

# ECOSynBio—Energy and Carbon Optimized Synthesis for the Bioeconomy

## PROJECT DESCRIPTIONS

---

### LanzaTech, Inc. – Skokie, IL

*Carbon-Negative Chemical Production Platform - \$4,160,262.57*

LanzaTech, Inc., (LT) will create transformative technology to enable direct conversion of carbon dioxide (CO<sub>2</sub>) to ethanol, a building block for low carbon intensity fuels and chemicals, at 100% carbon conversion efficiency to products. The LT team will develop a gas fermentation process that leverages affordable, renewable hydrogen (H<sub>2</sub>) to capture and fix CO<sub>2</sub> directly into valuable fuels and chemicals. At commercial scale, the inputs to the proposed “carbon refinery” process are carbon-free renewable energy, water, and CO<sub>2</sub>. The carbon-optimized conversion technology developed under this project can be integrated with multiple CO<sub>2</sub> sources, such as corn grain ethanol refining (near-term) and direct air capture (mid-long term).

### National Renewable Energy Laboratory— Golden, CO

*Formate as an Energy Source to allow Sugar Fermentation with no net CO<sub>2</sub> Generation: Integration of Electrochemistry with Fermentation - \$2,838,575.64*

The National Renewable Energy Laboratory (NREL) working with industrial partners (Genomatica and DeNora) will develop a biorefining concept that uses electrochemically generated formate as a universal energy carrier to facilitate a carbon optimized sugar assimilation fermentation to synthesize fatty acid methyl esters (FAME) without release of CO<sub>2</sub>. This process allows all the carbon within the sugar to be converted to FAME, which will allow these lipids—used to produce sustainable aviation jet fuel (SAF)—to be generated at lower cost and with fewer feedstock, resource, and land requirements.

### University of Wisconsin-Madison – Madison, WI

*Acetate as a Platform for Carbon-Negative Production of Renewable Fuels and Chemicals- \$3,421,197.10*

The University of Wisconsin-Madison aims to eliminate CO<sub>2</sub> release in the production of chemicals by integrating the unique and efficient capabilities of two microorganisms. The first produces acetate from CO<sub>2</sub> and H<sub>2</sub> while the second upgrades acetate to higher-value chemical products. The CO<sub>2</sub> released in the upgrading process is recycled internally to produce more acetate. Thus, this carbon utilization process is designed to operate with zero carbon dioxide release. The process avoids photosynthesis and bypasses the barriers created by using biomass as primary chemical feedstock. Further, this platform can be scaled to match existing sources of CO<sub>2</sub> emissions and can be located anywhere renewable H<sub>2</sub> can be provided.

### Stanford University – Stanford, CA

*Disruptive Technology for Carbon Negative Commodity Biochemicals— \$2,582,672.23*

Stanford University seeks to replace carbon- and energy-inefficient fermentation unit operations for commodity chemical production with innovative cell-free processes. Instead of releasing CO<sub>2</sub> into the

atmosphere, this new approach will enable utilization of atmospheric CO<sub>2</sub> as well as glucose obtained from cornstarch to produce renewable fuels and chemicals. Succinic acid will be the first product, but the technology can easily be adapted to produce a broad range of other biochemical products. The new cell-free technology introduces a biosynthesis platform that increases process yield, conversion rate, and energy efficiency to encourage investment in distributed production facilities located in rural communities.

### University of Delaware – Newark, DE

*Bioenergy production based on an engineered mixotrophic consortium for enhanced CO<sub>2</sub> fixation –\$2,752,577.08*

The University of Delaware aims to develop a platform technology based on synthetic syntrophic consortia of *Clostridium* microbes to enable fast and efficient use of renewable carbohydrates to produce targeted metabolites as biofuels or chemicals. In this syntrophic microbial consortium, two or more microbes are co-cultured, allowing the different species to divide individual bioconversion steps and reduce their individual metabolic burden. The goal is to achieve complete utilization of glucose substrate carbon and electrons, while also using additional CO<sub>2</sub> and electrons from H<sub>2</sub> to generate improved yields of products such as isopropanol.

