



# **Novel Biological Conversion of Hydrogen and Carbon Dioxide Directly into Biodiesel**

**Cooperative Research and Development Final Report**

**CRADA Number: CRD-10-408**

NREL Technical Contact: Pin-Ching Maness

**NREL is a national laboratory of the U.S. Department of Energy  
Office of Energy Efficiency & Renewable Energy  
Operated by the Alliance for Sustainable Energy, LLC**

This report is available at no cost from the National Renewable Energy Laboratory (NREL) at [www.nrel.gov/publications](http://www.nrel.gov/publications).

**CRADA Report**  
NREL/TP-2700-62148  
June 2014

Contract No. DE-AC36-08GO28308

## NOTICE

This report was prepared as an account of work sponsored by an agency of the United States government. Neither the United States government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States government or any agency thereof.

This report is available at no cost from the National Renewable Energy Laboratory (NREL) at [www.nrel.gov/publications](http://www.nrel.gov/publications).

Available electronically at <http://www.osti.gov/scitech>

Available for a processing fee to U.S. Department of Energy and its contractors, in paper, from:

U.S. Department of Energy  
Office of Scientific and Technical Information  
P.O. Box 62  
Oak Ridge, TN 37831-0062  
phone: 865.576.8401  
fax: 865.576.5728  
email: <mailto:reports@adonis.osti.gov>

Available for sale to the public, in paper, from:

U.S. Department of Commerce  
National Technical Information Service  
5285 Port Royal Road  
Springfield, VA 22161  
phone: 800.553.6847  
fax: 703.605.6900  
email: [orders@ntis.fedworld.gov](mailto:orders@ntis.fedworld.gov)  
online ordering: <http://www.ntis.gov/help/ordermethods.aspx>

*Cover Photos: (left to right) photo by Pat Corkery, NREL 16416, photo from SunEdison, NREL 17423, photo by Pat Corkery, NREL 16560, photo by Dennis Schroeder, NREL 17613, photo by Dean Armstrong, NREL 17436, photo by Pat Corkery, NREL 17721.*



Printed on paper containing at least 50% wastepaper, including 10% post consumer waste.

## 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-10-408

**CRADA Title:** Novel Biological Conversion of Hydrogen and Carbon Dioxide Directly into Biodiesel

**Parties to the Agreement:** OPX Biotechnologies

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

<b>Estimated Costs</b>	<b>NREL Shared Resources</b>
Year 1	\$ 636,485.00
Year 2	\$ 718,778.00
Year 3	\$ 534,007.00
TOTALS	\$ 1,889,270.00

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

OPX Biotechnologies, Inc. (OPX), the National Renewable Energy Laboratory (NREL), and Johnson Matthey will develop and optimize a novel, engineered microorganism that directly produces biodiesel from renewable hydrogen (H<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>). The proposed process will fix CO<sub>2</sub> utilizing H<sub>2</sub> to generate an infrastructure-compatible, energy-dense fuel at costs of less than \$2.50 per gallon, with water being produced as the primary byproduct. NREL will perform metabolic engineering on the bacterium *Cupriavidus necator* (formerly *Ralstonia eutropha*) and a techno-economic analysis to guide future scale-up work. H<sub>2</sub> and CO<sub>2</sub> uptakes rates will be genetically increased, production of free fatty acids will be enhanced and their degradation pathway blocked in order to meet the ultimate program goals.

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

To improve H<sub>2</sub> uptake, NREL had over-expressed the transcriptional regulator *hoxA* in *C. necator*. The outcomes lead to a two-fold improvement in the soluble hydrogenase activity and five-fold improvement in the membrane-bound hydrogenase activity. Moreover, we improved the CO<sub>2</sub>-fixation activity by 15-fold via using a stronger promoter and deleting a terminator for the Rubisco enzyme when the changes were expressed from a plasmid. The changes have also been integrated into the chromosome leading to six-fold improvement. Both hydrogenase and Rubisco improvements have been integrated into a single *C. necator* host. Thus far all the improvements were verified based on *in vitro* assays. OPX is testing the effects of improved upstream substrate uptake on downstream enhanced production of fatty-acid fuels in scale-up reactors.

**Subject Inventions Listing:** None

**Report Date:** March 18, 2014

**Responsible Technical Contact at Alliance/NREL:** Pin-Ching Maness

**This document contains NO confidential, protectable, or proprietary information.**