Development of Economical Food Products employing Synthetic Biology

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Abstract

Considering global issues pertaining to rapidly growing population, resource scarcity, and economic inequality, the search for affordable food products is increasing. Food production could be transformed by synthetic biology, a rapidly growing field that combines biology and engineering. Designing and engineering biological systems using synthetic biology enables more sustainable and efficient food products and ingredients. This includes specially adapted cultures with improved nutritional composition, optimized production methods and genetically modified microbes for precise fermentation. These technological advances have the potential to reduce production costs, reduce resource use and improve environmental impact, all of which help reduce food prices for people around the world. However, issues of safety, regulation and public acceptance must be resolved before synthetic biology can be widely used in the food sector. This chapter emphasizes the necessity for a balanced strategy that considers both the advantages and disadvantages of this technology.

I. Introduction

In today's rapidly evolving world, emerging trends and contemporary challenges are propelling biology into an exciting new era where effective and tailored solutions are the focus. Beyond our fundamental understanding of life, biological sciences have expanded their scope, embracing established principles and cutting-edge technologies to foster interdisciplinary collaboration among biology, chemistry, physics, engineering, and computational sciences. At the forefront of this convergence lies synthetic biology, a discipline dedicated to ushering in a new technological age where these diverse fields seamlessly merge and complement one another. Synthetic biology, in essence, involves designing and constructing novel biological components, devices, and systems that do not naturally exist while also reshaping existing biological systems to perform specific functions. What sets synthetic biology apart from conventional genetic manipulation methods is its ability to achieve high-throughput construction and the engineering of organisms with genotypic traits that govern the synthesis of intricate, biologically inspired systems, showcasing functionalities that are unparalleled in the natural world (1).

The need of the hour is to produce food products via synthetic biology because of certain reasons such as rapidly rising global population indirectly increasing the food demand in the 21st century. Another global challenge is ensuring sustainable food for the entire human population. Recent events, such as the disruptions in supply chains caused by the SARS-CoV-2 pandemic and geopolitical instability, have underscored the urgency of this issue. It is likely that these challenges will persist and intensify in the face of climate change. Addressing this complex problem requires collaboration among researchers, policymakers, and politicians to devise solutions that can provide sustainable food to everyone on Earth. However, these challenges extend beyond food production alone. Factors like food preservation, packaging, and logistics also play crucial roles and present opportunities for synthetic biology to contribute to the creation of more sustainable foods. It is a delicate balancing act to meet the needs of a growing population while also addressing issues like sustainability, environmental effects, and resource constraints. Science and innovation have stepped in to offer fresh ways for navigating this difficult environment. Synthetic biology is one such ground-breaking area that has enormous potential (2).

Synthetic biology allows the improvement of food production by using programmed monoclonal cell factories, engineered microbial consortia or cell free biosynthesis platforms. By applying synthetic biology technology in future, it may be possible to get rid of the limitations of traditional agriculture and animal husbandry in addition to improving resource conversion efficiency for food products. Overall, the synthetic biology driven food industry has the potential to address the challenges of sustainable food supply in the future. In this chapter, we will discuss the current and future food revolution guided by synthetic biology through three different sections. First section deals with the ways as to how synthetic biology can improve traditional food production and manufacturing. Second, how would this synthetic biology be able to improve food nutrition or add new functions. Third, how synthetic biology will be able to transform the traditional fermentation food production style using engineered microbial communities. In addition, the major synthetic biology-based biotechnologies expected to be used in future food are also introduced. Finally, the challenges and prospects of synthetic biology for future food are discussed.

II. Synthetic Biology: Foundations and Concepts

A. Definition and Evolution

Synthetic biology (syn-bio) is a vast field that needs to be explored to avail its maximum benefits in the current scenario. It is an advanced field of biotechnology that combines genetic engineering, computer science, mathematics, and engineering modeling to create new biological parts that do not exist in nature. It aims to develop ideas for creating new biological parts, devices and models, thus imitating nature and creating them in the laboratory. Redesigning models from the trash is the main driving force of syn-bio. This could include genetically modified life forms, spanning the fields of genetics and genomics from systems biology to synthetic applications. Award-winning advances in medical problem solving, metabolic and energy engineering, environmental restoration, materials science, and plant science have been made possible by synthetic biology.

The term synthetic biology was coined in 1970 by famous geneticist Waclaw Szybalski. During the 1970s, fundamental work was done to enable the development of DNA synthesis and sequencing technologies (5). The origins of synthetic biology date back to the 1960s. Francis Jacob and Jacques Monod provided the assembly of regulatory systems from molecular components.

