SBE SPECIAL SECTION:

INDUSTRIAL BIOTECHNOLOGY

Industrial Biotechnology Inspires and Innovates

The industrial biotechnology community spans industry, national laboratories, academic institutions, and investors in the private equity and venture capital (VC) space. From small startups to established multinationals, companies in this field are pushing boundaries and developing game-changing innovations. These organizations are creating functional replacements and drop-in alternatives to traditional petrochemical inputs and products, as well as improving the sustainability of agriculture and food production. They are commercializing a wide array of product types, from small molecules for biofuels and specialty biobased chemicals, to proteins, new foods, novel textiles, and biopolymers.

The third Commercializing Industrial Biotechnology (CIB) conference took place in Los Angeles, CA, on May 2019. The conference highlighted and emphasized the maturation of a new wave of products, bioprocess technologies, global markets, production capacity, and organizations in this sector.

A few of the many inspiring presentations are summarized in this special section of *CEP*.

In the first article, Joško Bobanović from Sofinnova Partners offers valuable context on starting a biotechnology company, communicating its value proposition, and developing it to the point of commercial-scale manufacturing and marketing. He describes some of the challenges startups will face in building and leveraging strategic and financial partnerships.

As consumers call for animal-free and humane alternatives to the textiles and fabrics that we wear, biotechnology companies are striving to meet these demands. Alex Patist, Ritu Bansal-Mutalik, and Jeroen F. Visjager give an illuminating tour of Bolt Threads' production platforms for Microsilk fiber and Mylo leather-like material. These sustainable mimics of silk and leather have a smaller environmental footprint and generate less waste than their traditional counterparts.

The next article in the special section discusses how industrial biotechnology is making an impact on agriculture. Kelly Smith from AgBiome presents a compelling vision for using and optimizing naturally occurring soil microbes (and communities of them) to potentially replace chemical pesticides. Her article describes how AgBiome's novel crop-protection practices offer better performance and yields than industry-standard chemical products, with less probability of resistance.

In an article on the food sector of industrial biotechnology, Elliott Swartz from the Good Food Institute describes the scaling and industrialization of mammalian cell culture to address meat demand. His article raises the question: Can we use stem cells and cell differentiation to produce meat without the agriculture and land-use requirements that drive current production?

Finally, Rachel Brenc of LanzaTech describes her company's platform for the production of biofuels and chemicals from waste-gas feedstocks. The article leads readers through LanzaTech's technical and commercial development. The strong focus on safety, sustainability, teamwork, feedstock flexibility, and global deployment draws attention to the perseverance and flexibility needed to grow a company in this sector.

These five unique perspectives give readers a glimpse into the many ways industrial biotechnology is transforming agriculture, fashion, food production, and the chemicals industry. From changing how meat is produced to improving crop yields with microbes, and from producing fuels from recycled waste gas to developing novel materials, these technologies are at the cutting edge of the industrial biotechnology revolution. A key ingredient to the success of any new company, technology, or industry sector is the availability of financing and strategic partnerships, which is also addressed in the special section.

The CIB 2019 conference included a panel session in which attendees discussed the importance of workforce diversity, equity, and inclusion. Participants reached a consensus that individuals of differing genders, backgrounds, nationalities, and life experience will be needed to raise the profile and strengthen the foundation of industrial biotechnology across the globe. The Society for Biological Engineering (SBE) and future CIB organizers are committed to continuing this dialogue at future conferences.

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Building Successful Startups in Industrial Biotechnology

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Industrial biotech startups are typically founded by technical individuals — engineers and scientists with little experience in the world of business. This article describes ways to avoid some of the pitfalls that budding startups face.

Starting a company in the industrial biotechnology field requires an entrepreneur to develop varied skills that contribute to its success, such as communication and negotiating skills. Although success is not entirely defined, we often consider a successful startup as one that creates an innovative product, demonstrates its economic viability, and then either sells the product and becomes profitable, or partners with a large company to jointly commercialize the product. At some point in time, this process leads to a so-called liquidity event for the company's shareholders, such as a public listing or acquisition of the startup by another company for cash or shares.

Sofinnova Partners has been helping entrepreneurs build startups for more than 45 years and has invested in more than 550 companies over that period. The goal of our industrial biotech practice is to develop new products that can address multiple societal challenges. Fundamentally, we believe that biology can provide the world with an unprecedented ability to develop more sustainable, better, faster, and cheaper solutions to pressing problems in the agriculture, food, materials, chemicals, and energy sectors.

When we started our efforts in industrial biotech a few years ago (after focusing the entire firm on life sciences), we leveraged our experience in building pharmaceutical biotech companies and expertise in working with early-stage entrepreneurs. Currently, we have 15 investments in the industrial biotech sector. Our objective is to tap into a new and growing market to leverage mature biotechnology tools and apply them in an industrial context.

The key advantage of an investment firm (over an entrepreneur) is that it often invests in a portfolio of companies, allowing it to have a helicopter view of the sector and rapidly apply knowledge and learnings it gains from one company to its dealings with others. Whenever possible, Sofinnova Partners attempts to generalize some of these teachings and develop a working set of guidelines for us and for our companies. This article shares some of the lessons that we have learned and describes some of the common challenges that young startups face. The article also describes how startups can communicate value and develop partnerships, as well as raise capital and generate liquidity for investors.

Make your message easy to understand

Most technology startups are founded and initially managed by technical people. These engineers and scientists are capable of changing the world with their technologies and inventions. By founding a startup, they are charting a path

Communicating the value of a startup's offering must begin on the day the startup is conceived and must continue in perpetuity.

that few people have traveled before. Their innovative spirit, and the technological and financial promise of the startup, is what motivates investment firms to join these entrepreneurs to help them realize their dreams.

The entrepreneurs take pride in their cutting-edge technology, portfolio of patents in development, and potential for growth and further technical achievements. To entrepreneurs, and often investors, the value of the product is given — they understand what it does and how it works. But, trying to explain the elegance of the technical invention to the outside world can be a problem.

Most of society does not speak in technical terms or understand science, let alone complicated science that may involve microorganisms, genetic engineering, or fermentation. Even other industry professionals and organizations may not be excited by the technical invention — larger companies speak the language of market share, margin, and product-market fit. They want to know if the startup has a go-to-market strategy and an idea of potential earnings.

Entrepreneurs often wonder why other organizations and society cannot see the value in their idea — value that, to them, is self-evident.

Communicating the value of a startup's offering must begin on the day the startup is conceived. Although founded on some kind of technical idea or discovery, startups (and their investors) need the discipline and skill to develop an easily understandable story that communicates what they do and why their product is important.

Take an everyday example such as mobile phones, which are used by billions of people because they make their lives easier and more entertaining, and allow them to work virtually anywhere. Every phone is packed with cutting-edge technology. Yet, you rarely hear about the nanoscale fabrication techniques required to cram billions of transistors on a small chip or the material science advances that make the glass screen nearly unbreakable.

Technologies such as nanoscale fabrication required years of research and development (R&D). Investors may not be eager to fund such seemingly mundane technologies. But, without such investments, we would not have the sleek mobile phones we carry around today. Entrepreneurs must learn how to communicate and translate their technologies into the applications that end-users care about. Although the utility of the technology is obvious to the entrepreneur, it may not be obvious to others.

Startups need to craft their story from day one. This should include testing the message with the outside world, gathering feedback, and then modifying, simplifying, evolving, and sharpening the story. And, just when an entrepreneur thinks they have the right message to accurately convey the value of their discovery, inevitably, the market will shift. Thus, this work of finding the best way to communicate the value of a startup must continue in perpetuity.

Entrepreneurs often struggle with this seemingly monumental task, and many startups have failed because their founders did a poor job of explaining the value of their cornerstone technology. Investment firms can shoulder some of the blame in these failures. Investors often understand the technology these startups offer, but do not force the startups to be clear in the messaging of the true impacts of the technology for the market, society, and/or environment.

You cannot do it alone

Most small companies look for partners to leverage their experience, scale, knowledge, or access to capital. This is a normal course of development for a startup and not necessarily exclusive to industrial biotechnology.

However, industrial biotech differs slightly from other fields in that scaling up the technology can be a major challenge. Typically, a startup excels in its creativity, ability to develop novel products or solutions, and react quickly when something is not working. Once developed in a lab, these solutions need to be scaled up to test how they work in a semi-industrial and eventually industrial environment. This effort requires a completely new skillset from that of the startup company. At this point, startups often look for outside help to leverage the knowledge of a larger company.

Potential partners are often very large multinational companies looking to take a peek at what's brewing in the startup world. These partners are typically working on multiple projects in parallel. The startup's founders think (often rightfully so) that they have developed the best possible product. However, the potential partner sees it as another lab-based technology that has not yet proven its economic viability and that must be scaled — which will most likely be a difficult task.

Even though the small company and the potential partner will not see eye-to-eye on every issue, they will need to figure out how to work together and develop a partnership.

A large industrial partner may have several collaboration projects with different companies in the hope that some of them become successful. For the startup, forming a partnership with a large industrial player represents its critical (and only) path to success. By partnering, the startup is putting its survival on the line. Entrepreneurs must understand this asymmetry in order to better design their relationship with an industrial partner.

The startup must go into the partnership with a clear understanding of partner motivation and their potential commercial relationship, if any, in the future. The entrepreneur should try to determine whether the partner is seeking to "try before they buy" (*i.e.,* they are serious about investing but want to verify that the technology meets specific criteria) or if they are simply gathering competitive intelligence to develop options.

Before going into negotiations, entrepreneurs should do some research on the partner. For example, what is the partner's track record in working with other startups? Is there a way to carve out one market/application dedicated to this particular relationship while keeping other options open?

Discussions and negotiations with a potential partner are often long, onerous, and unpleasant for entrepreneurs, yet they could pave the way to their success or oblivion.

The question that often gets debated at length is: Should the partner invest in the startup and become a shareholder? There is no simple answer to this question, as there are many types of relationships.

If the partner is looking only to invest to be closer to a startup and not form other relationships or ask for special rights, this may be beneficial to the startup. However, if the partner wants to become involved commercially or in R&D, this transcends the regular shareholding relationship and may create a divergence of interests between the partner and other (purely financial) shareholders.

Most importantly, in case the commercial or R&D project does not work, what is the mechanism for separating from the partner and what kind of signal does this send to the outside world? The tendency is to interpret such a separation as a vote of no confidence by the partner in regard to the startup's technology, but that is rarely true. Partnerships, like in life, do not work for myriad reasons — large companies change strategic direction under new leadership, abandon R&D projects, run into financial difficulties and need to cut spending, etc. Therefore, a solid prenuptial-like contract is critical for a good partnership of this kind.

Ultimately, it's all about money

Access to capital to finance development is critical to ensuring a startup's success. Although technologies are, in some cases, developed with government grant financing or customer prefinancing, most of the time startups raise capital from standard sources — starting with friends and family, technology incubators, and then moving on to institutional investors.

