

# Exploring Microbial Proteins for Bioremediation Capabilities

## Challenge/Need

Microbes live in our soil, water, and bodies — and they are among the tiniest, oldest living creatures on Earth. They may also be a cost-effective, natural tool for cleaning up toxic wastes.

For the development of bioremediation strategies, the U.S. Department of Energy (DOE) is focusing on the metabolic capabilities of microbes that reduce metals as part of their energy metabolism. Although the complete genome sequence can be used to predict metabolic functions based on the presence of genes encoding specific proteins, scientists need laboratory methods to verify those capabilities and explore their regulation.

The metabolic capabilities of any cell depend on the proteins expressed, their structure, the cofactors they require, and their interactions with other proteins. Existing methods for the global assessment of proteins are useful for identifying which proteins are expressed, but they do not allow analysis of function or protein associations. Therefore, researchers need to develop methods for the functional analysis of proteins in complex mixtures.

## Argonne's Answer

Argonne scientists are developing a nondenaturing two-dimensional electrophoresis protocol that can separate proteins and yet retain their biological activity and interactions. This protocol would provide researchers with a tool for the global functional analysis of complex protein mixtures.

To evaluate the protocol's effectiveness, scientists are using it to analyze proteins in *Geobacter sulfurreducens* (Figure 1) and *Shewanella oneidensis*, microbes that can reduce a variety of electron acceptors, including fumarate (a salt of fumaric acid), iron (III), manganese (IV), uranium (VI), cobalt (III), and technetium (VII). Both microbes are included in DOE's Microbial Cell Program because of their potentially useful metal reduction capabilities in bioremediation.

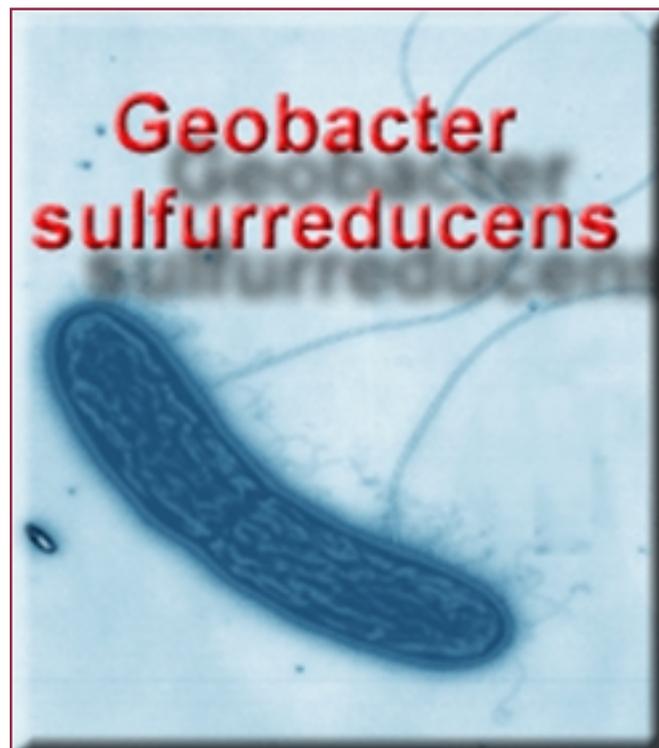


Figure 1. *Geobacter sulfurreducens*, a microbe that may be a cost-effective, natural tool for bioremediation.

## Approach

At Argonne, high-resolution protein separation of nondenatured proteins is achieved by first separating proteins by differences in isoelectric point and subsequently by differences in charge-to-mass ratio within a polyacrylamide matrix. The Argonne Protein Mapping Group detects the proteins by using a variety of protein-specific dyes (Coomassie Blue, silver), enzymatic activity assays (malate dehydrogenase), and staining methods dependent upon specific chemical groups (heme), and by the presence of metals (x-ray fluorescence). Comparative analysis is done to detect altered protein expression in microbes responding to different growth conditions (e.g., the presence or absence of specific metals). Proteins are identified by peptide mass spectrometry.

Scientists are exploring methods for using x-ray fluorescence (XRF) at the Advanced Photon Source (APS) to detect and characterize metalloproteins in complex protein mixtures after the proteins have been separated by means of Argonne's separation technique (Figure 2). Metal-containing proteins are critical to many of the chemical reactions involved in cellular energy metabolism (Figure 3); therefore, the regulation of their expression is essential to the correct response of cells to changes in their environment.