### University of California, Davis – Davis, CA

*A Microbial Consortium Enables Complete Feedstock Conversion - \$1,574,966.00*

The aviation industry requires energy-dense, carbon-based fuels, which are difficult to achieve biologically because of poor carbon conversion efficiency and low yields. To reach near 100% carbon conversion efficiency, the University of California, Davis, will engineer a novel microbial consortium approach. Single organism can be limited by CO<sub>2</sub> loss from core metabolic reactions; thus, coupling a heterotroph with an autotroph will enable CO<sub>2</sub> recycling and complete feedstock conversion. The proposed systems will use a heterotrophic production strain to convert sugar substrates into biofuels via a carbon conserving synthetic metabolism and will be co-cultured with a phototrophic strain engineered to be chemotrophic to enable CO<sub>2</sub> utilization and recycle CO<sub>2</sub> released during the sugar fermentation.

### INvizyne Technologies, Inc. – Monrovia, CA

*Carbon Negative Chemicals with Synthetic Biochemistry- \$1,657,763.25*

Existing technologies can efficiently capture CO<sub>2</sub> by converting it into simple chemicals such as formate (via electroreduction) and ethanol (via fermentation)—molecules that could become carbon-neutral feedstocks to replace petroleum-derived fuels and chemicals. To realize the potential of CO<sub>2</sub> capture and conversion, INvizyne Technologies, Inc., will demonstrate the usefulness of a cell-free biocatalytic platform for carbon-neutral production of several platform compounds that could be cost-competitive with petrochemicals. In the short term, INvizyne's approach can diversify existing bio-ethanol markets while the carbon-neutral CO<sub>2</sub> utilization technologies ramp up. If successful, this project will provide a springboard for additional cell-free developments, creating a robust carbon-neutral utilization capacity for the chemical industry.

### University of California, Irvine – Irvine, CA

*Carbon-Efficient Conversion of Carboxylic Acids to Fuels and Chemicals- \$1,841,394.00*

University of California, Irvine – Irvine, CA

Carbon-Efficient Conversion of Carboxylic Acids to Fuels and Chemicals- \$1,841,394.00

Compared with grain-derived sugars, carboxylic acids could be produced in large quantities from food and industrial wastes, serving as a more scalable and economical feedstock for biofuel and biochemical production. But natural biological pathways for carboxylic acid conversion suffer from a low carbon yield. The University of California, Irvine, proposes a cell-free enzymatic process to address this challenge. The system uses a unique, stronger-than-nature reducing equivalent carrier to overcome a thermodynamic hurdle in carboxylic acid utilization and product upgrading using a novel C1 elongation pathway. If successful, it will be

the first biological platform to convert carboxylic acids into a broad range of fuels and commodities with greater than 100% carbon efficiency.

### **The Wyss Institute at Harvard University – Boston, MA**

*CIRCE: Circularizing Industries by Raising Carbon Efficiency - \$2,985,025.23*

Some of the most economical and sustainable feedstocks for commodities such as fuels are gases (H<sub>2</sub>, O<sub>2</sub>, CO, CO<sub>2</sub>). Gaseous feedstocks are far less land-intensive and could potentially be more cost effective for biorefining than traditional biomass feedstocks. Further, gaseous feedstocks have fewer restrictions to delivery in large volumes and overall can have much smaller carbon footprints. Harvard University seeks to produce carbon-neutral fuels and related chemicals by integrating externally derived reducing power with precision fermentation. Harvard's technical approach will enhance, direct, and modify the capabilities of its microbial chassis with sophisticated genetic engineering and adapted fermentation technology. The goal is to shift the bioproduction paradigm and generate highly reduced carbon compounds from simple, inexpensive feedstocks.

### **University of Minnesota – Twin Cities, MN**

*Cell-free bioelectrocatalytic platform for carbon dioxide reduction - \$1,110,526.00*

Renewable electricity is now competitive with and in many instances less expensive than fossil fuel-derived electricity, but its storage remains challenging. Energy storage in chemical bonds through electricity-driven carbon reduction offers higher energy densities and greater safety and transportability than batteries. But the efficient use of electricity to reduce kinetically and thermodynamically stable CO<sub>2</sub> into a range of chemicals requires significant innovation. The University of Minnesota will design a cell-free biocatalytic system that will reduce CO<sub>2</sub> efficiently into formate, a C1 feedstock, with energy supplied from electricity. This project aims to develop a robust bioelectrocatalytic technology platform that will deliver a portable CO<sub>2</sub> capture technology and formate as a stand-alone chemical or for integration into longer chain chemical products.