The first institutional capital available to startups comes from venture capital (VC) investors, like Sofinnova Partners. The number of VC firms interested in industrial biotechnology has varied over time. In the mid-2000s, a relatively large number of firms invested in biofuel applications in response to the U.S. drive for energy independence. A majority of these investments led to disappointment. The main reason many of these companies failed was their attempt to rapidly industrialize technologies that needed time to mature and descend the cost curve. By going after fuels markets, which are huge in volume but cheap in terms of price, entrepreneurs attempted to counter classical technology development cycles — where early products are high-price and low-volume in niche applications, allowing for the development of economies of scale and improvement in cost over time. Some investors took those failures as a signal to look for their fortunes elsewhere, others applied the lessons learned and continued their efforts.

Overall, the number of VC firms investing in the field is still relatively small, for various reasons. The VC industry, like many, suffers from a mentality where many investors follow what is in fashion. For example, a few years ago, cryptocurrencies and virtual reality were in fashion. More recently, it is artificial intelligence and machine learning.

The industrial biotechnology ecosystem of startups and investors has not been able to create sufficient buzz to attract a large number of players. The financial results have not followed and, subsequently, only some investors with a long-term view persist and are available as sources of funding for industrial biotech startups.

For startups, this represents a major obstacle, as they need to become highly creative when looking for different sources of financing. This often means tapping into family offices, hedge funds, and large institutions. Some of these entities are not equipped to invest in startups or to understand their challenges. Building long-term syndicates that are able to support a company through several investment rounds is difficult, and this puts an additional strain on both startups and their early investors.

The moment a startup takes an external investor, the expectation for realizing the investment starts. Venture capitalists provide financing in the interest of generating a return through an eventual exit event, such as the company

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Developing the Next Generation of Sustainable Biomaterials

Jeroen F. Visjager
Ritu Bansal-Mutalik
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Bolt Threads

Inspired by nature, one company is using advanced biotechnology techniques to develop new textiles and materials that raise the bar for sustainability.

Silk and leather are valuable materials with a multibillion-dollar global market. Their production is part science and technology and part art, with roots dating back thousands of years. Today, technological and scientific insights are allowing engineers and scientists to produce these materials while keeping in mind the critical sustainability needs of our world.

Biotechnology company Bolt Threads has developed two platforms for making silk and leather sustainably. This article describes the production of its MICROSILK, a high-performance fiber produced via fermentation and traditional textile production techniques. It then describes the company's platform for manufacturing MYLO material, which looks, feels, and behaves like handcrafted leather, but is made from mycelia, the root structure of mushrooms.

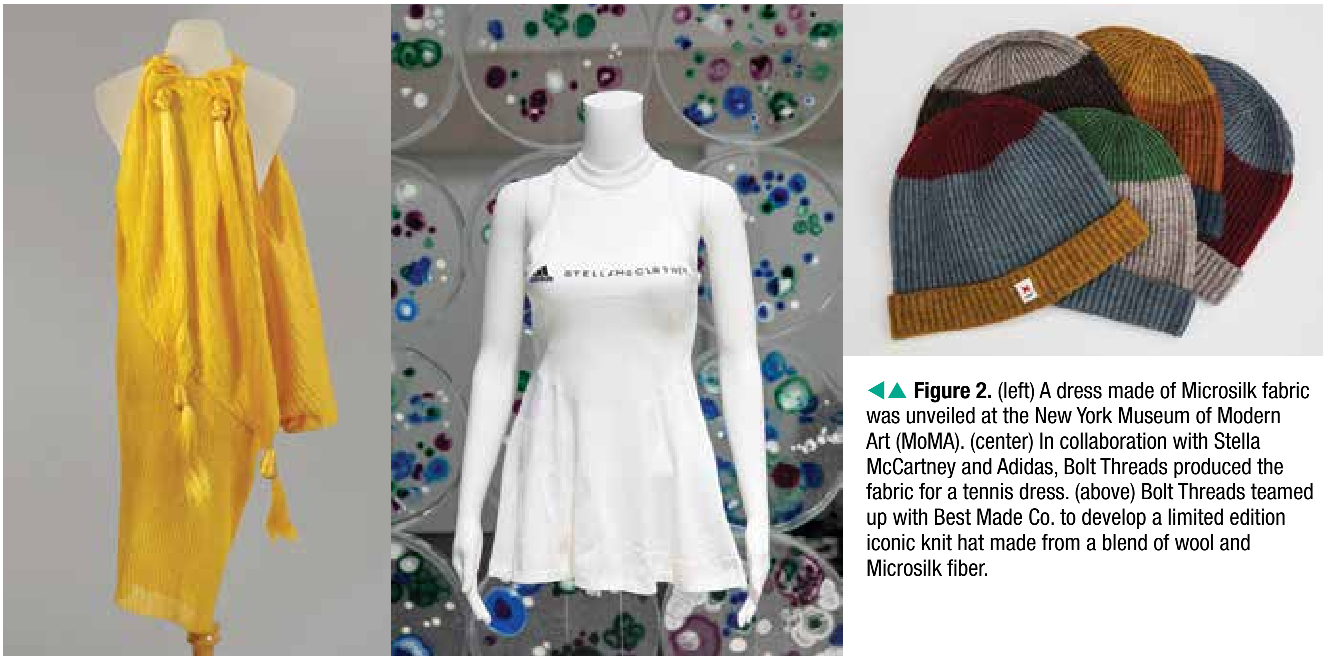
Combining biology and textile production

To create Microsilk fibers, Bolt Threads is using a yeast host capable of producing silk proteins similar to those created by spiders when spinning webs, draglines, and egg sacs. A liquid fermentation process produces these silk proteins at large scales. The fermentation process is followed by a series of steps to recover, purify, and dry the silk protein powder, which is further processed via wet spinning to create silk fibers. Those silk fibers can be knitted into fabrics and garments.

Microsilk fibers have generated positive buzz in the fashion community for their softness, durability, and sustainability. The first commercially available product made of spider silk — a Microsilk tie (Figure 1) — was launched in March 2017, and it sold out almost immediately. In October 2017, Bolt Threads collaborated with designer Stella McCartney to create a dress made of Microsilk fabric (Figure 2), which was unveiled at the New York Museum of Modern Art (MoMA).

Mylo material production begins with mycelium. Scientists grow mycelium in a type of solid-state fermentation process on beds of agricultural waste and byproducts. Billions of cells form a branching, interconnected network of mycelium. The mycelium is compressed into a mat or sheet and is dyed, plasticized, and finished (in accordance with the

▶ **Figure 1.** The first commercially available product made of spider silk — a Microsilk tie — was launched in March 2017.



◀▲ **Figure 2.** (left) A dress made of Microsilk fabric was unveiled at the New York Museum of Modern Art (MoMA). (center) In collaboration with Stella McCartney and Adidas, Bolt Threads produced the fabric for a tennis dress. (above) Bolt Threads teamed up with Best Made Co. to develop a limited edition iconic knit hat made from a blend of wool and Microsilk fiber.

product specifications) into a material similar to leather.

Stella McCartney made a prototype of her iconic Falabella bag of Mylo material and premiered it in April 2018 at the Fashioned from Nature exhibit at the Victoria & Albert Museum in London. Chester Wallace created the first commercially available bag made of Mylo material — a project fully funded through a Kickstarter campaign — in October 2018.

What is special about spider silk?

Silk has impressive properties; for example, it is tougher than any other natural or manmade fiber. Silk fibers from spiders and silkworms are some of the strongest fibers in nature, and they are fully compatible with the human body for use in medical devices and other such applications *(1)*.

Unlike silkworm silk, spider silk cannot be commercially farmed and harvested. This is due, in part, to the aggressive and territorial nature of spiders. And, spider silk is difficult if not impossible to chemically synthesize because it is made of polymer proteins with high molecular weights (*i.e.,* polypeptides on the order of ~300 kDa). These factors make biosynthetic production the most cost-effective and viable path to creating (and commercializing) spider silk textiles.

Spiders have several different types of glands (Figure 3) that produce silks with varying mechanical properties *(2)*. A single species of spider creates many fibers, each of which is used for different functions such as creating draglines and web capture spirals, prey immobilization, and egg sac protection *(3)*.

The fibers are named for the gland where they originate; the polypeptides that make up that type of fiber are labeled with the gland abbreviation — for example, "Sp" for spidroin (short for spider fibroin). In orb weaver spiders, other examples include major ampullate (MaSp), minor ampullate (MiSp), flagelliform (Flag), aciniform (AcSp), tubuliform (TuSp), and pyriform (PySp).

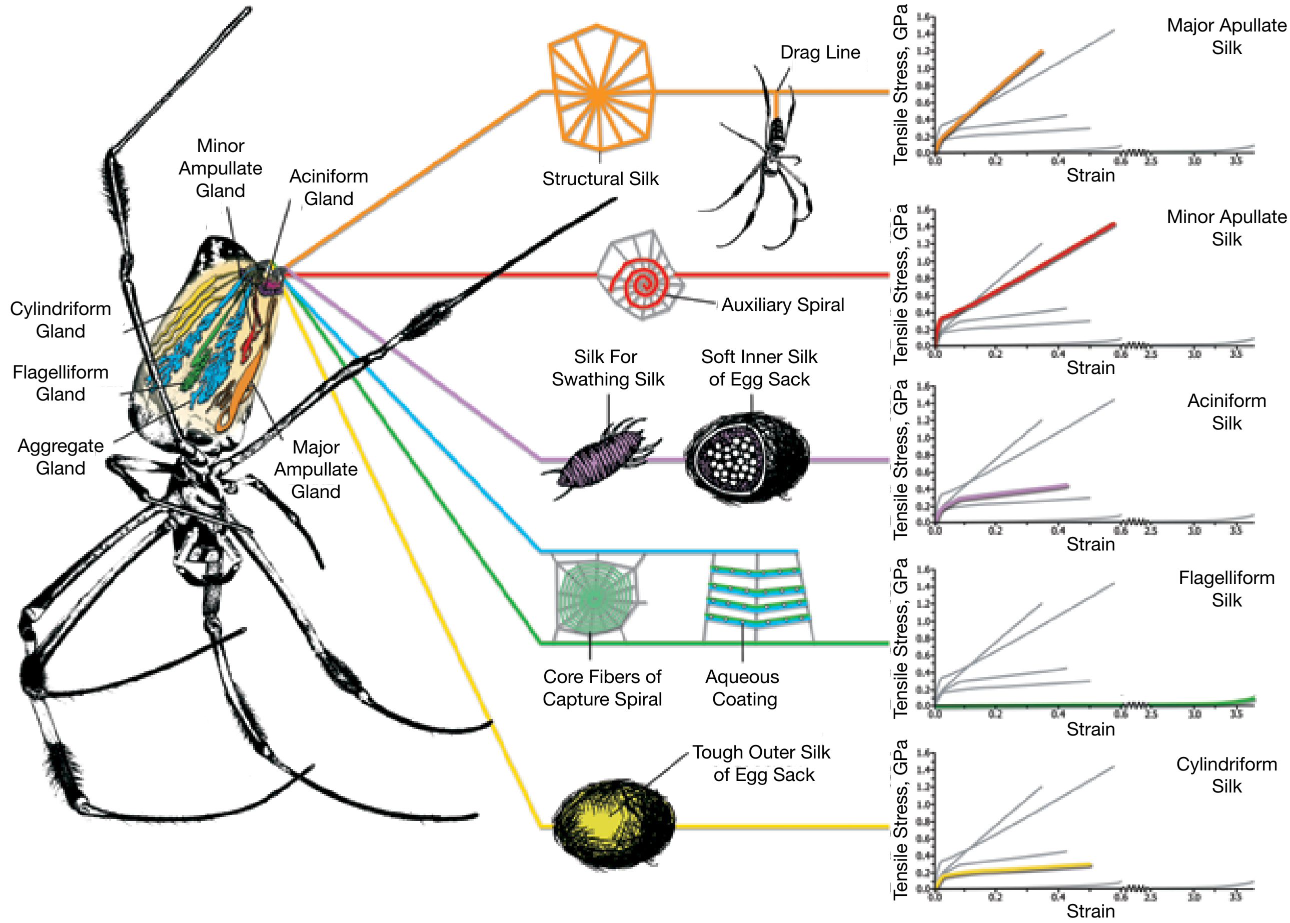
Amino acid composition and protein structure vary considerably between types of fiber and species of spider. The amino acid composition and sequence, as well as the fiber formation, affect the mechanical properties of the fiber.

