Organic Process Research & Development **2011,** *15,* 175–179

Facts, Growth, and Opportunities in Industrial Biotechnology

Rina Singh

Industrial Biotechnology and Environmental, Biotechnology Industry Organization (BIO), 1201 Maryland Avenue, SW, Suite 900, Washington, DC 20024, United States

Abstract:

The revolution in synthetic biology has enabled innovative manufacture of biofuels and the development of biological processes for the manufacture of bulk and fine chemicals. This short review gives some examples of recent progress.

Introduction

There exists today a desire to advance the global bioeconomy through creating integrated biorefineries which serve to produce sustainable biochemicals from flexible feedstocks. Biomaterials and biopolymers from renewable chemicals are a market reality and have consumer demand, more so now than ever before, from new innovative approaches which lower the carbon footprint.[1] Newly constructed biorefineries deploy innovative, sustainable technologies to complement the traditional energy, chemical, and plastics industries. Manufacturing facilities are producing first-generation biofuels, technical approaches are being sought to produce second-generation renewable biofuels from cellulosics, and research continues to accelerate in advanced biofuels which would give rise to the possibility of more stable molecules having improved performance properties beyond bioethanol. The U.S. Renewable Fuel Standard (RFS) for transportation fuels set minimum levels of renewable fuels that must be blended into gasoline and other transportation fuels from 2006 to 2022. Specific requirements for blending advanced biofuels,[2] including cellulosic biofuels and biomass-based biodiesel fuel, begin at 0.6 billion gallons per year in 2009 and rise to 21 billion gallons in 2022. The RFS levels for advanced biofuels production will drive the creation of a major new industry, creating a foundation for future technology development and commercial growth.[3] Algae-based fuel technology is advancing rapidly toward commercial-scale viability. Commercial production of algae-based fuels will greatly enhance our country's energy and environmental security.

The Synthetic Biology Revolution

A revolution occurred in the field of chemistry[4] in the "golden years, 1950s," referred to as the "synthetic chemistry revolution." It was during that period that methods for production of novel organic chemicals, including high performance engineering thermoplastics and thermosets, such as nylon, Teflon, and Derakane, were invented. This revolution extended into agriculture and pharmaceuticals, creating tremendous economic value and significant employment in the regions that led this revolution, in particular the United States, Europe, and Japan. We are in the early period of a new revolution referred to as the "synthetic biology" revolution. Synthetic biology is an extension of recombinant DNA technology, which was invented in the 1970s. The early period of the modern revolution in biology started with the development of recombinant DNA technology. At the dawn of this new era, researchers in the field agreed to develop guidelines to ensure the safe practice of the technology. There is growth in the United States research and development private sector, where small start-up companies are forming important partnerships to expand applications of biotechnology using synthetic biology technology in food ingredients, synthesizing and designing new pharmaceutical intermediates for the Health Care Industry, biobased specialty chemicals, and biofuels. These partnerships are formed to reduce the time to market and enhance the bioeconomy. Engineered microorganisms are being used to synthesize chemicals and polymers that are used in our everyday lives to produce everyday products.[5] Similar to the past revolution in chemistry, this new revolution in biology will broadly impact the chemical industry, and will extend into agriculture and pharmaceuticals. In the chemicals sector, production of chemicals using engineered microorganisms and enzymes could generate global revenue of $1.8 trillion (USD). The Utah-based life sciences company Beachhead Consulting estimates that synthetic biology research global market (currently worth around $600 million) has the potential to grow to $3.5 billion[6] over the next decade, while estimates by Lux indicate that one-fifth of the chemical industry (now estimated $1.8 trillion total) could be dependent on

