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

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); dimethylallyl pyrophosphate is metabolized into isoprene by the isoprene synthase enzyme. 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 microorganisms can express plant enzymes through gene transfer, it is a long and cumbersome process. Plant genes contain introns and their sequences are not optimized for microorganisms. Synthetic biology allowed the construction of a gene that encodes the same amino acid sequence as the plant enzyme but that is optimized for expression in an engineered microorganism.

DNA synthesis and DNA sequencing enabled the construction and rapid characterization of metabolically engineered microorganism strains that produce isoprene. This method provided massively parallel throughput, which 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 a $10 billion global market.

OPX Biotechnologies, Inc. (OPXBIO) is developing renewable biobased acrylic to match petro-acrylic performance and cost. BioAcrylic from OPXBIO will also reduce oil-dependence, increase energy security, and offer more stable prices.

The key to realizing these benefits, as with any bio-based product, is a highly productive and efficient microbe and bioprocess able to use renewable sources of carbon and energy (for example sugar from corn, cane, or cellulose). OPXBIO is applying its proprietary EDGE® (Efficiency Directed Genome Engineering) technology to improve naturally occurring microbes 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, and is planning to bring BioAcrylic to market in 2017.

Beyond BioAcrylic – New Fatty Acids

Fatty acids today are sourced from plant oils such as palm, palm kernel, and coconut, and from animal fats. The worldwide market is valued at $40 billion with typical uses in home and personal cleaning products and industrial fluids. There is a growing interest among customers in more sustainable and economic sources of fatty acids and to access new types of fatty acids offering improved cost-performance.

The power and versatility of OXPBIO's EDGE technology is demonstrated by its successful application to engineer microbial strains and bioprocesses to economically produce fatty acids with specific, precise numbers of carbon atoms using renewable sugar feedstocks. OPXBIO is working with Evonik Industries in a joint development program targeting a particular specialty fatty acid product.

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).

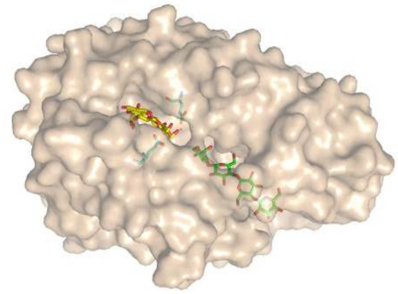
> M. Pete He, PhD., Senior Research Fellow, Corporate Sustainability, Dial, Henkel of America says: "Sustainable chemistry will be a major driver of U.S. economic growth, and I look forward to more of this type of impressive work, which is a win-win, combining academic

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.

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." 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.

Developing a Suite of Biobased Products and Services



Royal DSM, the Netherlands-based global Life Sciences and Materials Sciences Company, has a rich history in industrial biotechnology. Much of this heritage came into its hands after the acquisition of Gist Brocades - the first in the world to produce recombinant chymosin (patented in 1988), and also 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 for the first time employed enzymes designed by a combination of directed evolution and rational design strategies and replaced a 13-step classical chemical synthesis route, resulting in significant cost and energy savings. Similar impressive results were obtained with the replacement of a chemical process to riboflavin (Vitamin B2) with a biotech fermentation process enabled by improved microorganisms and process innovations.

DSM has gone on from these early days of biotechnology and exploration of the bioeconomy to create new businesses within its Biobased Products & Services unit. 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. DSM has developed a cocktail of thermo-stable enzymes to break down (hemi)cellulose from agricultural residues into simple C5 and C6 sugars. Advances in synthetic biology have enabled DSM to develop recombinant yeast capable of co-fermenting these C5 and C6 sugars. DSM introduced enzymes from native xylose-assimilating organisms to *S. cerevisiae*, allowing co-fermentation of xylose and arabinose along with glucose. These products are now introduced globally into the market. One example is the US joint venture with the major ethanol producer POET, from Sioux Falls (SD), for the commercial development, demonstration and licensing of cellulosic bio-ethanol. Other examples that apply DSM technology include the first commercial cellulosic bio-ethanol operation by GranBio in Brazil as well as several demonstration plants in Europe, such as Inbicon.

Starting in 2007, the use of synthetic biology methods also allowed DSM to develop proprietary yeast that can operate in a low pH fermentation system to cost-effectively produce high-quality biobased succinic acid, without the need to free up the acid from and create a salt by-product. This patented process is now in production on a commercial scale at Reverdia's (JV of DSM and Roquette) 10 kiloton plant in Italy, and new markets are expected to open with this 4-carbon chemical building block.

