Algae-Based Hydrocarbon Production

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Abstract:

This presentation focuses on characterization of growth and hydrocarbon production from *Botryococcus braunii* as part of an overall project to develop the tools to migrate the unique metabolic capacity of this organism for the production of C30+ isoprene hydrocarbons to other algae and higher plants. This work compliments our ongoing photobioreactor design work to achieve maximum photon use efficiency at minimum power input for algae growth systems. Utilizing stoichiometrically designed growth medium and semi-continuous growth conditions, we demonstrate growth to cell concentrations greater than 20 grams dry weight per liter and producing more than 1.5 grams dry weight per liter per day. Cell density drops off rapidly at higher dilution rates, but biomass produced stays relatively constant. Under the high light conditions used in this work, there is a very significant increase in aggregate size as the periodic steady-state density drops, and this is reflected in an order-of-magnitude variation in optical density (OD_{550}) to dry weight ratio which is problematic for bioreactor monitoring. Strategies and progress on genetically engineering hydrocarbon synthesis pathways into *Chlamydomonas reinhardtii* will be noted but details will be presented in a poster session. Effects of nitrogen availability on productivity have also been examined. Oil content and composition data will be available shortly.

Background:

Algae are getting considerable attention for their ability to capture CO_2 emissions and turn them into biofuels. Instead of the typical approach to producing fatty acids for biodiesel production, *Botryococcus braunii* (**Figure 1**) grows in colonies that contain large quantities of isoprene hydrocarbons in their matrix. This study was undertaken to understand the control of this metabolic pathway under high-intensity growth conditions. Since the culture did not tolerate either high nutrients or high light very well, the cultures had to be 'adapted' using fed-batch techniques leading to subsequent semi-continuous production.

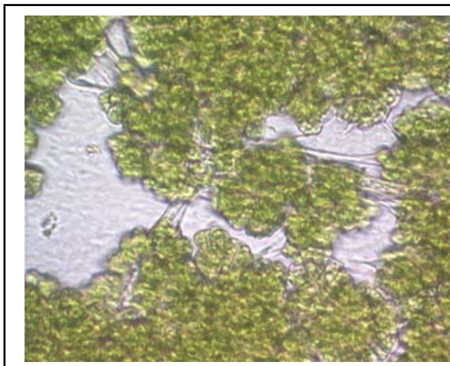

Figure 1: Photomicrograph of colony growth habit of *Botryococcus braunii* and surrounding isoprene matrix.

Results

Figure 2 shows a semi-continuous growth evaluation of *Botryococcus braunii* at various effective dilution rates where a proportion of the culture was drawn off and replaced every other day. Media was designed based on historic defined media for algae and plant cell culture to avoid accumulation of unused nutrients.

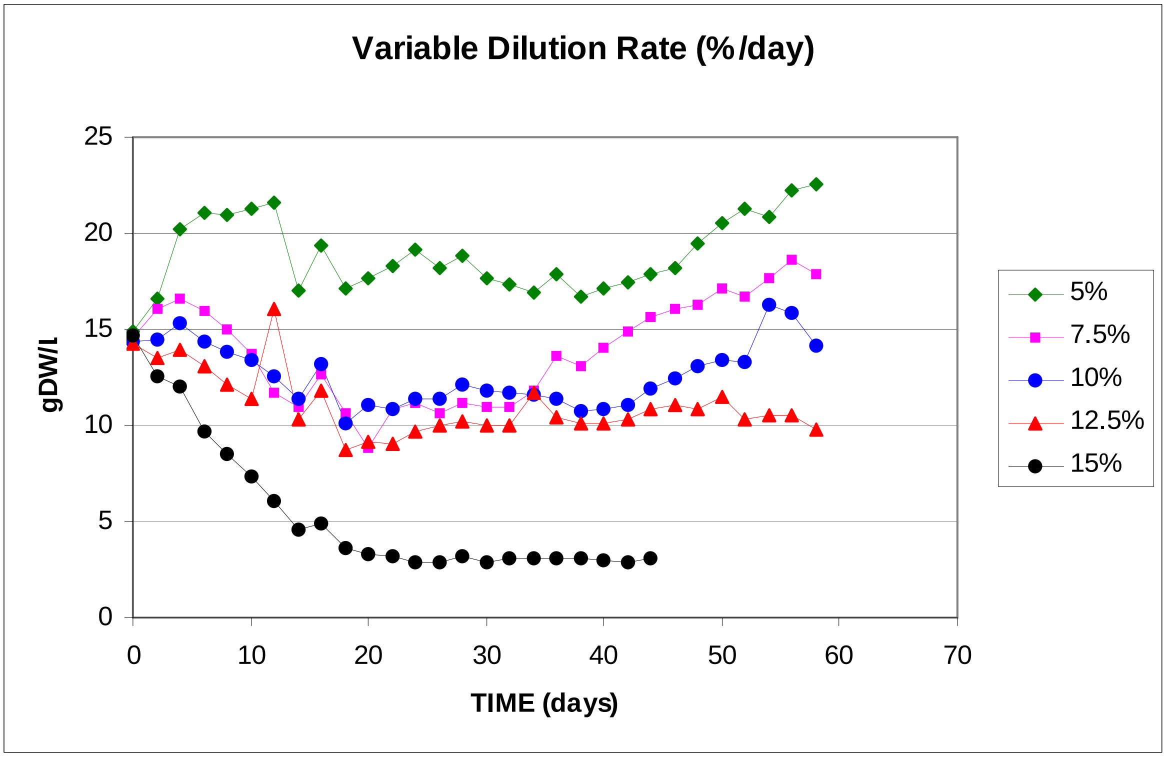

Figure 2: Growth of *B. braunii* in semi-continuous culture. Numerous modifications to media and nutrient balancing were implemented while gaining experience with long-term high-density culture.

Initial approximate methods of balancing nitrogen input based on measurements of dry weight and optical density were refined to reflect the large differences in optical density to dry weight ratio. At high dilution rates (near washout) the algae formed very large aggregates approach a millimeter in size. The large variation in culture dependent aggregate size results in a 3-fold variation in OD/DW ratio which is not observed in typical micro-algal culture.

Despite the decline in periodic steady-state cell density at different effective dilution rates, the volumetric productivity of the cultures was surprisingly constant reflecting a likely limitation by photon flux. Samples are currently being subjected to biochemical analysis including oil content and parameters to reflect biologically active biomass since a significant portion of the dry weight can be hydrocarbon & isoprene matrix.