Effects of Nutrient Limitation, Light Quantity and Quality on Growth Dynamics and Physiology of *Synechococcus* sp. PCC 7002

Allan E. Konopka*[1] (email: allan.konopka@pnnl.gov), Grigoriy E. Pinchuk[1], Eric A. Hill[1], Leo A. Kucek[1], Matthew R. Melnicki[1], Trang Vu[2], Jennifer L. Reed[2], and **Alex S. Beliaev[1](PI)**

[1]Pacific Northwest National Laboratory, Richland, WA; [2]University of Wisconsin-Madison, Madison, WI, USA

Project Goals: The PNNL Biofuels Scientific Focus Area (BSFA) conducts fundamental research of microbial photoautotrophs with specific emphasis on photosynthetic energy conversion, reductant partitioning, and central carbon metabolism. As a model system, we utilize unicellular prokaryotic organism *Synechococcus* sp. PCC 7002, which exhibits one of the fastest growth rates known among cyanobacteria and which is also remarkably tolerant to high light intensities. Understanding the genetic and physiological bases of these properties could provide fundamental new insights that are broadly applicable to the optimization of other biological systems for biofuels development. To that end, we are interrogating fluxes through central metabolic pathways to define the major constraining factors (*i.e.* metabolic and regulatory controls) governing carbon partitioning through the metabolic subsystems of cyanobacteria that can be manipulated to increase yields of specific molecules that are either precursors or fuel molecules themselves.

The initial experimental phase, which consists of analyses of photosynthesis and carbon fixation modules, employs turbidostat cultivation technology which allows the organisms to grow at their unrestricted, maximum growth rate. This approach is suited particularly well for photoautotrophs since attenuation of light by self-shading and mass transfer of CO_2 must be taken into account. We are utilizing a custom-designed photobioreactor (PBR) to rigorously control the physiological state of *Synechococcus* 7002, and analyze the properties of both wild type (and subsequently) discrete mutant strains. PBR illumination is provided by light-emitting diodes (LED) generating 680 nm and 630 nm light for the preferential excitation of chlorophyll *a* and phycobilin pigments. The continuous culture system was modified to control medium input to maintain constant turbidity (turbidostat); this produces a steady state in which growth rate is unrestricted by dissolved nutrient concentrations, but determined by either light irradiance, or cellular capacity (at saturating irradiances). *Synechococcus* 7002 grew when irradiated with only 680 or only 630 nm light, with the latter resulting in higher growth rates; notably, the growth rate differences were greatest at the lowest irradiances. Steady-state dissolved O_2 concentrations were linear functions of irradiance; the slope was 2.3-fold greater when a low amount of 680 nm irradiance was combined with 630 nm light than in cultures grown under 630 nm irradiances alone. When irradiance values were changed, the culture experienced either an increase (shift-up) or decrease (shift-down) in growth rate. After shift-downs, the new steady-state

growth rate was usually achieved within 3 h. In contrast, several shift-up transitions required >10 h to reach the new growth rate. The physiological consequences of shifts in light quality and quantity have been investigated using PAM fluorometry to calculate the maximum capacity for photosynthetic electron transport (rETR) and quantum yield of photosystem II (F_v/F_m).

Steady-state chemostat cultures have also been generated and are undergoing analysis of cellular composition, in conjunction with constraint-based modeling and global expression analyses. Significant differences in macromolecular composition have been found among unrestricted growth and light, carbon, or nitrogen-limited cultures and are consistent with previous understanding of macromolecular composition under these conditions. Protein content varied from 33% (N limitation) to 67% (light limitation). Under N limitation, polysaccharide comprised 61% of biomass, but was 10-17% under all other conditions. These measurements will be used to formulate the organism-specific biomass equation and incorporated into developed metabolic model of *Synechococcus* 7002 (*i*Syp612). The initial model was updated with additional genome annotation from pathway/genome databases (PGDBs) and includes 672 genes and 572 reactions. The new information from PGDBs resulted in the modifications of existing gene-protein-reactions associations (GPRs) due to addition of isozymes. A few (~20) new reactions were added to the pathways involved in quinone biosynthesis, carbohydrate, amino acid and nucleotide metabolism. Using the developed model, we will estimate the growth and non-growth associated ATP requirements (GAR, and NGAR, respectively) using the data described above for cultures grown under various light intensities. Model predicted growth, uptake, and secretion rates will be compared with experimental values for cells grown under different chemostat conditions.

This research is supported by the Genomic Science Program (GSP), Office of Biological and Environmental Research (OBER), U.S. Department of Energy (DOE), and is a contribution of the Pacific Northwest National Laboratory (PNNL) Biofuels Scientific Focus Area.