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Principal Investigator: Dr. Ming Tien
Project Title: Biochemical Mechanisms and Energy Strategies of Geobacter Sulfurreducens

To provide the scientific understanding required to allow DOE sites to incorporate relevant biological, chemical, and physical processes into decisions concerning environmental remediation, a fundamental understanding of the controls on micro-organism growth in the subsurface is necessary. Specifically, mobility of metals in the environment, including chromium, technetium and uranium, is greatly affected by the process of dissimilatory metal reduction (DMR), which has been shown to be an important biological activity controlling contaminant mobility in the subsurface at many DOE sites. Long-term maintenance of DMR at constant rates must rely upon steady fluxes of electron donors to provide the maintenance energy needed by organisms such as Geobacter sulfurreducens to maintain steady state populations in the subsurface. At present, we lack fundamental knowledge concerning how to answer the following questions: what influxes of electron donors are required to maintain a growing versus a non-growing population of Geobacter? How do changes in nutrient availability or other environmental conditions such as pH and ionic strength affect such required electron-donor influxes? How are these electron-donor influxes controlled by availability and nature of electron acceptors? Under survival conditions where availability of electron acceptors and nutrients are extremely low, what physiological and genetic modifications occur in Geobacter to achieve long-term survival? How do these changes affect the required electron-donor influxes? What approaches can be developed to relate metabolic activities characterizing Geobacter under survival mode conditions to the observed needs for electron-donor influxes under those conditions? What conceptual models can be used to predict maintenance energies of Geobacter under different phases of growth when measured under different environmental conditions? How can these models help the DOE predict the long-term evolution of contaminated sites?

In the proposed research, we outline a strategy to tackle these questions and lay the framework for DOE to make long-term predictions concerning DMR in the subsurface. We will test the following hypothesis:

Geobacter requires different fluxes of substrate for metabolism when existing under growth, stationary, or survival modes, and these substrate fluxes can be related to specific metabolic activities utilized under different geochemical conditions.

Our project has three specific objectives: i) measurement of maintenance energies using chemo- and retentostats; ii) determination of whether a survival mode adaptation of Geobacter includes a response characterized by high rates of mutations; iii) characterization of metabolic activities of Geobacter during stationary and survival modes using microarrays in order to compare the expression of genes to the measured maintenance energies. While site-specific studies are necessary for site-specific remediation, our work will provide the fundamental knowledge necessary for long-term stewardship of all DOE sites where contaminant mobility is affected by dissimilatory metal reduction.

The Initial Research Objectives

Below, I have listed the research objectives from the funded proposal and following this section, I have provided what we accomplished and the papers published.

<u>Objective 1.</u> Determined the maintenance energy of Geobacter. Growth yield (Y) and maintenance energy (ME) will be determined for cells that are growing under nutrient-rich or near nutrient-limiting conditions. To determine the upper and lower range of ME values that can be obtained with the chemostat, we will vary the following (with expected results in

parentheses):

a. High carbon, limiting Fe(III) (higher ME)

b. High Fe(III), limiting carbon (lower ME)

c. High Fe(III), high carbon, limiting nitrogen (higher ME)

ME and growth yield will be determined with soluble electron acceptor Fe(III)-citrate. We will also attempt chemostat studies with Mn(IV) pyrophosphate. During the first year will will also determine ME and Y for insoluble terminal electron acceptors ferrihydrite, hematite, goethite, birnessite and pyrolusite.

Objective 2. Determine the survival characteristics of *Geobacter* including whether the species demonstrates a survival (long-term stationary) phase.

Objective 3. Characterize the physiological response of *Geobacter* in the three phases (growth, stationary, survival) using DNA microarray chips and relate these responses to the measured substrate fluxes.

Progress from the objectives

We were able to construct a chemostat and we then initiated Y and ME calculations. These studies did not result in any publications because the finidings did not yield any relevant information to the survival strategy of Geobacter under the various growth limitation conditions. Our most productive and informative efforts were on Objectives 2 and 3 above where we investigated the survival strategy of Geobacter under starvation conditions.

List of accomplishments

We have documented for the first time:

i) that *Geobacter* sustains a stable population under starvation conditions for over a year,

ii) that prolonged stationary cultures of *Geobacter* acquire a competitive advantage over fresh cultures (see GASP below) *iii)* using SOLiD resequencing of the whole genome from 354-day old starved culture of *G. sulfurreducens,* identified a number of mutations found in the majority of surviving cells

iv) by iTRAQ analysis, found increased expression of proteins involved in membrane integrity

v) and electron transport during prolonged starvation.

We were also able to:

i) developed methodology to isolate sufficient RNA from a limited amount of starved cells to

ii) perform DNA microarrays and

iii) constructed a computer-controlled chemostat system with six reactors for studies on anerobic growth yield and maintenance energy.

Publications

We have published two papers from this grant.

1) Helmus RA, Liermann LJ, Brantley SL, Tien M Growth advantage in stationary-phase (GASP) phenotype in long-term survival strains of Geobacter sulfurreducens. Fems Microbiology Ecology 79: 218-228

2) Bansal, Reema; Helmus, Ruth; Stanley, Bruce; Zhu, Junjia; Liermann, Laura; Brantley, Susan; Tien, Ming (2013) “Survival During Long Term Starvation: Global Proteomics Analysis of Geobacter sulfurreducens under Prolonged Electron Acceptor Limitation" Journal of Proteome Research (in press).