Innovation trends in industrial biotechnology

Citation for the original published paper (version of record):
Nielsen, J., Tillegreen, C., Petranovic Nielsen, D. (2022). Innovation trends in industrial biotechnology. Trends in Biotechnology, 40(10): 1160-1172.
http://dx.doi.org/10.1016/j.tibtech.2022.03.007

Trends in Biotechnology

CellPress OPEN ACCESS

Opinion

Innovation trends in industrial biotechnology

Jens Nielsen, [1,2,4,5,*] Christian Brix Tillegreen, [1] and Dina Petranovic [2,3]

Microbial fermentations are used for the sustainable production of a range of products. Due to increasing trends in the food sector toward plant-based foods and meat and dairy product substitutes, microbial fermentation will have an increasing role in this sector, as it will enable a sustainable and scalable production of valuable foods and food ingredients. Microbial fermentation will also be used to advance and expand the production of sustainable chemicals and natural products. Much of this market expansion will come from new start-ups that translate academic research into novel processes and products using state-of-the art technologies. Here, we discuss the trends in innovation and technology and provide recommendations for how to successfully start and grow companies in industrial biotechnology.

Highlights

Microbial fermentations are widely used for the production of molecules used as pharmaceuticals, for foods and beverages, food ingredients and supplements, nutraceuticals, perfumes, monomers, solvents, and biofuels.

The creation and optimization of microbial cell factories and fermentation processes can enable the development of many novel solutions that could address several societal challenges (e.g., for creation of a sustainable food supply with reduced greenhouse gas emissions or petroleum-independent liquid fuels, solvents, and materials).

The complexity of biology can result in a variety of solutions to a given problem, thereby enabling several companies to enter a given area.

Academic entrepreneurship and start-up-driven innovation are important contributors to developments of new bioindustrial solutions.

Key criteria for success in start-ups are: addressing a need (zero-to-one); pivoting when needed; and aiming for a minimal viable product as early as possible.

Industrial biotechnology: the glorious past, the challenging present, and a bright future

Industrial biotechnology mostly relies on manipulating and growing different types of bacteria, yeast, and filamentous fungi. Controlled microbial fermentations used by humans have been around since the dawn of civilization for the production of fermented foods and beverages [1]. During the early 20th century, the first industrial-scale fermentation processes were established for the production of chemicals, including acetone, butanol, and citric acid. Production of citric acid in 1919 by *Aspergillus niger* was a particular breakthrough as it was the first aerobic fermentation process and, hence, required the establishment of technologies that could ensure the provision of sterile air in large quantities to support the production process. This advancement paved the way for the aerobic industrial-scale production of penicillin during World War 2; shortly after the war, several novel processes were established for the production of a range of antibiotics. During the 1960s and 1970s, production of several different chemicals through microbial fermentation was established, such as for amino acids used in food and feed, and for industrial enzymes with a wide range of applications. With the introduction of genetic engineering during the early 1970s, the biotech industry was established for the production of proteins for pharmaceutical use [1]. This industry has grown substantially over the years and most top-selling drugs are now produced by fermentation (including cell cultures), with several being produced through microbial fermentation [2], including insulin and other hormones [3]. With the ability to engineer microorganisms, the idea of developing cell factories for production of an even wider range of products emerged. Several large-scale ventures were established, such as by the chemical company Eastman, which invested heavily in establishing the commercial production of dyes, including indigo. However, the technology for engineering microorganisms was not sufficiently mature, and most of these ventures did not deliver financially or at scale. With the genomics revolution of the early 2000s, based largely on shotgun genome sequencing and development of modern 'omics analytics and data analyses of microorganisms, more data were obtained relating to microbial cells, which led to better understanding of their metabolic networks and physiology [4,5]. Based on these genomic data, it became possible to develop mathematical models describing the metabolism, first of the bacterium *Escherichia coli* [6] and then of the yeast *Saccharomyces cerevisiae* [7], two widely used cell factories and models for

[1]BioInnovation Institute, Ole Maaløes Vej 3, DK2200 Copenhagen N, Denmark
[2]Department of Biology and Biological Engineering, Chalmers University of Technology, SE41296 Gothenburg, Sweden
[3]Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, DK2800 Kgs. Lyngby, Denmark
[4]www.bii.dk
[5]www.biosustain.dtu.dk

*Correspondence:
nielsenj@chalmers.se (J. Nielsen).

bacterial and eukaryal biology, respectively. It also became possible to further develop these genome-scale metabolic stoichiometric models (GEMs) beyond descriptive functions and to start using different additional data and constraints that yielded better predictions that could be confirmed experimentally [8]. In parallel, methods in genetic and genome engineering developed and gave rise to **metabolic engineering** (see Glossary) and later **synthetic biology** disciplines. All these conceptual, computational, and experimental approaches gave rise to cell factories currently used for the production of valuable chemicals, solvents, monomers, pharmaceuticals, nutraceuticals, antibiotics, and so on.

The challenges of industrial biotechnology have been partially due to limitations of scientific knowledge and available molecular and analytical tools, and partially due to social, political, and market forces. Conceptually, the challenge is still in the engineering itself: we do not yet know what 'parts' a cell has (all genes, all RNAs, all proteins, and all metabolites in conditions relevant for production) and how all the 'parts' work individually and interact together; in addition, we cannot predict how the 'parts' or the 'whole' will behave when the system is perturbed either genetically or environmentally (i.e., during the production process). Neither can we efficiently perform all potential modifications, and those that we can, such as by using CRISPR/Cas systems, cannot always be automated and scaled.

By contrast, the societal context in which these cell factories are supposed to perform has also been tumultuous. Much focus over the past 20 years has been on developing sustainable production processes for the replacement of petroleum-based or derived fuels, chemicals, and materials. Several large chemical companies, such as BASF, DSM, BP, and Total, established substantial projects and collaborations in the area of metabolic engineering. Furthermore, several start-up companies were established with the objective of developing novel bio-based production processes for **sustainable chemicals**. Efforts so far have often been on the production of **commodity chemicals** that could replace the key building blocks used in the chemical industry, as a result of the report published in 2004 and updated in 2010 by the US Department of Energy (DoE) [9]. This report provides a list of chemicals that could fit this need. Even though this has resulted in the establishment of a few large-scale processes for the production of sustainable chemicals (Box 1), the impact of these efforts has been relatively minor in transforming the petroleum-based chemical industry into a bio-based chemical industry. An example is the bioproduction of succinic acid: extensive academic research has been directed at engineering cell factories for succinic acid production by microbes [10] and several promising companies were established (Box 2). To the best of our knowledge, these research and commercial activities have been either terminated or production is at a very low level solely to support niche markets (Box 2). So far, industrial-scale production has only been established for two of the chemicals on the DoE list, namely lactic acid and itaconic acid, with lactic acid first added to the 2010 list after large-scale production had been established. Lactic acid is currently produced on a very large scale with an estimated market value exceeding US$2.5 billion, with most being used for the production of polylactate.