Dragline silks have exceptional mechanical properties. They are very strong for their weight and diameter, and also exhibit a combination of high extensibility (*i.e.,* the ability to be stretched) and high ultimate tensile strength. The silk proteins made by Bolt Threads mimic the dragline silks produced by the European wasp spider (*Argiope bruennichi*).

Recombinant protein production and fermentation

Currently, recombinant silk fibers are not commercially available and, with a handful of exceptions, are not produced in microorganisms outside of *Escherichia coli* and other gram-negative prokaryotes. Previous efforts have been able to produce only small amounts of recombinant silk polypeptides; those production methods cannot be scaled to match conventional textile fiber production outputs. In the past, researchers developed experimental production hosts capable of generating silk polypeptides, including transgenic goats, transgenic silkworms, and plants *(4, 5)*. Those hosts have yet to enable commercial-scale production of silk, presumably due to slow engineering cycles.

Bolt Threads developed a method to produce recombinant proteins derived from spider silk in yeast and bacte-



▲ **Figure 3.** A single species of spider can create a variety of fibers, each of which is employed for a different function. On the right, typical stress-strain curves are shown for each of the five fibrous silks spun by the Nephila golden silk spider. Image reproduced with permission from and credit to Prof. F. Vollrath and Prof. C. Holland of the Oxford Silk Group.

rial hosts. We use diverse strain engineering approaches to modify these hosts to express these silk proteins at high rates and yields.

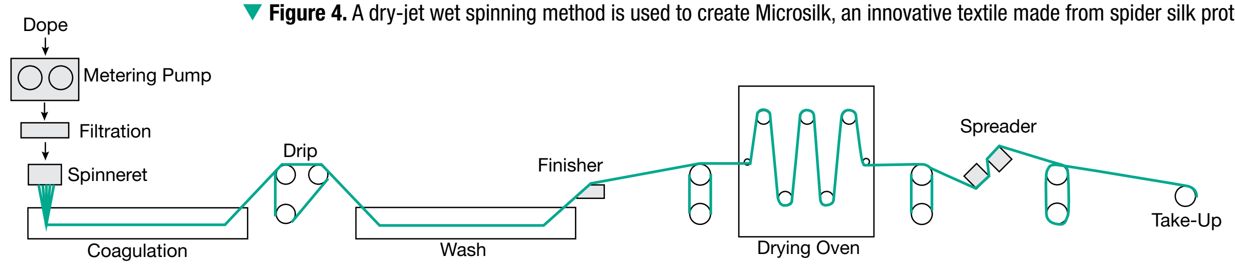
We initially explored different microbes as hosts for a wide variety of silk proteins. Transgenically modified *Pichia pastoris* as well as *E. coli* were two of the most promising hosts *(6, 7)*. *Pichia pastoris* secretes silks into the extracellular aqueous media, which simplifies downstream processing (DSP), making it an attractive host for commercial process development. However, *E. coli* is easier to manipulate and easier to modify with genetic engineering tools. Hence, even though most silks from *E. coli* are produced within the cell wall — requiring additional cell-disruption unit operations — we prefer *E. coli* as the screening host.

A suitable fermentation media comprises sugars, carbon sources, nitrogen sources, salts, minerals, and trace metals. Growth conditions — such as temperature, pH, dissolved oxygen, induction time, and the type and concentration of inducer — are optimized to obtain the highest possible silk titers using the minimum cell mass. After fermentation, silks are extracted in suitable solvents. Extraction is followed by further purification steps, which are mostly based on centrifugation and/or filtration. The final step is spray drying to obtain silk powders ready for wet spinning.

Because spider silk proteins are considered a specialty chemical product (unlike proteins for pharmaceutical applications), chromatographic operations are generally not used for recovering silks at commercial scale. However, chromatography can be used to make smaller quantities of purer silks for research purposes.

Bolt Threads researchers have found a strong correlation between the purity and the contaminant profile of a silk powder and its fiber properties. For example, silks with higher protein purity and a smaller amount of specific contaminants had a higher tensile strength. Hence, designing fermentation and DSP steps around purity targets is critical in balancing the cost and quality of the process.

One of the major challenges that dominates all aspects of silk processing is the insoluble nature of spider silk proteins *(8)*. Most protein purification processes are designed for



▼ **Figure 4.** A dry-jet wet spinning method is used to create Microsilk, an innovative textile made from spider silk proteins.

water-soluble proteins. In addition, the fiber spinning process requires the starting material — called a dope — to be a solution. However, other than strong chaotropes like guanidine salts and urea, most other aqueous or organic solvents cannot solubilize spider silk. These chaotropes are difficult to work with at large scales due to their hazardous nature and/or high waste disposal cost. Bolt Threads created several methods of solubilizing spider silk and filed patent applications covering the innovative solvents.

Wet spinning to make Microsilk fibers

After purification, the silk protein is subjected to dry-jet wet spinning. First, a homogenous solution (*i.e.,* dope) is prepared by dissolving silk protein powder in N-methylmorpholine N-oxide (NMMO) within a kneader-reactor mixer. (If silk-protein/cellulose-blend fibers are desired, cellulose can also be dissolved in the NMMO solvent.) Up to 99% of the NMMO used to dissolve the protein can be recovered and reused in the manufacturing process. Next, the dope is filtered and pumped through a spinneret to produce fiber strands (Figure 4) and subsequently coagulated and washed *(9)*.

After the coagulation and wash steps, a fiber finish (lubricant) is applied as a processing aid for future steps, such as carding and spinning into yarn. The fibers then undergo a drying step. The entire spinning process produces a continuous filament of silk, which can be cut into staple fibers or a specific length before being converted into yarns for apparel and other applications. This wet-spinning process has produced silk-protein/cellulose-blend fibers and 100% silk protein.

Leather-like material without animals

Like Microsilk fibers, Mylo material also represents a promising step forward for renewable, sustainable textiles.

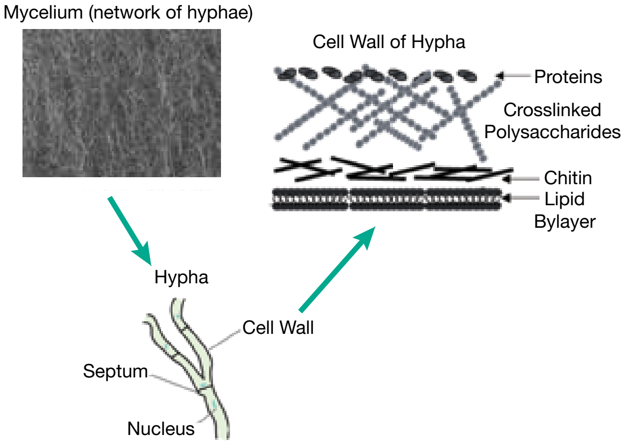
Bolt Threads has developed a process that converts mycelia — the root structure of mushrooms — into a non-animal leather-like material. This technique has a much lower environmental impact than raising cattle for beef and leather. Mylo material can also be processed, colored, and finished using more eco-friendly dyes and plasticizers (including completely animal-free products, if desired) than the chemicals typically required for leather tanning. This helps circumvent some of the negative environmental effects associated with the leather tanning industry *(10, 11)*.

Why use mycelia? Mycelia (plural of mycelium) are the branching underground structure of mushrooms. Due to mycelium's bioefficiency, mechanical strength, and small environmental footprint, its popularity has grown *(12)*. Various organizations have researched methods of growing mycelium both on its own and as a composite material (*e.g.,* enmeshed with particles, fibers, networks of fibers, or nonwoven lamina).

Mycelia are composed of a network of branching filaments called hyphae. The building blocks of each hypha are cells (Figure 5) *(13)*. The cell wall consists of polysaccharides, including glucans and chitin. Chitin comprises up to 5–7% by dry weight of dried mycelia.

The rigid chitin network gives the mycelia its characteristic rigidity and structural strength. Beneath the chitin layer is the typical cell membrane made of a lipid bilayer. On the outer side, chitin is tightly associated with glucans and other polysaccharides. The outermost layer consists of mannoproteins.

Hide and mycelia have very different compositions. While structural proteins, mostly collagen, constitute ~33 wt% of hide, proteins constitute only 3–4 wt% of



▲ **Figure 5.** Mycelium (the root structure of mushrooms) is composed of a network of branching filaments called hyphae. The building blocks of each hypha are plant cells. The cell wall consists of a chitin nanofiber network and a lipid bylayer.

mycelia. On a dry mass basis, mycelia consists of 75–80% polysaccharides, out of which 10–20% are the tough and strength-bearing chitin, while the remaining polysaccharides are mostly glucans and mannans. There is also a huge difference in the density of hide and mycelia. The density of hide is in the range of 0.9–1.2 g/cm^3 *(14)* — several times higher than the density of the starting dried but not pressed mycelia for Mylo material.

Solid-state fermentation for mycelia

Despite the big differences between hide and mycelia, Bolt Threads has developed a process to replicate leather-like properties in mycelia. We partnered with another startup that had been developing mycelium as a potential packaging material to replace Styrofoam to help reduce the amount of packaging waste that piles up in landfills and oceans.

