41. Genome-scale Reconstruction of Metabolic Networks from Microbial Communities at Sites Undergoing Natural Attenuation of Uranium

Jiao Zhao[1*] (jiao.zhao@utoronto.ca), Kelvin Li[2], Monika Bihan[2], Radhakrishnan Mahadevan[1], and **Barbara A. Methé [2]**

[1]Department of Chemical Engineering and Applied Chemistry, University of Toronto, Ontario, Canada.
[2]J. Craig Venter Institute, Rockville, Maryland, USA.

Project Goals: As part of a larger research project using 'metaomics' approaches to study subsurface microbial processes and their role in cycling of carbon, nitrogen and metals (see also "The Study of Microbial Communities using 'Metaomic' Approaches at Naturally Reducing Subsurface Sites") the objectives of this computationally focused work are: 1) to use data from 'metaomic' approaches to reconstruct metabolic networks of microbial communities at the Rifle Integrated Field Research Challenge (RIFRC) site undergoing natural attenuation of uranium, 2) provide insights into the potential mechanisms that lead to numerical dominance of specific bacterial taxa under different environmental conditions, 3) study the interaction and collaboration among members of the microbial community and 4) integrate gene expression data characterizing metabolic activity into genome-scale models to describe microbial activity in sub-optimal states.

Reductive immobilization of soluble U(VI) to the insoluble U(IV) is an efficient remediation strategy of subsurface groundwater contaminated with uranium. While U(VI) can be reduced to U(IV) by stimulation of indigenous bacteria with amendments of organic carbon, there are zones of natural attenuation of U(VI) at the RIFRC site, where U(VI) can be both reduced and remobilized in the absence of biostimulation. The elucidation of the potential mechanisms of microbial community structure and metabolism under these conditions of natural attenuation can facilitate more efficient remediation design and management strategies.

In order to undertake computational modeling approaches of microbial metabolic potential we first investigated the microbial community structure at a site previously identified as undergoing natural attenuation of uranium at the RIFRC ('JB01-05' site) using metagenomic data. This examination showed that in terms of relative abundance, β-proteobacteria dominate the community (45.2%), followed by Actinobacteria (17.9%), α-proteobacteria (14.8%) and γ- proteobacteria (13.3%). This result is in sharp contrast to the community structure seen in sites undergoing biostimulation through amendment with acetate in which δ-proteobacteria (especially members of the genus *Geobacter*) dominate with relative abundances as high as 99%. Due to a similarity in genome content between subsurface microbial community members assigned to the same taxonomic class at our study site, we pursued a pan-genome-scale approach to subsequently analyze metabolic potential at the class-level. A statistical analysis of the functional profiles from the JB site indicated that within the numerically dominant taxonomic classes there is an abundance of enzymes related to CO_2 fixation (e.g. Rubisco in the α- β-, and γ- proteobacteria, and PEPCase in the Actinobacteria). In contrast, in acetate amended sites within the numerically dominant δ-proteobacteria, there is a high abundance of enzymes related to N_2 fixation (e.g. Nitrogenase). Collectively, these results reveal different community structures and metabolic functions mediating C and N cycling under these contrasting environmental conditions.

Using metagenomic and reference genome datasets, pan-genome-scale metabolic networks were reconstructed for α- , β-, γ- and δ-proteobacteria, and Actinobacteria, respectively. The models were optimized and gaps filled to ensure that they are capable of growth in geochemical conditions similar to that of the RIFRC site. These class-level models were then integrated into a Dynamic Multi-species

Metabolic Modeling (DMMM) framework for investigating the interaction and collaboration among community members. The model analysis indicates that *Thiobacillus denitrificans* may dominate the community at the JB site due to its ability to use inorganic electron donors for energy and fix CO_2 as its major carbon source. Through electron transport with cytochrome bc1 complexes and NADH-Q oxidoreductase, a tight coupling between Fe(II) oxidation and NO_3 reduction can be established to support use of CO_2 as the main source of carbon. Similarly, reduced inorganic sulfur compounds may be oxidized to sulfate by ferricytochrome c with reduction of nitrate as a terminal electron acceptor and fixation of CO_2 as the major carbon source.

Interaction and collaboration of microbial community members were quantitatively estimated through the DMMM approach. While competitive interactions mainly occur in the community for electron donors and acceptors, and carbon sources, the simulations indicate that there are potential syntrophic interactions between β-proteobacteria (e.g. *Thiobacillus denitrificans*) and Actinobacteria (e.g. *Streptomyces*). *Streptomyces* may use the products of sulfur oxidation (e.g., sulfate) from *Thiobacillus denitrificans* as the final electron acceptor for CO_2 fixation under anoxic conditions.

Subsurface microorganisms may grow within either optimal or sub-optimal states depending on ever changing environmental conditions. Hence, application of a flux balance analysis (FBA) that seeks to maximize or minimize an objective function may not be always appropriate for describing and predicting microbial activities in the subsurface. Therefore, we are currently developing methods to integrate metatranscriptomic (gene expression) data into the genome- scale models to better identify the metabolic states of various community members, thereby elucidating functional mechanisms of the community indicative of, and relevant to, sub-optimal states. These combined models will be incorporated into the DMMM framework to improve the predictive capability of the genome-scale models. Further, the metatranscriptomic data will also be used for reconstructing the metabolic network of specific individual organisms of interest in the microbial community.