

47. Bioenergy and Biogeochemical Cycling in Elkhorn Slough Hypersaline Microbial mats

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Project Goals: The LLNL Biofuel SFA investigates systems biology of complex microbial communities. Specific goals in this work are to develop an integrated analysis of energy flow in complex microbial communities by combining multi-scale approaches including biogeochemical, stable isotope probing, metagenomic/transcriptomic, proteomic/metabolomic and computational analyses, to understand nutrient cycling and potential biofuel production in complex microbial communities. Our ultimate goal is the development of multi-scale models that can predict ecological and biochemical relationships within multi-trophic microbial systems.

Marine hypersaline cyanobacterial mats are diverse laminated microbial assemblages, thought to represent life on the early earth. The mats at Elkhorn Slough, CA and Guerrero Negro, Mexico have intrinsic relevance to biofuels systems biology because they produce significant nighttime fluxes of hydrogen gas and other potential biofuels as fermentation byproducts (Figure 1), and they cycle carbon, oxygen, sulfur, and nitrogen on a millimeter scale. The overall goal of these studies is a systems-level understanding of the partitioning of light and geochemical energy into biomass and potential biofuels in these complex microbial communities. Past studies in Elkhorn Slough have shown production largely from *Cyanobacteria* and consumption from sulfate-reducing bacteria (SRB).

However, the mechanisms and magnitude of hydrogen cycling are not well understood. The overall goal of the present work is a systems-level understanding of the partitioning of light and geochemical energy into biomass in microbial mat communities.

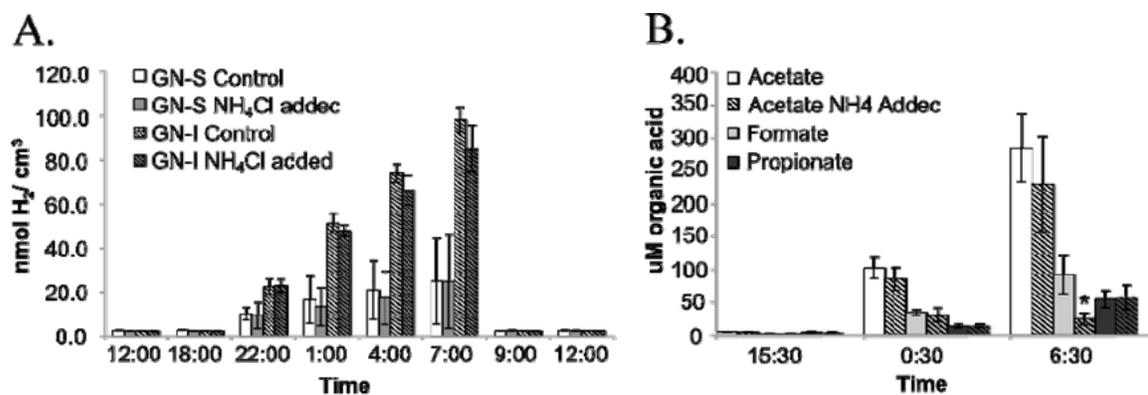


Figure 1. Hydrogen net flux over a diel (A), and net flux of common organic acids (B) in GN-I and GN-S mats.

Mats from Guerrero Negro, Mexico - permanently submerged *Microcoleus* microbial mats (GN-S), *Lyngbya* intertidal microbial mats (GN-I) - were used in microcosm diel manipulation experiments (with ammonium chloride) to determine mechanisms responsible for hydrogen cycling between mat microbes. H₂ production occurred under dark anoxic conditions with simultaneous production of a suite of organic acids (Figure 1). H₂ production appears to result from constitutive fermentation of photosynthetic storage products (glycogen) over the day night cycle. Comparisons of accumulated glycogen and CO₂ flux indicated that in the GN-I mat, fermentation released a majority (~90%) of the carbon fixed via photosynthesis during the preceding day, primarily as organic acids. In parallel, incubations with ¹³C-acetate and nanoSIMS isotopic imaging indicated higher uptake in both *Chloroflexi* and SRBs relative to other filamentous bacteria. These manipulations and diel incubations confirm that *Cyanobacteria* were the main fermenters in Guerrero Negro mats and that the net flux of nighttime fermentation byproducts (mostly acetate) was largely regulated by the interplay between *Cyanobacteria*, SRBs, and *Chloroflexi*. These data suggest that light energy partitions primarily into the *Cyanobacteria* (stored glycogen photosynthate) and is then released at night into EPS (extracellular polymeric substances), perhaps as organic acids. Ongoing experiments will examine how this photosynthate is partitioned and the fate of carbon released into the extracellular matrix.

Preliminary analyses of the genome of ESFC-1 has also been conducted. This taxa is an important member of the Elkhorn Slough system and a newly described lineage of filamentous diazotrophic cyanobacteria. We have identified EPS proteins and characterized EPS composition in both ESFC-1 and Elkhorn Slough mats. The most abundant of the extracellular proteins are predicted to be involved in protein and sugar degradation and putative structural components. This suggests that cyanobacteria may facilitate carbon transfer to other groups through degradation of their EPS components.

To determine which organisms were responsible for glycogen formation and storage and which organisms were involved in organic acid uptake, a combined metagenomic / metatranscriptomic study was completed to track the transcript response of mat-associated organisms over the course of a day as energy passed from sunlight into fixed carbon and nitrogen and subsequently into nighttime fermentation products. A total of 4 metagenomes and 9 metatranscriptomes (over 9 time points of the day) were sequenced. Future experiments also include metaproteome analyses over a diel cycle and tracing of ¹³C – labeled EPS.

In summary, this work contributes to our understanding of the partitioning of major pathways of energy from sunlight into various hydrogen and carbon utilizing microbes in complex multi-trophic microbial systems.

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