Report as of FY2006 for 2006MA58B: "Perchlorate Reduction in Groundwater Using Elemental Sulfur"

Publications

- Articles in Refereed Scientific Journals:
  - Conneely T., A.K. Sahu, S.J Ergas, and K. Nüsslein, Investigation of Sulfur Dependent Perchlorate-Reducing Consortia, (In preparation for Applied and Environmental Microbiology).
  - Sahu, A.K; S.J. Ergas, Perchlorate Reduction Using Elemental Sulfur, In preparation for Environmental Science and Technology.

Report Follows

1. Problem and Research Objectives

Perchlorate (ClO_4^-) release in groundwater has affected drinking water to over 15 million people in the south west of the United States and groundwater contamination has been recorded in over 40 US states (Urbansky, 2000, MADEP, 2006). Perchlorate is used in the manufacture of missiles, fireworks, leather industries (US Environmental Protection Agency (USEPA), 2005). Trace levels of ClO_4^- has known to affect uptake of thyroid hormone in the thyroid glands and other health effects have also been recorded (Zoeller, 2005). Since ClO_4^- is an extremely stable and soluble anion in water, it readily transports with water and is difficult to remediate using conventional treatment technologies. Present full scale technologies for ClO_4^- remediation include Ion Exchange (IX), Granular Activated Carbon (GAC) adsorption, and biological reduction (USEPA, 2005).

The USEPA has addressed ClO_4^- as an emerging contaminant but no national standards have been established (USEPA, 2002). The Commonwealth of Massachusetts has adopted a perchlorate standard of 2 μg/L ClO_4^- (MADEP, 2006) and other states (eg: CA, TX) have set an advisory levels of 4-18 μg/L ClO_4^- (USEPA, 2005).

This study investigated biological ClO_4^- reduction using elemental sulfur as an electron donor by sulfur oxidizing bacteria. Elemental sulfur is a waste byproduct of oil industries, is inexpensive and serves as an excellent packing material both in bioreactors and for *in situ* groundwater treatment in permeable reactive barriers (PRBs).

The objectives of this research were to:

1. Quantify ClO_4^- reduction using batch cultures.
2. Operate a series of bench scale packed bed reactors using realistic ClO_4^- concentrations.
3. Reduce the empty bed contact times (EBCT) and investigate the reactors for effects of recirculation rates, presence of dissolved oxygen.
4. Investigate ClO_4^- reduction by sulfur oxidizing bacteria in the presence of co-contaminants such as NO_3^--N and TCE.

2. Methodology

Batch cultures for ClO_4^- reduction were enriched from dentirification zone of a wastewater treatment plant containing elemental sulfur (4 mm) as an electron donor, oyster shells as an alkalinity source and 5 mg/L ClO_4^- with trace elements. Synthetic feed was prepared from a groundwater collected from a nearby farm. On acclimatization, these enriched cultures were inoculated in one liter acrylic column bench scale reactors. The reactors contained elemental sulfur and oyster shells in the volume ratio of 3:1. The reactors were designed with side ports at intervals of 10 cm to obtain sample for concentration profiles. The synthetic feed water to the reactor was sparged with nitrogen to maintain anoxic conditions in the bioreactor, this was quantified using Resazurin, a chemical that changes color in the presence of oxygen.

Perchlorate was monitored using the standard USEPA 314.0 method using an Ion Chromatograph with detection limits of 4 μg/L ClO_4^- (USEPA, 1999). pH and sulfate were also monitored on a periodic basis.

3. Principal Findings and Significance

Batch cultures

Enriched batch cultures showed that 5 mg/L ClO_4^- could be reduced to less than 0.5 mg/L ClO_4^- in 20 days; this trend was observed after repeated spiking with fresh perchlorate solution for more than one year. These enriched cultures were termed SUPeRB (Sulfur Utilizing Perchlorate Reducing Bacteria) by the research team. Perchlorate reduction by SUPeRB was investigated using other electron donors (acetate, elemental iron, ferrous iron, hydrogen). In addition, the effect of salinity, aerobic conditions, and inoculum source on perchlorate reduction was investigated. The results showed that SUPeRB could use other electron donors including acetate and ferrous iron for ClO_4^- reduction but could not reduce ClO_4^- using H_2 and elemental iron as sole electron donors. SUPeRB also showed the potential to reduce high levels of ClO_4^- (5-20 mg/L) at salt concentrations in the range of 30-45 g/L. The cultures did not reduce ClO_4^- under aerobic conditions. The inoculum source (mixed liquor from the denitrification zone of a wastewater treatment plant) was re-tested for and showed reproducible results. Autoclaved controls showed no ClO_4^- reduction.