Why has the bio-based large-scale production of commodity chemicals lagged behind expectations? There are at least five main reasons for this: (i) commodity chemicals are needed and used in large quantities, which is a scientific and technological challenge in itself because the creation of very efficient cell factories that can produce at large scale is not yet a predictable engineering discipline; (ii) commodity chemicals are characterized by low prices, typically US$1-2/kg, which makes it hard even for efficient cell factories to be competitive with very well-established and, in some cases, subsidized processes of oil-based production; (iii) most chemical plants used for the production of commodity chemicals are fully depreciated, giving petrochemical production a

Glossary

Bioindustrials: products that are produced based on microbial fermentation.
Commodity chemicals: chemicals that are produced in very large quantities and are used by the chemical industry as building blocks for production of solvents and materials.
Metabolic engineering: targeted genetic modifications of cell factories with the objective of producing novel chemicals and/or improving the product yield.
Minimal viable product (MVP): product that can be tested in the market or by key business partners.
Precision fermentation: production of novel food ingredients through fermentation of genetically engineered microorganisms.
Sustainable chemicals: commodity chemicals produced from renewable feedstocks (e.g., plant materials or carbon dioxide).
Synthetic biology: field of science that involves redesigning organisms for useful purposes by engineering them to have new abilities.
Target product profile (TPP): defines how the final product would look like and which market it aims to address.
Zero-to-one concept: technology or product that is so novel that it disrupts an industry.

Box 1. Bio-based production of commodity chemicals by large chemical companies

We illustrate here three examples of large companies successfully developing bio-based processes for the production of commodity chemicals. We could have also chosen lactate, which has seen a significant expanded production due to its use in the production of polylactate, as well as its classical application in the food sector. Much of this expansion came from Cargill establishing a novel process using an engineered yeast that could enable production of lactic acid at low pH. In addition, production of ethanol, by far the largest fermentation product by volume, has undergone a significant expanded production over the past 10–20 years due to its increased demand not only as a biofuel, but also for use as a chemical for the production of, for example, polyethylene.

DuPont, one of the largest chemical companies in the world, developed the process for the production of 1,3-propanediol using an engineered strain of *Escherichia coli* [33]. 1,3-Propanediol is one of the key chemicals in the production of the polymer Sorona®, which is used for the manufacturing of fabrics, carpets, and a range of plastic-based materials. The details around the costs associated with the development of this process have not been disclosed, but given that Dupont is the sole producer of Sorona, they had a strong incentive to develop this process.

DSM, a large Dutch chemical company that, for many years, was a dominant antibiotic producer (penicillins and other β-lactams) used a chemical process to convert penicillin derivatives to 7-ADCA, which is a precursor in the production of cephalexin, a widely used antibiotic with a growing market. During the early 2000s, DSM engineered the filamentous fungus *Penicillium chysogenum*, which is used for the production of penicillins, to produce 7-ADCA directly, thereby replaced their chemical synthesis route with a direct fermentation route. This resulted in significant savings in terms of not only materials and energy, but also variable costs. Given the very large production of β-lactams (more than 50 000 tons annually), these changes have had significant environmental impacts. In 2010, DSM sold its β-lactam business to Sinochem Corporation and, in 2018, DSM-Sinochem was purchased by Bain Capital and renamed Centrient Pharmaceuticals.

In 1990, BASF, the largest chemical company in the world, launched a complete biotech route for the production of the vitamin riboflavin, which is used as dietary supplement, colorant, and in animal feed. The earlier process relied on several chemical synthesis steps, but through optimization of a fermentation process with the filamentous fungus *Ashbya gossypii*, BASF developed a biotech route that resulted in both a reduction in raw materials and energy usage. Therefore, BASF terminated the chemical production of riboflavin in 1996 [34].

Box 2. From the DoE list: succinic acid production

Succinic acid is one of the chemicals on the DoE list of bio-based chemicals, because it has a range of applications if it can be produced at low costs (e.g., in the production of polymers, fibers, solvents, surfactants, and detergents). Thus, several companies have attempted to develop bio-based production of this chemical. In late 2010, Myriant Technologies announced that they would build a US$80 million plant for the production of succinic acid using sorghum as a feedstock and using an engineered strain of *Escherichia coli* as the cell factory. The plant was to produce 15 000 tons of succinic acid per year [35]. At the same time, several other companies initiated activities, including the start-ups BioAmber and Reverdia, a joint venture between DSM and Roquette. Both processes relied on yeasts: BioAmber used an engineered strain of a low pH-tolerant yeast species (licensed from Cargill, which developed this yeast platform for their lactic acid production process) and Reverdia used technology based on an engineered strain of *Saccharomyces cerevisiae*. BASF also established a plant for bio-based succinic acid production based on a newly isolated bacterium from the rumen of cows, in collaboration with Corbion.

Now, 10 years later, none of these processes are operating, or only at very low levels. The Myriant plant (now PTTGC Innovation America) has been idle for several years, as is the BASF-Corbion plant [35]. BioAmber filed for bankruptcy in 2018 and the Reverdia plant is operating only at a low level to supply succinic acid for niche markets [36]. These efforts illustrate the difficulties of establishing bio-based production of a commodity chemical. Even using four different technologies, relying on two different bacteria and two different yeasts, it has not been profitable enough to establish a large-scale bioprocess for succinic acid production at low commodity costs. Success would have required a combination of some of the key traits of the different technologies (e.g., a combination of the very high conversion rates of sugar to succinic acid provided by the engineered bacteria and the tolerance to low pH provided by the yeasts). It is favorable and cost-reducing to produce organic acids at low pH because it allows for direct production of the acid form (not the salt form), which reduces downstream processing costs.

