Project Title: **Armored Enzyme Nanoparticles for Remediation of Subsurface Contaminants**

Final Report Date: **February 2007**

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Number of Graduate Students Actively Involved in the Project:	**1**
Number of Undergraduate Students Involved (part-time) in the Project:	**0**
Number of Post-Doctoral Scholars involved (part-time) in the Project:	**0**
Number of Ph.D. degrees granted involved in the Project:	**0**
Number of M.S. degrees granted involved in the Project:	**0**

Research Objective

The remediation of subsurface contaminants is a critical problem for the Department of Energy, other government agencies, and our nation. Severe contamination of soil and groundwater exists at several DOE sites due to various methods of intentional and unintentional release. Given the difficulties involved in conventional removal or separation processes, it is vital to develop methods to *transform* contaminants and contaminated earth/water to reduce risks to human health and the environment. Transformation of the contaminants themselves may involve conversion to other immobile species that do not migrate into well water or surface waters, as is proposed for metals and radionuclides; or degradation to harmless molecules, as is desired for organic contaminants. Transformation of contaminated earth (as opposed to the contaminants themselves) may entail reductions in volume or release of bound contaminants for remediation.

Effective methods to carry out these transformations are required. One approach is microbial bioremedition, however this is not trivial. The microbes must be capable of living in the contaminated environment of concern, they must express the enzymes or enzyme systems of interest, and they must do so in competition with other microorganisms in the same subsurface environment. Highly contaminated soil and groundwater may be inhospitable to microorganisms, which would limit bioremediation to the periphery of the contamination and preclude bioremediation of the source. In addition, bacteria provide two barriers for uptake of organic contaminants: the cell wall and the inner cytoplasmic membrane.

An alternative approach is remediation with isolated enzymes instead of microorganisms. However, direct addition of the required enzymes to bioreactors, engineered landfills, or *in situ* is not feasible if the enzymes are too short-lived to be effective. Enzyme stability is a critical technical gap in the enzymatic alternative to bioremediation. Furthermore, stability is a significant component of the cost of using enzymes. The technical ability to produce enzymes on

a large scale has progressed dramatically in the past two decades, and further advances can be expected as attention shifts from genomics to proteomics. However, methods to stabilize enzymes in suitable forms for practical use remain an important issue.

Thus, the most fundamental scientific challenges are two-fold: to develop new methods of increasing the stability of enzymes and to do so without sacrificing the effective activity of those enzymes by limiting mass transfer to the active sites. ***We began to address these issues in the funded research project by developing armored enzyme nanoparticles.*** The nanometer scale dimensions of the "armor" will provide much less mass transfer limitation to enzymatic activity compared to other immobilization methods such as micron scale particles with entrapped or immobilized enzymes. The armored enzyme nanoparticles will be nano-bio-composites, combining the soft bio-organic enzyme core with an inorganic silicate-containing polymer network as the armor. This network will be porous to small or medium sized molecules.

Research Progress and Implications

This report summarizes research performed at Rensselaer in collaboration with colleagues at Pacific Northwest National Laboratory. The research effort is being directed at stabilization of enzymes as nanostructured enzyme-containing composites, and development of a dehalogenase enzyme composite as a stabilized enzyme form for the transformation of chlorinated organic contaminants. In addition, research is being performed on methods to modify enzymes for active and stable inclusion into these nanoscale composites.

The primary enzyme nanostructure developed was the armored enzyme nanoparticles. As these contain a single enzyme molecule, we also refer to them as single enzyme nanoparticles, or SENs. The preparation of SENs represents a new approach that is distinct from immobilizing enzymes on the surfaces of solids or encapsulating them in sol-gels, polymers, or bulk composite structures. Converting free enzymes to SENs can result in significantly more stable catalytic activity, as we have demonstrated for chymotrypsin as a model hydrolase enzyme. The nano-scale structure of the SEN does not impose a serious mass transfer limitation on substrates. At the same time, the synthesis of SENs is also different from conventional enzyme modifications such as surface amino acid modifications or polymer attachment, which generally do not provide as great a long-term enzyme stabilization. The process for the preparation of SENs begins from the surface of the enzyme molecule, with covalent reactions to anchor, grow, and crosslink a composite organic/inorganic network around each separate enzyme molecule. The reactions are carried out so that crosslinking is largely confined to individual enzyme surfaces, yielding discrete nanoparticles rather than the bulk solids that would result from interparticle reactions. SENs were observed using high resolution transmission electron microscopy (TEM). Individual nanoparticles with seemingly hollow centers and a contrasting outer structure could be resolved. The dimensions of the transparent core containing the protein are consistent with the size and shape of the enzyme. Kinetic measurements showed that the SENs were quite stable in contrast to their native free enzyme precursors, and that there was little mass transfer limitation.

Research at Rensselaer focused on the development of haloalkane dehalogenase as a critical enzyme in the dehalogenation of contaminated materials (ultimately trichloroethylene and related

pollutants). A combination of bioinformatic investigation and experimental work was performed. The bioinformatics was focused on identifying a range of dehalogenase enzymes that could be obtained from the known proteomes of major microorganisms. This work identified several candidate enzymes that could be obtained through relatively straightforward gene cloning and expression approaches. The experimental work focused on the isolation of haloalkane dehalogenase from a *Xanthobacter* species followed by incorporating the enzyme into silicates to form biocatalytic silicates. These are the precursors of SENs. At the conclusion of the study, dehalogenase was incorporated into SENs, although the loading was low. This work supported a single Ph.D. student (Ms. Philippa Reeder) for two years. The project ended prior to her being able to perform substantive bioinformatics efforts that would identify more promising dehalogenase enzymes. The SEN synthesis, however, was demonstrated to be partially successful with dehalogenases. Further work would provide optimized dehalogenases in SENs for use in pollution remission.