Current Uses of Synthetic Biology for Renewable Chemicals, Pharmaceuticals, and Biofuels

Biotechnology Industry Organization

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 the isoprene synthase enzyme 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. DuPont, 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 DuPont'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 biobased acrylic to match petro-acrylic performance and cost but with a 75 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 biobased 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 economical production of BioAcrylic.

OPXBIO has advanced its BioAcrylic production process from pilot to large demonstration scale. The company has established a joint development agreement with The Dow Chemical Company, the largest producer of petro-acrylic in the United States, to bring BioAcrylic to market by 2016.

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 for use 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."

Life Technologies Provides a Comprehensive Workflow for Vaccine Development

Demand is growing in developing and developed countries around the world for cost-effective vaccines to prevent infectious diseases. But development of new vaccines is a time consuming undertaking, requiring the identification of antigens – such as weakened viruses or bacterial toxins or other pathogens – and the development, purification and production of immunogens that might help prevent or treat diseases.

Life Technologies has a proven track record in vaccine development. It provides the molecular engineering tools and services necessary to sequence genetic information to formulate vaccines and other treatments in a more efficient and timely manner than current practices, allowing researchers to save time.

Synthetic biology enables Life Technologies to design, synthesize, test and deploy antigens and variants with rapid results, high expression and capacity. It also enables Life Technologies to develop immunogens engineered for efficacy and high titer and produce rapid assays for purification of the immunogens.

Life Technologies scientists developed the custom gene constructs that serve as the basis for HIV vaccine candidates. The gene sequences were custom-designed by scientists at GeneArt® – which merged with Life Technologies in December 2010 – and the University of Regensburg, and then tested in a phase I clinical trial by the EuroVacc Foundation. The trial proved the prophylactic vaccine to be safe and well tolerated, triggering a strong and lasting immune response in 90 percent of the candidates. Additional trials are ongoing. In 2009, GeneArt was awarded a contract by the HIV Vaccine Consortium (UK) to design and produce two HIV vaccine candidates based on the HIV gene sequences used in the 2008 trial.

In May 2009, the GeneArt gene synthesis and assembly platform was employed to create synthetic H1N1 genes, and the product was delivered within a 5-day period. GeneArt created an additional ten H1N1 viral coat protein constructs for the Robert Koch Institute (the central federal institution responsible for disease control and prevention in Germany).

Developing a Suite of Biobased Products and Services

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 enzyme-encoding genes for a one-step direct fermentation of adipoyl-7-ADCA, which could then be converted into Cephalexin via two enzymatic steps. The new process replaced a 13-step chemical process, resulting in significant cost and energy savings. DSM has gone on to build a business in antibiotics, vitamins, enzymes, organic acids, and performance materials within one of its emerging business areas called Biobased Products and Services.

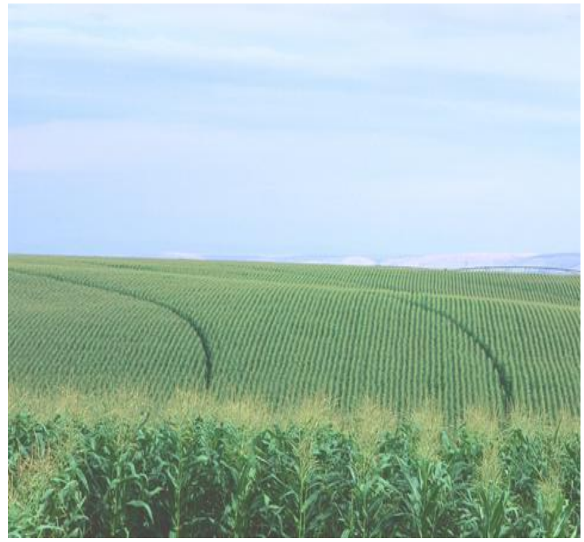
Major biotechnology advances are opening up opportunities in the production of biofuels and renewable chemicals as well as materials made from different types of renewable biomass. Recent DSM breakthroughs include a cocktail of enzymes that break down the lignocellulose from agricultural residues to simple C5 and C6 sugars. 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 co-fermentation of xylose and arabinose along with glucose. Recently DSM announced a 50/50 joint venture with a major ethanol producer, POET for the commercial development, demonstration and licensing of cellulosic bio-ethanol.

Similarly, starting in 2007, the use of synthetic biology methods allowed DSM to develop proprietary yeast that can operate in a low pH fermentation system to cost effectively produce high-quality biobased succinic acid. This patented process in collaboration with Roquette Frères will be scaled in a 10 kiloton plant in 2012 and new markets are expected to open with this 4-carbon chemical building block. More recently, similar work using both biotechnology and chemistry synergistically is resulting in the development of renewable adipic acid, a 6-carbon diacid that is a key monomer for applications in engineering plastics, textiles, resins, and polyurethanes. The value proposition for the industry with these new routes are cost advantaged economics, renewable feedstock flexibility, and a significant improvement in the carbon footprint measured by Life Cycle Analysis.

In summary, DSM's long track record in anti-infectives and vitamins combined with an ever growing experience base in synthetic biology have strengthened DSM's overall capabilities to work with partners and industry stakeholders along the emerging value chains.

Engineering Low-Cost Sugars for Petroleum Substitute

Sugars from non-food biomass can be used as building blocks to manufacture a wide variety of biofuels and renewable chemicals that are currently produced from expensive and price-volatile petroleum feedstocks. The advanced biofuels market is estimated to grow to 21 billion gallons by 2022, based on the U.S. Renewable Fuels Standard (RFS) under the Energy Independence and Security Act of 2007.



