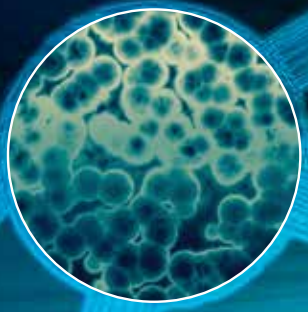
SBE SPECIAL SECTION:

SYNTHETIC BIOLOGY



Synthetic Biology: Fueling Industrial Biomanufacturing

The year 2022 marks ten years since the publication of the landmark study by Jennifer Doudna and her colleagues that pointed to a new revolutionary method for editing DNA. Today, CRISPR — and its associated gene-editing techniques — is one of the most well-known tools of synthetic biology and has paved the way for new advancements in this field. Synthetic biology has made great strides since *CEP* last published a special section on this topic in September 2016.

This special section takes an in-depth look at some of the companies innovating in the synthetic biology space, and discusses what will need to happen to maintain the talent pipeline for this industry to further spearhead such discoveries. Many of the articles in this special section are based on presentations given at the 2022 Synthetic Biology: Engineering, Evolution & Design (SEED) Conference, hosted by the Society of Biological Engineering (SBE) in May 2022.

The first article in the special section (pp. 24–29), written by Matthew Lipscomb, describes the technology being commercialized by DMC Biotechnologies. Their Dynamic Metabolic Control (DMC) platform has the ability to manufacture a wide variety of chemicals used in consumer and industrial products. DMC has engineered a microbe and fermentation process that decouples the growth of the microbe from product formation, minimizing the time and cost to go from the lab to commercial-scale production. Such a platform holds promise for reducing greenhouse gas (GHG) emissions from the U.S. chemical industry — an industry that has traditionally relied on petroleum feedstocks — and alleviating supply chain concerns by decentralizing the production of chemicals.

Sarah Richardson similarly emphasizes the importance of biomanufacturing in moving society away from its dependence on fossil fuels. In her article "Domestication is the Ancient Past and Imminent Future of Biomanufacturing" (pp. 30–34), she explores how humans have traditionally leveraged bacterial capabilities for a variety of applications. She believes that the dependence of synthetic biologists on a few chassis microbes have limited our ability to deploy biomanufacturing more widely. As a result, she founded MicroByre in 2017 to domesticate bacteria that are underleveraged in biomanufacturing. By genetically engineering bacteria that are already predisposed to make a certain product, the company is working to more readily produce chemicals in a sustainable yet cost-competitive way.

Engineers at Novome are also harnessing previously underleveraged bacteria — which happen to be sourced directly from the human gut. In the third article of the special section (pp. 35–39), Lauren Popov and Liz Shepherd describe Novome's approach to cell therapy to treat human diseases. Novome is engineering defined therapeutic activity into a single gut commensal bacterial genus called *Bacteroides*. Today, there are no U.S. Food and Drug Administration (FDA)-approved engineered live bacterial therapeutics on the market; Novome is looking to be the first company to reach this goal with its genetically engineered microbial medicine (GEMM) to treat enteric hyperoxaluria (EH). Patients with EH often suffer from recurrent calcium oxalate kidney stones, kidney damage, and end-stage renal disease. The company advanced its EH treatment to Phase 2a clinical trials in 2022, results of which are anticipated in 2023. The article offers a fascinating look at the future of therapeutic modalities and how synthetic biology will have direct and meaningful impact on human lives.

Training the workforce required to drive the bioeconomy and bring new biotechnologies to fruition will be no easy task. The final article in the special section (pp. 41–45), by Thomas C. Tubon and Jim DeKloe, describes some of the steps that must be taken to grow and sustain the skilled biomanufacturing workforce. These tactics include promoting new career paths, championing non-traditional learning pathways such as credentialing and skills retooling, and engaging underserved and underrepresented groups.

Synthetic biologists continue to harness a wide array of cutting-edge tools such as CRISPR-based gene editing, high throughput DNA sequencing, and DNA synthesis to aid various sectors of industrial biomanufacturing. By continuing to develop and improve these technologies, research in the field of synthetic biology will act as a springboard into a more sustainable future and better way of life.

Emily Petruzzelli, Editor-in-Chief, CEP

Precision Fermentation Can Lead the Way to Sustainability in the Chemical Industry

Matthew Lipscomb ▪ DMC Biotechnologies, Inc.

Industrial biotechnology offers an avenue for large-scale production of chemicals without requiring petroleum feedstocks. One company's innovations in this space hold promise for meeting decarbonization goals in the chemical industry.

Public attention has largely focused on automobile exhaust and coal-fired power plants as pollution sources, but less scrutiny has been given to the manufacture of chemicals needed to make everyday products as a source of greenhouse gas (GHG) emissions.

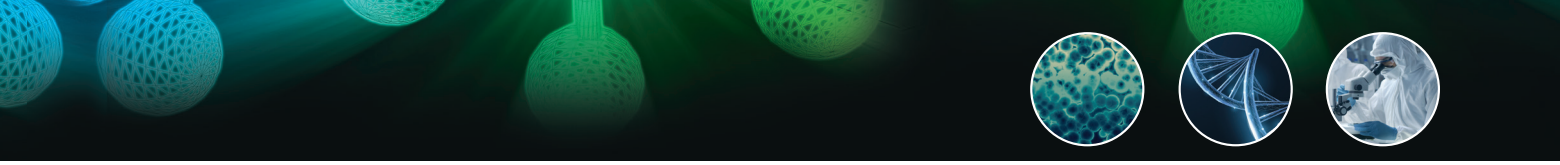
The footprint of the U.S. chemical industry's GHG emissions is over 200 million m.t. of carbon dioxide equivalent ($MtCO_2e$) per year — a little less than a third of the 617 m.t. released by passenger cars — making the industry a significant contributor to the nation's GHG emissions total *(1, 2)*. Moreover, this number is expected to double over the next 30 years if abatement measures are not implemented *(1)*.

The threat of climate change has given rise to initiatives to decarbonize by reducing GHG emissions. A growing coalition of countries, cities, businesses, and institutions have pledged to achieve net-zero emissions by 2050. More than 70 countries, including the biggest polluters — China, the U.S., and the European Union — have set a net-zero target, which would cover about 76% of global emissions *(3)*.

This target is increasingly being reinforced by regulations that are driving the adoption of sustainable practices, such as the landmark Inflation Reduction Act in the U.S., which is the most aggressive action to combat the climate crisis in the country's history, and Europe's recently approved Carbon Border Adjustment Mechanism (CBAM), a tariff on carbon-intensive products imported by the European Union.

The net-zero target is also being reinforced by consumer demand for sustainability, which is at an all-time high *(4)*. In the face of these pressures, customers for chemical products are increasingly focused on environmental, social, and governance (ESG) strategies, which are among the factors also considered by investors in measuring the sustainability of an investment in a specific company.

For the chemical industry, the decarbonization of chemical production presents a challenge — and an opportunity *(1)*. Successfully developing low-carbon products and solutions will require embracing new technologies and moving production closer to end markets. It also holds the potential to address the threat of climate change, to capture additional value, and to drive long-term growth. A recent Deloitte report *(1)* highlights how developing a sustainable product portfolio will be at the heart of a successful chemical company, noting that companies who don't adapt their manufacturing processes will face increasing



pressure from end-market consumers.

One way of achieving decarbonization is through innovative, next-generation industrial biotechnology. The application of biotechnology to chemical synthesis uses microbial organisms to produce chemical products from the fermentation of feedstocks like renewable agricultural sugars. Products include intermediate and specialty chemicals for human nutrition, animal feed, sweeteners, personal and home care products, materials, monomers, and more.

Today, industrial biotechnology is attracting increased interest because it doesn't rely on petroleum feedstocks, which are finite resources that can only be expected to become more expensive and are associated with GHG emissions. Producing more chemicals using industrial biotechnology could reduce the environmental impact of the chemical industry, while also lowering dependence on foreign petroleum.

The biobased manufacture of chemicals has historically required exorbitant costs and timelines to commercialize. DMC Biotechnologies has developed a proprietary precision fermentation process that addresses the barriers that have challenged the industry for decades. By making the development of biobased chemicals more affordable, the company offers an exciting opportunity to move them into the mainstream.

DMC's technology, Dynamic Metabolic Control (DMC), holds the prospect of harnessing biotechnology for the manufacture of a wide variety of chemicals used in everyday consumer and industrial products — chemicals that are economically attractive, have a lower environmental impact, and support local economies with a distributed manufacturing model.

This article will explore how DMC is accelerating the adoption of sustainable practices and goals in the chemical industry.

The opportunity for stronger supply chains

The COVID-19 pandemic and the war in Ukraine have exposed the fragility of global supply chains. Because many chemicals used to make everyday products are manufactured in China, chemical customers have been forced to cut back on production, which has affected products ranging from paint to plastic bags.

In addition to reducing the industry's carbon footprint, the ability to make biobased chemicals at a cost that is competitive with conventional petroleum-based products would support the re-establishment of chemical manufacturing in the U.S. and Europe, allowing chemical customers to strengthen the resiliency of their supply chains. This, in turn, would lower prices, create jobs, and promote national security.

The on-shoring of biobased chemical manufacturing would also help reduce GHG emissions because feedstocks would come from sustainable plant sources rather than from petroleum, as in China. In addition, the reduction of GHG emissions associated with transporting chemical products from distant offshore production sites would be reduced.

The difficult path to commercialization

Historically, the path to commercialization for biobased chemicals has been costly and challenging. In synthetic biology, the metabolism of microorganisms such as bacteria and yeast are manipulated so that they will grow and produce the desired molecule at the same time. But the engineering of biology is a notoriously complex business.