To create Mylo material, mushroom roots are grown on a heterogenous substrate made of agricultural byproduct plant biomass (Figure 6). Substrates used for exotic mushroom production, including corn stover, hickory, and beech sawdust, have all been used successfully. The substrate is designed and formulated to provide additional fats, protein, or mineral nutrients to ensure the growth of the mycelium *(12)*.

During the growth process, environmental conditions are controlled to ensure that the fungus grows in a manner amenable to producing a mycelium mat that can be processed into the final goods. Similar to growing mushrooms for consumption *(15)*, conditions such as temperature, moisture, and metabolic gas content (*e.g.,* oxygen and carbon dioxide) must be regulated. Certain conditions will suppress sexual fruiting and the production of secondary metabolites, and ensure homogeneity of growth.

While the process can be considered a solid-state fermentation, it also closely resembles the production of mushrooms on plant-based substrate for human consumption. Once the mycelium is fully grown, the mycelium mat is separated from the substrate and sent to downstream processing.

Mylo material DSP. Although the low density of mycelium makes it much more fragile than hide and poses challenges in processing, its high porosity offers several advantages that can be leveraged in the dying, plasticizing, and tanning steps.

Due to the nonporous nature of hide, the dyes, plasticizers, and other chemicals used in tanning (*e.g.,* chromium and fat liquors), take a long time to diffuse into the material, which necessitates long treatments with huge volumes of solutions loaded with chemicals *(16)*. Most of these chemicals are not absorbed and end up in wastewater streams *(10, 11)*. The porous nature of mycelium allows chemicals and biochemicals to be absorbed more easily and quickly, generating less waste and reducing processing times, potentially to hours rather than days for hides.

Mylo material combines processing innovations with age-old techniques from the leather industry, producing a revolutionary material with the feel, texture, flexibility, and performance similar to or better than animal-based leather.

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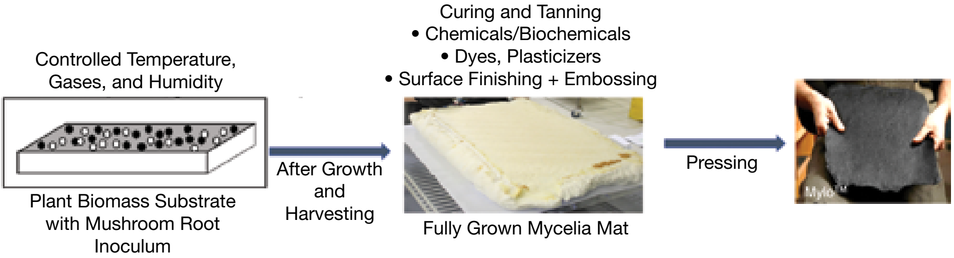
RITU BANSAL-MUTALIK, PhD, is the former technical director of Bolt Threads. She led a cross-functional team of process engineers, scientists, and researchers toward meeting downstream processing challenges for development, scaleup, and commercialization of both Microsilk fibers and Mylo material. Her main focus was on making these products and processes as economical, sustainable, and robust as possible. She has an interdisciplinary background with an MS and PhD in chemical engineering (with a focus on bioprocess technology and downstream processing of proteins) from the Institute of Chemical Technology (UDCT), Mumbai, India. She conducted postdoctoral research in biochemistry at the Univ. of California, Berkeley.

ALEX PATIST, PhD, is Vice President of Process Development and Manufacturing at Bolt Threads. He has more than 20 years of experience in directing new product, process development, and scaleup in the food, nutraceutical, biochemical, and biofuel industries. He has been a catalyst in mainstreaming a whole process approach to deliver economic advantaged bioprocesses, previously as Technology Director at Cargill (Minneapolis) and more recently as Senior Director at Genomatica in San Diego, CA. He holds a BS from Hogeschool Utrecht, an MS from TU Eindhoven, and a PhD from the Univ. of Florida, all in chemical engineering.



▶ **Figure 6.** To create Mylo, a substrate made of plant biomass is inoculated with mycelium cells. After the growth phase, the mycelium mat is peeled away from the substrate and subjected to a curing and tanning process. The chemically treated mat is pressed to make the Mylo material.

Closing thoughts

After billions of years of evolution, nature has been able to provide many elegant solutions to our most vexing problems. By combining the latest advances in biotechnology, which have unlocked the potential to design and tune material properties, a new generation of sustainable biomaterials has become reality, of which Microsilk fibers and Mylo material are great examples. Just as consumers are demanding animal-free meat and milk options, Bolt Threads is creating animal-free textiles and materials that generate less waste and have a lower environmental impact.

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"Building Successful Startups..." by J. Bobanović continued from page 29

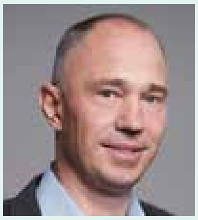
completing a merger and acquisition (M&A) or listing on the public markets through an initial public offering (IPO).

Apart from a short-lived wave of industrial biotech startups that took to the public markets in 2011, such as Amyris, Gevo, and Solazyme, we have not seen public listings or large acquisitions in the sector. The IPOs of 2011 were, in most cases, premature in terms of technology development. However, these IPOs were opportunistic because they captured market interest, and they provided the companies that survived continued access to financing, which gave them time to reshape their businesses to better fit current market needs.

More importantly, we have not seen any significant M&A activity in the industrial biotechnology sector in the past five years, save for a few smaller acquisitions (*e.g.,* Virdia, OPX Biotechnologies, and LS9). This suggests that the startups in the sector have not yet been able to prove themselves or their technologies indispensable for large players. The indication is that with the expanding footprint of industrial biotech applications, this is going to change rapidly. But for the moment, the lack of M&A activity remains an obstacle for the whole community's success.

Closing thoughts

Although the investment value chain is well established in other sectors, the industrial biotech field still has a long way to go. It all starts with building successful companies that make money for entrepreneurs and their investors. Although financial returns are not the only measure of success, they are an important measure. Until such time when we, as a community, can point to successful startups that are creating value, we will have to fight hard for every dollar raised and work even harder to convince other players in the ecosystem that this effort is worthwhile.



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New Tools from the Microbial World for Agriculture and Beyond

Kelly Smith
AgBiome

By 2050, crop production will need to increase by more than 70% to sustain the world's growing population. To meet this target, novel crop protection strategies must be developed and implemented. One biotech company is leading the charge in generating biological products for disease and pest control in crops.

Modern crop-protection practices employ high-quality agronomics, chemistries, and germplasm techniques to control damage due to pests and diseases. Despite these efforts, growers continue to experience crop yield losses of approximately 30% per year, and are faced with the rapid development of resistant pests and diseases after the introduction of each new tool.

Agricultural biotechnology company AgBiome discovers solutions to combat these problems based on a large and expanding core collection of fully sequenced microbes from the plant-soil microbiome. Using our innovative platform, both the microbes and their genome sequences are employed in the discovery of new traits and biological products for disease and pest control in crops. This article explains why new crop-protection strategies are desperately needed and describes AgBiome's approach to creating the tools that will help growers reduce crop damage and enhance yields.

The importance of agriculture

Since everyone needs to eat, it may seem obvious that agriculture is a globally important industry. However, the role of crop protection products in agriculture may be less clear.

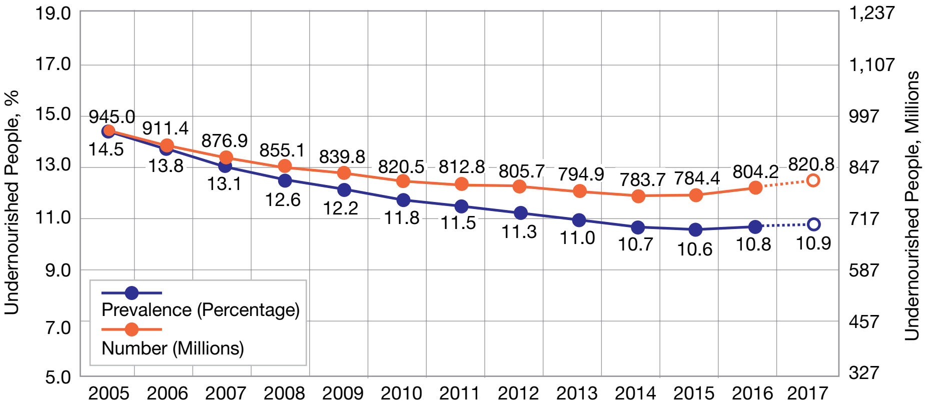
Agriculture is the world's largest and most important market; it is a multitrillion dollar business globally. Estimates vary, but most agree the agriculture industry is larger than the global pharmaceutical industry by severalfold. Abundant, affordable, healthy food is required for civilization to exist as we know it. However, food insecurity is a worldwide concern. More than 800 million people in the world regularly go to bed hungry, or do not know where their next meal will come from (Figure 1) *(1)*.

The Boston Globe referred to the uprisings in 2010 and 2011 known as the Arab Spring as a "revolution of the hungry" *(2)*; they were sparked in part by a doubling of wheat prices. Many other examples of social unrest in the Middle East and elsewhere can be traced to food insecurity *(3)*.

Good nutrition is the basis for all human health. However, lack of nutrition is not just a problem in the developing world. It was recently reported that one in five children under the age of 15 has limited access to food in the U.K. *(4)*. In the U.S., we speak about access to good and affordable healthcare — that same issue exists on an even greater scale for food.

For the first time in 200,000 years of human history, we are taxing our resources to their limits to produce food. Very little of our planet can support agriculture — only 10% of the Earth — and most of the productive land is already in use. Agriculture already consumes about 70% of useable freshwater. (Our planet contains very little useable freshwater to begin with — only 2.5% of all water on the planet is freshwater, and available freshwater is less than 1% of that, with the rest locked up in ice, permanent snow, or relatively inaccessible groundwater.)

This is not just a population growth problem, although we are adding over 80 million people to the world each



▲ **Figure 1.** The prevalence and number of undernourished people globally — defined by the Food and Agriculture Organization of the United Nations (FAO) as those having insufficient dietary energy consumption — has decreased over the past decade. However, more than 800 million people still go to bed hungry at night.

year. As billions of people in China and India enter the middle class as their economies grow, they demand more and higher-quality proteins, such as chicken, fish, beef, and pork. This places more demand on crops to feed these livestock. Therefore, agricultural production needs to increase 70% by 2050 and double by 2100 *(5)*. More crop output will be required to increase production at this scale, within the constraints we have on water and land.

Investment does not equal food security. Awareness of these challenges is increasing, as evidenced by the mounting press coverage of agricultural technologies. Investing in the agricultural sector is popular, with a record $6.9 billion invested in 2018. However, most of it is in socially driven areas that are unlikely to have broad impact on our ability to increase food production globally — such as organic produce. In the U.S., organic produce makes up only 5% of the market and is priced significantly higher than conventionally grown produce. And, organic agriculture uses significantly more land to produce the same amount of food, due to the lower yields inherent to open-pollinated crop varieties *(6, 7)*.