Traditional sugar fermentation processes to produce biofuels and renewable chemicals use either sucrose from sugarcane or starch from corn, sorghum, or wheat. Agrivida's engineered biomass provides greater price stability for raw materials, uses less energy in producing biofuels and renewable chemicals via fermentation and enables production with dramatically lower greenhouse gas emissions.

INzyme™ technology from Agrivida, a novel approach to synthetic biology, provides processors and biorefiners the ability to directly control dormant biodegrading enzymes that have been engineered into the biomass. After harvest, these enzymes are activated in a way that greatly reduces the energy, chemical and other pretreatments traditionally required to convert the plant material to sugar.

Agrivida's INzyme™ technology decreases a critical bottleneck in bioproducts production, allowing significantly improved production, reducing the cost and quantity of enzymes needed to produce cellulosic biofuels and renewable chemicals and reducing production facility capital and operating costs.

Ultimately, INzyme™ technology from Agrivida will allow consumers to fully realize the potential of a replacement for petroleum-based biofuels and renewable chemicals that have significant benefits in greater national security through reduced dependence on imported petroleum, lower greenhouse gas emissions and significant agricultural and manufacturing jobs creation. Importantly, Agrivida anticipates that its technology platform will provide consumers a considerable cost savings between 70 and 80 cents per gallon of biofuels produced.

Creating Economic Advantage for a Commonly Used Chemical

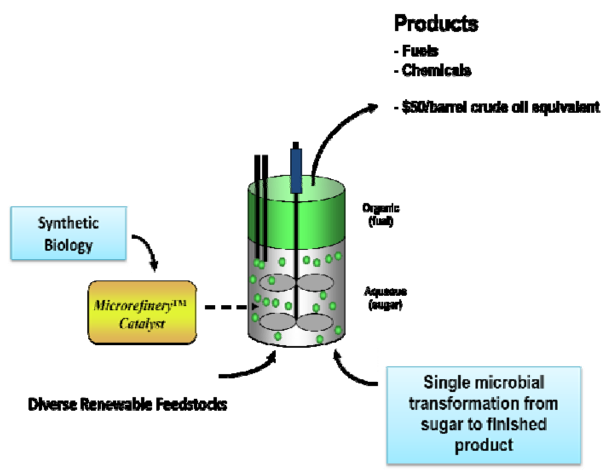
Adipic acid is a valuable chemical intermediate used in production of nylon for well-established markets like automotive parts, footwear, and construction materials. The current market for adipic acid is approximately $5.2 billion. Current petrochemical processes for the production of adipic acid generate as much as 4.0 tons of CO_2 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 Renewable Chemicals as Petroleum Alternatives

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 teraphthalate (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.

Producing a Building Block for Everyday Products

Succinic acid is a linear 4-carbon saturated dicarboxylic acid. Myriant's bio-succinic acid produced from renewable and sustainable raw materials is chemically identical to succinic acid produced by petrochemical routes, with a purity level equal or superior to the highest quality petroleum-based succinic acid. This enables Myriant's bio-succinic acid to be dropped in to any process currently using petroleum derived succinic acid. In addition, its chemical structure allows it to replace several other building block chemicals currently produced from petrochemical feedstocks – including maleic anhydride and adipic acid, which are used in the production of everyday consumer items from plastics to clothing fibers and biodegradable solvents, to food and pharmaceutical products. The market potential of succinic acid is estimated to be $7.6 billion per year.

In the conventional petrochemical process, benzene or butane is oxidized to produce maleic anhydride – releasing roughly half of the raw material as CO_2 – which is then converted to succinic acid through hydrolysis.

Myriant has developed a more eco-friendly method for the production of bio-succinic acid that addresses the supply, cost and environmental issues associated with the use of fossil fuels as feedstocks. Myriant's technology platform uses proprietary microorganisms in a fermentation process designed to produce high-value bio-based chemicals with high yields and productivity. The biocatalysts are non-genetically modified (non-GMO) microorganisms with altered metabolic pathways that grow and simultaneously produce target bio-based chemicals from a variety of renewable feedstocks. Our highly efficient biocatalysts are able to flourish in minimal growth medium without the use of expensive, complex nutrients, enabling us to bring renewable chemicals to market that are cost-competitive with their petroleum based counterparts.

Myriant's biocatalysts are able to consume a variety of readily available low-cost sugars from non-food sources, such as glucose from sorghum and cellulosic sugars from waste biomass. These raw materials are not subject to the extreme price volatility or supply availability issues associated with petroleum-based feedstocks. Additionally, Myriant's anaerobic process consumes CO_2, resulting in a reduced carbon footprint.

Bio-succinic acid can be used in many applications, such as plasticizers, coatings, polymers, esters, and polyester polyols. As an example, Myriant has developed Myrifilm® solvent, which can be used to manufacture coatings with superior functional performance. Myrifilm® also brings additional benefits to the coatings formulator, as it contains no volatile organic compounds (VOC), is free of hazardous air pollutants (HAPS), and is odor-free.

In 2009, Myriant received a $50 million grant from the U.S. Department of Energy to build the nation's first bio-succinic acid commercial production facility in Lake Providence, La. The plant is scheduled to start-up in the first quarter of 2013 and its 30 million pound per year production capacity is already sold to customers around the world.