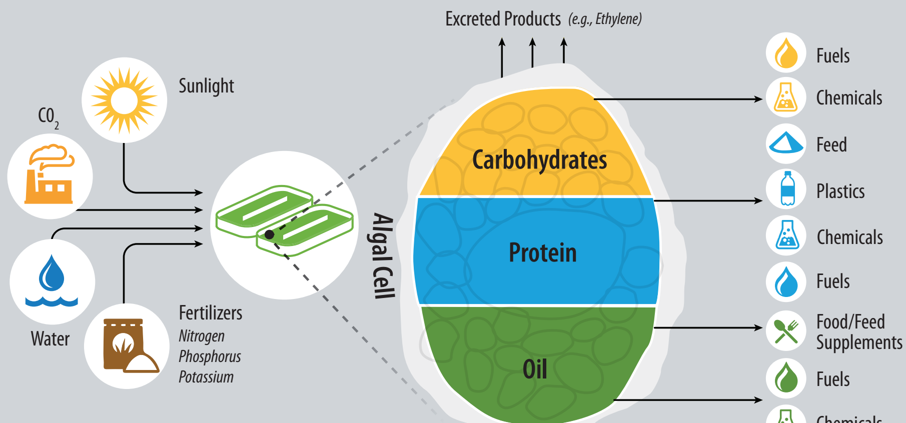
NREL Algal Biofuels Projects and Partnerships

NREL is engaged in several algal biofuels research and development projects focused on improving the economics of the algal biofuels production process

NREL is involved in several algal biofuels projects with national and international partners funded through the U.S. Department of Energy's (DOE's) Bioenergy Technologies Office (BETO) and NREL's Laboratory Directed Research and Development (LDRD) program. These projects are addressing the challenges that accompany the algal biofuels production process.

Microalgae—photosynthetic microorganisms capable of converting atmospheric CO_2 to biomass containing oil and other valuable components—offer great promise to contribute a significant portion to our renewable fuels and meet the mandate to dramatically reduce our dependence on the world's dwindling fossil energy resources.



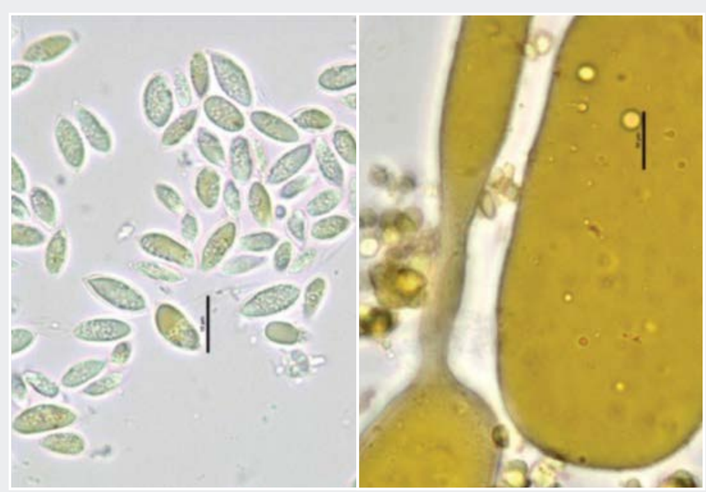
Multiproduct Algal Biorefinery Production Process.

Despite its huge potential, the technology of using microalgae as biomass feedstocks for advanced biofuels faces major challenges from both technical and economic barriers. These include developing suitable algal strains and cultivation parameters, developing processes to harvest the biomass and convert it to fuels and products, and rendering the entire process economical.

NREL's current algal biofuels program builds on the expertise and knowledge acquired from the research into microalgal biofuels done by the Aquatic Species Program, which was established in 1978 under funding from DOE.