(1) Biobased Chemicals and Products: A New Driver for Green Jobs; http://bio.org/ind/20100310.pdf.
(2) As defined by the Energy Independence and Security Act of 2007, advanced biofuels are renewable fuels, other than ethanol derived from corn start, that have lifecycle greenhouse gas emissions that achieve at least a 50% reduction over baseline lifecycle greenhouse gas emissions.
(3) *U.S. Economic Impact of Advanced Biofuels Production: Perspectives to 2030*, Report commissioned by BIO and written by Bio Economic Research Associates (bio-era); http://bio.org/ind/advbio/EconomicImpactAdvancedBiofuels.pdf.
(4) Landau, R.; Arora, A. The chemical industry: from the 1850s until today; October 1999. Retrieved from Business Services Industry: http://findarticles.com/p/articles/mi_m1094/is_4_34/ai_56973853/?tag=content;col1.
(5) Everyday products, synthetic biology report; *Current Uses of Synthetic Biology for Chemicals and Pharmaceuticals*; Biotechnology Industry Organization: Washington, DC; http://bio.org/ind/syntheticbiology/Synthetic_Biology_Everyday_Products.pdf.
(6) De Guzman, D. Green Chemicals reached $1.63 B. *Monitoring the development of green within the chemical industry* ; **2009** (March 19). Retrieved from Green Chemicals: http://www.icis.com/blogs/green-chemicals/2009/03/green-chemicals-reached-163bn.html.

10.1021/op100312a
Published on Web 12/07/2010

synthetic biology by 2015. Over 200 firms and universities in the United States are engaged in synthetic biology research, development, and commercialization. Synthetic biology is a transformative technology that has the potential to fundamentally change the way we make and use chemicals and materials, and it has the potential to accelerate and transform our economy. A group of pioneering chemical companies is using synthetic biology to create a wide range of basic products, which include companies that have commercial products in the pipeline, and others are developing products. Examples of some of these companies include:

• Genencor is developing BioIsoprene, a biobased chemical made from microorganisms used in rubber and adhesive products. BioIsoprene offers an alternative to using natural rubber or petroleum derived isoprene. 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.

BioIsoprene™ Rubber Tire

• NatureWorks produces Ingeo from polylactic acid (PLA) using the biotech process of fermentation. PLA-based materials use less energy and release less pollution during their production as compared to similar petrochemical-based materials. For example, greenhouse gases are reduced by 75% when compared to polyethylene terephthalate (PET) and 90% when compared to Nylon 6; both these polymers are used in making synthetic fibers and other materials. The applications for PLA-based materials are packaging, plastic flatware, fabrics, and carpets.

• DuPont won a Presidential Green Chemistry Challenge Award for their development process of obtaining 1,3-propanediol (Susterra) from corn-derived glucose in recombinant *Escherichia coli*. This process is used to create the biotech fiber Sorona. The applications for Sorona include apparel, carpet, resins, and biofiber composites.

• Genomatica developed a process to convert sugar into 1,4-butanediol (1,4-BDO). This process decreases fossil fuel use by 50% and reduces carbon dioxide and greenhouse gas emissions.

• Solazyme creates oils and chemicals from algae. This algae is capable of manufacturing thousands of gallons of oil and hundreds of tons of chemicals, which are used in a diverse range of products from oleochemicals (analogous to petrochemicals) to cosmetics and food.

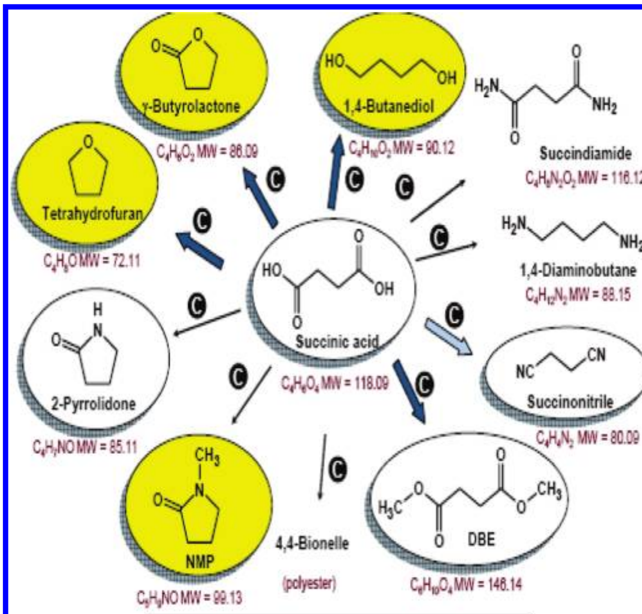
• Agro-food giant Archer Daniels Midland partnered with Metabolix, a small industrial biotech company, to produce polyhydroxyalkanoates (PHA), a versatile family of bioplastics branded as Mirel, at a biorefinery in Clinton, Iowa. The facility began making its commercial product available for customers in the first quarter of 2010 and is designed to produce 110 million pounds of Mirel annually. Mirel has broad applications (molded products, films, foams, and fibers), and is considered greenhouse-gas neutral. Metabolix is bringing new, clean solutions based on highly differentiated technology, to the plastics, chemicals, and energy industries. 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.