Although we used the example of succinic acid here, a similar example is the attempted bioproduction of 3-hydroxypropionic acid (3-HPA) as a commodity. Production of 3-HPA was also the focus of several large-scale initiatives, but all efforts have been terminated and there is no commercial-scale production of this chemical available for similar reasons to those seen with succinic acid.

further advantage in cost competition, compared with fermentation plants, which are expensive and have to be built from scratch; (iv) there needs to be a growing market for the commodity chemical to justify the investment in a new plant for bio-based production; and (v) the above factors are significantly impacted by large fluctuations in the price of oil, making it difficult to make financial predictions for how novel bio-based production plants can compete with oil-based production. Despite these challenges and apparent failures in developing the massive bio-based production of commodity chemicals, metabolic engineering and synthetic biology have significant potential, as illustrated by cases in which the focus has not been on the production of chemicals directly competing with oil-based derivatives. Most of these developments have been driven by small start-up companies. However, with the current strong political pressures to build societies that are not dependent on fossil fuels, there may be renewed interest in the development of the bio-based production of commodity chemicals and biofuels.

The bio-based chemical market was valued at ~US$59 billion in 2018, is expected to keep growing with a compound annual growth rate (CAGR) of 10.3%, and is predicted to reach ~US$130 billion by 2026 [11]. This is driven by the biological replacement of chemicals in a vast number of consumer products as well as the high growth segments of food applications and biofuels. Large companies, such as BASF, Novozymes, DSM, Corbion, and Evonik, are continuously adding technologies and capacity to their biomanufacturing and infrastructure. In addition, they appear to be shifting their focus from commodity chemicals to production of ingredients for food, feed, healthcare, and agriculture. This is exemplified by the Danish enzyme producer, Novozymes, which recently announced a commitment of US$315 million to increase its fermentation capacity in the USA to meet the demand for the production of food ingredients in plant-based 'meats'. In the transition toward the bio-based production of chemicals, large chemical companies have not only the advantage of having significant R&D capabilities, but also, more importantly, extensive engineering competence for manufacturing on a very large scale and depreciated fermentation plants.

Here, we discuss how innovation in the field of industrial biotechnology has impacted the growth of the field. Whereas an early focus on the translation of metabolic engineering and synthetic biology was on developing cell factories for the production of commodity chemicals and biofuels, focus has shifted to produce higher value-added chemicals, and we argue that this has resulted in significant growth of the industry. Although there have been some successes in the development of novel bioprocesses for commodity chemicals, it is through opening new markets that industrial biotechnology can offer new solutions and products. We end our review of the field by providing recommendations for innovation in industrial biotechnology.

Successful fermentation biomanufacturing start-ups

With the rush to develop novel processes for the bio-based production of commodity chemicals 15–20 years ago, several start-up companies entered this field. Here, we illustrate this development, mainly through two case stories.

Genomatica[i] was founded in 1998, and its original business model was to provide a computational platform for strain design (e.g., to companies that wanted to develop bio-based production of commodity chemicals). That has been and, still is, a challenge to build a solid business model around service to an industry that has very low margins. There have been a few successful previous cases; for example, Panlabs successfully managed to be a key provider of improved strains of *Penicillium chrysogenum* for penicillin production to several key producers in the field. In this case, Panlabs used a subscription model in which they delivered improved strains to several different companies; these improved strains could be used directly for production and provided a

direct measurable impact on the financials of penicillin production. If one aims to provide a technology that can be used for designing better strains, these designs need to be evaluated at scale, which poses a specific set of challenges for each individual strain and process/product. Zymergen (2013)[ii] and Ginko Bioworks (2009)[iii] were founded with a model to provide integrated services (i.e., offering a complete technology platform for strain design and construction). These companies probably also faced problems with a business model relying on delivering service to the fermentation industry and, therefore, later focused on development of bioprocesses for their own products, either internally or in close collaboration with a partner, as an addition to their Contract Research Organization (CRO) business. Due to the difficulties with growing a business based on service, Genomatica pivoted early on toward their own development of novel bioprocesses using their computational platform as a technological advantage in the strain design process. This enabled the company to engineer *E. coli* for the efficient production of 1,4-butanediol to be used for making plastics, elastic fibers, and polyurethanes [12]. With this engineered *E. coli* strain, Genomatica moved on to establish a 30 000 metric tons per year plant for this chemical in collaboration with Novamont, in Italy. More recently, this process has also been licensed to Cargill and Helm, which are building a US$300 million plant in Iowa, USA, for the production of 1,4-butanediol. Genomatica has further used its technology platform to develop a scaled process for 1,3-butanediol, used in the fragrance industry, and for the production of 1 ton of caprolactam, a precursor for Nylon 6 production. This enabled Genomatica to raise US $118 million in a series C financing round during mid-2021. Key lessons from this success have been the use of a very strong technology platform for the development of cell factories that address novel markets and using partnerships to ensure that large-scale processes are being established. Genomatica was also opportunistic when they observed an increase in investments in the production of synthetic cannabinoids, and spun out the company Creo, which received key intellectual property (IP) from Genomatica to start the production of high-valued cannabinoids for the cosmetics industry.

Another company that has survived thanks to its agility and multiple pivots is Amyris[iv], which was established in 2003. Through two grants from the Bill and Melinda Gates Foundation totaling more than US$50 million, the Keasling laboratory at University of California Berkeley and Amyris engineered yeast to produce the antimalarial drug artemisinic acid [13]. Much of the final strain optimization was performed by Amyris [14], which also developed a scaled production process that could enable commercial production. The technology was licensed under not-for-profit conditions via One Health (a requirement for obtaining the original grant) to Sanofi, which then produced millions of doses for the treatment of malaria. Even though Amyris did not profit financially from the project, it enabled the company to develop a yeast strain that could efficiently produce sesquiterpenes, a group of natural products to which artemisinic acid belongs. Amyris used this platform yeast strain for the production of other valuable sesquiterpenes (Figure 1A). In one case, they initiated a larger project in collaboration with Total for the production of farnesene, which can be used as a blend-in biofuel for diesel and jet fuels. Through further engineering of the yeast strain [15] and process development, Amyris established a commercial process in Brazil for the production of farnesene, which could be used as a biodiesel. However, it was difficult to compete on costs with traditional diesel and, therefore, the collaboration with Total was terminated and the plant in Brazil was sold. Nevertheless, the ability to produce farnesene at relatively low costs did enable Amyris to convert this chemical to squalene, which is an ingredient used in cosmetics. Amyris also used the same platform cell factory for the production of other valuable chemicals. Through a partnership with Firmenich, a large producer of flavors and fragrances, they developed a process for the production of fine fragrances, such as Clearwood, a patchouli-scented product, and Dreamwood, a santalene-derived product, both launched in 2014. Production of these chemicals by yeast had earlier been demonstrated in collaboration