To further investigate the characteristics of SUPeRB, bacterial community analysis using PCR and isolation of a sulfur oxidizing perchlorate reducing strain was accomplished in collaboration with Dr. Klaus Nüsslein and Teresa Conneely of the Department of Microbiology. The isolation of SUPeRB showed the bacteria belongs to the *Delftia sp*. Further characterization of this isolate is ongoing.

The batch culture results showed that SUPeRB can be enriched from a wastewater seed using elemental sulfur as an electron donor and perchlorate as an electron acceptor. These bacteria are autotrophs and are slow growing and hence produce very little sludge. There is no addition of carbon substrate needed since they use inorganic carbon as their carbon source. The salinity results show that SUPeRB can be used for treatment of IX brines which have high ClO_4^- concentrations. The brine concentrations chosen in this study are similar to IX brines used commercially. IX technology has become a cost effective method for treatment of perchlorate contaminated drinking water since the process is relatively inexpensive, can be done in a smaller reactor volume than biological process, (Martin *et al*., 2006) and there are no biological residues carried over to the product water. The problem arises when these resins are regenerated with brine solution, as these brines cannot be disposed of in wastewater treatment plants as high brines lead to upsets in wastewater operations, require higher doses of coagulant for settling and the effluent contains high brine which cannot be leached into the open fields. SUPeRB cultures can be inoculated in a hybrid system with IX and a packed bed reactor to treat these brines.

Bioreactor Performance

SUPeRB cultures were inoculated in one liter continuous bench scale bioreactors operated in upflow mode. At high concentrations (5-8 mg/L ClO_4^-) and with intermittent recirculation, ClO_4^- was reduced to less than 0.5 mg/L. EBCT was reduced from 100 hours to 13 hours over a period of 120 days. Average ClO_4^- removal efficiency was 88%. The contents of this reactor were divided to form two new bioreactors to treat ClO_4^- concentrations in the range of 80-100 μg/L. Reactor performance was investigated at varying EBCT, recirculation rates and with the presence of the co-contaminant, NO_3^-. EBCT was reduced from 30 hours to 8 hours with an average removal efficiency of 96%. Reactor operation with little or no recirculation showed the best ClO_4^- removal than at higher recirculation ratios. The presence of NO_3^--N did not inhibit ClO_4^- reduction. Concentration profiles showed that both ClO_4^- and NO_3^--N reduction was occurring in the first 10 cm of the reactor. The presence of dissolved oxygen in the feed water did not inhibit ClO_4^- reduction or bioreactor removal efficiency.

Perchlorate can be treated in *ex-situ* processes such as packed bed bioreactors with little recirculation. A little recirculation may be needed during the initial stages of operation of the reactor to improve mass transfer of ClO_4^- to the biofilm. Higher recirculation rates could scour off the biofilm which leads to decrease in ClO_4^- removal efficiencies as these bacteria take a longer time to grow. High ClO_4^- concentrations (5-10 mg/L) have been recorded in military ranges while low ranges of ClO_4^- (80-100 μg/L) have been found at many local and industrial sites (USEPA, 2005). These ranges of ClO_4^- concentrations could be reduced by SUPeRB at EBCTs ranging from 8-13 hours. Both ClO_4^- and NO_3^--N have been found at many military sites as co-contaminants (Clausen, 2006). This is one of few studies to show both ClO_4^- and NO_3^--N can be reduced simultaneously.

Based on the above results and findings, ClO_4^- reduction using elemental sulfur as an electron donor is a promising technology for ClO_4^- treatment in groundwater. Further tests will be required at pilot scale to prove its treatment potential and commercial aspect of the technology. The elemental sulfur pellets can also be used as a packing material for permeable reactive barriers (PRBs). Though this project did not investigate any PRBs, the researchers envision the use of SUPeRB in PRB for *in situ* groundwater remediation.