The microbes used in fermentation are typically sensitive to process conditions, which has meant that each time a new strain is created or advanced to a larger scale, the process must be re-optimized for each process variable, such as oxygen concentration, pH, temperature, medium composition, feed rate, and more. As a result, many cycles of process development are typically required for every new strain and scale. Importantly, the cost and the time required to conduct this process development work increase exponentially with scale.

A major impediment to early efforts to produce biobased chemicals at commercial scale, which date to the early 2000s, was a lack of tools for engineering microbes. The sequencing of the human genome in 2003, which marked the dawn of the genomics age, has brought a dramatic reduction in the cost of DNA sequencing and the emergence of new tools for editing and manipulating the microbial genome.

Though the coupling of advances in genomics with those in computing, data processing, and artificial intelligence (AI) has fueled a wave of biobased innovation *(5)*, microbial engineering largely remains a trial-and-error process, which will be discussed in more detail below.

Despite the challenges in the early phase of the industry, however, some notable successes have been achieved:

• NatureWorks (www.natureworksllc.com), a joint venture of Cargill and the Dow Chemical Co., was formed in 1997 to manufacture Ingeo polylactic acid (PLA). The building block of Ingeo is lactic acid (made by fermentation of plant sugars), which is transformed into a family of packaging polymers used to make a variety of consumer products like coffee capsules and yogurt cups. The Ingeo plant is located in Blair, NE, and came online in 2001 *(6)*. Based in Minnetonka, MN, NatureWorks is now jointly owned by Cargill and PTT Global Chemical, a Thai state-owned company. Last year, they announced plans to build a second plant in Thailand *(7)*.

• Dupont and Tate & Lyle Bio Products successfully commercialized 1,3 propanediol (1,3 PDO) *(8)*, a monomer used in a variety of industrial products, in 2006 at a manufacturing

plant in Loudon, TN. Their 1,3 PDO is sourced from plant-derived starch in place of petroleum. This success has led to at least one plant expansion and notable products including Dupont's Sorona (https://sorona.com), a polymer fabric line. Given the state of biotechnology tools available in the field at the time, it is not surprising that it required many years and hundreds of millions of dollars. The lessons learned from this pioneering venture would subsequently be leveraged by the team at Genomatica, but the technology itself was not able to be leveraged into other products.

• Genomatica launched its first commercial plant for the production of renewable 1,4 butanediol (1,4 BDO), a chemical intermediate that is formulated into a variety of consumer products including spandex and plastics, with Novamont in Italy in 2016. Genomatica announced last year that it is licensing its technology to Cargill, which will participate in a joint venture called Qore with the German chemical company HELM to build a second plant in the U.S. *(9)*.

Although synthetic biology has made technological progress over the last decade, significant limitations still exist. These early successes are not platform technologies: they are artisanal in nature, resulting from arduous efforts in technology development and market creation that required significant time and money.

The high cost of product development means that, broadly speaking, products manufactured through fermentation of agricultural feedstocks remain more expensive than those derived from petroleum *(5)*. But it is important to keep in mind that the petroleum refining industry has nearly a 170-year head start on the field of biotechnology and has benefited from a variety of federal subsidies for almost the entirety of that time *(10)*. The production costs for new technologies are frequently greater than the incumbent that they replace — at least initially. Consider the introduction of the automobile to replace the horse-drawn carriage as the preferred mobility solution. Initially, it was much more costly to own an automobile, but the net advantages in the long term resulted in the end of the era of the horse-drawn carriage.

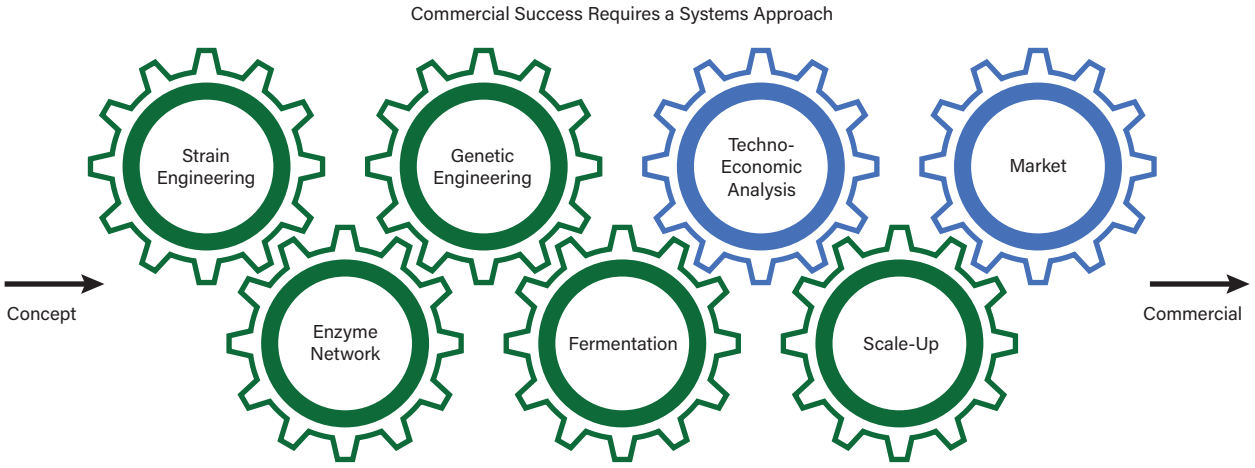
The development of Dupont and Tate & Lyle's 1,3 PDO pathway, which required 15 years and 575 person-years of effort *(11)*, offers a case in point. Indeed, it has taken on average seven years and $75 million per product to get to commercial performance metrics through traditional approaches — and that's without the cost of building a plant *(12)*.

If U.S. biobased chemical manufacturing is to achieve commercial success and concomitantly have a positive impact on decarbonization, the costs of development and production will have to be dramatically reduced.

Current challenges in biobased chemicals

In the past decade, AI and machine learning (ML) have been increasingly integrated within biotech. This effort has been fueled by the extraordinary success of tech companies (and an entire generation of venture capital companies with successful exits eager to deploy their capital) that have been willing to invest huge amounts of money — more than $3 billion — in biotech research and development (R&D).

The confluence of biotech and tech has focused on what many consider the main reason for the failure to economically produce biobased products: the inability to complete enough design-build-test-learn (DBTL) cycles in strain



▲ **Figure 1.** In the past decade, the predominant approach to biotechnology has assumed that the completion of more design-build-test-learn (DBTL) cycles in strain engineering would result in advancement of more projects to commercialization. To enable more DBTL cycles and to synthesize the massive amount of data being generated, machine learning (ML) approaches have dominated the discussion. But strain engineering DBTL cycles are just one part of the entire system required to get to commercialization. New technologies that are specifically designed to enable efficient commercialization are required to realize the potential of biotechnology to decarbonize the chemical industry. DMC has demonstrated one such approach that is intentionally designed to address the historical barriers, integrate multiple systems (as illustrated by the green "cogs" in this figure), and enable a standardized and systematic approach to biomanufacturing.

development *(13)*. DBTL is an approach based on the military's observe, orient, decide and act (OODA) loop *(14)*, a decision-making tool in which a hypothesis is made (design), a strain is created (build), the hypothesis is empirically tested (test), and the outcome of the hypothesis testing is incorporated into a model that informs the next cycle (learn).

The prevailing hypothesis in the field of biotechnology over the past decade has been that the limiting step in the DBTL cycle was the learn step; by applying ML, researchers assume that completing a greater number of test cycles will yield greater progress. But this hypothesis fails to appreciate the scale of experimentation required (Figure 1). When ML is applied to building a smartphone app, the developers typically benefit from the ability to obtain their training datasets at zero cost. But in biology, every single one of those experiments has a cost. For example, one approach to combine AI/ML with biology involves engineering every nucleotide in the genome for every other nucleotide to see what happens (a screening process known as single nucleotide polymorphisms).

To put the scale required for this approach into perspective, the relatively simple microbe, *E. coli*, for example, has about 4,000 genes. If each gene is treated as a simple two-factor interaction (ignoring higher-order interactions), the potential design space would exceed 10^{24} (or a septillion). This is roughly equivalent to the number of stars in the known universe. In other words, it is a brute force approach requiring massive empirical experimentation (and associated cost).

The limitations of this approach can be explained by contrasting it to that of SpaceX and Blue Origin. These tech-backed aerospace companies did not launch thousands of rockets in the hopes that an AI would figure out how to successfully put a satellite into orbit. Rather, they took advantage of more than a century's worth of knowledge and experience in astrophysics to identify the critical challenges and define the specific engineering approaches to overcome them.

Further complicating the picture is the fact that, to the chagrin of those in the biotechnology field, manipulating life is not as easy as engineering hardware: the biology gets in the way of the engineering *(15)*.

"[A] major challenge exists because of our incomplete knowledge of how life works, the daunting complexity of cells, the unintended interference between native and synthetic parts, and — unlike typical engineered systems — the fact that cells evolve, have noise, and have their own agenda such as growth and adaptation," writes one expert *(11)*. "The guiding question, therefore, is how do we develop a new way of engineering in the face of these unique and complex features of biology?"

Bio-design automation, as the ML approach to biotechnology is called, has enabled higher throughput and reduced human error. But there has also been a misunderstanding of the difference between doing work and making progress. With regard to the DBTL cycle: Companies are now able to complete more DBTL cycles than ever before with AI and ML. But where are the products?

