
Current Uses of Synthetic Biology for Chemicals and Pharmaceuticals

Biotechnology Industry Organization

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Naturally Replicating Rubber for Tires

Isoprene is an important commodity chemical used in a variety of applications, including the production of synthetic rubber. Isoprene is naturally produced by nearly all living things (including humans, plants and bacteria); the metabolite dimethylallyl pyrophosphate is converted into isoprene by the enzyme isoprene synthase. But the gene encoding isoprene synthase has only been identified in plants such as rubber trees, making natural rubber a limited resource.

Currently, synthetic rubber is derived entirely from petrochemical sources. Genencor®, a Division of Danisco U.S. Inc., together with The Goodyear Tire & Rubber Company, is currently working on the development of a reliable, high-efficiency fermentation-based process for the Bioisoprene™ monomer, and synthetic biology has played an important role in making this undertaking a reality.



Although plant enzymes can be expressed in microorganisms through gene transfer, it is a long and cumbersome process, as plant genes contain introns and their sequences are not optimized for microorganisms. DNA synthesis and DNA sequencing have enabled the construction and rapid characterization of metabolically engineered microorganism strains to produce isoprene. Synthetic biology has enabled the construction of a gene that encodes the same amino acid sequence as the plant enzyme but that is optimized for expression in the engineered microorganism of choice. This method has provided massively parallel throughput which has made it possible to identify and track genetic variation among the various strains, providing insights into why some strains are better than others.

Continued use of synthetic biology should help refine Genencor's biocatalyst for the production of Bioisoprene™ monomer.

Delivering Economic, Renewable BioAcrylic

Acrylic is an important petrochemical used in a wide range of industrial and consumer products. Acrylic ingredients make paints more durable and odor-free, adhesives stronger and longer-lasting, diapers more absorbent and leak-proof, and detergents better able to clean clothes. Today, petroleum-based acrylic is an \$8 billion global market.

OPX Biotechnologies (OPXBIO) is developing renewable bio-based acrylic to match petro-acrylic performance but with lower cost and an 85 percent reduction in greenhouse gas emissions. BioAcrylic from OPXBIO also will reduce oil-dependence and offer more stable prices.



The key to realizing these benefits, as with any bio-based product, is a highly productive and efficient microbe able to use renewable sources of carbon and energy (for example corn, sugar cane, or cellulose) in a commercial bioprocess. A microbe that meets these criteria for BioAcrylic has not been found in nature, so OPXBIO is applying its proprietary EDGE™ (Efficiency Directed Genome Engineering) technology to redesign a natural microbe to achieve these goals. With EDGE, OPXBIO rapidly defines and constructs comprehensive genetic changes in the microbe to optimize its metabolism for low-cost production of BioAcrylic.

OPXBIO is already producing BioAcrylic at pilot scale in advance of opening a demonstration plant in 2011 and a full-scale commercial plant in 2013. OPX has a commercial target of 50 cents per pound for BioAcrylic production costs to match the industry standard for petroleum-based acrylic.

Making “Green Chemicals” From Agricultural Waste

Surfactants are one of the most useful and widely sold classes of chemicals, because they enable the stable blending of chemicals that do not usually remain associated (like oil and water).

Today, nearly all surfactants are manufactured from either petrochemicals or seed oils, such as palm or coconut oil. Worldwide production of surfactants from petrochemicals annually emits atmospheric carbon dioxide equivalent to combustion of 3.6 billion gallons of gasoline. Production from seed oil is greener, but there is a limit to the amount of seed oil that can be produced while protecting the rainforest. To address this problem, Modular has developed microorganisms that convert agricultural waste material into useful new surfactants. Dr. P. Somasundaran of the University Center for Surfactants (IUCS) at Columbia University finds that Modular’s surfactant is 10-fold more effective than a similar commercially available surfactant.

M. Pete He, PhD., Senior Research Fellow, Corporate Sustainability, Dial, Henkel of America says: “Sustainable chemistry will be a major driver of U.S. economic growth, and I look forward to more of this type of impressive work, which is a win-win, combining academic achievement with industrial implications.”

Modular has developed an engineered microorganism that converts soybean hulls into a surfactant that can be used in personal care products and other formulations. The hull is the woody case that protects the soybeans, and it cannot be digested by humans or other monogastric animals, such as pigs. The U.S. produces about 70 billion pounds of indigestible soy carbohydrate annually, and Modular seeks to upgrade this underutilized material by converting it into a variety of useful new chemical products. Modular's surfactant program is partially supported with funds from the New Uses Committee of the United Soybean Board (USB), which seeks to expand soybean markets through the development of technology that enables the conversion of soy-based materials into new products.