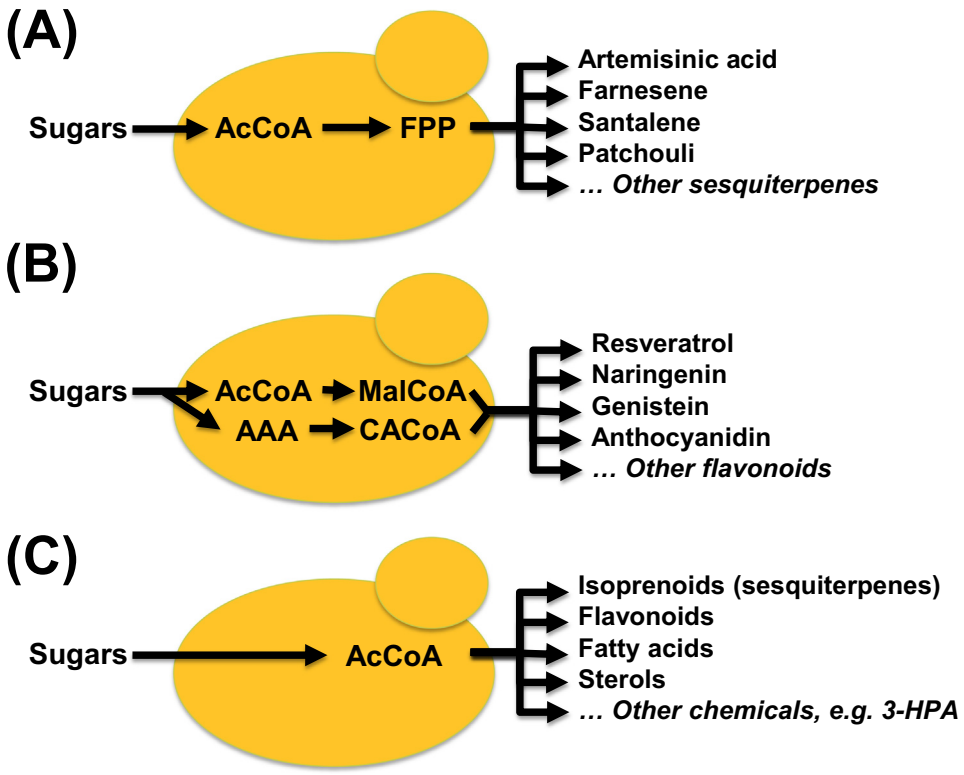

Figure 1. The concept of platform cell factories (here illustrated with yeast cells) can enable pivoting of business focus toward different markets using the same base strain engineered for efficient production of the key precursor for several different products. (A) Illustration of how a yeast strain optimized for the production of one sesquiterpene can be used for the production of a range of other valuable sesquiterpenes because they are all derived from farnesyl-pyrophosphate (FPP). FPP is produced by the mevalonate pathway, in which acetyl-CoA (AcCoA) serves as precursor. (B) Illustration of how a yeast strain optimized for production of coumaric acid and its activated form, coumaryl-CoA (CACoA), can be used for the production of a range of valuable flavonoids and polyphenols via the aromatic amino acids (AAAs) tyrosine and phenylalanine. Biosynthesis of flavonoids and polyphenols also requires malonyl-CoA (MalCoA) as a precursor. MalCoA is produced from AcCoA through a carboxylation reaction. (C) Illustration of how a yeast strain that can efficiently provide AcCoA can be used as a platform cell factory for the production of many different products, including in addition to isoprenoids, a large group of chemicals to which sesquiterpenes belong, and flavonoids, encompassing not only fatty acids and sterols, but also many other chemicals, such as 3-hydroxypropionic acid (3-HPA).

with Firmenich [16]. However, Amyris is not the sole company to address the US$40 billion flavors and fragrance market. BASF added nootkatone and nine other fermentation-derived molecules to its portfolio of synthetic aroma chemicals by acquiring the Dutch start-up Isobionics in 2019 [17]. At the same time, BASF also acquired technology for the production of fermentation-derived vanillin from Conagen [17]. There are also several other players in this space, and Givaudan, another large producer of flavors and fragrances, established a collaboration with Manus Bio, another USA based start-up [17].

The Amyris business case demonstrates how a company can afford to be pathway centric as long as derivatives of this pathway address sufficiently large markets. Sesquiterpenes represent a broad group of chemicals with many different applications, but this concept can be extended to other chemical groups, such as flavonoids, which are all derived from coumaric acid (Figure 1B). Flavonoids are another group of natural products with a range of applications as cosmetics,

nutraceuticals, dietary supplements, and food ingredients due to their antioxidant properties. Flavonoids are also part of the US$40 billion flavors and fragrance market, and their own market value is ~US$1.5 billion[v]. Given that flavonoids are all derived from coumaric acid, a yeast strain engineered for the high-level production of this chemical [18] can serve as a platform cell factory for the production of many different flavonoids and polyphenols [19], including isoflavonoids, a group of commercially interesting chemicals [20]. However, this concept can be traced even further back in their metabolism by engineering yeast for the high-level production of acetyl-CoA, which serves as a precursor for the production of fatty acids, terpenes (including sesquiterpenes), and flavonoids [21] (Figure 1C).