The thesis that simply completing more DBTL cycles will naturally result in making improvements in a desired bioprocess performance is like running on a treadmill. You can accumulate miles and vertical gain on a treadmill, but if your goal is to stand on top of the mountain, the treadmill won't get you there. The fact that there is little to show for the massive screening approach to biobased manufacturing isn't a surprise; it simply isn't possible in any reasonable amount of time or with any reasonable amount of money to screen through the impossibly large design space, even with AI as an enabler.

I think we can be confident that the approach of conducting a massive amount of experimentation and letting a computer figure it out has not addressed the key barriers in the field.

The DMC technology solution

DMC's technology uses a standardized, two-stage fermentation process that, in combination with gene silencing and targeted proteolysis, decouples the growth of the microbe from product formation (Figure 2). The technology limits the ability of the microbe to respond to the environment, enabling a standard process that is independent of product or scale.

In the growth stage, the microbe is grown at the maximum theoretical rate and yield, which achieves the desired biomass concentration at a rate equivalent to a wild-type strain. In the production stage, the metabolic network is dynamically "re-wired" using pre-programmed genetic ele-

▲ **Figure 2.** DMC's technology uses a two-stage fermentation process that decouples the growth of the microbe from product formation. Key benefits include standardization, strain robustness, and predictable performance.

Microbes have evolved over millennia to respond to their environments. While that may be advantageous for survival in the wild, it has created the need for significant work in process development in the industrial environment.

ments (*e.g.,* gene silencing and targeted proteolysis) from its original state to a minimal metabolic network that is optimized for the conversion of feedstock to product.

The impacts of network minimization include:

• reducing the complexity of engineering biology by dramatically limiting the relevant design space

• reducing potential adverse impacts of the product on the microbe (such as growth inhibition due to product toxicity)

• reducing the microbe's response to the process environment, which increases robustness across a broader range of process conditions.

To go back to the previous *E. coli* example, network minimization reduces the design space from 10^{24} to roughly 50. The minimal network can then be experimentally explored with statistical confidence in a matter of weeks.

The key features and benefits of the DMC technology include:

• *Standardization*. The DMC technology further simplifies biomanufacturing by using a standardized set of equipment, operations, microbes, and feedstocks. In traditional bioprocessing, the individual strains used for each production process require unique process development and scale-up and are sensitive to even minor changes in the physical properties of the fermentation broth such as temperature, pH, and dissolved oxygen (DO), which typically occur at different scales.

With the DMC technology, the strain is more resilient to these changes, eliminating the need to develop a new process for each new strain, product, or scale. As a result, biobased chemicals can be commercialized faster and with significantly lower R&D costs than with traditional approaches, enabling the profitable biological production of a larger variety of products, including specialty chemicals and even commodities.

• *Robustness*. Robustness is defined as a relative lack of responsiveness to the environment. Microbes have evolved over millennia to respond to their environments as a means of survival. While that may be advantageous for survival in the wild, it has created the need for significant work in process development in the industrial environment. With the DMC technology, bioprocesses can tolerate a greater range of industrial process conditions, resulting in fewer lost batches and better economics than other approaches.

The dynamic deregulation of metabolism using two-stage dynamic control results in improved strain and bioprocess robustness. Microbial strains have been engineered for the improved scalability of important industrial chemicals at scales ranging from 200-µL plates to 85,000-L fermentation tanks and larger. Many pathways and product chemistries have been advanced to different stages of commercial readiness, including but not limited to: alcohols, diols, polyols, amino acids, organic acids, esters, monoterpenoids, sesquiterpenoids, and carotenoids.

By engineering robustness, or insensitivity to process conditions, a standardized process can be developed independent of product *(16)*. This allows DMC researchers to evaluate a larger number of genetic variants, enabling challenges to be overcome with metabolic engineering strategies rather than process changes.

• *Predictable performance*. In traditional industrial biotechnology, results obtained from high-throughput or bench-scale experiments often do not translate, even in the same microbe, to a larger scale. The net result of standardization and process robustness is predictable performance, as demonstrated by DMC technology *(16)*. The frequent failure of small-scale screening studies to readily translate to larger-scale production processes has been a major factor inhibiting the commercialization of fermentation processes.

By contrast with traditional approaches, the DMC technology has led to a rapid acceleration of the development and commercialization of biobased chemicals. By 2020, DMC Biotechnologies had produced four products with commercial performance metrics and demonstrated scale (3,000-L scale fermentation). It has also brought its lead product, L-alanine, to 85,000-L scale fermentation, and has a full pipeline of products with markets such as intermediate chemicals, nutrition, and personal and home care, among others.

Furthermore, all of this was accomplished with an investment of less than $15 million, which is orders of magnitude faster and more economical than any other technology in the field. DMC Biotechnologies has achieved the creation of a true platform technology with demonstrated capability to produce a diversity of chemistries, a library of chassis microbes that is ready for deployment, and a standardized fermentation process that works for every product and at every scale.

Rethinking the paradigm

Chemicals derived from fossil fuels have been the building blocks of modern life for as long as any of us can remember. But their use is contributing to one of the major threats to life as we know it: climate change. By making biobased chemical manufacturing more affordable and sustainable, biotechnology has the potential to revolutionize how we

make chemicals and how we feed a growing population.

But the successes need to be faster and more cost effective. DMC Biotechnologies' versatile technology platform addresses key barriers by transitioning the approach to product development from one based on random screening to an engineering discipline with standardized, predictable, and robust methods, shortening development times and reducing development costs for a wide range of products.

Processes enabled by biotechnology are potentially more sustainable than incumbent manufacturing approaches, which can help reduce the industry's carbon footprint. For example, an independent, third-party analysis demonstrated that DMC Biotechnologies' production of L-alanine in Europe will reduce CO_2 emissions by more than 90% over imported L-alanine, which is the equivalent of removing 62,000 cars from the road each year.

In addition to producing more sustainable, affordable products, biotechnology holds the potential to benefit the economy by promoting the onshoring of chemical production, reducing the risk of global supply chain disruptions, supporting regional agricultural production, and creating new biomanufacturing jobs.

The field of metabolic engineering was launched in the late 1990s by applying engineering principles to biology. The application of tech to biology in the 2010s spurred what would come to be known as synthetic biology. Today, we stand at the cusp of the next evolution in the field. But harnessing precision fermentation to remake countless everyday materials will require rethinking the industrial biotechnology paradigm to embrace innovative technologies and overcome the key barriers upon which the field has historically stumbled. CEP

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Domestication Is the Ancient Past and Imminent Future of Biomanufacturing

Sarah Richardson ▪ MicroByre

Domestication is the most successful bioengineering project humans have ever undertaken. To address the climate emergency, we must deploy the principles of domestication to onboard new microbes that can bring an economic challenge to petroleum supremacy.

No organism is an island; there is no such thing as a successful single-species ecosystem. This is true for wildlife, for human agriculture, and for corporate boardrooms. Diversity is how we survive, and it is an equilibrium that successful ecosystems seek. Every multicellular organism lumbers around with an entourage of bacteria that has a direct and measurable impact on its existence. Every plant cultivates its own crop of roommate microorganisms, encouraging helpers and stamping out freeloaders. And, every organism across every biological kingdom coexists with a staggering number of viruses, most of which do not cause disease.

While organisms specialize and cooperate, their genetic makeups reflect this by changing, adding, and discarding genes over time in response to success or failure at their new tasks. Humans know how to take advantage of this mechanism and have successfully deployed the skills of our planetary cohabitants to great advantage. But when it comes to microbes, we have not fully embraced the lessons or philosophy of such domestication.

This article explores symbiotic relationships and discusses how humans have traditionally leveraged bacterial capabilities for an unending variety of applications. It also discusses a key stumbling block of the synthetic biology approaches of today, and describes how one company is changing the paradigm by harnessing previously undomesticated bacteria for specialized tasks.

Exploring a long history of domestication

Agriculture has always been genetic engineering. Bioengineers have worked for thousands of years without the title and so their work has lacked the label of biomanufacturing. The Greeks and Romans began the process of turning wild cabbage into kale by selecting wild cabbage for leaf production. In the 1500s, the same plant was adapted to broccoli by selecting for flower buds and stems and cauliflower for just flower buds. Cabbage was selected for terminal leaf buds, brussels sprouts for lateral leaf buds, and kohlrabi for the stem. Gai lan and broccoli hybrids were established in the 1990s and marketed as broccolini *(1)*. This was a concerted and deliberate collaboration with a plant that had no other reason to diverge so wildly from its found state; humans gave it space to specialize and worked to protect and propagate it, and it changed our diets (Figure 1).

Humans are not exceptional; if you look around nature you cannot help but find organisms directly modifying each other's behavior. Ants and termites farm fungus, while other ants farm aphids *(2)*. Damselfish farm algae that coral conservation experts would prefer not to have around *(3)*; they actively weed other algae species and spit the chunks away at the edges of their gardens. They defend their patches aggressively, and as a result, their algae is usually found only near them. Monkeys in Japan have been spotted riding deer by offering them food *(4)*.

Plants and charismatic megafauna are not the only participants in this phenomenon; microbes are prime targets for domestication. Many adaptations have taken place over millions of years as organisms grew to depend on each other. For example, ruminants depend on their microbiota to break down their cellulose-rich feed *(5)*. Humans depend on our gut microbiota to produce vitamins. In mammals in general, the aerotolerant *Escherichia coli* may be contributing to reducing the amount of oxygen present, which enables more specialized, but oxygen-intolerant, bacteria to continue to do their job of breaking down food and feeding us nutrients *(6)*. Over millions of years, mammals have negotiated with gut bacteria; the bacteria may give up some independence and hone their specializations, and we may share our food and offer protection. Both sides may alter their genetic content to facilitate and perpetuate the mutual benefit.