### **Massachusetts Institute of Technology – Cambridge, MA**

*Zero-Carbon Biofuels: An Optimized Two-Stage System for High Productivity Conversion of CO<sub>2</sub> to Liquid Fuels - \$2,108,532.00*

The Massachusetts Institute of Technology (MIT) has engineered the oleaginous yeast *Yarrowia lipolytica* to produce biodiesel-like lipids and alkanes. MIT proposes to reduce or eliminate CO<sub>2</sub> generation during lipid production by (1) engineering *Y. lipolytica* with the enzymes necessary to generate **reducing equivalents** from hydrogen, formic acid, or methanol, and (2) installing a carbon conserving equivalent to glycolysis, called non-oxidative glycolysis. When combined, these modifications will enable **almost** stoichiometric conversion of glucose and acetate to lipids and alkanes. The system's commercial competitiveness is currently limited by the rate of CO<sub>2</sub> fixation in the first stage. MIT recently showed that synergistic substrate co-feeding drastically enhances CO<sub>2</sub> fixation rates and will explore additional co-substrate pairs.

### **Ohio State University – Columbus, OH**

*A Novel Integrated Fermentation Process with Engineered Microbial Consortia for Butanol Production from Lignocellulose Sugars without CO<sub>2</sub> Emission - \$1,611,940.63*

Ohio State University is designing, modeling, and constructing synthetic microbial consortia consisting of three bacterial species to maximize carbon conversion and butanol production with a 100% theoretical product yield from glucose and zero or negative CO<sub>2</sub> emissions, aided by the addition of electrochemically reduced formate. With a 50% higher product yield from glucose compared with current acetone-butanol-ethanol (ABE)

fermentation with corn, biobutanol can be produced at prices that compete with gasoline, bioethanol, and the existing ABE fermentation technologies, but by a more carbon-efficient method.

### **ZymoChem, Inc. – San Leandro, CA**

*Development of a Bio-electrochemical Hybrid Fermentation Technology for the Carbon Conserving Production of Industrial Chemicals - \$1,053,000.00*

Many consumer goods and high-performance materials are manufactured with polymers made from precursors that derive from petroleum. ZymoChem, Inc., has created fermentation processes that convert sugars into polymer precursors using microorganisms with novel enzyme-based pathways that avoid the loss of the sugar's carbon as CO<sub>2</sub>. During this project, ZymoChem aims to develop next-generation bioprocesses that combine (1) inexpensive metal catalysts for converting electricity and CO<sub>2</sub> into formate, and (2) electricity-compatible fermentation systems that enable microbes to co-utilize formate and sugars for the production of valuable chemicals.

### **University of Washington – Seattle, WA**

*Self-Assembling Cell-Free Systems for Scalable Bioconversion – \$1,664,297.00*

The University of Washington aims to develop cell-free platforms that produce functional multi-enzyme systems that will enable the cost-effective bioconversion of CO<sub>2</sub> into industrial chemicals. Rather than incurring the high costs and process inefficiencies associated with producing and purifying multiple enzymes in separate steps, the team will engineer technologies to de-risk approaches for producing complex, multi-enzyme systems in situ with stoichiometries optimized for CO<sub>2</sub> bioconversion. The team will create a self-assembling system that electrochemically regenerates formate-reducing equivalents in real time and assimilates formate into malate, an industrially-relevant di-acid, without carbon loss. The proposed system will capture CO<sub>2</sub> in the process of making malate at a cost competitive with more carbon-intensive microbial bioproduction.

### **ZymoChem, Inc. – San Leandro, CA**

*Development of Carbon-Conserving Biosynthetic Systems Co-Utilizing C1 and Biomass Derived Feedstocks- \$3,177,642*

ZymoChem, Inc., aims to develop carbon- and energy-efficient biocatalysts capable of co-conversion of C1- and biomass-derived substrates to a high-volume platform fuel and chemical intermediate. The team will demonstrate a carbon-conserving process decoupling growth and production. The process will enable improved carbon efficiency during the growth phase and 100% carbon-efficient production of bioproducts during the production phase, using biomass-derived sugars and methanol as a reducing equivalent carrier. If successful, this project will demonstrate the improvement of carbon efficiencies beyond current theoretical maximums during the growth and production phases of a bioprocess.