Breakthroughs in metabolic engineering and synthetic biology

Faster development of novel biotech processes for the production of various chemicals has been enabled by many technological advancements in industrial biotechnology, metabolic engineering, and synthetic biology. These advancements fall into four main areas: (i) novel synthetic biology tools that have enabled faster strain construction; (ii) capabilities for the fast production of synthetic genes and even whole genomes. This has resulted in the establishment of new start-ups, such as Twist Bioscience and Codex DNA, which rely on servicing the synthetic biology community with synthetic DNA; (iii) data analyses (e.g., 'omics) and integration, which have enabled more detailed phenotypic characterization of engineered strains; (iv) improved quantitative description of metabolism using advanced metabolic models have enabled better prediction of various design strategies; and (v) implementation of robotics and automation for enabling multiplexing and high-throughput strain construction and characterization. The field has also seen the establishment of companies that provide platforms that can ensure more efficient workflows, such as Benchling, which has generated a strong information technology (IT) platform with electronic notebooks specifically fitted for synthetic biology.

Synthetic biology arose from inspiration from electronics and with the objective to develop specific regulatory circuits using biological components that could be applied as plug-and-play in various design strategies [22]. The application to strain design rapidly made this an interesting approach for the field of metabolic engineering [23]. This resulted in the development of a range of novel techniques for engineering cell factories as well as of tools that have enabled automation of experimental steps in the design–build–learn–test cycle of synthetic biology (i.e., genome editing and testing) [24]. Thus, through the development of many new genome-editing technologies, it has become possible to multiplex the construction of many different strains using well-plates. CRISPR/Cas9 and related technologies have also added to this portfolio, because they have enabled faster as well as more efficient genome editing, especially of organisms that are traditionally more difficult to modify, such as non-conventional yeasts and filamentous fungi, both used in many bioprocesses. The development of biosensors and other assays that can enable high-throughput evaluation (or testing) of strains, has resulted in the rapid generation of large data sets, which provide predictive learnings on how different genome-editing strategies impact product formation, and improved design. Automation and multiplexing have significantly reduced the time and cost for developing new cell factories, and these have also been identified as business opportunities by companies such as Zymergen and Ginko, which have built large biofoundries with the capacity to service not only internal research projects, but also external clients. Amyris has also developed a strong automation platform that is now being offered to external clients, but many universities and research organizations are also developing their own biofoundries, which they also offer to external clients [25], such as the Agile BioFoundry funded by the DoE or the CFB2.0 Biofoundry in Denmark, funded by the Novo Nordisk Foundation. Such biofoundries can not only contribute to an expansion of the bioeconomy as predicted by the Organization for Economic Co-operation and Development (OECD) [26], but also assist in responding to future pandemics [27].

In recent years, there have been significant advances in 'omics data analyses of key cell factories. Several studies of multi-omics interpretation and integration have been performed on *S. cerevisiae* and *E. coli*, resulting in new insights and increasing our understanding of the physiology and metabolism of these organisms. Advances in quantitative proteomics have been particularly important [28,29], because these data integrate well with mathematical modeling of metabolism and significantly improve the predictive strength of such models [30], such as modeling overflow metabolism in both *E. coli* and *S. cerevisiae* [31] and new insights into the Crabtree effect in different yeast species [32]. Even though mathematical modeling is not completely integrated in most metabolic engineering efforts, it has been used to support design strategies and for data analysis. We expect that, with improved models and data quality (under different conditions and at different scales), the performance and predictions will enable wider use. This will result in another major reduction in time and costs for cell factory and bioprocesses (including scale-up) development.

The advances in metabolic engineering and synthetic biology mentioned here have significantly reduced the required time and, thus, the cost, associated with developing novel cell factories, and this has resulted in a new boom of biotech start-ups that use microbial fermentation for production of various chemicals. Table 1 provides a list of examples of start-up companies that have been established in recent years in the **bioindustrial** space and that all rely on microbial fermentation for production.

Table 1. Examples of recently founded bioindustrial companies

Company (year of set-up), website	Product focus	Total raised to date (US$)	Technology basis	Refs
Antheia (2013) www.antheia.bio	Developer of plant-inspired medicines created to tap full potential of nature to discover new medicines, such as opioids	101 million	Yeast synthetic biology platform for reconstruction of complex plant biosynthetic pathways	[37]
Berkeley Yeast (2017) http://berkeleyyeast.com	Beers produced using engineered yeast with hoppy flavors	2 million	Yeast synthetic biology platform for production of monoterpenes that are flavor compounds of hops. Spun out from the Keasling laboratory at University of California, Berkeley	[38]
Chrysea (2020) www.chrysealabs.com	Production of spermidine and other natural products with validated health claims	11 million	Yeast synthetic biology platform for production of spermidine and other natural products	[39]
Demetrix (2015) http://demetrix.com	Developer of fermentation system designed to create natural medicines of cannabinoids	62.5 million	System uses genetics and computing-enabled deciphering technology to produce coevolved and bio-inspired cannabinoids medicines and nutraceuticals via genetics and synthetic biology	[40]
Perfect Day (2014) http://perfectday.com	Producer of animal-free milk substitutes and proteins intended to offer protein nutritionally identical to that of cow's milk	714 million	Yeast synthetic platform of plant-based sugars; includes ingredients that are sustainable, vegan, and devoid of antibiotics, cholesterol, and lactose, providing nutrient-dense and environmentally safe dairy alternatives	
Pivot Bio (2010) www.pivotbio.com	Developer of microbial nitrogen fertilizers intended to replace synthetic nitrogen fertilizers	686 million	Fertilizers help farmers to grow crops that can capture and metabolize atmospheric nitrogen, reducing need for petrochemical fertilizers, enabling farmers to reduce costs of farming, improve health, and create a future with cleaner water and air	[41]
The EVERY Company (2015) http://theeverycompany.com	Manufacturer of protein food products intended to offer animal-free proteins (former Clara Foods)	97 million	Uses advanced yeast engineering and fermentation technologies to selectively cultivate yeast for production of proteins for food sector	
Geltor (2015) http://geltor.com	Offers a selection of designer proteins with biocompatibility, functionality, and durability	120 million	Products are animal proteins produced by microbial fermentation (e.g., collagen and elastin used in cosmetics and food)	

Mega-trends

The significant advances in our ability to engineer and characterize cell factories rapidly and precisely coupled with abilities associated with data capture, analyses, and integration, have led biotechnology closer to classical engineering disciplines in terms of rational and predictable design. Although metabolic models serve as a strong platform for integrating different kinds of data, these models still have limitations. Machine learning (ML) and artificial intelligence (AI) are being increasingly proposed as a necessity to improve computational biotechnology. We are also convinced that the next frontier in ensuring the engineering of biology will require ever more high-quality data, metadata, ML, and AI, and a true relationship between genotype and phenotype will probably require modern computing approaches, such as quantum computing.