Novel Microalgal Production and Downstream Processing Technologies for Alternative Biofuels Applications

NREL is investigating alternative conversion pathways to obtain the most value from all components in algal biomass. A novel fractionation process allows for recovery of separate lipid, carbohydrate, and protein streams. Specific tasks include investigating catalytic upgrading of the lipid stream, biochemical conversion of the carbohydrates to biofuels and bioproducts, and exploration of novel coproducts from proteins. NREL is also testing the physical and chemical properties of the novel fuels and fuel intermediates with respect to compatibility with existing fuel infrastructure. In this way, we will develop a conversion process that optimizes yields and reduces production costs.



Physical appearance of algae before and after biochemical conversion, showing large oil droplets formed during the reaction. *Photo by Nicholas Sweeney, NREL*

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

Algae Testbed Public Private Partnership (ATP^3)

To accelerate the commercialization of technologies based on algal biofuels, NREL is part of a team led by Arizona State University to fill data gaps for algal cultivation and processing and to provide access for technology developers to its five testbed sites. NREL leads the Integration and Analysis directorate, which includes oversight of experimental design, data management, techno-economic analysis, and analytical method harmonization.

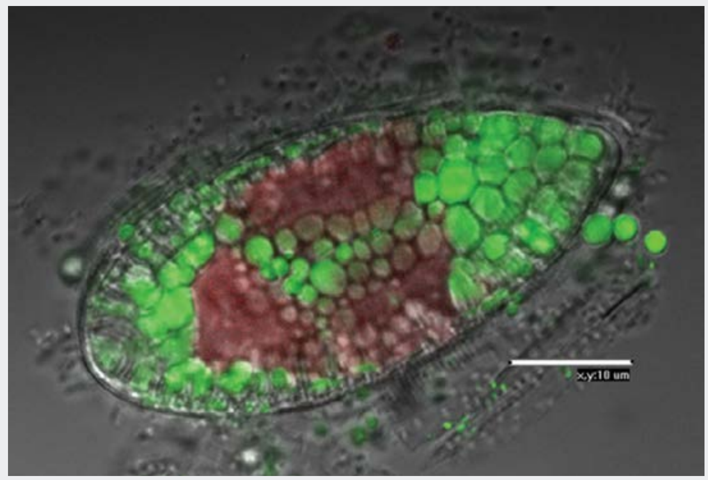
Direct Conversion of CO_2 to Ethylene Using Cyanobacteria

NREL is developing cyanobacterial strains that photosynthetically convert CO_2 to ethylene, a versatile chemical and fuel intermediate. This BETO-supported research has generated new insights into the regulation of photosynthesis and carbon metabolism in the model cyanobacterium *Synechocystis* 6803. The progress has shown that the cyanobacterium can be genetically engineered to continuously convert CO_2 to ethylene, and that the process can be supported by seawater. As a gas, ethylene leaves the culture by itself, which stimulates photosynthesis such that the culture fixes more CO_2 than the wild type strain. This work has received recognition including an R&D 100 Award and Editor's Choice Award.

Extracted algae oil. *Photo by Dennis Schroeder, NREL 18230*

Development of Robust and High-Throughput Characterization Technologies

NREL researchers are developing technologies for the analysis of biofuel process-relevant components in algal biomass. These methods will allow researchers to assess the efficiency of the overall algal biofuels production process through the use of techno-economic analyses. This will keep NREL at the forefront of compositional analysis of novel feedstocks and allow for accurate quantification of important constituents in the process. In addition, scientists are also developing high-throughput spectroscopic-prediction methods to rapidly measure the composition of algal biomass from model organisms and culture collection strains in minutes rather than days.



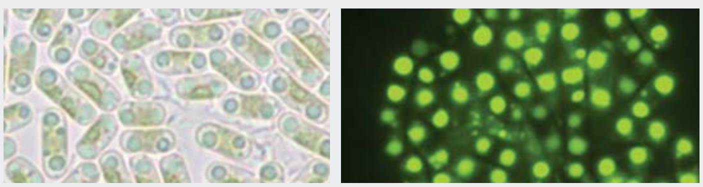
High lipid strain from NREL culture collection. *Photo by Lee Elliott, Colorado School of Mines*

