate was reduced from 84 µg/L to 13 µg/L over the entire length of the column. This profile was taken only a few days after the start of the experiment, suggesting that in the early stages of biofilm growth the entire reactor is utilized for perchlorate reduction.

Figure 4. Column profile of packed bed reactor operated at low (0.08-0.1 mg/L) perchlorate concentration for reactors 2 (4 mm) and 3 (0.85 mm)
5. CONCLUSIONS

This research investigated a novel biological process for treatment of perchlorate contaminated water using S\textsuperscript{0} as an electron donor and the microbial community carrying out the perchlorate degradation. A culture enriched from sludge from the denitrifying section of a wastewater treatment plant with S\textsuperscript{0} and oyster shell media was able to reduce perchlorate from 5 mg/L to less than 0.5 mg/L in approximately 15 days. SUPeRB cultures were subsequently inoculated into bench-scale upflow packed bed bioreactors filled with elemental sulfur and oyster shell. High levels of perchlorate (5-8 mg/L) were successfully reduced to less than 0.5 mg/L in the bioreactor at an EBCT of 13 hours. Low levels of perchlorate (80-120 \textmu g/L) were reduced to less than 4 \textmu g/L at an EBCT of 8 hours. Increased recirculation ratios resulted in decreased perchlorate removal efficiency, possibly because of removal of microbial biomass from the packing media.

REFERENCES

United States Environmental Protection Agency (USEPA) 1999. Method 314.0: Determination of perchlorate in drinking water using ion chromatography.