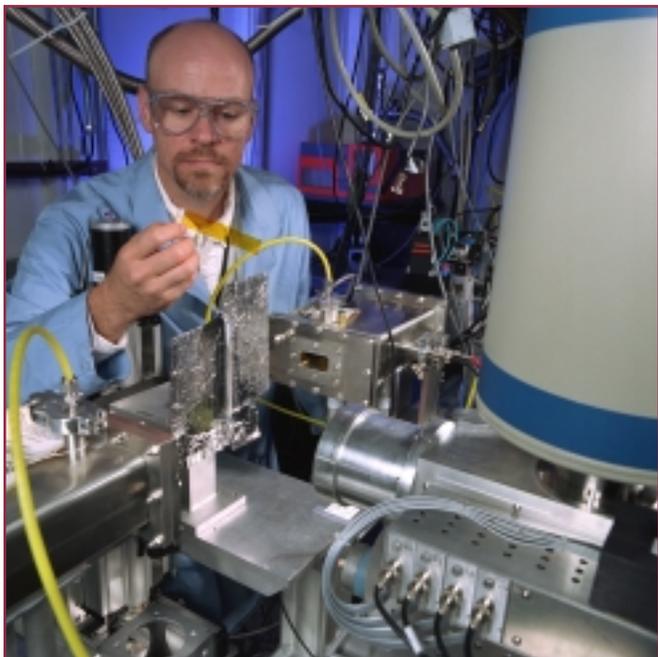


Figure 2. X-ray fluorescence analysis of metalloproteins at Argonne's Advanced Photon Source.

## Accomplishments

Although the work is still in the research phase, the results are promising. For example, Argonne scientists have

- Identified hundreds of proteins from the soil metal-reducing *G. sulfurreducens* and the aquatic metal-reducing *S. oneidensis* by using nondenaturing methods.
- Detected proteins containing heme groups associated with iron (which are implicated in metal reduction) in both microbes by using a colorimetric assay for heme groups.
- Detected the enzyme malate dehydrogenase by using an in-gel assay of its functional activity.

- Identified protein complexes, such as ATP synthase (which consists of multiple distinct protein components), as intact entities within the polyacrylamide matrix.
- Demonstrated the feasibility of detecting metal-containing proteins by using the nondenaturing electrophoresis method to separate pure proteins (such as cytochrome c and catalase) and then using XRF to detect the metals.

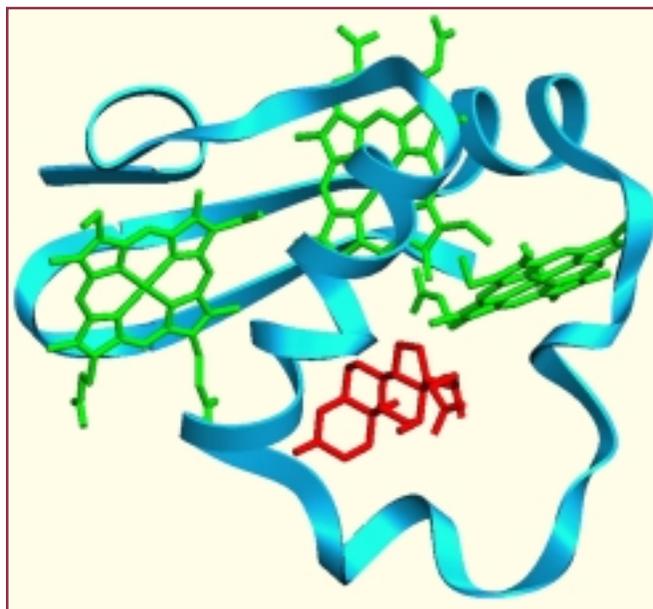


Figure 3. An example of a known metalloprotein expressed by *Geobacter sulfurreducens*: the 3D structure of cytochrome c7.

Data from these studies will become part of the public database of gel images and protein identifications that Argonne is developing (<http://ProteomeWeb.anl.gov>).

## Partners

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## Sponsor

U.S. Department of Energy

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