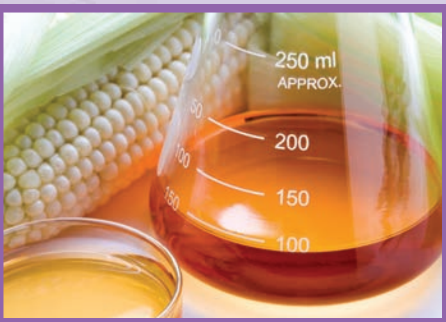
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# *Metabolic engineering:*
# The new manufacturing cell

*IE tools could help build scalable alternatives for producing biofuels, industrial chemicals and biopharmaceuticals*

By Devin Leake

With the growing interest in synthetic biology during the last 15 years, scientists have been seeking to use metabolic engineering to create powerful manufacturing plants out of individual biological cells or simple organisms. The concept of cells as factories is not new, but the ability to use predictable engineering strategies to improve yield, production and quality is still developing.

This means that now and in the future, industrial engineers could see new opportunities in translating their skills from the factory floor to manufacturing at a cellular level.

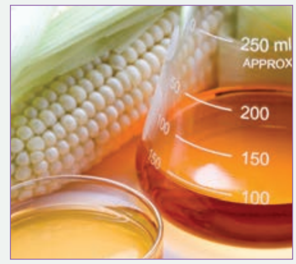
Although aspects of metabolic engineering have existed for more than 25 years, synthetic biology, or the use of customized biological building blocks to alter an existing organism (or even to create a new one), has advanced since that time to become more sophisticated in its molecular approach. One demonstration of this, the genetic toggle switch to turn a gene on or off on command, was described in the journal *Nature* in "Construction of a Genetic Toggle Switch in Escherichia coli." Another example is an oscillator switch that could automatically turn a gene on or off based on the state of other genes or proteins in the circuit, detailed in *Nature's* "A Synthetic Oscillatory Network of Transcriptional Regulators."

Since those early days, synthetic biologists have designed thousands of such tools, many of which are publicly available through the Registry of Standard Biological Parts and meet technical standards established by the BioBricks Foundation. These parts can be inserted into various organisms to effect a desired change, such as up- or down-regulating specific genes, halting transcription of a gene or changing the location of a protein.

With the emergence of robust engineering tools, many industries are now exploring the use of metabolic engineering to boost the manufacturing potential of microorganisms or cells for a range of applications, such as targeting the replacement of petroleum-based products with environmentally responsible processes. Synthetic biology has enabled the use of rational design, allowing scientists to be more efficient and effective in their attempts to target important pathways and increase product yield. Already, this approach has been used to manufacture biological therapeutics and industrial chemicals more efficiently and to generate biofuels from nonedible plants.

In this review, we will look at three areas of interest to industrial engineers.

All three use metabolic engineering to make cells or organisms better at processing or producing important materials: biofuels, industrial chemicals and biopharmaceuticals. These fields have emerged as the leading edge in the adoption of synthetic biology and metabolic engineering for use in manufacturing. As the science is evolving so rapidly, we restrict our review to just a few examples of recent research advances to provide readers with the latest developments in a very new area of industrial engineering. We also look ahead to implications of this manufacturing approach and existing best practices that may be applicable.




## Biofuels

Biofuel development was underway long before the advent of synthetic biology, but early attempts at corn-based ethanol led to concerns about repurposing valuable farmland to produce fuel instead of food. The importance of metabolic engineering for biofuel production cannot be overstated, as it offers a sustainable alternative to fossil fuels without risking much-needed farmland. Many scientific projects have used engineered strains of algae, E. coli or yeast to produce elements needed to create alternate fuels.

One goal is to tune organisms to process fatty acid feedstock from sunflower, palm or nonedible crops and convert them into biofuels. In recent work, scientists from Rensselaer Polytechnic Institute and the Massachusetts Institute of Technology were able to increase fatty acids production in E. coli with a genetically encoded metabolic switch that allows for optimized pathway expression by redirecting resources toward fatty acids biosynthesis, reported "Improving Fatty Acids Production by Engineering Dynamic Pathway Regulation and Metabolic Control" in Volume 111, No. 31 of the *Proceedings of the National Academy of the Sciences (PNAS).*

FapR, a transcriptional regulator that occurs naturally in E. coli, was rewired to achieve optimal control of resources involved in processing of malonyl-CoA, a carbon donor. The metabolic switch's ability to redistribute resources resulted in a "balanced metabolism between cell growth and product formation and significantly improved fatty acids production," according to the report. "The synthetic malonyl-CoA switch engineered in this study opens up new venues for dynamic pathway optimization and efficient production of malonyl-CoA-derived compounds."

Fatty acids production for the engineered E. coli was 15.7 times higher than a naturally occurring strain of the organism.

Another recent development was reported by scientists at the Oak Ridge National Laboratory in *PNAS*. They engineered Caldicellulosiruptor bescii, bacteria that can break down biomass, to produce ethanol from switchgrass, an abundant but nonedible plant. Pretreatment for switchgrass and other plants can be necessary in biofuel development, but it is a cumbersome and expensive process.

Using high-temperature fermentation, the researchers were able to convert switchgrass to ethanol without pretreatment. Through their work, C. bescii was engineered to remove lactate dehydrogenase by integrating a plasmid containing genetic material from the related organism, Clostridium thermocellum.