The technological advancements over the past 10–20 years have lowered the entry requirements for exploration of novel ideas. It is now affordable to read, write, and edit gene and genomes and collect a reasonable amount of relevant data. Metabolic models have been constructed for many industrial microorganisms and are available in free public repositories. This can mean that getting to a **minimal viable product (MVP)** or proof of concept (PoC) has become a realistic possibility, which has unleashed creative potential in early-stage start-ups as well as in academic laboratories.

Two areas that have particularly seen a boom in development are agricultural biotech (ag-tech or agro-tech) and novel foods. In ag-tech, the concept of vertical farming is a rapidly growing business and, with opportunities for up to 40 harvests per year for some crops, this concept can also become a platform for advancing research in plant science, such as in plant health, crop protection, and plant–soil–microbiome interactions, in which controlled conditions and large amounts of data remain scarce. This approach may enable the development of solutions that can significantly reduce the costs of production, reduce pollution, reduce land usage and so on, while still having the potential to scale up and be competitive with traditional farming. Therefore, we expect to see impactful innovation in this area. An example is the technology for microbial-based nitrogen fixation offered by the US Pivot Bio. This technology could eliminate the need for nitrogen fertilizers, which have been produced for decades through the classical Haber–Bosch process, which accounts for ~5% of the total carbon dioxide emission globally. Another interesting technology is based on a yeast cell factory that produces insect pheromones, which can be used for pest control, potentially eliminating the need for toxic pesticides, developed by the Denmark-based company BioPhero[vi].

The area of novel food is particularly interesting and is in synchrony with the societal mega trend of reducing or eventually removing the dependency on animal-based products, such as meats, eggs, and dairy products. This societal trend is fueled by the fact that as the number of wealthy, educated, and informed customers around the world increases, so too does the willingness and ability to pay for products that are healthier for both the individual and the environment (including impacts on climate change) and more ethical. Using plant-based meat substitutes offers more than a 90% reduction in direct greenhouse gas emissions and land use (which is directly translated to increased biodiversity, which then directly translates to increased carbon capture), a 99% reduction in water use, and an almost 50% reduction in energy use. Furthermore, such a switch would eliminate the use of antibiotics in animal farming and husbandry and directly reduce world-wide antimicrobial resistance, which is a major health hazard in many countries, and could become the next big biological threat. A switch to plant-based and microbial food products will, for some market segments, require targeted production of specific ingredients, such as hemoglobins or other heme proteins and caseins to mimic better ‘meat’ and ‘dairy’ organoleptic properties. This in itself is an opportunity to develop microbial production processes for supplying these ingredients by using so-called ‘**precision fermentation**’. This goes beyond the production of proteins because it may also be necessary to add specific fats and other ingredients that ensure the proper

texture, flavor, and color of the novel foods. These trends are well illustrated by companies that, since mid-2010, started to bring plant-based food substitutes to the market, such as Impossible Foods[vii], Clara Foods (now The EVERY Company), and Perfect Day. These companies have, in total, raised more than US$3.5 billion in financing. Even though plant-based burgers and fungal meat replacements (e.g., Quorn) have existed since the 1980s, they have not had a dramatic impact on consumers; thus, we could consider these new technologies 'zero-to-one'-type companies in the food biotech sector.

Our recommendations

Although it is hard to define the exact requirements for building successful industrial biotechnology start-ups, we offer here recommendations based on our own experiences from establishing, advising, financing, and supporting start-ups. We think there are four key categories: (i) keep the 'zero-to-one' concept in mind; that is, think about simple ideas that result in many changes; (ii) be ready to pivot at opportune times; that is, do not be emotionally attached to your original idea but evaluate it rationally and change direction if needed; (iii) have the end product in mind from the start; for example, think about the **target product profile (TPP)** or aim for a MVP as soon as possible because this will teach you about the challenges of a profitable project; and (iv) fail often and as soon as possible, with the aim to learn as much as you can from it and cut losses when needed, making yourself available for change or a new enterprise.

The **'zero-to-one' concept** is well known from the internet-based technology sector, where companies have launched new concepts or platforms for provision of services such as transportation (Uber), accommodation (Airbnb), payments (PayPal), and music (Spotify). These companies built new software platforms that, to a large extent, have disrupted traditional markets [i.e., those provided by taxis, hotels, banks, and physical entities (CDs)]. In life sciences, it is harder to completely disrupt existing markets because it is more difficult to scale at the same rate, and most valuable products are biological entities and processes, rather than services, platforms, or databases. The complexity of biology makes biological R&D costly and time-consuming, and service companies that handle data and information can be profitable, but the true transformation of the industry will not come from that. Additionally, biological discovery leads to a variety of solutions to a given problem (as illustrated by the case of succinic acid production, where several different cell factories could be engineered), which means that the first movers do not necessarily have an advantage and complete IP protection even if they have spent significant resources. This can represent a challenge for investors because they will have to focus on rapidly advancing start-up companies to a level where they can position their products in the market to become market leaders. An illustrative example of how complexity in biology can result in multiple solutions comes from medical biotechnology, with the identification of immune check-point inhibitors, which have transformed cancer treatment. This discovery, which has resulted in the development of antibody-based drugs that have completely transformed the treatment of certain cancers, was made by James P. Allison and Tasuko Honjo, who shared the 2018 Nobel Prize in Medicine. The use of antibodies targeting check-point inhibitors has enabled the complete eradication of cancer in many patients with multiple melanoma. However, even though identification of check-point inhibitors as a target for cancer treatment enabled significant improved treatments, this has not resulted in one dominant provider who controls the market, because it is possible to identify different targets (proteins and their epitopes), generate different antibodies, and formulate different delivery modes. Additionally, cancers are not all the same; thus, the right approach for one would not necessarily work for another type and, to obtain drug approval, it would be necessary to run many different clinical trials. The result is that almost all large pharmaceutical companies that focus on anticancer drugs have

developed immune check-point inhibitor drugs, and a single key leader has not emerged from this important discovery. This is a very different result compared with internet-based tech.

