



# **Establishment of a Bioenergy Focused Microalgae Strain Collection Using Rapid, High- Throughput Methodologies**

**Cooperative Research and Development  
Final Report**

**CRADA Number: CRD-07-248**

NREL Technical Contact: Philip T. Pienkos

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## **Cooperative Research and Development Final Report**

In accordance with Requirements set forth in Article XI.A(3) of the CRADA document, this document is the final CRADA report, including a list of Subject Inventions, to be forwarded to the Office of Science and Technical Information as part of the commitment to the public to demonstrate results of federally funded research.

**CRADA Number:** CRD-07-248

**CRADA Title:** Establishment of a Bioenergy-Focused Microalgae Strain Collection Using Rapid, High-Throughput Methodologies

**Parties to the Agreement:** Colorado Center for Biorefining & Biofuels (C2B2)

### **Joint Work Statement Funding Table showing DOE Commitment:**

<b>Estimated Costs</b>	<b>NREL Shared Resources</b>
Year 1	\$ 00.00
Year 2	\$ 00.00
Year 3	\$ 00.00
TOTALS	\$ 00.00

### **Abstract of CRADA Work:**

This project is part of the overall effort by and among NREL, Colorado State University, University of Colorado, and Colorado School of Mines known as the Colorado Center for Biorefining and Biofuels. This is part of a larger statewide effort provided for in House Bill 06-1322, establishing a Colorado Collaboratory that envisions these four institutions working together as part of the state's energy plan.

This individual project with Colorado School of Mines is the first of many envisioned in this overall effort. The project focuses on development of high throughput procedures aimed at rapidly isolating and purifying novel microalgal strains (specifically green alga and diatoms) from water samples obtained from unique aquatic environments.

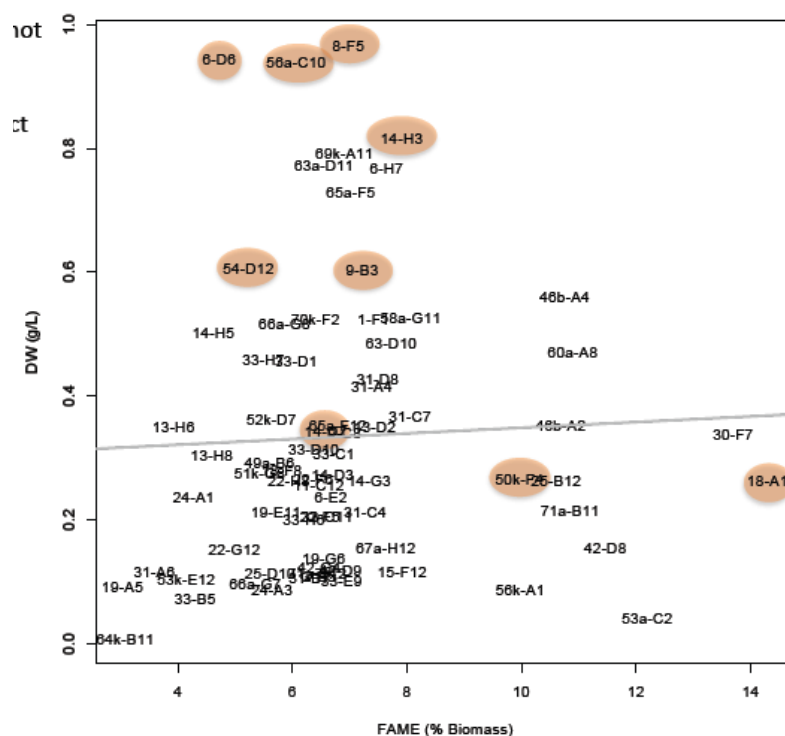
### **Summary of Research Results:**

A promising renewable energy scenario involves using photosynthetic microalgae for the production of biomass that can be converted into fungible, energy-dense fuels. Microalgae transform the energy in sunlight into a variety of storage products, including triacylglycerols, which can be readily transformed into diesel fuel surrogates. To develop an economically viable algal biofuels industry, it is critical to maximize the accumulation of targeted bioenergy carriers in selected feedstock strains.

In an effort to identify promising production isolates we developed, evaluated, and optimized contemporary high-throughput cell-sorting techniques to establish a collection of microalgae isolated from highly diverse ecosystems near geographic areas that are potential sites for large-scale algal cultivation in the Southwest United States. These efforts resulted in the isolation of 360 distinct

microalgal strains. We have conducted initial screening studies of the culture collection to identify important biofuel phenotypes including neutral lipid accumulation and rapid growth rates. As part of this undertaking we determined suitable cultivation media and evaluated cryopreservation techniques critical for the long-term storage of the microorganisms in this collection. This rich source of biodiversity represents a valuable resource that can be leveraged for the selection of promising bioenergy feedstock strains, as well as for providing fundamental advances in our understanding of photoautotrophic diversity, biology, and metabolism.

In subsequent work, we developed a small scale photobioreactor system to allow us to grow multiple strains in the light under controlled conditions (including air and CO<sub>2</sub> inputs) to evaluate these strains for growth and lipid productivity. One hundred promising strains were chosen for testing and 74 provided enough biomass after 15 days of cultivation for analysis of dry weight and lipid content (by fatty acid methyl ester (FAME) concentration. Cells at this point in the growth phase were believed to be nutrient replete and therefore expected to have high lipid content. The figure below summarizes the relationship between growth rate (gdcw/L at 15 days) and lipid content.



This work was discontinued due to lack of follow on funding. We have attempted to market the culture collection in various partnership efforts and have included it as an aspect for a proposal to the U.S. Department of Energy, with no success. We have attempted to revive strains from cryopreservation from time to time with variable results. It will require further funding to determine the viability of all of the strains, and so at this point, we are uncertain of the future potential for this work.

**Subject Inventions Listing:**

NREL Record of Invention No. 11-109

**Report Date:**

9/26/13

**Responsible Technical Contact at Alliance/NREL:**

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