Vertical farming has also received much attention and investment. Although it can produce small amounts of expensive leafy greens in urban areas, vertical farming is not the solution to increase productivity globally. It will be difficult to scale up these operations to meet production requirements, and even as these operations are scaled, energy requirements will increase substantially *(8)*.

Other trending technologies, such as artificial intelligence (AI), precision farming, big data, and autonomous vehicles, will have a substantial impact on global food production. But we are still years from seeing broad impact on productivity or on farmers' bottom lines.

Meanwhile, all of this investment and attention have neglected a current and growing need in agriculture. Although 2018 was the biggest year ever for investment in agricultural technologies, almost none of those funds went into crop protection. The bottom line is that we have to protect crops from pests and diseases, or none of that other technology matters.

Crop protection and increasing resistance. Crop protection is required to grow food. Figure 2 shows a cucumber field infested with powdery mildew *(9)*. Nearly 100% of the crop was lost in the nontreated rows, on the left. Fungicide treatment (treated rows are shown on the right) gives the grower a better chance at successful cucumber harvest.

Without crop protection, the yield for many crops would be nothing. Even with the best seed, agronomic practices, and chemistries, it is common to see crop losses of 20% or more. On top of this, evolution ensures that pathogens, bugs, nematodes, and weeds develop resistance to crop-protection agents, and we are seeing resistance developing for every herbicide, insecticide, and fungicide that is on the market today. Figure 3 maps the resistance to herbicides occurring globally *(10)*.

The development of resistance is an evolutionary treadmill — we must continually develop new crop protectants to counter the presence of resistant populations and newly emerging pests. This is analogous to antibiotic resistance in human pathogens, where the resistant populations spread as the use of the products increase, and eventually the products lose all practical efficacy.

Technological developments for increased yields. Socioeconomic factors also play a role in determining the amount

▲ **Figure 2.** Crop protection is a crucial part of producing food. Powdery mildew can severely damage cucumber plants (left); treatment with a fungicide can protect cucumber plants and keep them healthy *(9)*.

of food we can produce. Figure 4 shows increasing corn yields in the U.S. over time *(11)*. The introduction of hybrid seed in 1940 triggered huge gains in yield.

Open-pollinated corn can be saved and planted the next year — so there is no financial incentive for breeders to improve the seed (they can only sell it once). Henry Wallace, the founder of Pioneer Hi-Bred seed, developed the first hybrid corn, which yields far better than the open-pollinated variety. However, the plants that grow as a result of the hybridization process produce seeds that are infertile and cannot be saved and replanted. Thus, farmers must buy new seed each year. As a result, breeders are incentivized to make better products that farmers will buy each year.

As these technologies have improved, developing countries have been left behind, which impacts their ability to feed themselves and to contribute to the increase in yield that the world needs for the future. Increasing yields in these countries, even to the level obtained in the U.S. by 1960, will require them to make sweeping, complex transformations in their politics, government, and infrastructure.

Industry response to food insecurity

The modern pesticide industry had its beginnings around World War II. Until the late 1990s, it regularly introduced synthetic chemical active ingredients with new modes of action (MoAs). In the early 2000s, the rate of discovery decreased significantly. The last major new MoA chemicals are now almost 20 years old, and all major agrochemicals are subject to resistance in the field.

Massive industry consolidation has stifled innovation. As recently as 1990, there were 13 significant players in the global agrichemicals and seeds business, each with global research and development (R&D) activities. As this article is being written, there are now only three.

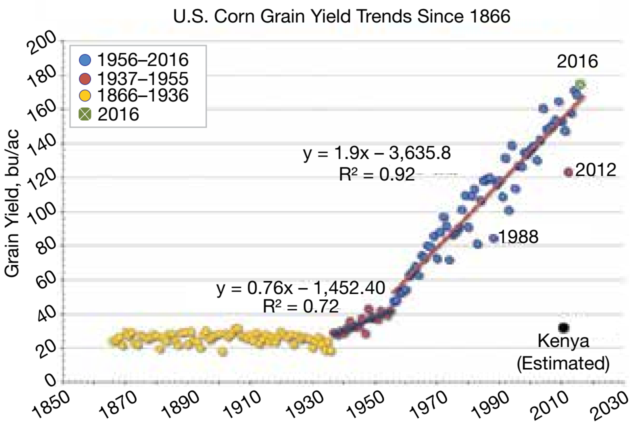
It is evident that the world needs leadership in pest and disease control to protect all of the other investments being made, and to protect our ability to produce food in the future. And, like the biotechnology and biopharmaceutical industries, that innovation is going to come from nimble, dynamic, and innovative agricultural-biotech companies.

AgBiome's mission is to develop the best new MoA biologicals (*i.e.,* live bacteria that are applied much like agrichemicals), biochemicals (compounds produced by microbes), and crop traits. Our core business is finding new ways of protecting crops to combat the effects of insect pests and plant diseases.

Technology offerings

AgBiome's products are based on microbes. Microbes are a largely untapped source of active ingredients. We currently have more than 60,000 completely sequenced, novel microbes for which we know every gene in each strain. Our data suggests that we will continue to discover new isolates and new genes as we increase the size of our collection, rather than starting to rediscover the same ones. We are on track to grow our fully sequenced microbe collection to 1,000,000 novel isolates by 2021. This seems like a big number, but we are not even scratching the surface of planetary microbe diversity, which is estimated to be around

▲ **Figure 3.** The number of herbicide-resistant weed species is increasing. On this map, the darker the red shading, the larger the number of weed species resistant to herbicides that have been recorded *(10)*.



▲ **Figure 4.** Corn yields in the U.S. increased sharply with the introduction of hybrid seed in 1940 *(11)*.

Table 1. Microbial-based insecticides have been on the market for many years. These are a few examples.

Active Ingredient	Example Brand Name	Source
Bt spores and crystal toxin	DiPel	*Bacillus thuringiensis*
Cry1F	Herculex	*Bacillus thuringiensis*
Spinosad	Tracer	*Saccharopolyspora spinosa*
Avermectin	Zephyr	*Streptomyces avermitilis*

10^{12} different bacterial strains, each of which contains approximately 5,000 genes *(12)*.

Of course, finding crop protectants in the microbial world is not a new concept. As Table 1 illustrates, several successful products that are based on microbes or microbial proteins have been on the market for many years. AgBiome has a unique opportunity to add to this set of tools for growers by finding novel pest-control active ingredients as we continue to screen our collection.

We are a product-focused company, and our first biological products are Howler for agricultural uses and Zio for nonagricultural uses, such as turfgrass and ornamentals. (Howler is distributed by AgBiome Innovations, and Zio is distributed by SePro.) In a trial conducted by Purdue Univ., researchers applied Howler as a seed treatment to control disease in soybeans (Figure 5). Howler provided better disease control than the most widely used chemical seed treatment (Azoxystrobin), as evidenced by the lack of live plants in the rows that received chemical treatment.

The photo on the right of Figure 5 is an electron micrograph demonstrating how Howler works. Soybean leaf (green) infected with Asian soybean rust (orange) is treated with Howler (blue). Howler appears to act as a pathogen to the fungus. The result is the highest efficacy for a biological product ever seen.

In addition, the product has a wide spectrum of activity against a variety of plant diseases. These include fungi such as *Rhizoctonia spp*. and oomycetes such as *Pythium spp*. The product can therefore be used in early-season, late-season, and post-harvest applications. It is Organic Materials Review Institute (OMRI)-listed and holds the lowest re-entry interval (REI) allowed by the U.S. Environmental Protection Agency (EPA) — 4 hr. REI is the time interval workers must wait after application of a pesticide before they can safely re-enter the area to tend or harvest the crops.

AgBiome's product strategy is designed to incorporate the strength of our proprietary biologicals and utilize the power of synthetic chemistry to solve plant protection problems for growers. We have three product lines (Figure 6):

- Valsyn — synthetics or nonbiologicals that we acquire at a low cost because they are off-patent
- Finaer — a robust pipeline of biological active compounds (*e.g.,* Howler), which are patent-protected
- Connate — a product line that combines ingredients from the other two lines to broaden the number of different diseases controlled and increase the number of different MoAs that are supplied, while reducing the amount of synthetic chemical residues on the plant.

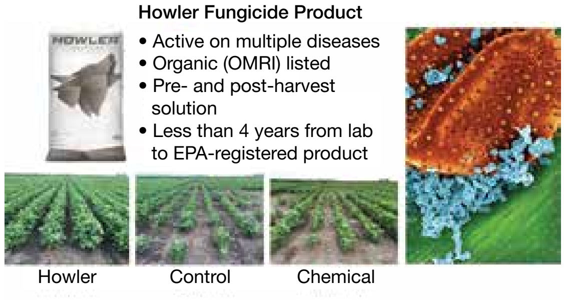
A small business with a unique structure

AgBiome has three strategic investors — Bayer, Syngenta, and Novozymes — with additional funding from venture funds such as Polaris, Arch, UTIMCO, Fidelity, Innotech Advisors, and the Bill & Melinda Gates Foundation. The support of this group indicates a high degree of confidence in our ability to deliver valuable new products in this industry.

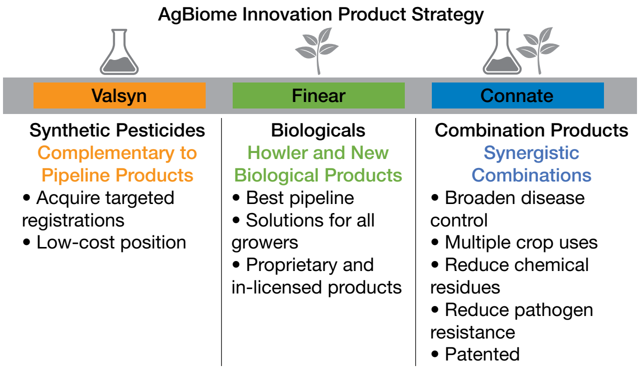
The parent company of AgBiome Innovations and AgBiome, Inc., is an LLC. We can take advantage of this structure by spinning off companies that can utilize our research platform and our unique database of microbial genes and strains for various business opportunities in the animal health, human health, and genome editing sectors. For example, we established a subsidiary called LifeEdit to discover and commercialize new genome editing tools (Figure 7). We are actively seeking partnerships to commercialize the hundreds of novel, patent-protected gene editing tools that we have discovered in our microbe collection.

We are also seeking partnerships to discover and commercialize new products for human health and animal agriculture from our collection. Because the collection contains more than 350,000 biosynthetic pathway genes, it is highly likely that some of these will produce useful proteins or small molecules for these industries.

In the animal-agriculture industry, direct-fed microbials,



▲ **Figure 5.** Howler is a novel fungicide that performs better than an industry-standard chemical product. On the right, soybean leaf (green) infected with Asian soybean rust (orange) is treated with Howler (blue). Howler involves a unique mode of action (MoA) in which it acts as a pathogen to the fungus.