Our centuries-long development of fermentation for food is a highly visible form of microbial domestication not frequently labeled as such. For instance, the flavoring agent monosodium glutamate (MSG) — and its cousin amino acids used as animal feed additives — are produced in *Corynebacterium glutamicum (7)*. Xanthan gum is produced in *Xanthomonas campestris*. Many varieties of hot sauce are fermented by bacteria *(8)*. You can brine any vegetable and call it a pickle, but some of the best pickles are fermented by lactic acid bacteria, and you can do this safely at home *(9)*. The same lactic acid bacteria are used to ferment meat so it can be stored safely (without requiring massive additions of salt), thereby producing salami *(10)*. There is no cheese without fermentation, and there is no yogurt without microbes; today, the rennet enzymes that make the cheese coagulate are often produced by genetically modified fungus instead of cow stomachs *(11)*.

A few more examples of our mastery of fermentation: You can use acetic acid bacteria to convert any carbohydrate to vinegar, which explains the variety of vinegars you find in the grocery store. The Romans began fermenting otherwise inedible olives in alkaline brine to leach the bitterness out and add flavor *(12)*. Chocolate pods grow on trees and are colonized by yeast and bacteria. They are picked and packed into dark, moist crates, where the yeast make so much alcohol that they kill themselves, and then the bacteria take over to convert the alcohol to acid and flavor molecules *(13)*. Every coffee cherry has to be pulped to extract the bean from the skin and fruit. The bean is covered in a thick gooey substance called mucilage, and the easiest way to remove it is fermentation: the microbes add flavor while breaking down the mucilage so it can be rinsed off *(14)*. When cleaning clothing, three enzymes are useful: an amylase to break down starches, a protease to break down proteins, and a lipase to break down fats. These three are fermented in bacteria and then added to your Tide Pods *(15)* — that's why grass stains aren't as difficult to remove today.

Bacteria are capable of much, much more than the delicious souring of foodstuffs. Every ecosystem depends upon them to break down biomass and to run the carbon, nitrogen, and oxygen cycles. Bacteria produce chemicals and materials that are highly desirable, but in some cases, inaccessible by petrochemical synthesis. Scientists are still uncovering microbes that surprise us with their unexpected capabilities, honed by selection in niches that range from in our noses to deep under the earth.

For example, *Magnetospirillum magnetotacticum* is a magnetotactic bacterium that lives in fresh water and mineralizes metal for purposes we haven't quite identified *(16)*. *Scalindua* is an example of an anammox bacterium that can make hydrazine from nitrogen-rich environments *(17)*. *Frankia* fixes nitrogen for a surprisingly broad range

Cabbage
Selected for:
Terminal Leaf Buds

Kale
Selected for:
Leaf Production

Broccoli
Selected for:
Flower Buds and Stems

Cauliflower
Selected for:
Flower Clusters

◀ **Figure 1.** Cabbage, kale, broccoli, and cauliflower all originated from the same plant species. Humans "engineered" these vegetables by selecting the native plant for its terminal buds, leaf production, flower buds and stems, and flower clusters, respectively.

Good bioengineers realize that cells are neither modules nor machines; they are not inherently standardizable.

of plants; similar bacteria protect plants without the need to add pesticides, herbicides, or synthetic fertilizer. *Thermoanaerobacter thermohydrosulfuricus* is a heat-loving bacterium that makes an S-layer, a type of self-assembling mesh nanomaterial that is more consistent than similar materials we have tried to synthesize. *Cyanobacteria* are responsible for at least half of our atmospheric oxygen *(18)*. *Clostridium thermocellum* is one of the species of bacteria that breaks down the celluloses found in grasses and rough biomasses, deep inside in your compost pile.

We recognize the value and the potential of these bacterial capabilities, but they have not been domesticated. In fact, the word "domesticated" rarely comes up when discussing any bacterial species, even the ones that have been our loyal companions for centuries or even millennia. When we catch organisms in nature cooperating, we call it symbiosis. When humans work with bacteria with tools like CRISPR, we call it genetic modification. No matter what you call it, if two or more organisms are making a mutually beneficial pact and changing each other as a result, it is domestication. But if you don't think of the process as a two-way street, you are apt to offer your potential partner an untenable deal. If you think you don't have to adapt your methods for different species, you are apt to offend — *e.g.,* a dog may wag her tail to allow you to approach; a cat swishes hers to warn you away.

When it comes to microbes, many biotechnologists have discarded our rich history of collaboration for a philosophy of control. They tend to threaten with sticks rather than entreat with carrots — they apply the lethal selective pressure of antibiotics rather than a reward for deeper specialization. This is not how you ever approach an animal, and it is not how you successfully approach microbes, either.

Scientists and engineers may not recognize that the small handful species they consider fully genetically manipulable are the result of domestication! Those bacteria were not simply laying around ready to work with people; they spent 70 years in the laboratory adapting to those conditions. If you think of bacteria as "chassis" and DNA as a "program" that runs within them, you have strayed far from biology and the precedents that would help you realize scalable success. You are thinking like a computer scientist, a chemical engineer, or a physicist. Bioengineering must not borrow the wrong lessons from other engineering disciplines.

Good bioengineers realize that cells are neither modules nor machines; they are not inherently standardizable (although some of the tools we use with them are). They are self-replicating cells that make imperfect copies of themselves, and the emergent property of their growth is something that looks like choice. Order bacteria around and they will choose not to work with you. Embracing and leveraging their stochastic choices makes you a bioengineer. Ignoring or fighting them sets you up for failure.

MicroByre's new paradigm

In 2017, I founded a company, MicroByre, dedicated to the domestication of bacteria that are underleveraged in biomanufacturing. Domestication is deeply embedded in our corporate culture. Our name was chosen to respect the old efforts: byre is an old English word for cowshed, and the source of the word barn, so we are the bacteria barn.

We believe that the biggest bottleneck in deploying biomanufacturing more widely is the lack of flexibility in which organisms we can grow and manipulate, and how comfortable we are expanding that stable. Thus, at MicroByre, we get to know as many organisms as deeply as we can. We focus on those that offer promise to stalled areas of chemical development. We find species that cost less to ferment because they naturally secrete the products of interest, or they happily grow on biomass much less refined than the old standbys demand. We seek species that still work in existing hardware, but have inborn chemistries that were never successfully engineered into the model organisms.

Of course, it is not enough to merely find or know these bacteria — and many have been known for decades (if not to the level of detail that MicroByre demands). If these bacteria could be tamed into economic feasibility at scale, they would have been, just like the food-producing microbes I named. What's missing for most of MicroByre's favorite bacteria are the genetic tools that allow gain-of-function genetics, which is an advantage that bioengineers take for granted in *Yarrowia lipolytica*, *Saccharomyces cerevisiae*, *Bacillus subtilis*, or *Escherichia coli*.

When you ask the synthetic biologists who adhere to the programming philosophy why they are not working in the organisms from which they draw their genes, they usually say that the infrastructure is not available, that they do not have growth protocols or genetic toolkits, and sometimes that they do not know even which organisms make sense for such an investment. But, as we have learned at MicroByre, it is easier to learn how to alter the genomes of capable but recalcitrant organisms than to engineer skills into friendly but inept ones.

MicroByre's mission: deploy the right bacteria for the job, whether it be mining, agriculture, bioremediation, or biomanufacturing. First, determine which ones have the inborn talent for the task, so that the biochemical means to do that task do not have to be engineered in from first

principles. Learn each bacterium's specialties and tastes extensively. Quickly establish how to genetically engineer them to deepen their specialization even more. Finally, adopt them out to loving industrial homes, companies who are more than happy to receive a friendly bacterium that likes its job and offers a low-capital, cost-competitive way to produce chemicals that were previously only available from petroleum-based feedstocks.

Closing thoughts

Language shapes thought and thought drives action *(19)*. The analogies we use to describe our work, to train our students, and to communicate with our sponsors affects the limits of our imaginations. Our metaphors (*e.g.,* cells as chassis) set biases and influence what we find feasible and fundable. The analogies of genetic control have led us to spend decades struggling to import designed and redesigned DNA into a small set of historically manipulable species. When we apply that analogy at the macroscale, its deficiencies become obvious. You would never fund a company that sought to genetically engineer dogs to produce large quantities of milk, or a research group redesigning goats to catch mice. You would ask, "We have cows, we have cats. Why aren't we starting there?" And if the answer was "We do not know how to shelter, breed, or train those animals," your first thought might be "Well, let's go learn." Instead, we try over and over, gene after gene, chasing worthy targets in medicine, agriculture, and chemistry, trying every genetic permutation but getting the same biological answer: "I am not the right animal to help you with this." Comforting metaphors and comfort with old technology both limit us.

Dropping ten genes from one capable bacterium into a merely manipulable one to save ourselves the trouble of learning to shelter, feed, or train new species has proven to be a difficult model to scale. Our attempts have not even begun to definitively answer the pressing industrial and environmental challenges that biotechnology has sworn itself to address. It will be difficult to shift our perspective, but it will be worth it. We can learn the preferences of bacteria other than our old standbys and we can build genetic engineering capabilities in them. We can form the scalable symbioses that leverage their skills against our problems. We will, and must, do this to have any chance of mitigating the adverse effects of the climate emergency.

