The Effect of Carbon Flux Topology and Synchronized Culture Growth on Microalgal Productivity

Mark Hildebrand[1] * (mhildebrand@ucsd.edu), Juergen Polle[2], Michael Huesemann[3]
[1] Scripps Institution of Oceanography, University of California, San Diego, San Diego,CA
[2] CUNY, Brooklyn College, Brooklyn, NY
[3] Marine Sciences Laboratory, Pacific Northwest National Laboratory, Sequim, WA

Project Goals

The overarching goal of this project is to understand and manipulate fundamental molecular mechanisms involved in maximizing growth rate and lipid accumulation in diverse classes of microalgae under authentic diurnal conditions to enhance production capabilities for biofuels. There are three primary targets to achieve this goal. The first is to advance the development of promising new model organisms relevant to biofuels production, which we are doing by focusing on highly productive strains of *Acutodesmus obliquus* and the diatom *Cyclotella cryptica*. The second is to clarify the features and organization of metabolic pathways in the study organisms, particularly with regard to carbon flux. The third is to evaluate growth and productivity under simulated outdoor cultivation conditions, to understand how cellular processes, particularly during synchronized cell division, affect productivity. Microalgae experience sinusoidal variation in light and temperature when grown outdoors, which is not typically considered in a lab setting, but which has a substantial influence on cellular processes.

Abstract

I. Elucidation and clarification of metabolic pathways in green algae and diatoms.

Using bioinformatic analysis and experimental approaches, we have gained substantial knowledge about carbon flux metabolism in our study organisms, which relates on a larger scale to two major classes of microalgae. We have generated the first metabolic map of core carbon metabolism in *A. obliquus*, which highlights the major routes of carbon processing. We have completed determination of the genome sequence of *C. cryptica*, and identified differences in carbon flux topology, particularly in processing of pyruvate, compared with the model species *Thalassiosira pseudonana*, that could explain the superior ability of *C. cryptica* to accumulate lipid. We have used a combined bioinformatic and experimental approach to clarify the process of photorespiration in *T. pseudonana*. Photorespiration is the second most important carbon flux pathway in algae. The data indicate that diatoms do not follow the pathway as elucidated in higher plants, and rather than recycle photorespiratory products back into the chloroplast to be re-fixed by RubisCO, they convert the products mostly into amino acids.

These analyses enabled us to perform comparative analyses of carbon flux pathways in different green algal species and different species of diatoms. Unexpectedly, predicted intracellular targeting data indicate substantial differences in carbon flux comparing six different green algal species that have sequenced genomes. One fundamental concept is that even though starch is stored in the chloroplast of most species, in some cases the pathways of synthesis and/or breakdown of starch have been relocated to the cytoplasm. This may affect the efficiency of carbon flux, and temporal preference to undergo cellular division. A comparative analysis of three diatom species also indicates substantial differences in carbon flux topology, which suggests active evolution to optimize arrangements by moving portions of pathways between different cellular compartments.

An overarching conclusion from these analyses relates to the preferred time of cell division of green algae and diatoms during the day; the former tend to divide at night because sufficient carbohydrate stores must be generated during the day, and the latter tend to divide during the day, because they rely more on direct photosynthetically fixed carbon for division than stored carbohydrate. In addition to the temporal effect on division, there are implications related to the extent of nighttime respiration, which also contributes to productivity.

II. Characterization of performance and productivity parameters under simulated outdoor conditions.

We have taken two approaches in this regard, performing 1) small scale experiments using Phenometrics photobioreactors (PBRs), and 2) large scale experiments using the PNNL simulated outdoor raceway ponds.

PBR experiments were done on *C. cryptica* using a 12:12 l:d cycle, 2000 uE maximum light intensity, with a temperature variation between 15-26^{o}C. The data indicate that cell cycle synchronization occurred, with the majority of the population dividing once per day beginning in the early afternoon. Changes in the optical density at 750 nm (OD750) preceded changes in culture density, indicating that OD750 is not a completely accurate proxy for culture density. It was an excellent proxy for ash free dry weight (AFDW). Lipid content, as triacylglycerol (TAG) increased until prior to mitosis, decreased as cell membranes were synthesized, then increased subsequently. The data indicate that optimal times of the day to harvest cultures for biomass or for lipid differed substantially.

We have completed two sets of runs in duplicate each of *C. cryptica* in the PNNL simulated outdoor raceway systems, with a focus on extensive sampling at two different times, 1) on a day when the cultures became limited for silicon, and 2) on a day when no limitation occurred. Conditions were 12:12 l:d cycle, 15-26^{o}C and maximum light intensity of 1200-1400 umol$\bullet$m$^{-2}\bullet$s^{-1}. The cultures synchronized, with division occurring during the day. Productivity during both runs was impressive, the average productivity from lag into stationary phase for run #1 was 18g AFDW/m^2/d, and for run#2 was 20 g AFDW/m^2/d. Productivity during the two peak days for each was 28-30 g AFDW/m^2/d for run #1, and 39-42 g AFDW/m^2/d for run #2. The sensitivity of productivity to mean light intensity was monitored, showing a relatively linear response. The mean light intensity and mean dissolved oxygen (DO) levels were highly correlated. DO measurements indicated that nighttime respiration was minimal, which could relate to the preferred division time during the day. Variation in cellular TAG content was monitored throughout the runs. Samples for RNA extraction were taken, and will be processed for transcriptomic analyses to investigate underlying cellular responses to progression through the diurnal cycle.

In comparison to *C. cryptica*, under identical pond cultivation conditions *A. obliquus* had a much lower average biomass productivity of about 12g AFDW/m^{-2}/d. This drastically lower productivity of the green alga *A. obliquus* may be attributable to the above mentioned species differences, separation of carbohydrate accumulation during the day from cell division at night, thus forcing the green alga to loose biomass due to respiratory activity for energy provision for the energetically costly cell division process during the night. Different temperature optimum for cultivation could also contribute to lower productivity for *A. obliquus.*

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