Category III - Water Quality

Perchlorate Removal in Groundwater by Perchlorate Reductases from the Perchlorate Respiring Bacterium, Perc1ace

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Executive 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 (Urbansky and Schock, 1999). Waste water generated from the manufacturing, maintenance, and testing of solid rocket propellants can contain NH_4 perchlorate in the grams per liter concentration range (Herman and Frankenberger, 1998). Perchlorate is recalcitrant in the environment and is potentially toxic to various forms of life, humans in particular (Lamm et al., 1999). California Department of Health Services adopted the action level of 18 ppb for perchlorate in portable water.

Physicochemical water treatment technologies (e.g. membrane and ion-exchange systems) have been considered for the ClO_4^- remediation but they are expensive and not practical for ClO_4^- removal from the ground water. Moreover, these processes produces 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 (Herman and Frankenberger, 1998; Logan, 1998). 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.

Perchlorate reductase enzyme was identified and isolated from the ClO_4^- aspiring bacterium, perclace. The biochemical properties of the enzyme were characterized. Biotechnological methods are being developed to mass produce cell-free recombinant enzyme to be used in designing and testing the enzymatic reactors for reduction of perchlorate in water.

The ClO_4^- respiring organism, perc1ace 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 nmol^{-1} min^{-1} mg protein^{-1}, indicating that it is a soluble enzyme. A ClO_4^- reductase from cell-free extracts of perc1ace 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 Vmax and Km of 1589.09 nmol ml^{-1} min^{-1} and 34.5 μM, respectively. Maximal activity was recorded at 25^{o}C and pH 7.5 – 8.0. Perc1ace ClO_4^- reductase is a dimer with molecular masses of 35.07 kda and 75.1 kda determined by SDS-PAGE and MALDI-TOF/MS. To study the genetic determinants of ClO_4^- reductase, the amino terminal sequences of 10 tryptic peptides of the approximately 35 kda ClO_4^- reductase subunit were obtained by electron spray mass spectrometry. GenBank Blastn analysis of the amino acid sequences revealed homology to reductases. In batch studies of in vitro reduction of perchlorate, perc1ace ClO_4^- reductase reduced perchlorate in water with either NADH or methyl viologen as electron donor. Addition of perc1ace ClO_4^- reductase to ion-exchange (IEX) brine impacted with ClO_4^- substantially enhanced ClO_4^- removal by salt tolerant bacteria.

Additional studies are focusing on the molecular characterization of the genetic determinants of ClO_4^- bioreduction by perc1ace 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 mass produced 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.