Today, most organic chemicals are derived from petroleum. Fredrick Frank, Vice Chairman, Peter J. Solomon Company, offers this perspective on the sustainable chemistry industry: "Several published reports have concluded that about two-thirds of those chemicals can be generated from renewable raw materials, rather than from oil. If so, sustainable chemistry potentially has a market size of about \$1 trillion. Less than 7 percent of organic chemicals are currently produced from renewable materials, thus there is an opportunity for long-term growth."

Creating Economic Advantage for a Commonly Used Chemical

Adipic acid is a valuable chemical intermediate used in production of nylon for well-established markets like automotive parts, footwear, and construction materials. The current market for adipic acid is approximately \$5.2 billion. Current petrochemical processes for the production of adipic acid generate as much as 4.0 tons of CO₂ equivalents per ton of adipic acid produced. A biobased process could reduce the production costs of adipic acid by 20 percent or more.

Verdezyne is developing a cost-advantaged, environmentally friendly fermentation process for adipic acid. The company's proprietary metabolic pathway can utilize sugar, plant-based oils or alkanes, and the company has completed proof-of-concept testing for fatty acids and alkanes. The potential benefit of this feedstock flexible approach is the ability to maintain a sustainable economic advantage regardless of future energy volatility and to reduce the environmental footprint for producing adipic acid.

Adipic acid is not produced in nature. Verdezyne's novel combinatorial approach to pathway engineering rapidly creates and harnesses genetic diversity to optimize a metabolic pathway. Rather than manipulating one pathway gene at a time, the company uses synthetic gene libraries to introduce diversity into a metabolic pathway. The company's unique computational and synthetic biology toolbox allows effective design, synthesis and expression of synthesized genes in a heterologous recombinant yeast microorganism.



Producing Biofuels and Chemicals That Can Directly Replace Petroleum

Diesel is the most widely used liquid fuel in the world. This energy dense fuel supports the transport of 70 percent of U.S. commercial goods and is in high demand in the developing world to support the heavy equipment (trucks, bulldozers, trains, etc) required for infrastructure development. Today there is no cost effective renewable alternative to diesel.

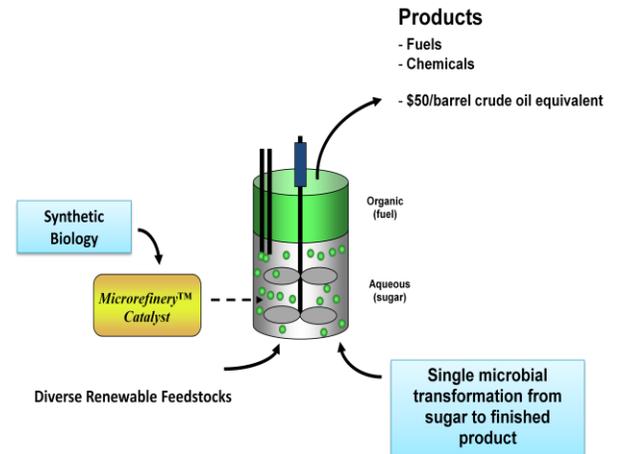
LS9 has developed a platform technology that leverages the natural efficiency of microbial fatty acid biosynthesis to produce a diversity of drop-in fuels and chemicals. Using synthetic biology, LS9 has developed microbial cells that can perform a one-step conversion of renewable carbohydrates (sugars) to two diesel alternatives, a fatty acid methyl ester (biodiesel ASTM 6751) and an alkane (ASTM D975).

The LS9 processes are unique in that all of the chemical conversions from carbohydrate to finished fuel are catalyzed in the cell, with the finished product secreted. The fuel forms an immiscible light organic phase that is non-toxic to the organism and is easily recovered from the broth through centrifugation. There is no need for further chemical conversion, and there is no requirement for hydrogen in the process. These simple processes enable the production of diesel from scalable renewable resources at a price competitive with petroleum (without subsidy).

Synthetic biology has been essential in engineering the LS9 microbial catalysts. The biosynthetic pathways to produce finished fuel products do not exist in the native *E. coli* host, and prior to our efforts alkane biosynthetic genes were unknown. LS9 designed the pathways, synthesized the genes encoding each enzyme in the pathway, and constructed multigene biosynthetic operons enabling production. To improve yield, productivity, and titer – the drivers of process economic efficiency – the biosynthetic pathways and host metabolism have required significant genetic optimization. LS9 developed capabilities for the computational design and automated parallel construction of gene, operon, and recombinant cell libraries that have enabled the rapid construction and evaluation of thousands of rationally engineered microorganisms. This capability in combination with state of the art screening, process development, and analytical methodologies has enabled LS9 in only a few years to advance from concept to a process slated for commercial-scale demonstration.