Polyhydroxyalkanoates (PHA), A Broad Family Of Biopolymers

• The Dow and Crystalsev joint venture creates bioethylene from the fermentation of Brazilian sugar cane. Bioethylene derivatives include polyethylene, ethylene oxide, vinyl chloride monomer, vinyl acetate monomer, and styrene. When compared to its petrochemical counterpart, bioethylene is less expensive to process, has a lower carbon footprint, and is walled off from crude oil volatility.

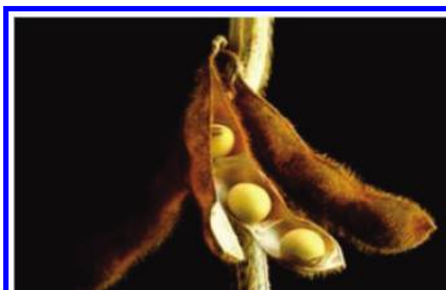
• OPX Biotechnology is developing a process for producing bioacrylic acid using its EDGE (Efficiency Directed Genome Engineering) technology platform. EDGE uses synthetic biology to convert sugar to bioacrylic acid. The process is targeting the production of economic and renewable bioacrylic acid which will match petrochemical-based acrylic acid performance but with lower cast and an 85% reduction in greenhouse gas emissions. Bioacrylic acid from OPX Biotechnology will reduce oil-dependence and offer more stable prices. Acrylic acid is an important petrochemical used in a wide range of industrial and consumer products. Acrylic acid 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.

• Succinic acid has successfully reached production quantities in a variety of companies. The DNP Green Technology and GreenField Ethanol partnership has built a succinic acid biorefinery to produce a new generation of environmentally friendly deicing solution that has a negative carbon footprint and is less corrosive than traditional deicers. Myriant's metabolic engineering technology produces highly pure succinic acid. Both PURAC/BASF and DSM/Roquette are developing a succinic acid to absorb instead of emit carbon dioxide.



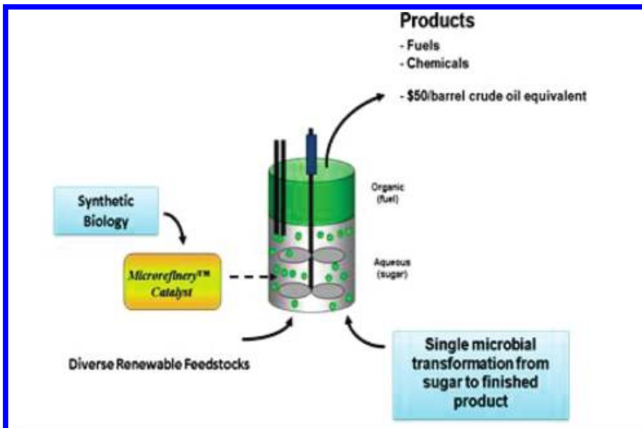
Succinic Acid Platform

• Modular Genetics has developed an engineered microorganism that converts soybean hulls into a surfactant that can be used in personal care products and other formulations. 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 (such as 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. 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 Genetics seeks to upgrade this underutilized material by converting it into a variety of useful new chemical products such as surfactants.