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

Engineering Fiber Digestion to Produce Food and Fuels

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. By enabling the use of biomass on a wide scale, the absolute amount of feedstock per acre will increase and enable significant amounts of grain and sugar to be repurposed for food production.

INzyme® technology from Agrivida 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 fundamentally enables alternative processes for making a variety of bioproducts, reducing the cost and quantity of enzymes needed to produce cellulosic biofuels and renewable chemicals and reducing production facility capital and operating costs. In addition, Agrivida's engineered biomass significantly improves ruminant performance when included in a total mixed ration, increasing beef and dairy productivity.

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.

Economic and Environmental Advantages for Commonly Used Chemicals

Dicarboxylic acids (diacids) are organic monomers used for the preparation of polyamides, adhesives, lubricants, and polyesters. These industrial chemicals are currently made from petroleum. Verdezyne is commercializing technologies that create a positive impact on the environment by providing sustainable alternatives, at a lower cost.

Verdezyne is a biotechnology company that produces industrial chemicals from renewable sources. The company specializes in using non-food co-products from low cost plant oil feedstocks such as soybean and palm to produce diacids via fermentation. Verdezyne produces adipic, dodecanedioic and sebacic acid using its proprietary yeast platform and downstream processing.

Adipic acid is a key component of nylon 6,6 and thermoplastic polyurethanes, with an annual global market value of over six billion dollars. Nylon 6,6 has myriad end uses including clothing, footwear, furniture, carpets and automotive parts. Current adipic acid production methods emit nitrous oxide, a potent greenhouse gas associated with ozone depletion. Verdezyne is currently the only company that has achieved pilot scale production of adipic acid via fermentation and demonstrated end-use products.

Dodecanedioic acid (DDDA) is used to make nylon 6,12, molding resins, lubricants, adhesives, powder coatings, as well as end products such as fishing line, toothbrush bristles and fuel lines. DDDA is currently made from petroleum-based intermediates. Verdezyne has produced approximately 1 metric ton of DDDA in demonstrating its process.

Sebacic acid is an intermediate used in the production of nylon 6,10, a plastic widely used in everyday household items like toothbrush bristles and fishing line. It is also found in coatings, adhesives and polyester resins. Sebacic acid is currently produced from a limited supply of castor oil.

Increasing Rates of Natural Fermentation for Polymers

Metabolix is bringing new, sustainable, performance-driven solutions to the plastics and chemicals industries based on its 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 that can efficiently transform natural sugars into PHA. The recovered polymer is made into pellets to produce proprietary PHA biopolymers. Metabolix has also developed a novel fast-acting, selective thermolysis (“FAST”) process to convert polymers to target chemical monomers.

Conventional plastic materials like polyethylene (PE), polyethylene teraphthalate (PET) and polypropylene (PP) are made from petroleum or fossil carbon. The PHA made by Metabolix through the fermentation of sugar can be biodegraded by the microbes present in natural soil or water environments. Although PHAs are produced naturally in many microorganisms, the 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 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 its PHA biopolymers for a range of applications. More recently, Metabolix has leveraged advances in its PHA technology to develop a product line of performance additives to enhance performance and processing of PVC (polyvinyl chloride) and PLA (polylactic acid), and the Company is also developing PHA latex coatings for paper and cardboard. In addition, Metabolix is focusing on the development and commercialization of biobased chemicals.

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 is bringing PHA-based and PHA-modified products to market at commercial scale.

A Sugar-Derived Building Block for Chemicals and Fuels

Isobutanol is a building block that can be used in solvents, plastics, polyester, rubber, and transportation fuels, each of which constitute a multi-billion dollar market. Through chemistry, isobutanol can be converted into feedstocks for the production of nearly 40 percent of traditional chemicals (such as butenes, toluenes and xylenes) as well as many transportation fuels. As a solvent, isobutanol appears in paints and cosmetics such as nail polish. The solvent, petrochemical and fuel markets are each worth several billion dollars.

Volatility in petroleum prices can have a significant impact on these markets. Bioisobutanol is a drop in replacement for petroleum-based products that offers greater stability in pricing and a potential cost savings.

Gevo has developed the Gevo Integrated Fermentation Technology® (GIFT®) that combines genetically engineered yeast with a continuous separation process to separate the isobutanol from the fermentation broth, allowing for higher yields of isobutanol and lower separation costs. Using synthetic biology, Gevo has engineered a yeast to concentrate on the production of isobutanol – which is a standard product of fermentation by brewer’s yeast, as in beer or wine making – by blocking the production of ethanol and acetic acid. Through this integrated process, Gevo has reached 94 percent of theoretical yields.