Techno-Economic Assessment of Algal Biofuels Processes

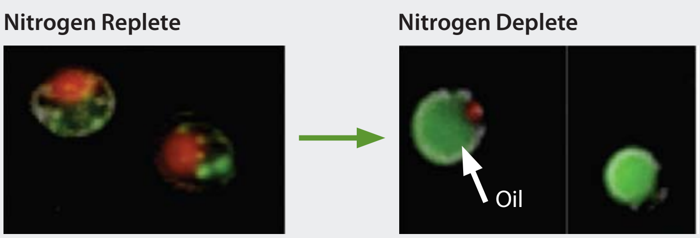
NREL supports DOE's efforts to establish cost targets and track progress toward meeting these targets through techno-economic modeling and analysis of biofuel production pathways. These models are leveraged to quantify R&D efforts from an economic standpoint and to provide peer-reviewed documentation of projected algal biofuel production costs. Over recent years, the NREL algae techno-economic assessment (TEA) team has published several high-impact design reports documenting process and cost projections for algal biomass production ("farming") as well as conversion to fuels and products via NREL's Combined Algal Processing pathway. Additionally, the team supports TEA evaluation of the cultivation data made available by ATP^3, and works in close partnership with collaborators at other national laboratories and universities to harmonize algae models across economic, sustainability, and resource analyses.

The Algal Biotechnology Partnership Identifies Robust, Halotolerant Algal Biocatalysts with Exemplary Deployment Characteristics.

Realizing the promise of sustainable and affordable algal biofuels will require genetic optimization and deployment of high-productivity algal strains with robust deployment characteristics. NREL, in collaboration with Los Alamos National Laboratory, Colorado School of Mines, and ATP^3, has identified and characterized a series of halotolerant algae strains with promising characteristics for summer and winter cultivation, including robust growth rates and carbon flux to fuel precursors, in saline media. We have initiated efforts to develop facile genetic toolkits for top-candidate strains, with the goal of public strain dissemination and data deposition to encourage widespread adoption and development of strains.



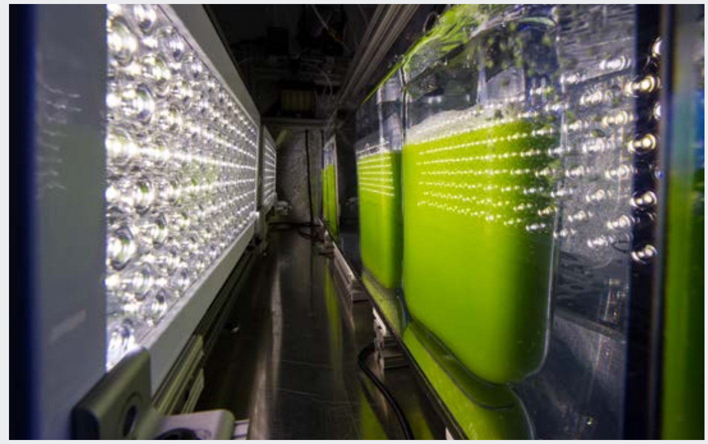
Examples of high lipid isolates from NREL culture collection. *Photo by Lee Elliott, Colorado School of Mines*



Increase of lipid content in *Chlorella vulgaris* with nitrogen starvation. *Photo by Michael Guarnieri, NREL*

Molecular Foundations of Algal Biofuel Production: Proteomics and Transcriptomics of Algal Oil Production

NREL is combining a proteomics and transcriptomics approach to fully characterize the molecular foundation of algal biofuels production with external environmental conditions in oil accumulating microalgae. The combined '-omics' approach will lead to a better understanding of the changes that occur during transition to high-lipid growth conditions. These technologies provide NREL with information that can be used to identify genes and pathways involved in biofuel production and to guide strain improvement strategies.