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Article continues on next page

More than 500 billion tons of carbon in biomass exists on Earth. Carbon is the currency of life, and bacteria are the middlemen moving it around. Humans used to make nearly all of our chemicals from biomass. In many ways, the shift to petrochemicals was one of convenience, but may have been facilitated by a misunderstanding of the importance and ubiquity of bacteria. We teach evolution as a tree with humanity at the top, with microbes at the foot as "primitive" or ancestral. This analogy is also misleading. The bacteria around us are as new as we are. They have tried a lot of chemistries and can teach us, but we won't learn if we don't cooperate with them.

The biotech industry as a whole needs a paradigm shift on the scale that was originally suggested by Thomas Kuhn in his work *The Structure of Scientific Revolutions (20)*. We cannot build on genetic control as a theory; it is incommensurable with domestication. We must drive a shift away from funding, policy, and infrastructure that set up bioengineering to be a struggle for dominance. Unfortunately, we do not have as much time to wage this campaign as we had to shift from Newtonian mechanics to quantum physics. The climate emergency, the contamination of our environment, and the mounting challenges of modern medicine are urgent. For scientists and engineers to do their part, we need to humble ourselves a little more before the clever solutions and evolutionary adaptations of microbes. CEP

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Living Medicines: Engineering Bacteria to Treat Disease

Lauren Popov ▪ **Liz Shepherd** ▪ Novome Biotechnologies

A new approach to cell therapy deploys engineered cells from the human gut microbiome to treat disease.

Remarkable progress in biomedical therapeutics has been achieved through the successive emergence of novel treatment modalities, with each new class of drugs opening up an exciting world of possibilities to address the diversity of human disease. In the 1980s and 90s, "biologics" came onto the pharmaceutical scene as an exciting new alternative to traditional small molecule drugs. To name just a few, biosynthetic insulin and antibody-based therapies, such as Humira and Herceptin, altogether revolutionized medical care for patients with metabolic disease, inflammatory conditions, and cancer. Similarly, the past two decades have followed with the invention of even more complex so-called "cell therapies," wherein patients are transplanted with functional, living human cells to treat a disease. For example, in CAR T-cell therapy, a patient's immune cells are removed from the blood, propagated, and genetically engineered in the laboratory to enhance their activity, and then transplanted back into the patient to fight against cancer. Such innovations have revolutionized the field of oncology and are now considered standard of care for a variety of blood cancers *(1)*.

At Novome Biotechnologies, we believe that cell therapies are the future of biomedical innovation. We are pushing the envelope for this class of drugs by engineering bacterial cells, not human cells, to transplant into patients. These bacterial cells live in the gut while treating disease and serve as the first engrafting, engineered microbial cell therapies to be developed.

Why use bacteria? The human colon is home to trillions of diverse, commensal (symbiotic) bacterial cells, and as such is arguably the most meaningful natural interface between the human body and foreign genetic material (Figure 1) *(2)*. This incredibly rich and dense bacterial community (*i.e.,* our microbiota) has a central — if still poorly understood — role in promoting health and disease outcomes. No two human microbiotas are exactly the same, and the activity of this complex internal ecosystem has so many important impacts on human health that some have referred to it as a "supporting organ." Despite the clear importance of our microbiota in shaping whether we develop a given

▲ **Figure 1.** The human large intestine is home to trillions of diverse microbial residents: predominantly bacteria but also fungi, parasites, and viruses. The sum total of this community is dubbed the "gut microbiota." The majority of these microbial residents are symbiotic, meaning both the bacteria and the human body benefit from their presence. Because the human gut microbiota factors into many diseases — impacting the gastrointestinal system and beyond — it is an ideal location to deploy engineered microbial cell therapies.

disease or respond to a particular drug therapy, the inherent complexity of this internal ecosystem has both obscured the biological details of how it functions and hindered attempts to selectively modify it.

Some companies, looking to leverage the power of our gut microbes as therapeutics, have adopted a holistic approach of transplanting entire naturally occurring communities from healthy individuals into patients with a particular disease, in a process called fecal microbiota transplant. Such donor-derived microbial therapies are inherently variable and attempt to promote health via largely unknown molecular mechanisms. Furthermore, introducing a new gut ecosystem wholesale requires initially displacing the patient's native microbiota with the use of broadly acting antibiotics, and even with such dramatic perturbations, the transplanted community may not persist over time *(3)*.

In contrast, at Novome, we are applying synthetic biology tools to engineer defined therapeutic activities into a single gut commensal bacterial genus, *Bacteroides*. We introduce our genetically engineered microbial medicines (GEMMs) into patients as a unique cellular therapy: a single bacterial strain that is rationally designed to engraft (or "colonize") into the gut and deliver its health-promoting effects with defined, controllable, and reversible activity. There are currently no FDA-approved engineered live bacterial therapeutics, and we are working to bring our vision of this new class of drugs to patients.

This article describes Novome Biotechnologies' approach to making engineered microbial cell therapies, including the key challenges and our solutions, real world proof of concept of this approach in ongoing clinical trials, and how we are expanding our platform to work in big disease spaces like inflammatory bowel disease (IBD).

Engineering non-model organisms to perform useful tasks

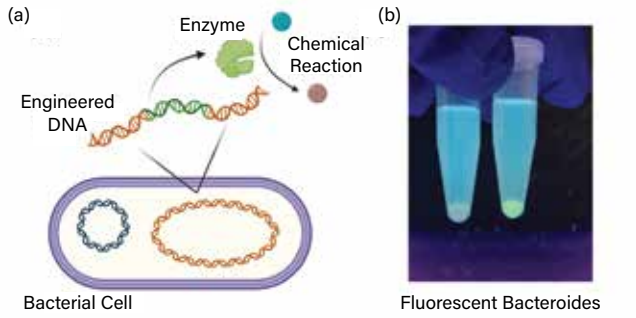
The first challenge in building therapeutic cells is to develop methods to engineer them. Most bacterial genetic engineering tools have been developed to work in *Escherichia coli*. This is the model bacterial organism in the lab as it grows easily in aerobic (oxygenated) conditions; however, *E. coli* is not abundant in the anaerobic (low-oxygen) gut environment. In contrast, the anaerobic genus *Bacteroides* constitutes the most abundant genus of symbiotic bacteria resident in the human gut microbiota, making up approximately 50% of total bacteria in the average American *(4)*. To take advantage of the abundance of the *Bacteroides*, we first set out to create basic genetic tools that would allow us to engineer these cells to perform useful tasks. Cells utilize proteins and enzymes, often in pathways that catalyze chain reactions, to produce molecules, break them down, or modify them. These proteins and enzymes are coded for in DNA, which we can manipulate in the lab and insert into our cells (Figure 2a).

As a proof of concept, we identified DNA sequences that would allow us to produce a protein in *Bacteroides* cells to turn them fluorescent green. We inserted this genetic construct into the DNA of our target *Bacteroides* strain and produced bright green cells (Figure 2b). With a basic DNA manipulation and protein expression system in place, we developed a method to produce genetic constructs and integrate them into our target cells in high throughput, allowing us to design and test many genetic modifications of interest at once *(5)*.

We continue to improve our ability to engineer *Bacteroides* strains both by expanding the types of genetic manipulations we can make, as well as the processes we use for making the genetic manipulations. We have recently incorporated robotic liquid handlers into our workflows, which allows for not only higher throughput production, but also automation and decreased demand on human inputs to our engineering protocols. This has expanded the types of strains we can produce, and the number of strains we can assay. We can now test large libraries of cells at once as opposed to a few strains or constructs at a time, enhancing our ability to design-build-test and generate new therapeutic strains.

Ensuring our engineered cells can reliably colonize the gut

With the ability to genetically engineer *Bacteroides* strains, we then needed to develop a strategy for getting them to engraft in the gut at appreciable levels. Given the complexity and variability of the gut microbiota across people, simply delivering our strain like you would a probiotic (such as the capsules you can buy at the drugstore) would not guarantee colonization and would result in variable density in the gut of individuals that did become colonized, demonstrated in Figure 3a.



▲ **Figure 2.** (a) Bacteria interact with their environment by expressing proteins encoded in DNA, many of which catalyze chain reactions. Novome researchers engineer *Bacteroides* cells to perform therapeutic functions by manipulating DNA in the lab; the DNA can then be re-inserted into the *Bacteroides* cells. (b) As a proof of concept, we engineered a strain of *Bacteroides* to fluoresce bright green.

To solve this problem, we developed a paired food source that only our strains could utilize (an exclusive prebiotic), giving them a competitive advantage relative to all the other microbes competing for commonly utilized food sources. We took a piece of DNA from a related strain of bacteria that enabled the cells to utilize porphyran, a carbohydrate from seaweed, and integrated it into our *Bacteroides*' genome. Porphyran is rarely consumed by other members of the microbiota, so the ability to metabolize it gave our strain an advantage in the competitive gut ecosystem. We tested the ability of our strain to colonize different human gut communities (modeled in mice) and saw that without porphyran, our strain engrafted to varying degrees, or not at all. When we administered porphyran in the diet of the mice, however, our strain not only colonized all the communities we tested, but it also grew to a high and comparable abundance across all communities (Figure 3a) *(6)*.

This exclusive metabolic niche concept has opened the way for engrafting live therapeutics in the gut microbiota. Future work on this portion of our platform will focus on expanding beyond porphyran as the exclusive food source, since some communities do have members that compete for it, and it's a naturally occurring product in the diet. Alternatives may be other rare, naturally occurring polysaccharides, or synthetic polysaccharides with engineered enzymes that break them down.