Gevo's production process was demonstrated at a commercially relevant scale with the retrofit of ICM's 1 million gallon per year plant in St. Joseph, Mo. The company is currently operating a corn ethanol plant in Luverne, Minn., to produce up to 18 million gallons per year of isobutanol, ethanol, or a combination of both. Gevo has demonstrated production of butyl rubber, butenes, solvents and lubricants through partnerships with chemical manufacturers Lanxess, Sasol and Toray. Gevo has also a biorefinery in Silsbee, TX that converts isobutanol into bio-jet fuel, paraxylene, and iso-octane. Gevo's bio-jet fuel has been purchased by the US Army, Navy and Air Force. Gevo's paraxylene will be sold to Toray for the production of PET and other polyesters. Gevo's isooctane has been used in race cars and high performance engines.

Turning Carbon Dioxide into Chemicals and Biofuels

Phytonix Corporation utilizes synthetic biology to enable the integration of 21st century scientific ingenuity with the ancient and elegant process of photosynthesis. Starting with the most energy and photosynthetically efficient natural microorganisms on Earth, cyanobacteria, synthetic biology allows us to substitute, in a bio -safe manner, a target chemical synthesis pathway for the natural food production pathway of the organism.

With this approach, we are able to create microbial chemical production platforms that can directly convert carbon dioxide as the sole, direct feedstock into valuable renewable chemicals and biofuels. CO_2 needs no longer to be seen as a waste product with dangerous environmental effects, but increasingly as a valuable feedstock for chemicals, fuels or polymers. This positive vision has been gaining momentum and is now emerging from research laboratories and new commercial ventures as a realistic alternative path to securing the constant supply of carbon atoms the industrial chemistry sector will continue to need for their production cycles, even in a future world where fossil resources may be completely depleted, all enabled by the evolving field of synthetic biology.

Combining nature and science in this manner holds great potential to provide for civilizations' future chemical and transportation fuel needs while alleviating the stress on Earth's environment for the betterment of all life that it supports.

Phytonix's product pipeline starts with the broadly used industrial chemical and potential "drop-in" fuel n-butanol, followed next by n-pentanol, a high energy density, five carbon alcohol. The traditional method for producing n-butanol uses the petroleum-based feedstock propylene, and is energy and carbon-intensive and not a sustainable process. Phytonix's process uses waste carbon dioxide as feedstock, preventing it from being released into the atmosphere, and gives off oxygen as a by-product.

Phytonix is also working to be a leader in biosafety, with proprietary, redundant bio-safe technology to prevent proliferation of engineered microbes into the environment. The Synthetic Biology Industry is self-regulated, and Phytonix believes that to maintain favorable regulatory status, companies in this emerging field can increase their competitiveness and the market adoption of their products through demonstrating ethical leadership by including bio-safe mechanisms into their engineered organisms.

Biological Conversion of Natural Gas to Fuels and Chemicals

The recent rise in domestic production of methane has driven the cost of natural gas to record lows. Calysta Inc. has developed a genetic engineering platform for host organisms (methanotrophs) capable of metabolizing this abundant domestic feedstock to a variety of biofuels and biochemicals. The genetic tools, together with innovative fermentation and bioprocess approaches, enable the rapid implementation of well-characterized pathways to utilize natural gas as a biological feedstock instead of sugar. Methane's 34x higher greenhouse gas contribution relative to CO2 implies that capturing these sources will have a significant environmental benefit. Longer term, biomass-to-methane strategies may eventually enable a fully renewable carbon cycle if 'green' methane-based technologies are developed.

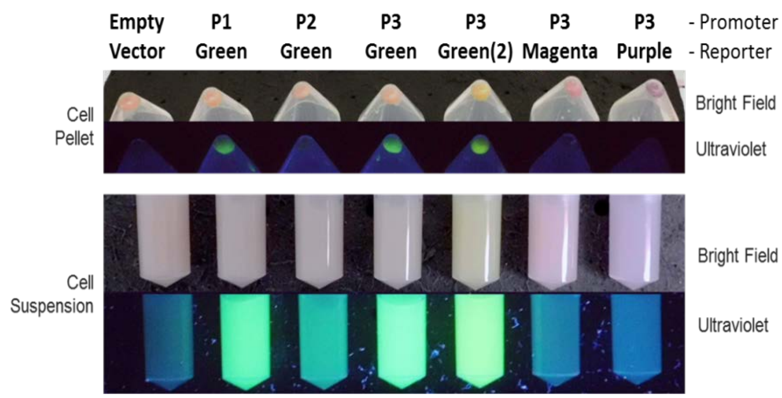


Figure 1: Demonstration of genetic manipulation in methanotrophs.