AgBiome Innovation Product Strategy

Valsyn	Finear	Connate
Synthetic Pesticides	Biologicals	Combination Products
Complementary to Pipeline Products	Howler and New Biological Products	Synergistic Combinations
• Acquire targeted registrations • Low-cost position	• Best pipeline • Solutions for all growers • Proprietary and in-licensed products	• Broaden disease control • Multiple crop uses • Reduce chemical residues • Reduce pathogen resistance • Patented

▲ **Figure 6.** AgBiome's product offerings fall into three categories.

which can be used as a type of probiotic in animals, have generated a great deal of interest. A recent U.S. Food and Drug Administration (FDA) directive restricted the number and type of antibiotics that can be used in animal feed, and the industry is searching for microbes that can provide some of the same activities. AgBiome has recently entered into an R&D partnership with Elanco Animal Health to develop animal health products as part of Elanco's comprehensive Antibiotic Stewardship Plan *(13)*.

A team with purpose. AgBiome prides itself on a culture where experts with the knowledge make decisions. We believe that the key to employee engagement is ensuring that each employee has autonomy, mastery, and purpose in their day-to-day work *(14)*.

We rely on self-organizing and self-managing teams rather than a management hierarchy. These teams organize around operational tasks, problems to solve, or products to commercialize, and disband when those tasks are complete. The result has been 300 patent applications covering more than 100 biological strains and more than 3,500 genes for traits, as well as an EPA-registered biological product less than four years after first laboratory work on the active ingredient began.

Closing thoughts

Pest control and crop protection are large, unmet needs in agriculture, and no amount of investment in new technologies for food production will allow the industry to feed the future global population unless these needs are addressed. AgBiome is poised to become the leading supplier of new crop protection products based on our large and growing collection of novel microbes and our database of complete genomes from these microbes.

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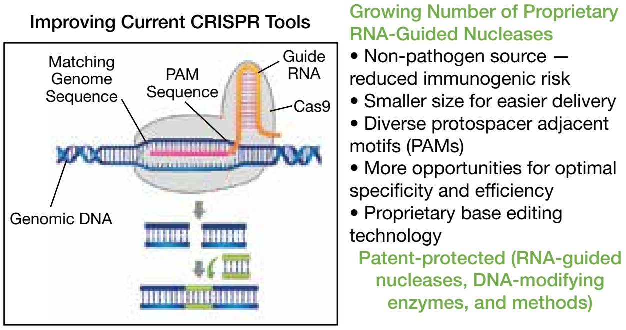


▲ **Figure 7.** LifeEdit's patent-protected tools, including diverse new protospacer-adjacent motif sequences (PAMs) and RNA-guided nucleases (RGNs), offer significant opportunities for new products in human health, animal health, and agriculture.

KELLY SMITH, PhD, is an experienced biotechnology innovator. She returned to the entrepreneurial community in 2014 after serving as Head of Pasteuria Bioscience for Syngenta Crop Protection. She was a co-founder of Pasteuria Bioscience, where she was principally responsible for development of the proprietary Pasteuria manufacturing process. At AgBiome, she founded the fermentation and formulation teams, led the early manufacturing development and registration of the Howler fungicide project, and currently serves as a research and innovation leader for all of the company's biological product research and development projects. She holds an MS and PhD in environmental engineering science from the California Institute of Technology and a BS in chemical engineering from Michigan State Univ.

Meeting the Needs of the Cell-Based Meat Industry

Elliot Swartz
The Good Food Institute

Commercialization of cell-based meat products at economically viable prices will require significant innovations, presenting new challenges and opportunities for industrial biotechnologists.

Cell-based meat (also referred to as clean or cultured meat) is genuine meat cultivated directly from animal stem cells rather than by raising and slaughtering animals (Figure 1). The meat is created through a bioprocess in which stem cells are extracted, isolated, and proliferated in bioreactors at high densities and/or in large volumes. These stem cells are subsequently differentiated, either in the presence or absence of scaffolding materials, into the principal cellular components of meat, including skeletal muscle, adipocytes, and fibroblasts of the connective tissues. The final product mirrors the structure, composition, and nutritional value of conventionally derived meat.

Advances in regenerative medicine and bioprocess engineering have made the creation of palatable prototypes relatively straightforward. However, scaling up the process while lowering costs will require innovations in cell line development, cell-culture-medium optimization, bioreactor and bioprocess engineering, and scaffold biomaterials.

A growing problem

The United Nations estimates that by the year 2050 there will be 9.7 billion humans on Earth. As this number grows, the socioeconomic status of residents in developing countries will continue to increase, and global demand for meat is expected to double *(1)*. This appetite for meat from industrialized animal agriculture is not without consequence.

Animal agriculture accounts for 14.5% of global greenhouse gas emissions *(2)* and is projected to account for 81% of the remaining carbon budget under the Paris Agreement by 2050 if current rates of production continue *(3)*. While 77% of habitable land on Earth is used to raise and feed livestock, this land use accounts for only 17% of the global caloric supply *(4)*. Industrial animal agriculture is the leading cause of global deforestation and biodiversity loss *(5)*, and it is a major contributor to foodborne illness and zoonotic disease outbreaks *(6)*. The volumes of antibiotics used to produce livestock and farmed fish is at least equivalent to that used in humans, and antibiotic use is expected to rise, making industrial animal agriculture a significant contributor to antibiotic resistance *(7)*.

The public awakening to the urgency of climate change and the negative externalities associated with industrial

▲ **Figure 1.** This meatball is formed from cell-based meat that was grown in a bioreactor from bovine stem cells, eliminating the need for livestock and the associated ethical and environmental challenges. Photo courtesy of Memphis Meats.

animal agriculture, including animal welfare, has made consumers more accepting of alternative meat products, such as plant-based and cell-based meat *(8)*.

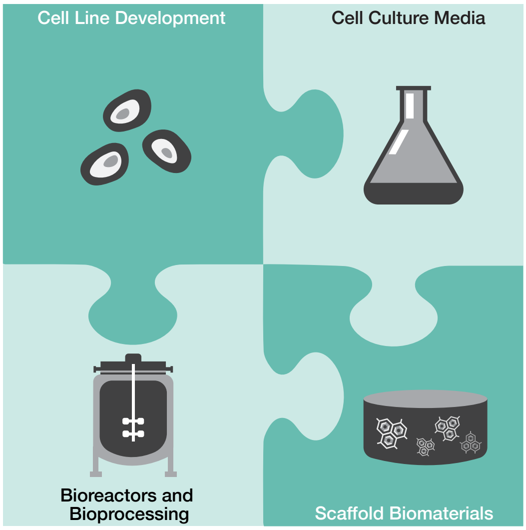
A potential solution

Growing crops to feed animals to produce meat is a vastly inefficient process, as most calories are expended for metabolism rather than creating edible meat. In 2013, Mark Post revealed the first cell-based hamburger, demonstrating that the animal could be cut out of the equation altogether. Since then, more than three dozen cell-based meat companies have formed across the world, aimed at dramatically reducing negative externalities of meat production while taking a bite out of the more than $1 trillion global market.

Preliminary projections estimate large gains in land use and energy efficiency and reductions in eutrophication (*i.e.,* nutrient runoff from fertilizers and manure that cause algal blooms and water dead zones) *(9)*, as well as curtailment of livestock-related biodiversity loss and zoonotic disease. At scale, preventive controls and monitoring methods adapted from existing biopharmaceutical bioprocesses enable antibiotic-free cultivation, lowering global antibiotic use while simultaneously reducing the incidence of foodborne illness. These benefits make cell-based meat a potential solution to many pressing problems.

Critical technology areas

To commercialize cell-based meat, four critical technology areas require further innovation: cell line development, cell culture media, bioreactors and bioprocessing, and scaffold biomaterials (Figure 2) *(10)*.



▲ **Figure 2.** To reach price parity with conventionally derived meat, engineering of cell lines and bioreactors is needed alongside smart selection of raw materials for cell culture media and scaffolding.

Cell line development

As starting material for cell-based meat, cells that can self-renew and differentiate into the cellular components of meat are isolated and selected. Companies in the cell-based meat space work with embryonic, induced pluripotent, mesenchymal, and adult stem cells such as myosatellite cells. The starting cell type ultimately influences many of the downstream variables of the bioprocess, such as timeline and differentiation strategy. Cell selection should be weighed alongside cost models and design requirements for the intended products.

Considerable work has been done using these cell types from bovine and porcine species, but substantially less work has been performed on the range of other species humans consume, especially sea creatures. Publicly available biorepositories of cell lines from commonly consumed species are needed to accelerate research and generate -omics datasets to facilitate development.

A variety of cell line engineering strategies can improve upon or optimize the bioprocess. However, future regulatory standards may dictate the extent to which engineering appears in final products. For example, strategies might include the creation of immortalized cell lines and cells that have high tolerance to shear stress, resistance to toxic metabolite buildup such as ammonia and lactic acid, suitability for suspension growth, and low growth factor concentrations. Engineered biosensors can assist in signaling hypoxic conditions, mechanical stress, or amino acid and glucose starvation *(11)*. Other strategies may be able to remodel metabolic or differentiation pathways, making them more efficient or favorable to low-cost cell-culture-medium ingredients, rather than expensive growth factors.

Researchers may pursue cell lines that inherently exhibit many of these properties, such as insect cell lines that are adaptable to suspension growth, tolerate nutrient starvation, and readily immortalize *in vitro,* or fish cells that can be grown at room temperature *(12, 13)*. Companies and researchers with experience in strain optimization or high-throughput genome editing are needed to support these efforts.

Cell culture media

The cell culture medium is the most important factor in maintaining cells *ex vivo*. Since the 1950s, virtually all basal cell culture media have consisted of variable buffered solutions of glucose, inorganic salts, water-soluble vitamins, and amino acids tailored to specific cell types. To achieve long-term maintenance and proliferation, insulin, transferrin, selenium, lipids, antioxidants, and other growth factors are included, typically in the form of animal sera such as fetal bovine serum (FBS).

FBS has been a mainstay in mammalian cell culture

because it is rich in growth factors and hormones, which supports a proliferative fetal-like state. However, FBS is not viable for use in cell-based meat because:

- it varies by region and batch
- it is a potential source of contamination
- it is misaligned with animal welfare
- not enough of it is available to supply the industry *(14)*.

While serum-free alternatives exist, they are expensive and often optimized for human cells in clinical settings or cell lines used in production of biologics under current good manufacturing practice (cGMP) guidelines. Estimates suggest that 55–95% of the marginal cost contribution of a cell-based meat product will come from the medium. Thus, the cell-based meat industry will likely require optimized serum-free formulations for a variety of cell types, at price points below $1.00 per liter to become economically feasible at industrial scales *(15)*.