This same technology platform has been leveraged for the production of surfactants for use in consumer products in collaboration with Procter & Gamble. The ability to exchange biosynthetic parts and leverage the core host “chassis” has enabled the development of this chemical product line much faster, achieving in months what had taken years for the earlier products.

LS9 intends to continue to leverage the power of synthetic biology to further advance these and future products as quickly and cost effectively as possible. We feel strongly these technologies are essential to the goal of weaning our dependence on fossil feedstocks and the further development of a world leading industrial biotechnology industry.



Increasing Rates of Natural Fermentation for Polymers

Metabolix is bringing new, clean solutions to the plastics, chemicals and energy industries based on highly differentiated technology. For 20 years, Metabolix has focused on advancing its foundation in polyhydroxyalkanoates (PHA), a broad family of biopolymers. Through a microbial fermentation process, the base polymer PHA is produced within microbial cells and then harvested. Development work by Metabolix has led to industrial strains of the cells, which can efficiently transform natural sugars into PHA. The recovered polymer is made into pellets to produce Mirel™ Bioplastics by Telles products.



Conventional plastics materials like polyvinyl chloride (PVC), polyethylene terephthalate (PET), and polypropylene (PP) are made from petroleum or fossil carbon. The PHA in Mirel bioplastics is made through the fermentation of sugar and can be [biodegraded](#) by the microbes present in natural soil or water environments. Although PHAs are produced naturally in many microorganisms, the cost and range of compositions required for successful commercialization dictated that PHA pathways had to be assembled in a robust industrial organism that does not naturally produce the product.

Metabolic pathway engineering was used to accomplish this task, relying on modern tools of biotechnology. These include DNA sequencing and synthetic construction of genes encoding the same amino acid sequence as in the donor strain, but optimized for expression in the engineered industrial host. These technologies provided rapid development and optimization of robust industrial production strains that would not have been feasible using classical techniques relying on isolation and transfer of DNA from one species to the other.

This has allowed Metabolix to successfully commercialize Mirel bioplastics. More than 50 years after it was first considered as a potentially useful new material and following several efforts by leading chemical companies to commercialize PHAs based on natural production hosts, Metabolix has made these products available at a commercial scale.

Increasing Efficiency in Bioprocessing of Pharmaceuticals

Sitagliptin, Merck's first-in-class dipeptidyl peptidase-4 inhibitor, is marketed under the trade name Januvia® as a treatment for type II diabetes. The chemical manufacturing route to Sitagliptin developed by Merck won a Presidential Green Chemistry Challenge Award in 2006, but there were still several opportunities for improvement. Codexis and Merck collaborated to develop a novel, environmentally benign alternative manufacturing route. Using synthetic biology and its directed evolution technologies, Codexis discovered and developed a transaminase capable of enabling the new biocatalytic route, which is currently in scale-up towards commercial manufacture.

One common definition of "synthetic biology" is "the design and construction of new biological entities that do not exist in the natural world." In this instance, there was no known enzyme that could perform the reaction required to enable the biocatalytic



route. By designing and generating new enzyme variants, Codexis was able to identify a novel enzyme that provided detectable initial activity. This enzyme was then improved greater than 25,000-fold in order to generate the highly active, stable, enantioselective and practical enzyme from a starting activity that did not previously exist in the natural world. This work was awarded with the Presidential Green Chemistry Challenge Award in June, 2010.

Pioneering Bioprocesses for Antibiotics and Vitamins

DSM, a Life Sciences and Materials Sciences company headquartered in the Netherlands, was one of the first companies to utilize synthetic biology, dramatically improving an existing process for commercial production of Cephalexin, a synthetic antibiotic. Starting with a penicillin-producing microbial strain, DSM introduced and optimized two heterologous genes encoding acyl transferase and expandase respectively for a one-step direct fermentation of adipoyl-7-ADCA. This product was then converted into Cephalexin via two enzymatic steps, replacing a process requiring 13 chemical steps. The new process resulted in significant cost and energy savings. DSM has gone on to build a business in antibiotics, vitamins, enzymes, organic acids, and performance materials.



Advances in synthetic biology have enabled DSM to develop recombinant yeast capable of co-fermenting both hexoses and pentoses. DSM introduced enzymes from native xylose-assimilating organisms to *S. cerevisiae*, allowing fermentation of xylose and arabinose along with glucose. Similar uses of synthetic biology methods have allowed DSM to lower the cost of cellulose saccharification and, in collaboration with Roquette Frères, to develop a proprietary and unique technology for the production of a high-quality renewable succinic acid, a four carbon building block that is currently made from fossil fuel feedstocks. The new fermentation and recovery process is expected to have more favorable economics, which will expand existing applications and open up new uses as well as have a lower environmental footprint.