Methylococcus capsulatus was transformed using proprietary methods with plasmids containing a combination of promoters (P1, P2, P3) and reporter proteins (two versions of Green, Magenta, Purple). The two Green reporters are fluorescent in ultraviolet light, while Magenta and Purple absorb in the visible spectrum only.

Calysta's proprietary biological GTL platform utilizes genetically engineered methanotrophs, which are prokaryotes that utilize methane as their sole source of carbon and energy. Methanotrophs have been observed in a wide range of environments, including both aerobic and anaerobic, typically in association with natural methane sources such as degrading biomass or petroleum offgas. While methanotrophs are a logical starting point for the development of a biological methane conversion platform, a critical requirement for the development of a biotechnology platform is the availability of tools for the directed manipulation and modification of the host cell's metabolism. Although such tools are commonplace for model organisms (e.g. Escherichia coli or Saccharomyces cerevisiae), relatively little effort has been expended to develop similar capabilities in methanotrophs. Calysta has therefore developed a suite of tools for the expression of heterologous proteins in methanotrophs (Fig. 1), as well as tools for the efficient targeted manipulation of the methanotroph genome. As Calysta continues to improve the technical capability to modify methanotrophic organisms, we are also making the tools available at no cost to the academic research community to help build interest and critical mass in the field.

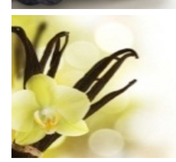
It is important to note that the central metabolism of methanotrophs is comparable to that of most model organisms, in that it proceeds through typical metabolites such as pyruvate and acetyl-coA. This means that metabolic pathways which have been developed, characterized, and validated in other host organisms can be readily adapted for use in methanotrophs. Using this approach, Calysta has successfully demonstrated production of a variety of chemicals from methane via engineered metabolic pathways.

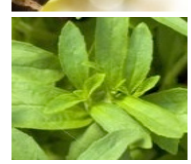
The Evolution of Yeast Fermentation

Biotechnology is enabling the production of new and more sustainable, flexible and functional ingredients for food, beverages, personal care, and wellness products. There are a vast number of really important ingredients in these products that have taste, cost, purity, reliable sourcing, and sustainability issues that significantly limit wider use. And, looking ahead, some of these ingredients will have even greater challenges because of climate change.

That’s where Evolva’s platform technology comes in. We are developing alternative routes for producing natural ingredients for consumer products via biosynthesis and yeast fermentation. Our ingredients make products more sustainable, affordable, and broadly accessible to consumers in all income brackets. Essentially, we are a 21st century specialist brewer. Our initial products are resveratrol, vanillin, stevia, and saffron.

Is it natural?





The word ‘natural’ means many things to many people: it’s not black and white. Regulatory experts in both Europe and the U.S. have stated publicly that ingredients produced like ours should qualify as ‘natural’. The average consumer views ‘naturalness’ as a spectrum, a sliding scale.

We are comfortable saying our products are ‘natural’ because they are made from natural ingredients (sugar), using natural processes (fermentation), and the end product is identical to the ingredients found in the original plants—and because we remove our tweaked yeast during production. We recognize, nevertheless, that some believe that if you tweak the genetics in your yeast it makes the end product less natural—even though genetics (albeit different genetics) have been used over centuries, indeed millennia, to develop garden plants, beer and bread.

Why yeast?

The DNA code of baker’s yeast (Saccharomyces cerevisiae) is one of the most well-studied genetic codes in nature. And, as it happens, yeast genetics have been tweaked for millennia by people to make products such as beer, bread, wine, etc.

We tweak a vanishingly small number of the yeast’s genes to, among other things, better leverage its production potential. In our case we tweak less than 1 percent of the genes in the baker's yeast so that it can make ingredients like resveratrol, vanillin, stevia and saffron.

Typically our yeasts will contain genes from several different plants. The plant genes we use will have been adjusted to work in yeast. Different organisms read their genes in slightly different languages: you might say that plants speak French while yeast speaks English.

What's the big deal about biosynthesis?

Perhaps better known in academe as "synthetic biology", biosynthesis is finding a growing number of real-world applications from medicines and vitamins to oil-eating microbes and palm oil substitutes. To get our yeast to produce, say, vanillin instead of a chemical like ethanol, it takes biosynthesis—and some very smart, thoughtful scientists.

We believe that the safety, societal impact, and ethical considerations of "synthetic biology" have been thoroughly evaluated by independent academic and government scientists around the globe, as well as by technology experts who assess the potential impacts of new technologies on the environment.

Still, there are some who feel that more "big-picture" discussions on "synthetic biology" are needed. There is plenty of room around the table for such a dialogue.