

## NREL'S CAPABILITIES IN ORGANISM DEVELOPMENT AND FERMENTATION SCIENCES RESEARCH AND DEVELOPMENT (R&D)



NREL is developing robust microbial strains and scalable fermentation technologies for converting biomass sugars to fuels and biochemicals, and one-carbon (C1) gaseous substrates (e.g., CO<sub>2</sub>, CO, CH<sub>4</sub>) to high-value products.

### Core Capabilities

- Fermentation optimization of aerobic, micro-aerophilic, and anaerobic cultivation processes of gas and traditional liquid fermentation R&D
- Gas mass transfer R&D and CFD modeling for improved reactor design and gas mixing
- Metabolic engineering in CRISPR editing tools to improve titer, rates, and yields
- Probing and optimizing carbon, redox, and energy flux using transcriptomics and fluxomic techniques
- Consolidated bioprocessing (CBP) using cellulose and hemicellulose directly to minimize pretreatment



Top Photo: One of NREL's bench-scale fermentation laboratories with 36 500-mL Sartorius stirred tank bioreactors. Photo by Dennis Schroeder, NREL 35821

Bottom Photo: NREL researchers discover how *Methylobacterium alcaliphilum* 20ZR, a methane-eating organism known as a methanotroph, operates. It was one of the first research efforts of its kind. Photo by Dennis Schroeder, NREL 49737

### Standard Bench-Scale Fermentation

NREL's bench-scale fermentation laboratories house a variety of fermentors sized from microbioreactors to 10-L benchtop units. All units have dissolved oxygen monitoring and control, pH and temperature control, and data acquisition. Most systems have off-gas analysis.

- Thirty-six 500-mL Sartorius BIOSTAT® Q-plus bioreactors with mass spectrometer for off-gas analysis
- Three Eppendorf 300 series fermentors with 5-L or 10-L vessels
- Six Applikon® In-Control series fermentors with 3L vessels with remote access/control and off-gas measurement using gas chromatography
- Seven New Brunswick 3000 series fermentors with 1-L to 10-L vessels with mass spectrometer for off-gas analysis
- BioLector Pro microbioreactor

### C1/Gas Fermentation

NREL has developed state-of-the-art gas fermentation capabilities from bench to pilot-scale demonstrating production of renewable fuels and chemicals from tailored ratios of hydrogen and C1 gaseous substrates (e.g., CH<sub>4</sub>, CO, CO<sub>2</sub>, and H<sub>2</sub>). All fermentor systems are fully instrumented with safety interlocks and off-gas analysis.

- Coming in 2020, a mobile lab-scale 10-L to 30-L pressurized (18 bar) bioreactor with integrated 10-kW to 15-kW PEM electrolyzer
- Hooded Applikon In-Control Series 200-mL for CO and H<sub>2</sub> fermentations with off-gas GC analysis
- Two hooded Electrolab 2-L fermentors for CO, CO<sub>2</sub>, and H<sub>2</sub> fermentations with off-gas GC analysis
- CH<sub>4</sub> fermentation capability in 50-mL small-format bubble reactors and three 500-mL Sartorius bioreactors with mass spectrometer for off-gas analysis

## Pilot-Scale Gas Fermentation

NREL operates a pilot-scale 700-L gas bioreactor with capabilities to convert renewable hydrogen and CO<sub>2</sub> to fuels and chemicals at pressures up to 18 bar. This state-of-the-art system has nutrient dosing pumps, heat rejection, and cell retention with in-house production of renewable hydrogen via 250-kW PEM electrolyzer. The system is fully instrumented with Coriolis mass flow meters, pressure/temperature/level sensing, GC analysis, pH monitoring paired with data acquisition, and control and data visualization capabilities.



The Energy Systems Integration Facility's 700-L pressurized gas bioreactor designed for hydrogen and carbon dioxide gas feeds.

Photo by Dennis Schroeder, NREL 58759

## Find Out More

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## Highlighted Publications

Brunecky, R., Subramanian, V., Yarbrough, J.M., Donohoe, B.S., Vinzant, T.B., Vanderwall, T.A., Knott, B.C., et al. "Synthetic Fungal Multifunctional Cellulases for Enhanced Biomass Conversion." *Green Chemistry*. December 18, 2019.

Gionet, A. "NREL 'Bug' Creates Renewable Natural Gas." *CBS 4 Denver*. March 3, 2019.

Henard, C.A., Akberdin, I., Kalyuzhnaya, M.G., and Guarnieri, M.T. "Muconic Acid Production from Methane Using Rationally-Engineered Methanotrophic Biocatalysts." *Green Chemistry* 21(24):6731–6737. 2019.

Lacey, S. "Are Ancient Bugs the Key to Storing Wind and Solar?" *Green Tech Media*. October 15, 2019.

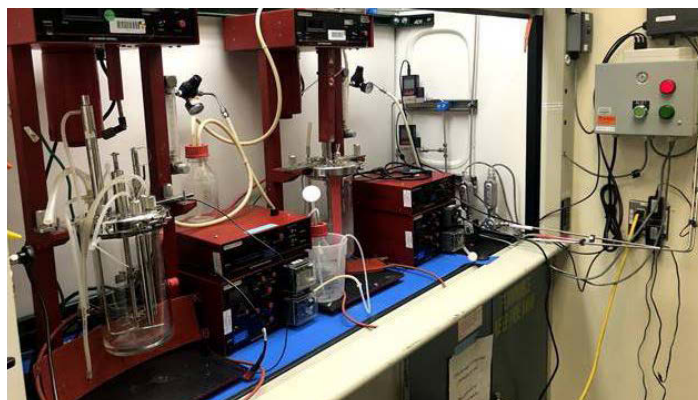
Marcano-Velazquez, J.G., Lo J., Nag, A., Maness, P.C., and Chou, K.J. "Developing Riboswitch-Mediated Gene Regulatory Controls in Thermophilic Bacteria." *ACS Synthetic Biology*. 8(4):633–640. April 19, 2019.

## KEY RESULTS

Recent efforts have demonstrated high product titers from biomass sugars, utilization of carbon dioxide to products and fuels, and CH<sub>4</sub>/CO<sub>2</sub> conversion to chemicals.

### Recent Accomplishments from Fermentation R&D

- Demonstrated 2,3-butanediol titer of 110 g/L from biomass sugars and scaled fermentation process to 100-L pilot fermentor.
- Commissioned the pilot-scale 700-L pressurized gas bioreactor to produce renewable CH<sub>4</sub> from CO<sub>2</sub> and renewable H<sub>2</sub>.
- Established CH<sub>4</sub>/CO<sub>2</sub> co-utilization capacity in microbial biocatalysts, which enable conversion of diverse gaseous waste streams including anaerobic digestion biogas and landfill gases.
- Developed CRISPR tools to edit microbial genomes and generated strains capable of co-fermenting cellulose and hemicellulose via CBP, fix CO<sub>2</sub> with high carbon-conversion efficiency and developed the first CRISPR editing system in methanotrophic bacteria.



Hooded Electrolab 2-L fermentors for CO, CO<sub>2</sub>, and H<sub>2</sub> fermentations.  
Photo by Lauren Magnusson, NREL



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