However, we do occasionally see truly innovative companies, such as Rubius, a company that uses transformed red blood cells as a drug delivery vehicle, and Moderna and BioNTech, which enabled the broad use of RNA vaccines, which became critical in rapid population-wide vaccinations against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Thus, even though the 'zero-to-one' concept may not apply precisely to the life sciences and biotechnology, it is still possible to learn from the concept, with some relevant examples.

Another key lesson is to be ready to pivot. Particularly in the bioindustrial space, it may be difficult initially to identify the exact path to market. For example, there may be regulatory constraints that can be hard to foresee, simply because you may be the first to pursue a certain path, or there may be market constraints based on how you define the application of your product (e.g., is it a biofuel, a perfume, or a food ingredient, all basically derived from the same molecule). Thus, the customer or even the product might not turn out to be what you originally had in mind at the start of the project and, therefore, you should be able to pivot and move in a different direction. To enable pivoting, it is important that you have a strong technology platform or scientific base. As described earlier, both Genomatica and Amyrsis pivoted several times and were successful due to their strong and unique science and technology platforms.

Last, think about defining the TPP early in development. It is when you first define this that you can go backwards and ask questions about how you would get this product to the market (e.g., how to produce it, how to get it approved, and how to ensure the supply to the market). If possible, you may even aim for an early launch of an MVP because getting early feedback from potential customers can lead to crucial insights. The MVP concept is well known for software solutions because it is relatively easy to make and test by customers and to use the feedback to improve the product. It may be more difficult to rapidly obtain an MVP in industrial biotechnology, but consider early on to scale your process and produce the first test batches of your product, because these are challenges in themselves and you should learn early on how to prepare for them. Many investors will see this as significant derisking step and would prefer it to spending resources on continuously optimizing the cell factory on a laboratory scale. Even though you may not have the perfect strain (close to the theoretical titer, rate, or yield) or process, the fact that you have an MVP makes you more attractive for investors. If it is difficult to scale your process early on, you should at least define the TPP and use this to map out the challenges to bringing the product to the market.

Concluding remarks

We are optimistic that future innovation in industrial biotechnology will be significant and transformative, despite its tumultuous past. As discussed, advancements in relevant wet-lab and dry-lab technologies are enabling faster and better development of cell factories and adjacent offerings. It has become possible to obtain key inflection points required for commercial investments more quickly, which has made it more attractive for many investors to enter into biotech, which is a new development (see Outstanding questions). However, it is important to carefully map out the product space to target (Figure 2) and provide a TPP or MVP early on. Based on such recommendations, pivoting while grounded in your technology platform should be used to form the basis for a new direction or a new start-up to target a different market segment than originally planned. If one can plan and learn from these key lessons with an ambition to reach for significant markets or disrupt current markets, the road to success should be well mapped and accessible.

Outstanding questions

How can the development of cell factories in academia be advanced further such that they can be rapidly scaled up for industrial use?

What are the key areas where industrial biotechnology could make a significant impact on society in terms of the sustainable production of foods and materials that can lead to reduced greenhouse gas emission?

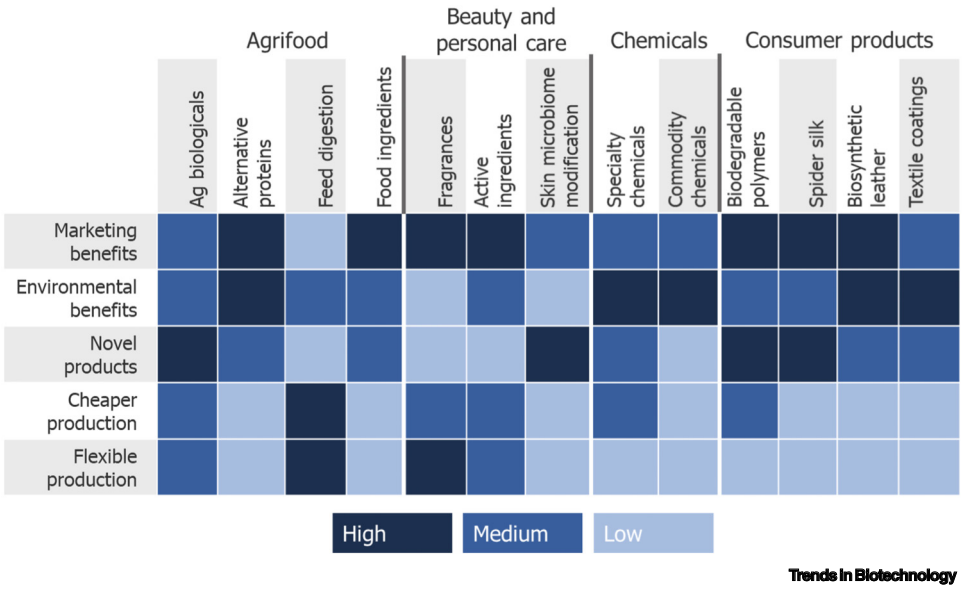

Figure 2. Overview of where opportunities lie i industrial biotechnology (or synthetic biology) in terms of building start-up companies. Marketing benefits include growing market or increased consumer interest in bio-based products. Abbreviation: Ag, agricultural.

Acknowledgments

We would like to thank the Novo Nordisk Foundation for generous funding to both the BioInnovation Institute (grant no. NNF21SA0074038) and the Novo Nordisk Foundation Center for Biosustainability (grant no. NNF20CC0035580). We also would like to thank our colleagues and collaborators Markus Herrgård and Bobby Soni for constructive comments to our work.

Declaration of interests

J.N. is a shareholder of Melt&Marble AB and Chrysea, Inc. The authors otherwise declare no conflict of interests.