Soybean Hull

• Adipic acid is a valuable chemical intermediate used in production of nylon for well-established markets such as automotive parts, footwear, and construction materials. 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 bio-based process could reduce the production costs of adipic acid by 20% or more. Current market for adipic acid is approximately $5.2 billion. 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 benefit from this flexible feedstock approach is the ability to maintain a sustainable economic advantage. 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.

• Diesel is the most widely used liquid fuel in the world. This energy-dense fuel supports the transport of 70% 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 nontoxic 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 *Escherichia coli* host, and prior to the LS9 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.



Biosynthetic Pathways to Finished Products

• 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.

• 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.

Synthetic Antibiotic

The Future

Synthetic biology holds promise for advances in many industrial biotechnology areas, including the development of renewable chemicals and bioproducts, carbon-neutral energy sources (biofuels), safer and improved pharmaceutical intermediates, and better environmental remediation technologies. The potential impact of synthetic biology in research and product development speaks directly to the ability to make and test prototype biological systems with a speed and complexity not presently feasible, or not presently cost-effective. As with most product development, innovation and competitiveness can often be tied to the ability to rapidly and predictably iterate through a solution space to obtain optimum performance outcomes. Synthetic biology offers this promise to academic research groups, government technology institutes, and to public and private corporations seeking to develop biological solutions to today's challenging needs.

One of the fundamental shifts afforded by synthetic biology is the ability to "write" genetic information on a scale heretofore impossible. The example of "writing" an entire genome *de novo*, as exemplified by the recent work at the J. Craig Venter Institute, culminates an evolutionary development of technology started when humans first understood that breeding for traits is, in effect, causing genetic information to assemble in a manner more conducive to man's interests, as in animal or crop domestication. At that time, science lacked the ability to cause DNA to assemble in a new manner, but it did understand the benefits of selecting for that assembly. As time and technology advanced, the ability to manipulate DNA at an increasingly direct level led to molecular cloning and, for the first time, to our ability to directly compose DNA in a sequence of our choosing. This started 40 years ago with small genes, such as that for human insulin, but has moved to pathways, chromosomes, and now to entire genomes. The ability to solve problems/challenges through technology, that otherwise would not have a solution, causing the production of something like human insulin in a controlled, renewable system has brought direct benefit to mankind, and was part of the tremendous biotechnology revolution that spawned entire industries, and changed forever the way one thinks about discovery of or production of a product. So in a sense, last century's technology accelerated in the chemical and pharmaceutical industries when we learned to "write" complex molecules synthetically, instead of relying solely on finding and extracting them from nature. The biotechnology revolution allowed us to begin the process of deliberate assembly of still relatively small amounts of DNA into systems that produced important products or facilitated fundamental research.

Conclusions

Today, synthetic biology holds the promise of allowing us to write entire pathways, or genomes, to create routes to production of valuable biochemicals, biopolymers, therapeutics, and performance materials. As with prior technology revolutions, it also should spawn entire new industries (as was the case with companies created to supply restriction enzymes or PCR kits), or drive innovation in adjacent space, such as in computational and information sciences, automation technologies, and analytical science, to name a few. Research and development based on synthetic biology technology needs to support the design of microorganisms which serve as powerful catalysts capable of synthesizing an ever wider range of biofuels (next-generation and advanced biofuels) and biochemicals from renewable feedstocks. It is extremely important to develop and implement new or improved lower-cost microbes (enzymes) for biorefining applications. The impeding factor in enzyme technology today is their cost and performance efficiency in converting renewable feedstocks (such as cellulosics) to value-added biochemicals and biofuels; thus, even more research and development is required to improve biochemical conversion processes to improve productivity of conversion mechanics (enzymes or fermentation organisms). Development and operating demonstration-scale and commercial-scale integrated biorefineries efficiency with multiple classes of feedstocks require enzyme discovery, characterization and modification to improve enzyme performance, as well as progress on cost effectively producing and applying enzymes to biorefinery processes. As innovations lead to product and market development, policy, legal framework, and public education need to occur in concert with these key inventions to reap the full benefits of a global biobased economy.

Received for review November 22, 2010.

OP100312A