"Whereas wild-type C. bescii lacks the ability to make ethanol, 70 percent of the fermentation products in the engineered strain were ethanol [12.8 mM ethanol directly from 2 percent (wt/vol) switchgrass, a real-world substrate] with decreased production of acetate by 38 percent compared with wild-type," according to the article.

These examples underscore the potential we see in using metabolic engineering to optimize simple organisms to produce biofuels, often through fermentation.

## Industrial chemicals

Fermentation and metabolic engineering are also important factors in manufacturing chemicals that are used on a massive scale for industrial purposes, such as solvents and paints.

One example is butanediol (BDO), a compound that in different variations can be a solvent, an ingredient for rubber and more. Global demand for just one form, 1,4-butanediol, is in the billions of dollars annually. Bio-based forms of butanediol offer an environmentally friendly alternative to typical butanediol production pipelines, which rely on petroleum for synthesis of the compound.

Many teams are working to design microorganisms that would produce ingredients for 1,4-BDO in a renewable manufacturing process. In one recent project, scientists from the Research Institute of Biotechnology in Korea engineered E. coli for high-level production of O-Succinyl-L-homoserine, an intermediate used in the fermentation process that yields butanediol. Compared to petroleum-based conversion of the compound, the researchers suggested in the *Journal of Industrial Microbiology & Biotechnology* that their bio-based workflow could reduce overall manufacturing costs for butanediol.

Other efforts have involved yeast instead of E. coli. In two published reports from scientists at the University of Illinois at Urbana-Champaign, Saccharomyces cerevisiae was engineered for the production of 2,3-BDO. Researchers designed a strain of S. cerevisiae with genes from a bacterium to produce 2,3-BDO from cellulosic sugars. Their initial process resulted in substantial amounts of an undesirable ethanol byproduct, according to an article in *Applied Microbiology & Biotechnology*. So they modified their design by introducing a deletion mutant and engineered another strain that produced 2,3-BDO without ethanol production.

In separate work described in *Metabolic Engineering*, researchers also adapted S. cerevisiae to produce 2,3-BDO efficiently and without generating ethanol, but their engineered yeast strain was able to consume glucose and galactose at the same time, which appears to be the first time this has been accomplished.

"The high titer and yield of the enantiopure R-BDO produced as well as the ability to co-ferment glucose and galactose make our engineered yeast strain a superior host for cost-effective production of bio-based BDO from renewable resources," the scientists wrote in "Metabolic Engineering of a Saccharomyces cerevisiae Strain Capable of Simultaneously Utilizing Glucose and Galactose to Produce Enantiopure (2R,3R)-Butanediol."

## Biopharmaceuticals

Yeast is used often for manufacturing biopharmaceuticals, which are therapeutics created by biological processes. Vaccines, proteins and other biopharmaceuticals can be made by inserting specific materials into a host organism, which grows or secretes the desired product.

While various organisms are used for these processes, the yeast Pichia pastoris is one of the most commonly used host organisms in biopharmaceutical development, so it is often the focus of metabolic engineering projects. A recent report from scientists at East China University of Science and Technology in Shanghai compares two strains of P. pastoris, each engineered with a different level of protein expression to compare their metabolism and production capabilities. Their results were reported in the *Journal of Biotechnology*. The findings, based on changes seen during the organism's growth cycle, show that the yeast can be genetically altered to optimize production of heterologous protein, an important type of biopharmaceutical.

For some therapeutic targets, the native host strain is necessary for growth but least optimal for scalability of molecule production. One example is nonribosomal peptides, natural products that are biologically interesting from a therapeutic perspective and are found in various bacteria.

However, this class of protein is difficult to scale in its native host strains. Recent work in *Applied Microbiology and Biotechnology* shows that E. coli can be modified to express the antibiotic valinomycin using highly controlled growth conditions. Further, this finding suggests potential scalable solutions in E. coli for other nonribosomal peptides, according to "Enhanced Production of the Nonribosomal Peptide Antibiotic Valinomycin in Escherichia coli through Small-Scale High Cell Density Fed-Batch Cultivation.

## On the Web

Industrial engineering's multidisciplinary practices have been involved in improving biomedical technology for a couple of decades, as detailed in "Regenerative Medicine Manufacturing" from the August 2013 issue of *Industrial Engineer*. Visit *www.iienet.org/IEmagazine/aug2013/Shirwaiker* for the story.

## Moving forward

As rational design from synthetic biology becomes more broadly applicable in industrial engineering, it is interesting to consider to what extent best practices in manufacturing might be applied to production at the microorganism or cellular level.

Lean manufacturing, for example, highlights the need to reduce byproducts, such as the ethanol created as part of the butanediol production by certain strains of yeast. It is also worth considering how to implement Six Sigma processes in a manufacturing workflow that relies on biological organisms, which are inherently more complex than petroleum-based processes that rely on chemical mechanisms.

Is it possible to standardize the biological processes to be measured, controlled and improved? Models already are being tested. One such strategy uses pumps to remove product and reduce buildup of toxic compounds. In addition, feedback loops enable a biological measure of performance as well as a means of regulation, work reported in "A Model for Improving Microbial Biofuel Production Using a Synthetic Feedback Loop" in *Systems and Synthetic Biology*.

Used with the proper caution, metabolic engineering appears to be an effective and scalable alternative to petroleum-based and other traditional types of manufacturing for important chemicals, compounds and energy. ❖

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