Containing colonizing GEMMs in the intended ecosystem

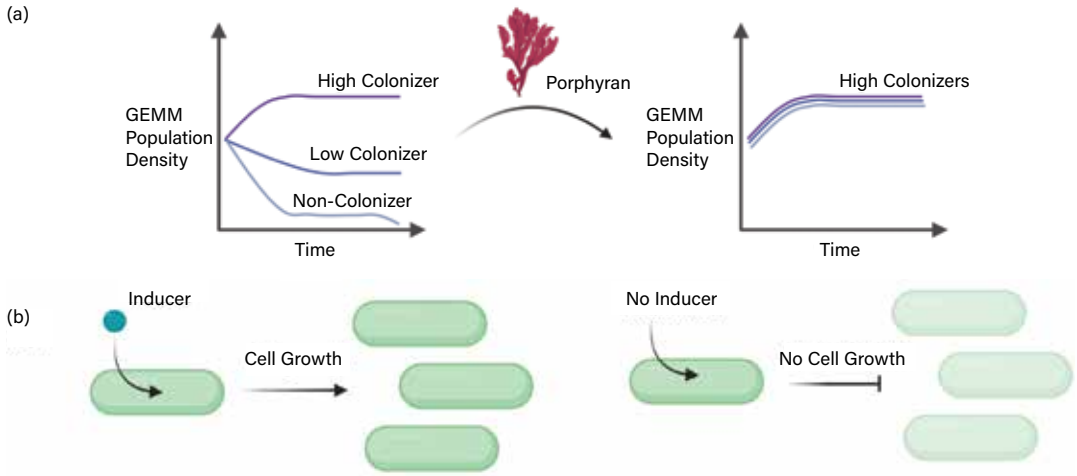
The third key to our platform for building GEMMs is reversibility. In order to safely deploy cells that are both engineered with therapeutic properties and designed to colonize and grow in the gut, we need to ensure that they will only live in the intended location, can be eliminated at will, and won't spread person to person through the environment. It may also be important to eliminate the strains from the gut if they were to mutate and stop acting therapeutically, so that we could replace them with efficacious strains.

To do this, we have put a gene that is essential for our cells' survival under inducible control. This means that without an inducer molecule, our cells cannot turn on this essential gene and thus can't grow, providing a genetic kill switch to our therapy (Figure 3b). To make things simple in our first-generation therapies, we are utilizing porphyran not only as the food source for our strains, but also as the inducer molecule that turns on the essential gene in our cells. In practice, patients colonized with our therapeutic bacteria consume porphyran daily so that our cells have an exclusive food source, and the porphyran turns on the essential gene that allows the bacteria to continue to grow in the gut. If patients were to stop taking porphyran, the cells would be unable to make their essential gene, would stop growing, and would be eliminated from the gut. Future work on biocontainment will focus on increasing redundancy of the biocontainment system to limit the ability of the strains to break the engineered system.

Novome GEMMs have been engineered to prevent kidney stones

With the tools in hand to engineer and reversibly transplant a bacterial strain in the gut, Novome identified enteric hyperoxaluria (EH) as an important disease where our GEMMs could deliver meaningful therapeutic activity.

Patients with EH suffer from an excess of the organic



▲ **Figure 3.** (a) The natural diversity of the gut microbiota across people results in variable engraftment of genetically engineered microbial medicines (GEMMs) when administered alone. The graph on the left shows mock data from testing our strain in mice harboring different gut microbiota, without giving porphyran. By administering porphyran alongside our GEMMs, which provides our GEMMs an exclusive food source inaccessible to other members of the microbiota, we can achieve high colonization regardless of the makeup of the pre-existing community (see graph at right). (b) To ensure our microbial cell therapies act only where intended, we have engineered them to grow only in the presence of an inducer molecule. Without it, the cells are unable to grow and will wash out of the gut.

molecule oxalate that is excreted in their urine. EH has been causally linked to recurrent calcium oxalate kidney stones, kidney damage, and end-stage renal disease. Oxalate has no known purpose in human biology, yet it is absorbed readily in our diet from a wide variety of foods and normally disposed of in urine and feces. Individuals with EH have a tendency to hyper-absorb oxalate from their diets, and thus are prone to subsequent kidney stones and kidney damage. There are currently no approved therapeutics for EH, and approximately 250,000 patients in the U.S. suffer from this chronic condition *(7)*.

Novome's EH therapeutic NOV-001 is engineered to consume oxalate present in the gastrointestinal tract and transform it into a harmless waste product (Figure 4). Because of our synthetic metabolic niche strategy, our therapeutic strain can grow to very high levels, which enables substantial removal of oxalate. In preclinical disease models of EH, animals colonized with our engineered therapeutic strain showed a 30% to 50% reduction in urine oxalate levels over controls. This magnitude of reduction in urine oxalate is anticipated to be clinically meaningful in reducing the occurrence of kidney stones in patients.

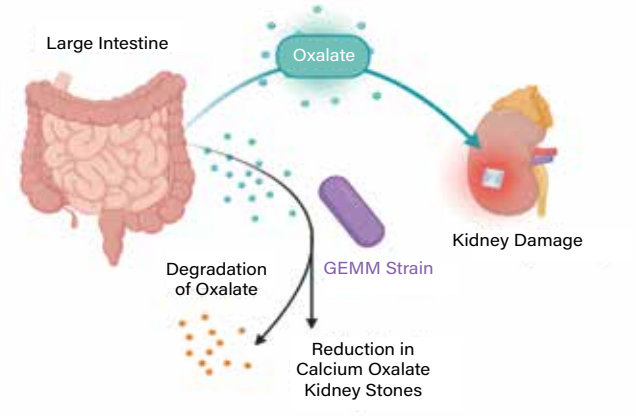
Novome completed a successful first-in-human Phase 1 study that demonstrated the ability to safely colonize the human gut with a therapeutically engineered microbe and control its abundance via once-daily dosing of a prebiotic control molecule. In this trial, Novome's microbial cell therapy was shown to be safe and well-tolerated in healthy subjects *(8)*. In 2022, Novome advanced NOV-001 into a Phase 2a study assessing the safety, tolerability, and early efficacy of NOV-001 in patients with EH. Results from this trial are anticipated in 2023.

Expanding the GEMMs platform to secrete proteins and treat IBD

In addition to our ongoing clinical trial in EH, Novome is developing its next generation of GEMMs through both internal research and partnerships to address challenges within important immunological and inflammatory diseases, such as IBD. IBD is a multi-factorial inflammatory disease where chronic inflammation of the lining of the digestive tract causes tissue damage. Common symptoms of IBD include diarrhea, chronic abdominal pain, bloody stool, and fatigue. In some cases, patients require surgical removal of portions of the intestine *(9)*. In 2021, Novome announced a multiyear strategic partnership with Genentech to develop bacterial strains that express and deliver specific therapeutically relevant molecules to targets in the human intestinal tract to treat IBD *(10)*. Many currently approved drugs for IBD are administered systemically, meaning the patient's entire body is exposed to the therapeutic molecule. A major potential advantage of using a colonizing bacterial cell to deliver anti-inflammatory therapeutics locally in the gut is that they may be more effective when delivered directly at the site of inflammation and limit side effects brought on by systemic administration.

While Novome's first GEMM aims to treat a disease by breaking down a toxic molecule present in the gut, battling localized inflammation to treat IBD will likely require delivering specific therapeutically relevant molecules in the gastrointestinal tract. Building and delivering protein molecules outside the confines of the microbial cell is something all bacteria do to influence their environment; however, getting bacteria to export sufficient amounts of non-native protein molecules presents a real technical challenge *(11)*. An obstacle for transporting protein out of *Bacteroides* cells is the presence of not one, but two lipid membranes that separate the inside of the bacterial cell from the outer environment. Recently, Novome developed a proprietary technology that overcomes this significant barrier and enables highly efficient protein delivery. The ability for our orally delivered GEMMs to introduce high amounts of therapeutic proteins to the gastrointestinal tract through continuous, controlled secretion represents a major breakthrough in the overall versatility of Novome's microbial cell therapy technology.

Since its founding in 2016, Novome has grown from an initial team of four to over 40 full-time employees headquartered in South San Francisco, CA. In September 2022, Novome raised $43.5 million dollars in Series B funding to continue advancing the next generation of bacterial cellular therapeutics, with a significant portion of that going toward advancing GEMM candidates for the treatment of IBD *(12)*.



▲ **Figure 4.** When too much dietary oxalate is absorbed from the gastrointestinal tract, it circulates to the kidneys where damaging calcium oxalate kidney stones can form. Novome's GEMM aims to treat an excess of oxalate by introducing genes that allow our engineered bacterial cells to robustly degrade oxalate into a harmless waste product. The presence of the GEMM is controlled by consumption of the porphyran control molecule. In preclinical animal models, our engineered GEMM reduces the amount of oxalate present in the urine between 30% and 50%. Novome is currently testing this microbial cell therapy in patients.

Closing thoughts

As the world of therapeutic modalities has greatly expanded since the early days of small molecule discovery to encompass such exotic medicines as engineered T cells and whole gut microbiota transplants, we strive to push the boundaries further and introduce the first genetically engineered bacterial therapies designed to engraft the human gut (Figure 5). The myriad diseases that are influenced by activities happening in the gut, many of which don't have existing effective therapies, are ripe for microbial cell therapy enabled by the development of GEMMs.