Several strategies could help achieve this goal. For example, protein-rich hydrolysates from plants, such as soy, wheat, pea, or organisms such as yeast and cyanobacteria, can support a proliferative environment for cells at low cost *(16)*. Machine learning or differential evolution algorithms could be used in tandem with *in silico* modeling or high-throughput microfluidic systems to accelerate the pace of formulation discovery *(17)*.

Production of commonly used recombinant proteins, such as insulin, transferrin, FGF2, TGFβ, and platelet-derived growth factor (PDGF), must be scaled to match production costs of food industry enzymes such as pectinase and cellulase, which can be purchased for less than $5.00 per gram. This may require additional host or protein engineering, as certain growth factors, such as TGFβ, are typically produced in mammalian expression systems rather than microbial host platforms. The growth factors themselves may also be engineered to create synthetic proteins with multiple bioactive domains or more-stable isoforms.

Recent demonstrations focusing on the optimization of growth factor production suggest that stem cell medium costs can be reduced by 97% or more *(18)*. Lower purification demands for food-grade production of basal and recombinant components may reduce costs further, but may also require new, nonpharmaceutical-grade manufacturing facilities. It is unclear whether regulations or the need for reproducibility will require chemically defined medium formulations; the answer may dictate the exclusion of medium constituents such as hydrolysates, which are chemically undefined.

Additional methods to reduce costs include the development of small molecules that can mimic the bioactivity of more-expensive growth factors. However, the safety profile of any residuals within a final product should be considered for this approach. Water and nonmetabolized medium components could be recycled using size-exclusion dialysis filters to reduce costs while simultaneously removing waste *(19)*. Efforts by the biopharma industry to move toward perfusion culture and continuous bioprocessing have driven the development of continuous monitoring systems and adaptive control with concentrated feeds, which could also help lower the cost of cell-based meats.

None of these strategies are technologically infeasible or require large scientific leaps. Rather, the demand being established by the ultra-large-volume cell-based meat industry is driving the effort to rethink the composition of cell culture media. New business opportunities abound for those equipped to scale recombinant protein production and rapidly iterate media formulations.

Bioreactors and bioprocessing

In order to scale beyond taste tests toward market readiness, standard 2D culture or miniaturized stirred flasks must be replaced by bioreactors capable of supporting high-density and/or large-volume cell cultures. Production of biologics using suspension-adapted cells in stirred-tank reactors has reached volumes of 20 m^3. But, the production of therapeutic off-the-shelf mesenchymal stem cells typically uses volumes less than 0.25 m^3, as these cells must be cultured on microcarriers or another solid surface to avoid a form of programmed cell death known as anoikis *(20)*. Cells used in cell-based meat are also anchorage-dependent and face similar challenges. Thus, significant developments are needed to scale cell-based meat to affordably and reproducibly produce batches upward of 10^{12} to 10^{15} cells.

Scaling up can require large capital expenditures and time. To increase scaling efficiency, miniaturized bioreactors or microfluidics can produce predictive models of process parameters. Once the process works at larger scales, development of real-time, online sensor systems can help enable continuous and/or perfusion bioprocessing methods that save money. *In silico* modeling of nutrient utilization and the buildup of inhibitory or stimulatory paracrine factors and/or toxic waste can inform feeding strategies, timelines, and perfusion rates *(21)*. The implementation of automation from the ground up, as opposed to retroactively replacing manual steps, can unlock additional cost savings. Dynamic cost-of-goods models can help identify bottlenecks that can be prioritized for automation or future research and development (R&D) efforts as the industry matures.

Proliferation of cells in a semi-continuous or continuous process can minimize processing times or increase the productivity of seed train processes. In a seed train process, cells are grown and used to inoculate sequentially larger, higher-volume vessels, capturing the greatest efficiencies at later cell doublings. For example, productivity can be increased by using a percentage of cells from the highest-

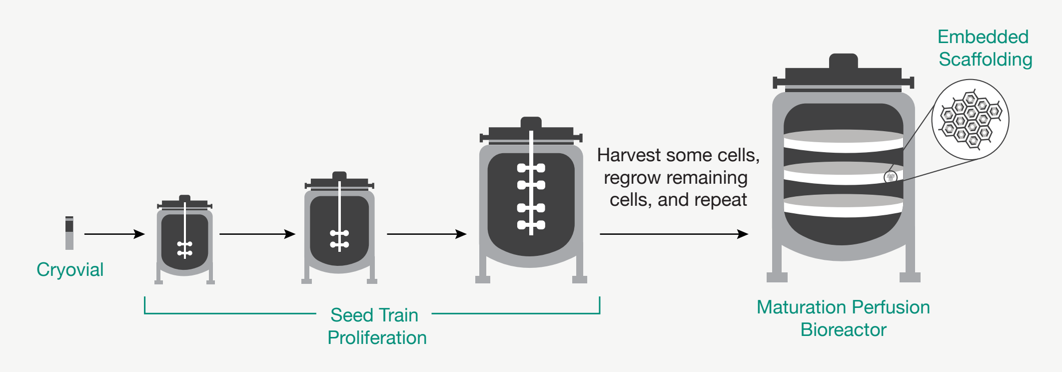


Figure 3. Seed train processes may be semi-continuous or continuous. In this process, cells are grown and used to inoculate sequentially larger vessels in higher volumes. Productivity can be increased by using a percentage of cells from the highest-volume vessel in the proliferation stage to directly inoculate a final, large-volume maturation perfusion bioreactor.

volume vessel in the proliferation stage to directly inoculate a final, large-volume maturation bioreactor (Figure 3).

Perfusion bioreactors, such as hollow-fiber bioreactors, can achieve higher cell densities in lower volumes and operate continually over months, making them an attractive conduit between proliferation seed-train stages. Additionally, larger-volume reactors can be directly inoculated using high-density cryobanking at greater than 10^8 cells/mL, lowering the time to achieve desired cell densities or numbers in seed trains *(22)*.

Innovations such as cell-laden core-shell hydrogels can achieve remarkably high densities of 5×10^8 cells/mL, permitting cellular proliferation in 3D microenvironments shielded from shear stress *(23)*. Creative approaches that entail thinking beyond what has worked for cell therapy may prove to be a valuable strategy for those moving into the cell-based meat space.

While cell therapy and cell-based meat both share the cell itself as the end product, the final stages of cell-based meat — differentiation and harvesting — will likely look quite different. Although unstructured meat products could themselves be composed of pressed cells, cells as additives, or even cells on edible microcarriers, structured products will require the use of a scaffold. Computational fluid dynamics (CFD) models are needed to understand how fluids in a perfusion bioreactor with embedded scaffolding behave. Online sensors can be used to adjust flowrates as the scaffold becomes cell-laden to protect the cells and scaffold itself from fluctuating shear forces. Bioreactor and bioprocess engineers are needed to create new bioreactor models that can support this culture strategy while integrating straightforward harvesting and sterilization processes.

Scaffolding biomaterials

A scaffold for cell-based meat ideally permits cells to attach and differentiate in a specified manner that mimics the 3D cytoarchitecture of an intended meat product. The cytoarchitecture must allow for continuous perfusion of media, analogous to the vascularization of real tissue. In tissue engineering, considerations of the porosity of the scaffold, mechanical properties, and biocompatibility are paramount; in creating cell-based meat, the use of cost-effective edible or biodegradable materials is just as important. However, cell-based meat does not require the same microscale precision as functional tissue. It merely needs to represent tissue structure sufficiently to replicate the appropriate texture and mouthfeel.

Further exploration of plant- or fungal-derived polymers as scaffolds is needed. These organisms may be engineered to express key cell adhesion domains used by vertebrates to boost biocompatibility *(24)*. Alternatively, a polymer-based scaffold could be enzymatically modified or embedded with growth factors to temper the dynamic cellular behavior following seeding. Chemical modifications can create a tunable scaffold that is responsive to simple external stimuli such as light or temperature *(25)*. These or other forward-thinking strategies related to preferred materials and how they may be sourced via existing or new supply chains can help encourage the development of cell-based meat.

Methods pioneered by tissue engineers can be adopted for the assembly of cell-based meat scaffolding but will need to be expanded upon. For example, extrusion and stereolithographic bioprinting are promising candidates, but these processes must be able to be run economically at large scales in parallel. Use of electrospinning and decellularization techniques can be informative from an R&D perspective, but may be difficult to implement at scale. Databases with information on plant, fungal, and microbial biopolymer mechanical properties, biocompatibility, anisotropy, viscosity, and other parameters can inform the selection of the most promising candidate methods and materials.

Looking forward

Cell-based meat is a nascent but rapidly growing field that may significantly benefit human, animal, and planetary health. It is a highly interdisciplinary field that presents fascinating scientific challenges, as well as potentially lucrative new market entry points. Challenges for cell-based meat are

not problems to be faced by the industry alone, but problems to be tackled in collaboration with other fields, such as cell therapy, regenerative medicine, and fermentation products, as the solutions will have a rippling effect.

To have the greatest impact on solving the world's toughest challenges, scientists, engineers, and biotechnologists should consider cell-based meat as an opportunity to apply their skillsets. An influx of talented scientists from across these fields will be needed to further drive the success of the industry.

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Scaleup and Commercialization of a Gas Fermentation Technology

Rachel Brenc
LanzaTech

A newly commercialized gas fermentation platform could be key to addressing climate change by enabling biological conversion of waste gases to products.

As our world's need to combat climate change becomes critically important, sustainable production of fuels and chemicals is at the forefront of the fight. In October 2018, the Intergovernmental Panel on Climate Change (IPCC) released a report on the necessity of limiting global warming to a maximum 1.5°C above preindustrial levels to reduce the risk of catastrophic climate change *(1)*. Of the 90 scenarios put forward by the IPCC to limit global warming, only nine have a 50–66% likelihood of limiting peak warming to below 1.5°C during the 21st century. The remaining scenarios involve some amount of temperature overshoot before dropping back to 1.5°C.

Negative emissions technologies (NETs) such as carbon capture and storage (CCS), afforestation, and bioenergy with CCS (BECCS) are among the solutions proposed to limit peak warming and to correct for any overshoot, although each solution would require rapid deployment on a massive scale, which may or may not be achievable *(2)*. Every scenario the IPCC proposed to keep warming below 1.5°C requires decarbonization of the electrical grid. Such decarbonization could be accomplished by transitioning to low-carbon technologies like wind and solar. IPCC also noted that such transitions need to be "large-scale and rapid" and that "all relevant companies, industries, and stakeholders would need to be involved to increase the support and chance of successful implementation *(1)*."

The transition to a fully decarbonized grid and the development and deployment of zero-emission vehicles is underway. Nevertheless, low-carbon fuels will continue to be required for road vehicles and sustainable plane and jet travel *(3)*. Therefore, both policies and technology development must drive a transition toward grid decarbonization, as well as lower emissions in light- and heavy-duty road, air, and marine vehicles.

LanzaTech was founded in 2005. Our proprietary process directs the waste gases from industrial facilities to a fermentation reactor in which microbes convert the carbon in the feed gases into valuable fuels and chemicals. Following downstream processing steps, the fuel or chemical product can be recovered and used or sold.