Photobioreactor used for carbon uptake and composition modeling. *Photo by Dennis Schroeder, NREL 25505*

Investigation of Cell Signaling Mechanisms Governing Lipid Accumulation

Scientists in NREL's Applied Biology section are investigating cell cycle and cell signaling mechanisms governing nutrient deprivation-induced lipid accumulation. This work is funded through NREL's LDRD program, with the goal of identifying and engineering gene targets for rapid induction of microalgal lipid accumulation in the absence of exogenous triggers. This approach has the potential to dramatically decrease costs associated with current algal cultivation strategies.

Valorization of Algal Biomass by Isolating, Purifying Substrates for Synthesis of Coproducts to Reduce Fuel Selling Price

NREL researchers have focused on the identification and isolation of coproducts that are compatible with an integrated algae biorefinery process to reduce the modeled fuel selling price toward $3/gallon gasoline equivalent. More than 30 natively produced products were identified in algae and selected based on the compatibility with a lipid extraction pathway at scale. NREL has demonstrated the successful isolation of isoprenoid alcohols as coproducts from an algae fuels process and synthesized novel surfactants, which can add to the overall revenue of the pathway and reduce the cost of fuels.

Controlled Cultivation for Carbon Uptake and Composition Modeling

NREL has a state-of-the-art photobioreactor system that allows for simulated cultivation of algae under mimicked outdoor conditions while monitoring CO_2 assimilation, nutrient uptake rates, and full biochemical composition of the resulting biomass.

Publications

Dong, T., S. Van Wychen, N. Nagle, P.T. Pienkos, and L.M.L. Laurens. "Impact of biochemical composition on susceptibility of algal biomass to acid-catalyzed pretreatment for sugar and lipid recovery." *Algal Research* 18 (2016): 69–77. *doi:10.1016/j.algal.2016.06.004*

Dong, T., E.P. Knoshaug, R. Davis, L.M.L. Laurens, S. Van Wychen, P.T. Pienkos, and N. Nagle. "Combined algal processing: A novel integrated biorefinery process to produce algal biofuels and bioproducts." *Algal Research* (2016). *doi:10.1016/j.algal.2015.12.021*

Markham, J.N., L. Tao, R. Davis, N. Voulis, L.T. Angenent, J. Ungerer, and J. Yu. "Techno-Economic Analysis of a Conceptual Biofuel Production Process from Bioethylene Produced by Photosynthetic Recombinant Cyanobacteria." *Green Chemistry* (2016). *doi:10.1039/C6GC01083K*

Davis, R., J. Markham, C. Kinchin, N. Grundl, E.C.D. Tan, and D. Humbird. *Process Design and Economics for the Production of Algal Biomass: Algal Biomass Production in Open Pond Systems and Processing Through Dewatering for Downstream Conversion.* Golden, CO: National Renewable Energy Laboratory, NREL/TP-5100-64772, 2016. *www.nrel.gov/docs/fy16osti/64772.pdf*

Laurens, L.M.L., N. Nagle, R. Davis, N. Sweeney, S. Van Wychen, A. Lowell, and P.T. Pienkos. "Acid-Catalyzed Algal Biomass Pretreatment for Integrated Lipid and Carbohydrate-Based Biofuels Production." *Green Chemistry* 17, no.2 (2015): 1145–1158. *doi:10.1039/C4GC01612B*

Xiong, W., J.A. Morgan, J. Ungerer, B. Wang, P-C. Maness, and J. Yu. "The plasticity of cyanobacterial metabolism supports direct CO2 conversion to ethylene." *Nature Plants* 1 (2015). *doi:10.1038/nplants.2015.53*

Davis, R., C. Kinchin, J. Markham, E.C.D. Tan, L.M.L. Laurens, D. Sexton, D. Knorr, P. Schoen, and J. Lukas. *Process Design and Economics for the Conversion of Algal Biomass to Biofuels: Algal Biomass Fractionation to Lipid-and Carbohydrate-Derived Fuel Products*. Golden, CO: National Renewable Energy Laboratory, NREL/TP-5100-62368, 2014. *www.nrel.gov/docs/fy14osti/62368.pdf*