A pivotal piece of Novome's platform is our ability to not only capitalize on new discoveries about the roles our gut microbes play in disease, but also to introduce completely novel functions into the gut environment. As we unravel the dynamic complexities of diseases such as IBD, the ability to do things like build reactive therapies — medicines that can go beyond static dosing and actually sense and respond to their environment — may be the solution that's needed for widespread patient efficacy. This is where engineered microbial cell therapy shines as the next frontier in medicine, taking us beyond single-target, static therapies and ushering in an era where multifactorial target engagement and dynamic, long-term treatment is possible. CEP

▲ **Figure 5.** Novome co-founders Will DeLoache and Weston Whitaker are pictured gathering a sample of sewage at a San Francisco Bay Area wastewater treatment facility in 2016. Key genes used to engineer our *Bacteroides* therapeutic strain were isolated from a naturally occurring microbe present in sewage.

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LIZ SHEPHERD, PhD, is a co-founder of Novome Biotechnologies, and currently serves as its Head of People. She received her PhD from the Microbiology and Immunology Dept. at Stanford Univ. where she trained with Justin Sonnenburg. Shepherd played an instrumental role in Novome's early platform technology development, and is the lead author of the *Nature* article that introduces the concept of using porphyran to create an exclusive metabolic niche. She is an inventor on numerous patents in Novome's intellectual property portfolio, and led Novome's animal research team prior to directing its People function.

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Building the Bioindustrial Manufacturing Workforce

Thomas C. Tubon, Jr. ▪ BioMADE

Jim DeKloe ▪ Solano Community College

A well-prepared, highly skilled technical workforce in bioindustrial manufacturing is essential to realize the full potential of the emerging bioeconomy.

The rapid pace of discovery, innovation, and commercialization of biologically produced commodity chemicals, textiles, energy, materials, and food products of modern society will require more than 1.1 million workers. These high-paying jobs will fuel a projected $4–30 trillion global bioeconomy in the near future *(1)*.

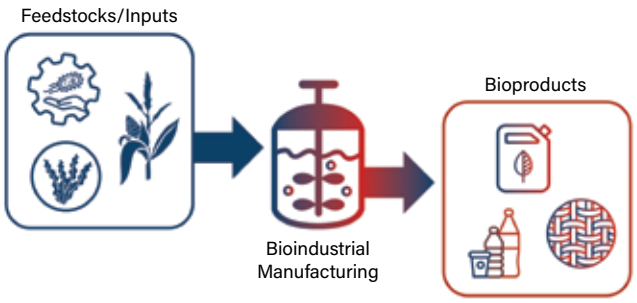
Recent advancements in engineering biology have led to a paradigm shift from petroleum-based chemical manufacturing to biologically sourced products. Such a shift can impact grand challenges such as climate change, food security, energy independence, and environmental sustainability. An estimated 60% of the materials in the global consumer product supply chain could, in principle, be produced biologically, which emphasizes the importance of commercial-scale engineering and sustainable production of biobased products *(2)* (Figure 1).

This article describes a concerted approach to building and sustaining the large and diverse workforce needed to fuel the growing bioeconomy.

Introduction

The U.S. has a wealth of biomass and feedstocks; such feedstocks offer an opportunity to create a sustainable and resilient bioeconomy by building out the industries where these resources are most abundant. The growing bioeconomy will have numerous direct societal benefits, including economic revitalization of rural communities through local processing of biomass, the large-scale bioproduction of commodity chemicals from renewable biological sources, and supply chain resiliency. In addition, the bioeconomy may impact climate change, with a near-term estimate of 10% net reduction of carbon dioxide emissions in the U.S., amounting to 450 m.t. annually *(2)*. The U.S. Dept. of Energy estimates that the U.S. has the capacity to produce more than 1.3 billion tons of biomass per year without negatively impacting food, animal feed, export demands, and the environment *(3)*.

We will need a well-prepared workforce to meet the demand and realize the potential of bioindustrial manufacturing. Jobs in the biomanufacturing sector are expected to grow a continued 20% on average over the next five years, with an annual salary range from $68,000 to



▲ **Figure 1.** Bioindustrial manufacturing takes biomass-based feedstocks and transforms them into commodity chemicals and other useful products.

$100,000 *(4)*. The growth of the bioindustrial manufacturing sector requires a talent pool of biomanufacturing professionals at all levels, from production technicians to bioprocessing engineers, research and development (R&D) scientists, and management. Developing a diverse and robust workforce to fuel the bioeconomy will require us to leverage what currently exists to support cross-disciplinary career pathways, recruit from non-traditional and underrepresented groups, and build a strong coordinated effort between public and private entities.

Dedicated U.S. government job and workforce training programs, including the National Science Foundation's (NSF's) Advanced Technological Education Program, and programs within the U.S. Depts. of Agriculture, Energy, Commerce, Education, Labor, and Health and Human Services, and the Economic Development Agency, are supporting efforts to drive our bioeconomic security *(1)*.

Catalyzed by the U.S. Dept. of Defense and specifically designed to address public-private partnerships in the engineering biology sector, BioMADE — the Bioindustrial Manufacturing and Design Ecosystem — was established in October 2020 *(5)*. BioMADE's mission is to enable domestic bioindustrial manufacturing at all scales, develop technologies to enhance U.S. bioindustrial competitiveness, de-risk investments in relevant infrastructure, and expand the biomanufacturing workforce to realize the economic promise of industrial biotechnology. BioMADE's vision is to build a sustainable, domestic, end-to-end biomanufacturing ecosystem.

Collectively, the first and foremost challenge in education and workforce development is to increase awareness of the bioindustrial manufacturing sector, the social and economic impacts of the technology, and the possible career pathways that exist.

Global efforts to address worker education and training in the bioindustrial manufacturing sector are evident. An increase in technical workforce readiness programs in biotechnology, biomanufacturing, biological and chemical engineering, process development, supply chain logistics, bioreactor fermentation, and related interdisciplinary training is available. However, these programs are woefully insufficient to meet the demands of the emerging bioindustrial marketplace.

Existing, well-established strategies for workforce training in the biopharmaceutical and biomedical sectors serve as a strong foundation to develop our efforts for bioindustrial manufacturing. Similar accelerants to workforce capacity-building existed within the petrochemical and chemical engineering industry base. There are, however, sector-specific competencies that require specific workplace training for biological and chemical engineers to facilitate career entry. Effectively scaling these resources will involve broader coordination of a distributed network of workforce training agencies above and beyond the traditional institutes of higher education. This raises the possibility for expanding academic training programs — traditionally at two-year and four-year colleges and universities — to engage high schools, federal and state workforce development agencies, and community-serving organizations to elevate awareness and career development pathways and opportunities.

In addition, broad dissemination of industry-validated training for bioindustrial manufacturing-specific skills and competencies can be achieved through online, hybrid, and in-person instruction via short courses, workshops, and technical certifications. Access and availability of these workforce-readiness programs are key components to ensuring that career development pathways are broadly accessible and lead to the support of a diverse, equitable, and inclusive workforce.

Promoting new career paths in biomanufacturing

Arguably, government and state support for workforce development is a major focus for creating education and training resources. The September 2022 Presidential Executive Order on "Advancing Biotechnology and Biomanufacturing Innovation for a Sustainable, Safe, and Secure American Bioeconomy" lists as a top priority to "train and support a diverse, skilled technical workforce and next generation of leaders from diverse groups to advance biotechnology and biomanufacturing" *(6)*. This Executive Order outlines a strategic blueprint that includes interagency collaboration and policy to support bioeconomic growth.

To inspire and recruit a diverse workforce, key bioindustry stakeholders will need to define bioindustrial manufacturing in a way that demonstrates real-world impact. A diverse workforce may include (but is not limited to): underserved and underrepresented groups, rural populations, veterans, incumbent and displaced workers, and persons who are differently abled.

The ability to successfully create a pipeline for a diverse and inclusive workforce is hinged on a deeper understanding of social and cultural needs that impact career pathway choice — a challenge that all science, technology, engineering, and math (STEM) disciplines face. Recognition of this allows us to use successful existing strategies, such as providing financial and social support for trainees, mentoring, multi-generational community outreach, and the use of universal design for learning and adaptive technologies, to more effectively increase awareness of and engagement with career opportunities in the biomanufacturing space.

By addressing social, cultural, and economic needs, we can shift the focus for many away from the barriers of engagement to opening the doors of opportunity to new career paths.

Training the workforce

Skilled workers in the bioindustrial manufacturing sector cover a broad range of positions, from entry- and mid-level technicians and production associates to bioprocess engineers and operations management. A small but growing number of exemplary workforce readiness programs for bioindustrial manufacturing currently exist, but many more are needed. These pioneering programs target a broad scope for impact through both vertical integration (*e.g.,* traditional credentialing pathways) and horizontal integration (*e.g.,* industry-based training, reskilling, and uptraining the incumbent workforce) (Figure 2).

Several notable programs exist, including one directed by Natalie Kuldell, CEO of the BioBuilder Foundation, who is leading efforts to develop bioprocess engineering pathways for underserved and underrepresented high school students. Linnea Fletcher, Executive Director of the NSF InnovATEBIO National Biotechnology Education Center, is leading the national effort to engage the community and technical college system in developing and disseminating bioindustrial manufacturing curricula.

Andy Ellington at the Univ. of Texas, Austin is leading efforts to build out early-career awareness and industry-anchored training in bioindustrial manufacturing through the university's well-established Freshman Research Initiative (FRI) model for student engagement.

Jason Ryder, Univ. of California Berkeley Chemical and Biomolecular Engineering Professor and CEO of Joywell Foods, has taken the lead to develop a Masters of Bioprocess Engineering (MBPE) program with Lawrence Berkeley National Labs (LBNL) and the Advanced Biofuels Production Demonstration Unit (ABPDU). This MBPE program provides students with access to mid-scale bacterial fermenters and specialized equipment, such as disc stack centrifuges, spiral membrane filtration systems, and fast protein liquid chromatography (FPLC) systems, which are commonly out of reach for many academic teaching institutions.