Our gas fermentation technology diverts CO_2 away from the atmosphere and incorporates it back into the market as fuel or another chemical product, extending the lifecycle of the carbon.

Feedstocks for gas fermentation

In a world where chemical prices fluctuate with the price of oil, imagine being able to decouple from the market, instead relying on a stable and predictable feedstock. By producing commodity chemicals from waste gases of a known and stable price, LanzaTech is making this a possibility.

The opportunities for carbon recycling via gas fermentation are numerous. For example, offgases from steel mills, refineries, and chemical plants all make good feedstocks for direct fermentation within the platform. Typical waste gases and syngases are a blend of carbon monoxide, carbon dioxide, hydrogen, and nitrogen.

The possibilities increase significantly when solid feedstocks — such as municipal solid waste (MSW, or unsorted garbage), agricultural residues (*e.g.,* nutshells and corn stover), and logging residues — can be gasified to generate syngas. Once the recyclable components are removed from an MSW stream, the remaining trash can be gasified, broken down into syngas, and remade into goods, fuels, or chemicals (Figure 1).

Key to the technology is the potential volume of feedstocks. If offgases and solid wastes from facilities all over the globe were fed through the process, we could produce approximately 500 billion gal of ethanol annually, averting 7% of global CO_2 emissions, which is the equivalent of taking 700 million cars off the road.

The ideal feedstock for the process is waste gas or fluegas that is released to the atmosphere or flared, a common occurrence at many steel mills, refineries, and chemical plants worldwide. Solid products for gasification can even be obtained at a negative feed price, as a tipping fee for taking what would otherwise be waste.

The gaseous feedstock is compressed, treated, and directed into a bioreactor, where it is consumed by a proprietary microbe (Figure 2).

The fermentation microbe

The LanzaTech microbe performs a water-gas shift reaction, enabling use of feedstocks with a variety of H_2:CO ratios. The microbe consumes CO_2 in the presence of H_2 (Figure 3). A range of feedstock compositions can be utilized without requiring expensive bulk gas separations.

The proprietary microbe is incredibly robust — able to withstand large fluctuations in feed gas composition, flowrate, and a wide range of common contaminants that would poison traditional catalysts. The microbes live in bioreactors, where they are maintained at a specific temperature, pressure, and pH. They utilize the carbon and energy in the feed gas as their main substrate, producing the target fuel or chemical in addition to growing additional microbes. Single-pass gas conversions (the fraction of reactive components consumed in the reactor) are up to 95%.

This biological process has several benefits over traditional catalytic processes. For example, it operates at a significantly lower temperature and pressure than a catalytic process. It is also flexible, in that if a more optimized microbe is developed, the new microbe can be used in the next run without a plant shutdown.

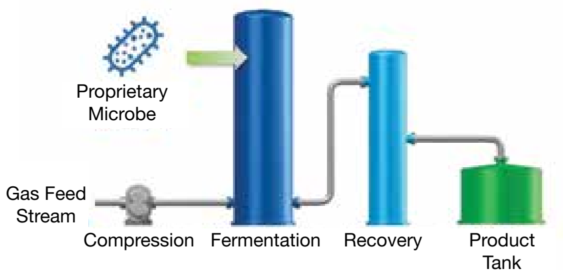
The LanzaTech microbe can produce more than 50 different products, natively and with genetic modifications, making it an excellent chassis. Given its anaerobic nature, it can be more robust than traditional chassis microbes like *E. coli* and *S. cerevisiae*.

The products

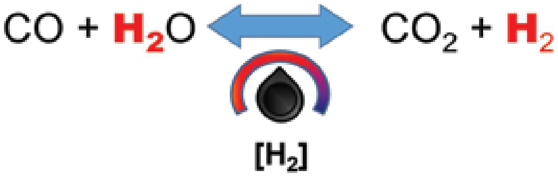
The flagship LanzaTech process produces ethanol, which is easily distilled and purified to fuel or chemical-grade ethanol. However, if the price of a different product becomes economically attractive, a new microbe can be introduced, utilizing the same infrastructure and reactors (with modified downstream processing steps) to take advantage of the market changes.

The process is continuous; broth containing the target product is continuously withdrawn from the unit and purified to generate a product stream. In addition to the main product, the platform produces several other value streams. The biomass, once spent, is useful as a high-protein animal feed. Non-biomass metabolites are digested and converted to biogas (methane). Unconverted gas is oxidized and the resulting heat is captured as steam energy for the process.

▲ **Figure 1.** Discarded products, such as unrecyclable waste plastic, can be broken down into building blocks, gasified, and transformed into valuable fuels and chemicals.



▲ **Figure 2.** The LanzaTech process is continuous and consists of a few simple process steps. A low-pressure waste gas is compressed and fed to the fermentation reactor. The product is then recovered and stored for shipment.



▲ **Figure 3.** The LanzaTech microbe is able to perform a biological water-gas shift reaction to consume CO_2. It utilizes hydrogen when available; otherwise, it generates its own from water.

Path to commercialization

LanzaTech was founded in 2005. In its infant years, the company researched ethanol production on a small scale, and it optimized and trained the microbe to produce higher yields at a better selectivity. The work quickly scaled from small-batch bottles to lab-scale continuously stirred tank reactors.

As milestones were hit, the company developed partnerships to build and operate pilot plants. A key first pilot plant was constructed at New Zealand Steel in Glenbrook, New Zealand, in 2007. The slipstream of the offgas available at the steel mill was diverted to a LanzaTech bioreactor. This plant was critical to the scale-up journey, as the microbes were exposed to a gas stream that fluctuated in flow and composition in real time. The robustness of the microbe was confirmed, and its stability was proven for potential commercial applications.

Subsequent pilot plants were quick successes, with milestones reached at the White Biotech pilot plant in Taiwan within two campaigns.

The final stepping stones prior to commercialization were two demonstration-scale facilities in 2012, including one with future commercial partner Shougang LanzaTech (SGLT) in Caofeidian, China. Demonstration-scale operation enabled full-unit integration, including distillation and wastewater treatment. Several campaigns over multiple years hit milestone productivity and run lengths, demonstrated water recycle capabilities, and demonstrated recovery of spent microbes as a potential product stream. Once all parameters necessary for scaling were validated, and the risk was sufficiently low, SGLT received key funding for construction of the first commercial plant.

Deployment and learning at scale

The first commercial plant with SGLT broke ground and started design work in 2016, and reactor construction followed in 2017. A commercial support team was deployed once plant construction was underway, while a small engineering team supported unit commissioning locally. The plant started up in May 2018, and fermentation scientists and analytical scientists joined the engineers on site once fermentation was live. These key personnel had many combined years of experience operating fermentation across all scales and they provided real-time training, solidified procedures, and gave advice as the unit started up (Figure 4).

This first commercial plant has a nameplate capacity of 46,000 metric tons (m.t.) of ethanol per year (~15 million gal/yr), which is currently being sold on the local fuel market (Figure 5). More than 9 million gal of ethanol has been produced to date, and selectivity and productivity targets have been met. Now production is primarily limited by feed gas availability. Additionally, biogas (methane) is generated during anaerobic digestion of process effluent.

LanzaTech uses a licensing model, whereby the gas fermentation technology is licensed and a portion of the revenue is collected as royalties, while the licensee owns and operates the plant.

A first-of-its kind commercial plant does not come without challenges. We had to shift our mindset from that of a research-based organization to a production-focused one. Plant construction and startup required rapid troubleshooting and took an all-hands-on-deck approach. We instituted a support roster so that the onsite team could call on experts to answer daily questions. It was important to communicate to all employees that a commercial plant is not a testing ground or training facility, and that changes should only be advised if there is supporting lab data and sufficient evidence.

The primary consideration throughout all decisions was safety. Developing evacuation plans, guidelines for vessel

▲ **Figure 4.** A team of scientists and engineers poses outside the newly commissioned Shougang LanzaTech (SGLT) commercial ethanol plant in 2018.

◀ **Figure 5.** At the SGLT commercial plant, ethanol is made in the bioreactors (left) and then distilled within the distillation unit (right).

entry, and maintenance of safety equipment was highest on the priority list and was required before our advisory work could begin at the plant.

An important part of our early operations support was troubleshooting mechanical and operational issues and close monitoring of fermentation performance so that quantitative recommendations could be provided. To make fast decisions, operating and sample data had to be easily accessible and comparable to other commercial campaigns, as well as operating data from prior small-scale operations.

We realized that data from lab devices, online field devices, and process data from the distributed control system (DCS) needed to be co-located in a single database, with the ability to plot data quickly for visualization purposes. This required specific expertise in this area, as it is likely that data from the different sources is in different formats or timescales, and also not in alignment with similar data from lab, pilot, and demonstration scales. An open-platform communications (OPC) server and database was a good solution for this type of data processing. We recommend a single full-time equivalent (FTE) employee with expertise in this area to manage this information for a first commercial implementation.

Another challenge facing all new process technologies, biological or otherwise, is selecting the proper analytical equipment to fully characterize the feed gas. To prepare for variations in gas feed and impurities from upstream process upsets, a robust and comprehensive set of analytical devices was necessary. In advance of the plant start-up, it was important to gather a full set of continuous gas data, as spot samples may not capture the natural fluctuations of the upstream process. With continuous sampling, peaks and important trends can be identified early and proper mitigation taken by adjusting process variables.

A final challenge associated with start-up was working across countries and time zones. To manage these challenges, a strong and experienced local team of native speakers was invaluable. This included local subsidiaries for billing and purchases, and administration to help with travel arrangements and accommodations. It was advantageous to line up a local laboratory, local freight forwarders, consultants, and other support crew prior to the plant start-up. Once these systems were in place, the plant could be supported smoothly, as the staff had the proper tools, suppliers, and technical support required.

The future

The journey does not stop at ethanol production. Both acetone and isopropyl alcohol have been piloted and are under optimization as a result of extensive development and scale-up work by our synthetic biology and fermentation teams.

We have planned a full pipeline of commercial projects to produce ethanol, including projects in Belgium with ArcelorMittal utilizing steel mill offgas, and in India with Indian Oil Corp. using refinery offgas (Table 1). We also have a planned project with Sekisui in Japan, where we will gasify municipal solid waste to produce ethanol.

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Table 1. LanzaTech's portfolio is growing, with several facilities in construction and design stages that are expected to be operational in the next few years.

Project/Client	Location	Feedstock	Capacity, m.t./yr ethanol	Status
Shougang LanzaTech	China	Steel offgas	46,000	Operating
ArcelorMittal Ghent	Belgium	Steel offgas	62,000	Construction
Indian Oil Corp.	India	Refinery offgas	34,000	Construction
Swayana	South Africa	Ferro-alloy offgas	52,000	Design
Aemetis	California, U.S.	Biomass syngas	35,000	Design