Eckert, C. W. Xu, W. Xiong, S. Lynch, J. Ungerer, L. Tao, R. Gill, P-C. Maness, and J. Yu. "Ethylene-forming enzyme and bioethylene production." *Biotechnology for Biofuels* 7, no. 33 (2014). *doi:10.1186/1754-6834-7-33*

Gerken, H.G., B. Donohoe, and E.P. Knoshaug. "Enzymatic Cell Wall Degradation of Chlorella vulgaris and Other Microalgae for Biofuels Production." *Planta* 237, no. 1 (2013): 239–253. *doi:10.1007/s00425-012-1765-0*

Guarnieri, M.T., A. Nag, S. Yang, and P.T. Pienkos. "Proteomic analysis of Chlorella vulgaris: Potential targets for enhanced lipid accumulation." *J Proteomics* 93 (2013): 245–253. *doi:10.1016/j.jprot.2013.05.025*

Bazilian, M., R. Davis, P.T. Pienkos, and D. Arent. "The Energy-Water-Food Nexus Through the Lens of Algal Systems." *Industrial Biotechnology* 9, no. 4 (2013): 158–162. *doi:10.1089/ind.2013.1579*

Ungerer, J., L. Tao, M. Davis, M. Ghirardi, P-C. Maness, and J. Yu. "Sustained photosynthetic conversion of CO2 to ethylene in recombinant cyanobacterium Synechocystis 6803." *Energy and Environmental Science* 5 (2012): 8998–9006. *doi:10.1039/C2EE22555G*

Davis, R., D. Fishman, E. D. Frank, M.S. Wigmosta, A. Aden, A.M. Coleman, P.T. Pienkos, R.J. Skaggs, E.R. Venteris, and M.Q. Wang. *Renewable Diesel from Algal Lipids: An Integrated Baseline for Cost, Emissions, and Resource Potential from a Harmonized Model*. Argonne, IL: Argonne National Laboratory, ANL/ESD/12-4; Golden, CO: National Renewable Energy Laboratory, NREL/TP-5100-55431; Richland, WA: Pacific Northwest National Laboratory, PNNL-21437, 2012. *www.nrel.gov/docs/fy12osti/55431.pdf*

Elliott, L.G., C. Feehan, L.M.L. Laurens, P.T. Pienkos, A. Darzins,and M.C. Posewitz. "Establishment of a Bioenergy-Focused Microalgal Culture Collection." *Algal Research* 1, no. 2 (2012): 102–113. *doi:10.1016/j.algal.2012.05.002*

Laurens, L.M.L., T.A. Dempster, H.D.T. Jones, E.J. Wolfrum, S. Van Wychen, J.S.P. McAllister, M. Rencenberger, K.J. Parchert, and L.M. Gloe. "Algal Biomass Constituent Analysis: Method Uncertainties and Investigation of the Underlying Measuring Chemistries." *Analytical Chemistry* 84, no. 4 (2012): 1879–1887. *doi:10.1021/ac202668c*

Guarnieri, M., L.M.L. Laurens, E.P. Knoshaug, Y-C. Chou, B.S. Donohoe, and P.T. Pienkos. "Chapter 8: Complex System Engineering: A Case Study for an Unsequenced Microalga." In *Engineering Complex Phenotypes in Industrial Strains*. Edited by R. Patnaik. Hoboken, NJ: John Wiley & Sons, Inc., 2012. *doi:10.1002/9781118433034.ch8*

Guarnieri, M.T., A. Nag, S.L. Smolinski, A. Darzins, M. Seibert, and P.T. Pienkos. "Examination of Triacylglycerol Biosynthetic Pathways via De Novo Transcriptomic and Proteomic Analyses in an Unsequenced Microalga." *PLoS ONE* 6, no. 10 (2011). *doi:10.1371/journal.pone.0025851*

Davis, R., A. Aden, and P.T. Pienkos. "Techno-Economic Analysis of Autotrophic Microalgae for Fuel Production." *Applied Energy* 88, no. 10 (2011): 3524–3531. *doi:10.1016/j.apenergy.2011.04.018*

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