Resources

[i]www.genomatica.com

[ii]www.zymergen.com

[iii]www.ginkobioworks.com

[iv]http://amyris.com

[v]www.alliedmarketresearch.com/flavonoid-market-A14262

[vi]www.biophero.com

[vii]www.impossiblefoods.com

References

1. Nielsen, J. (2019) Yeast systems biology: model organism and cell factory. *Biotechnol. J.* 14, 1800421
2. Huang, M. *et al.* (2014) Biopharmaceutical protein production by *Saccharomyces cerevisiae*: current state and future prospects. *Pharm. Bioprocess.* 2, 167–182
3. Nielsen, J. (2013) Production of biopharmaceuticals proteins by yeast. *Bioengineered* 4, 207–211
4. Macklin, D.N. *et al.* (2020) Simultaneous cross-evaluation of heterogenous *E. coli* datasets via mechanistic simulation. *Science* 369, eaav3751
5. Di Bartolomeo, F. *et al.* (2020) Absolute yeast mitochondrial proteome quantification reveals trade-off between biosynthesis and energy generation during diauxic shift. *Proc. Natl. Acad. Sci. U. S. A.* 117, 7524–7535
6. O'Brien, E.J. *et al.* (2013) Genome-scale models of metabolism and gene expression extend and refine growth phenotype prediction. *Mol. Syst. Biol.* 9, 693
7. Lu, H. *et al.* (2019) A consensus *S. cerevisiae* metabolic model Yeast8 and its ecosystem for comprehensively probing cellular metabolism. *Nat. Commun.* 10, 3586
8. Chen, Y. *et al.* (2022) Genome-scale modeling of yeast metabolism: retrospectives and perspectives. *FEMS Yeast Res.* 22, foac003
9. De Jong, E. *et al.* (2020) *Bio-Based Chemicals: A 2020 Update*, IEA Bioenergy
10. Ahn, J.H. *et al.* (2020) Enhanced succinic acid production by *Mannheimia* employing optimal malate dehydrogenase. *Nat. Commun.* 11, 1970

11. Verified Market Research (2021) *Global Bio-based Materials Market Size by Type, by Application, by Geographic Scope and Forecast*, Verified Market Research
12. McCoy, M. (2013) Myriant starts up succinic acid plant. *Chem. Eng. News* 91, 25
13. McCoy, M. (2019) The final chapter for succinic acid. *Chem. Eng. News* 97, 12
14. Paddon, C.J. *et al.* (2013) High-level semi-synthetic production of the potent antimalarial artemisinin. *Nature* 496, 528–532
15. Meadows, A.L. *et al.* (2016) Rewriting yeast central carbon metabolism for industrial isoprenoid production. *Nature* 537, 694–697
16. Scalcinati, G. *et al.* (2012) Combined metabolic engineering of precursor and co-factor supply to increase a-santalene production by *Saccharomyces cerevisiae*. *Microb. Cell Fac.* 11, 117
17. Bomgardner, M.M. (2021) Why the flavor and fragrance industry is embracing biotechnology. *Chem. Eng. News* 99, 5
18. Liu, Q. *et al.* (2019) Rewiring carbon metabolism in yeast for high level production of aromatic chemicals. *Nat. Commun.* 10, 4976
19. Li, M. *et al.* (2016) Engineering yeast for high-level production of stilbenoid antioxidants. *Sci. Rep.* 6, 36827
20. Liu, Q. *et al.* (2021) De novo biosynthesis of bioactive isoflavonoids by engineered yeast cell factories. *Nat. Commun.* 12, 6085
21. Krivoruchko, A. *et al.* (2015) Microbial acetyl-CoA metabolism and metabolic engineering. *Met. Eng.* 28, 28–42
22. Nielsen, J. and Keasling, J. (2011) Synergies between synthetic biology and metabolic engineering. *Nat. Biotechnol.* 29, 693–695
23. Nielsen, J. *et al.* (2014) Engineering synergy in biotechnology. *Nat. Chem. Biol.* 10, 319–322
24. Liu, Z. *et al.* (2022) Yeast synthetic biology advances biofuel production. *Curr. Opin. Microbiol.* 65, 33–39
25. Hillson, N. *et al.* (2019) Building a global alliance of biofoundries. *Nat. Commun.* 10, 2040
26. Philip, J. (2020) *A Roundup of Bioeconomy Work at DSTI*, OECD
27. Vickers, C.E. and Freemont, P.S. (2022) Pandemic preparedness: synthetic biology and publicly funded biofoundries can rapidly accelerate response time. *Nat. Commun.* 13, 453
28. Yu, R. *et al.* (2020) Nitrogen limitation reveals large reserves in metabolic and translational capacities of yeast. *Nat. Commun.* 11, 1881
29. Schmidt, A. *et al.* (2016) The quantitative and condition-dependent *Escherichia coli* proteome. *Nat. Biotechnol.* 34, 104–110
30. Lu, H. *et al.* (2022) Multiscale models quantify yeast physiology: towards a whole-cell model. *Trends Biotechnol.* 40, 291–305
31. Yu, C. and Nielsen, J. (2019) Energy metabolism controls phenotypes by protein efficiency and allocation. *Proc. Natl. Acad. Sci. U. S. A.* 116, 17592–17597
32. Malina, C. *et al.* (2021) Adaptations in metabolism and protein translation give rise to the Crabtree effect in yeast. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2112836118
33. Nakamura, C.E. and Whited, G.M. (2003) Metabolic engineering for the microbial production of 1,3-propanediol. *Curr. Opin. Biotechnol.* 14, 454–459
34. Revuelta, J.L. *et al.* (2017) Bioproduction of riboflavin: a bright yellow history. *J. Ind. Microbiol. Biotechnol.* 44, 659–665
35. Yim, H. *et al.* (2011) Metabolic engineering of *Escherichia coli* for direct production of 1,4-butanediol. *Nat. Chem. Biol.* 7, 445–452
36. Ro, D.-K. *et al.* (2006) Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature* 440, 940–943
37. Galanie, S. *et al.* (2015) Complete biosynthesis of opioids in yeast. *Science* 349, 1095–1100
38. Denby, C.M. *et al.* (2018) Industrial brewing yeast engineered for the production of primary flavor determinants in hopped beer. *Nat. Commun.* 9, 965
39. Qin, J. *et al.* (2021) Engineering yeast metabolism for the discovery and production of polyamines and polyamine analogues. *Nat. Catal.* 4, 498–509
40. Lou, X. *et al.* (2019) Complete biosynthesis of cannabinoids and their unnatural analogues in yeast. *Nature* 567, 123–126
41. Tamsir, A. *et al.* (2011) Robust multicellular computing using genetically encoded NOR gates and chemical 'wires'. *Nature* 469, 212–215