

Report for 2002CA3B: Perchlorate Removal in Groundwater Using Immobilized Cell-Free, Purified and Recombinant Perchlorate Reductases from the Perchlorate Respiring Bacterium, Perclace

- unclassified:
 - Articles in Refereed Scientific Journals Okeke, B.C., Giblin, T. and Frankenberger Jr. W.T., Reduction of perchlorate and nitrate by salt tolerant bacteria, Environmental Pollution, 118, 357-363, 2002. Giblin, T. and Frankenberger Jr. W.T., Perchlorate and nitrate reductase activity in the perchlorate respiring bacterium perclace, Microbiological Research, 156, 311-315, 2002. Losi, M.E., Giblin, T., Hosangadi, V. and Frankenberger, W.T., Bioremediation of perchlorate-contaminated groundwater using a packed bed biological reactor. Bioremediation Journal, 6, 97-104, 2002. Giblin, T., Losi, M.E., Hosangadi, V. and Frankenberger, W.T., Bacterial perchlorate reduction in simulated reverse osmosis rejectate. Bioremediation Journal, 6, 105-111, 2002. Book Chapter Giblin, T., Herman, D. C. and Frankenberger, W.T., An autotrophic system for the bioremediation of perchlorate from ground water. Pp. 199-211, 1999. Perchlorate in the Environment, Plenum Press. Other Publications Okeke, B.C. and Frankenberger Jr. W.T., Molecular analysis of a perchlorate reductase from a novel perchlorate respiring bacterium, perclace. In Preparation. Losi, M.E., Hosangadi, V., Tietje, D. Okeke, B.C., Zuromski, R. and Frankenberger, W.T. (2003). Field pilot testing of a dynamic suspended bed bioreactor for removal of perchlorate in ground water. In preparation.

Report Follows:

PROJECT SUMMARY

Perchlorate (ClO_4^-) is an important energetic component of solid rocket fuel. The major source of ClO_4^- pollution is the military, space program and supporting industries. Wastewater generated from the manufacturing, maintenance, and testing of solid rocket propellants can contain NH_4 perchlorate in the grams per liter concentration range. Perchlorate is recalcitrant in the environment and is potentially toxic. The California Department of Health Services adopted an action level of 4 ppb for perchlorate in potable water. Physicochemical water treatment technologies (e.g. membrane and ion-exchange systems) have been considered for ClO_4^- remediation, but they are expensive and not practical for ClO_4^- removal from ground water. Moreover, these processes produce high salt waste streams contaminated with perchlorate and require further treatment to remove residual ClO_4^- . Microbial reduction of ClO_4^- to environmentally-acceptable innocuous end products is currently an area of intense interest. Microorganisms that reduce perchlorate to chloride and molecular oxygen have been isolated. For designing an efficient biological-based ground water ClO_4^- remediation strategy, the biochemical and molecular data on the enzymatic reduction of ClO_4^- are needed. The ClO_4^- respiring organism, *perclace* when grown using either ClO_4^- or NO_3^- as a terminal electron acceptor produced ClO_4^- reductase to a significant extent. The ClO_4^- reductase activity appeared to be within the periplasmic space, with activities as high as $14,000 \text{ nmol}^{-1} \text{ min}^{-1} \text{ mg protein}^{-1}$, indicating that it is a soluble enzyme. A ClO_4^- reductase from cell-free extracts of *perclace* was purified 10-fold by ion-exchange and molecular exclusion fast protein liquid chromatography (FPLC). The ClO_4^- reductase catalyzed the reduction of ClO_4^- at a V_{max} and K_m of $4.8 \text{ Units mg protein}^{-1}$ and $34.5 \mu\text{M}$, respectively. Maximal activity was recorded at $25\text{-}30^\circ\text{C}$ and pH $7.5 - 8.0$. *Perclace* ClO_4^- reductase is a dimer with molecular masses of 35.07 kDa and 75.1 kDa determined by SDS-PAGE. Matrix-Assisted Laser Desorption Ionization-Time of Flight/Mass Spectrometry (MALDI-TOF/MS) analysis of the 35 kDa protein revealed several tryptic peptides (Fig. 1). To study the genetic determinants of ClO_4^- reductase, the amino terminal sequences of 22 tryptic peptides of the approximately 35 kDa ClO_4^- reductase subunit were obtained by electrospray mass spectrometry. GenBank Blast analysis of the amino acid sequences revealed similarity to reductases, dehydrogenases and heme proteins. In batch studies of in vitro reduction of perchlorate, *perclace* ClO_4^- reductase reduced perchlorate in water with either NADH or methyl viologen as an electron donor. Less enzyme activity was

observed with methanol and ethanol. Addition of perchlorate ClO_4^- reductase to ion-exchange (IEX) brine impacted with ClO_4^- substantially enhanced ClO_4^- removal by salt tolerant bacteria. Experiments showed that ClO_4^- reductase immobilized to Ca alginate reduced chlorate. Additional studies are focusing on: optimization of reaction conditions for perchlorate reduction by immobilized perchlorate reductases, molecular characterization of the overall genetic determinants of ClO_4^- bioreduction by perchlorate by cloning the genes using degenerate primers designed from the amino acid sequences of ClO_4^- reductase tryptic peptides and over-expression of recombinant ClO_4^- reductase. Such a recombinant enzyme available in large quantities can be immobilized and safely used for the treatment of perchlorate contaminated ground water on site. Treatment systems designed to employ cell-free enzymes catalyze the ClO_4^- reduction reaction without the production of biomass wastes. Moreover, the spent enzymes can be regenerated and reused, substantially reducing cost. Cell free perchlorate reductase immobilized on calcium alginate displayed ClO_4^- reductase activity.

STUDENT SUPPORT:

The project provided practical training for over 30 students enrolled in Environmental Science 155 through several hours of laboratory classes on “Bioremediation of perchlorate in ground water”. The project also supported in part, a post-doctoral fellow (Benedict C. Okeke) to carry out the study.