In response to the bioindustrial sector demand, new opportunities are underway to create comprehensive credentials in biomanufacturing that break the mold of traditional two-year college programs. Solano Community College (Vacaville, CA) and Mira Costa Community College (Oceanside, CA) have established industry-driven biomanufacturing BS programs that provide hands-on education and training that merge traditional biotechnology with key skills needed for bioprocess engineering.

Horizontal integration approaches include efforts by Angela Cosani (Bioscience Cores Skills Institute) and Tammy Mandell (Biotility), who are leading industry-driven national credentialing programs with micro-credentials, certificates, and the Biotechnician Assistant Credentialing Exam (BACE) that open employment and career opportunities.

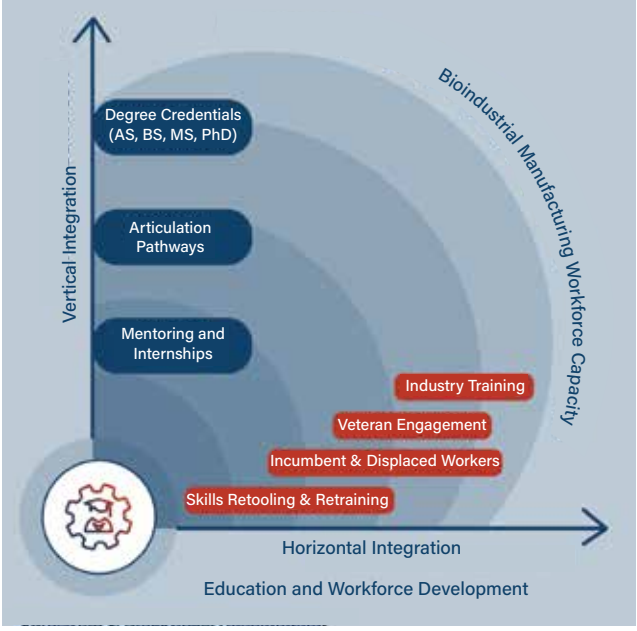
Worcester Polytechnic Institute offers skill building through one-day to week-long programs at their Biomanufacturing Education and Training Center (BETC). These focused experiences are designed to serve incumbent workers and company teams that are seeking to upskill. Likewise, North Carolina State Univ. offers short courses for skills training in advanced biomanufacturing that can be specifically tailored to meet industry needs through their Biomanufacturing Training and Education Center (BTEC), with a track record of training and education for over 5,200 individuals from more than 340 organizations worldwide.

Taken together, these cutting-edge programs are designed specifically to fill the need for workforce programming across multiple points of career entry and credentialing.

Addressing the industry pull

The emerging bioindustrial workforce will require a new and agile model for education and training. Although the bioprocess industry has developed benchmarks on training for associate scientists and process engineers, many graduating students do not meet these benchmarks *(7)*. Existing programs that lead to degrees and credentials in biotechnology provide critical core skills but fall short of meeting the industry benchmarks for the skills and competencies in demand for industrial-scale bioprocessing.

Traditional biotechnology programs are predominantly designed around research knowledge and skills, and afford little if any experience in critical engineering principles such as analysis, design, development, scale-up, and commercialization of biobased processes and products. Although bench-



▲ **Figure 2.** Current approaches to training the workforce focus on either traditional vertical or non-traditional horizontal pathways.

scale product purification and recovery separation through centrifugation, filtration, and column chromatography are taught in some programs, operational experience with scaling up the process to produce larger kilogram and metric ton quantities is lacking.

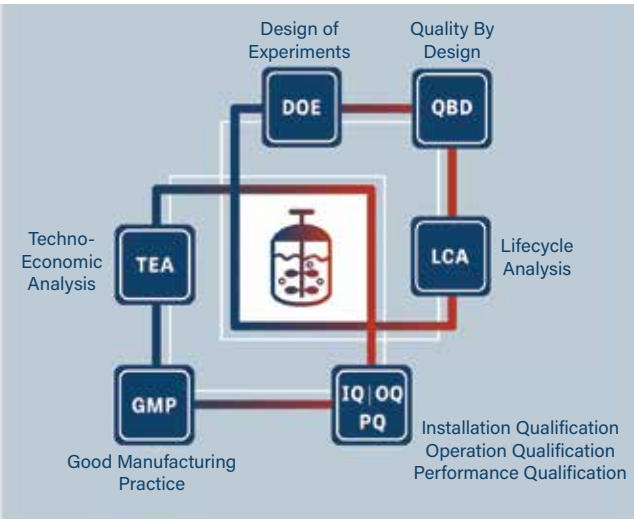
In addition to more programming in process optimization, other key concepts unique to industrial-scale manufacturing can help prepare the incoming bioindustrial manufacturing workforce (Figure 3). These include:

- upstream processing (USP) and downstream processing (DSP)
- quality by design (QBD)
- statistical design of experiments (DOE)
- good manufacturing practices (GMPs)
- lifecycle analysis (LCA)
- installation qualification (IQ), operation qualification (OQ), and performance qualification (PQ)
- techno-economic analysis (TEA).

Currently, most bioprocess engineers enter into the sector with a biochemistry or chemical engineering background, where these learning objectives are central to the program of study. Expansion of the talent pool to leverage the existing biotechnology program infrastructure will require the development and adoption of new opportunities to integrate key benchmarks for bioprocess engineering.

Opportunities and challenges

The recent forecasts of the size of the emerging bioeconomy cited in this article may sound like hyperbole; however, the bioeconomy has the potential to grow to a $30 trillion sector annually, and such growth will require an increase of the workforce in this developing industry by 1.1 million new workers *(1)*. Panelists at the White House Summit that accompanied the President's September 2022 Executive Order assured the audience that these estimates represent the true scale of their projections. The growth of this emerging field will create tremendous opportunities but will also present enormous accompanying challenges. The industry will provide careers that pay a livable wage with many opportunities for upward mobility. But these opportunities will only be available to workers who received the appropriate education and training.



▲ **Figure 3.** The incoming bioindustrial manufacturing workforce will need to know several key concepts not frequently taught in traditional biochemistry programs.

The first challenge will be to raise awareness of the field and to dramatically increase and expand recruiting. Currently, few high school students know about this field at all. Few undergraduate biology majors, chemistry majors, or chemical engineers know about the fields required to grow the bioeconomy. Major recruiting efforts must target underserved communities. Even colleges with diverse student bodies will have to initiate aggressive recruiting programs to expose their students to this field.

Whereas pharmaceutical biotechnology has been concentrated largely on the coastal states of the U.S., the new bioeconomy will be more geographically diverse as companies build bioindustrial manufacturing plants closer to their source of feedstocks. All communities and geographic regions in the U.S. must become involved in this revolution. Placing students from underserved communities into high-wage careers with high opportunity for upward mobility constitutes true equity work; this action makes a difference in the life of that individual and in their community.

The next challenge will be to recruit faculty with the right expertise. Many potential faculty members come from traditional PhD programs that teach research methods and have not been exposed to the skills and knowledge required in the production facilities of a mature industry. Faculty members who do have industry experience often get recruited by industry, with its dramatically higher wages. Faculty who can teach the engineering principles at the core of production are in short supply. The recruitment of faculty with the proper background represents a major challenge.

These faculty members must develop new curriculum and new delivery methods. Workers in the bioeconomy must understand and apply the engineering principles required for fermenter and bioreactor design and operation. Programs must include the principles behind upstream and downstream processing, product recovery, bioseparations, and purification strategies (Figure 4). Semester-long courses must be supplemented or even replaced by targeted and intensive short courses. And the value of specific knowledge and skills must be emphasized over formal degrees.

The funding of programs will challenge the field. High-quality instruction will require the ordering, set up, and operation of expensive equipment. New facilities will have to be

built all over the U.S. This revolution will require significant new investment by both national and state governments.

Educating the workforce will involve new delivery methods — both online and in person. Programs should emphasize specific training with targeted skills and knowledge, rather than the current emphasis on general degrees. A dramatic expansion of the students exposed to this training requires the elimination of artificial barriers — unnecessary prerequisites and unnecessary courses. Concepts, especially mathematical concepts, can be presented in a more contextualized way.

Building the bioeconomy will involve rethinking higher education and training. Education must emphasize subjects that are more applied, more geared toward industrial applications, and all programs must incorporate chemical engineering principles into their curriculum. As the bioeconomy grows and evolves, all members of society must be actively and aggressively recruited to become part of it. The development of the industry and the development of the workforce for that industry must occur in parallel or the effort will fail and the U.S. will fall behind other countries. This development presents great opportunities, but great accompanying challenges.

Closing thoughts

By working together, industry, government, and academia have the ability to harness the technologies that can drive our bioindustrial manufacturing capabilities to the next level. In order to achieve this, we need to create and sustain a strong, resilient, and well-prepared workforce. BioMADE, alongside our ecosystem of stakeholders in the public, private, and government sectors, stands ready to meet the challenge of building the technologies and workforce for the near and distant future. CEP

▲ **Figure 4.** Programs for training the next generation of industrial bioengineers must address principles behind upstream and downstream processing.

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JIM DEKLOE, PhD, is a professor, director, and founder of the Industrial Biotechnology Program at Solano Community College in the North San Francisco Bay Area. He has more than 35 years of experience in the biotechnology sector and is a widely recognized leader for developing and delivering industry-driven programming in the life sciences that incorporates the scientific, technical/engineering, quality, regulatory, and business aspects of the biomanufacturing field. He holds an MA and PhD in biology from the Univ. of California, Los Angeles.

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