

Understanding the thermodynamic Foundations of microbial Growth Efficiencies in the Lab and Field

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Project Goals: A key element of microbial growth and therefore microbial community assembly is how microbes partition the available resources between energy required for maintenance and growth. Using microcalorimetry and thermodynamic modelling, we gained a quantitative proxy for microbial growth efficiency under different growth conditions. As the next step, we aim to apply our methods to address the ecological framework guiding the partitioning of denitrification pathways at the Oak Ridge National Laboratory Field Research Site (FRC); through the establishment of microbial activity assays, isotope fractionation analysis as well as establishment of mass balances and stoichiometry for representative nitrate respiring isolates.

The assembly of microbial communities is determined by many factors, with the environment setting the stage via availability of electron acceptors, donors and carbon sources as well as with physical/chemical parameters such as temperature and salinity. In this framework microbes have to adapt to either stable or dynamic conditions and to either compete or share resources for survival. Also, bacteria need to balance constantly the division of available energy between maintenance of basic cellular functions and growth. Therefore, microbes with the most favorable ratio between maintenance and growth requirements should be more competitive compared to microbes with higher energy demands for maintenance and growth.

Maintenance energy levels as a proxy for microbial competitiveness are usually measured in chemostats near zero growth conditions. Here, we attempt to capture maintenance energies via microcalorimetry and a metabolite-dependent thermodynamic model as a quantitative proxy for microbial growth efficiency. Microcalorimetry offers a direct and highly sensitive method to assess the enthalpy-related terms of microbial growth in relation to the potential energy supplied by growth substrates. This in turn allows for a quantitative description of growth and maintenance

in thermodynamic terms under different growth conditions, based on the comparison of the metabolite profile at the start and end of growth.

We have examined the influence of temperature stress, simulated environmental dynamics and adaptation to salt stress on the growth efficiencies of different strains of *Desulfovibrio vulgaris* Hildenborough and *D. alaskensis* G20. These analyses quantified the cost of maintenance (survival) in relationship to increasingly suboptimal growth conditions, the cost of regulation in a fluctuating environment and the reduction in maintenance costs realized through adaptive evolution.

We aim to apply our growth efficiency and maintenance proxy to field conditions by addressing the ecological framework guiding the partitioning of denitrification pathways at the Oak Ridge National Laboratory Field Research Site (FRC). This site has a long contamination history with nitrate among a large variety of other contaminants. Thermodynamic modeling based on mass balances and stoichiometries of site-relevant nitrate respiring isolates will be associated with in situ measurements of nitrate respiration and nitrate stable isotope fractionation analyses to develop a predictive framework for microbial community assembly and activity.

As the next step, we are cultivating available nitrate respiring isolates (*Rhodanobacter*, *Acidovorax* and *Pseudomonas* spp.) under different C/N ratios and the application of the thermodynamic model established with metabolite profiles, we aim to record the energy requirements under different biogeochemical conditions. These profiles can then be put into context to actual biogeochemical field conditions as characterized by nitrate specific acetylene-block activity assays and the metabolic history as traced by nitrate stable isotope fractionation.

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