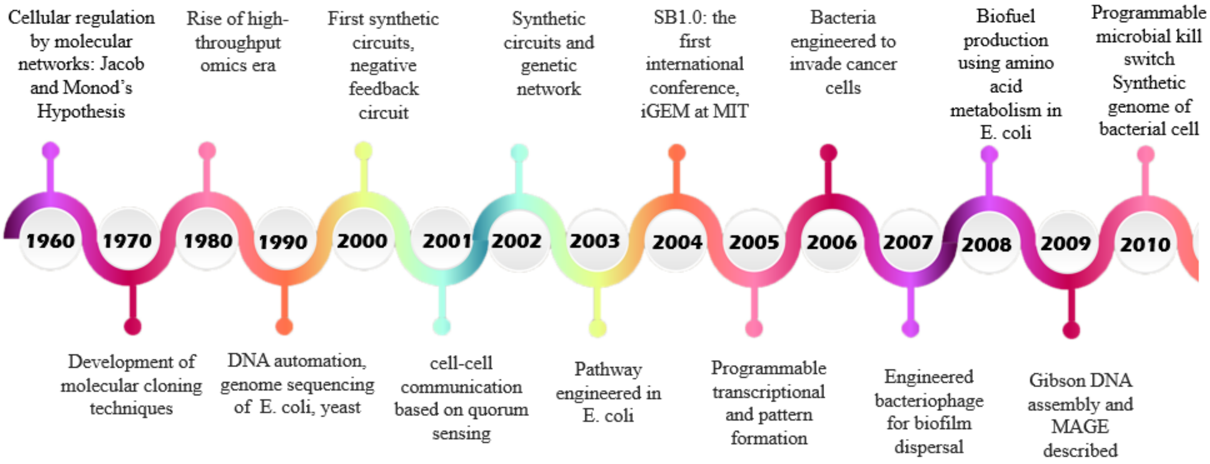

Figure 1- History of Synthetic Biology from Year 1960-2010

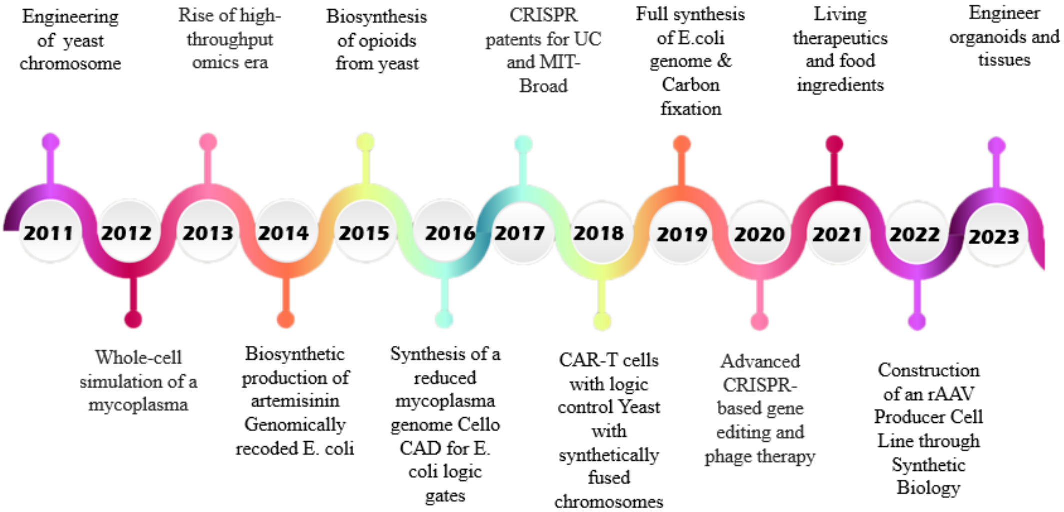

Figure 2- History of Synthetic Biology from Year 2011-2023- The figure 1 and 2 depict the evolving history of synthetic biology from year 1960-2023

B. Principles of Synthetic Biology

This section begins by illustrating the basic principles of synthetic biology to describe the basics of the same. This section focuses on how biological systems are viewed as programmable objects and how standardized genetic building blocks make it possible to construct new biological capabilities. To fully appreciate synthetic biology's disruptive potential in the food sector, it is essential to comprehend these ideas.

The following are the engineering principles of synthetic biology, allowing for quick prototyping and the easy exchange of designs across synthetic biologists operating in different parts of the world (6).

- **i.** **Modularity** - Modularity is a crucial principle in synthetic biology, enabling the design and construction of components that can be combined to create complex systems. This allows for quick assembly, troubleshooting, reuse of existing parts, safety when working with hazardous materials, and efficient data storage, making it a valuable tool for research and development.
- **ii.** **Standardization** - A key principle in synthetic biology is standardization, which enables more exact design and assembly of biological components and apparatus. Scientists can design systems that are more dependable and reproducible by standardising components like DNA sequences, proteins, enzymes, or other compounds.One such collection of standardised DNA components is the Registry of Standard Biological Parts (BioBricks), which can be assembled together like a set of building bricks to produce new biological systems (6).
- **iii.** **Abstraction** - Abstraction simplifies data storage by providing a common language for describing biological systems, enabling easy sharing of designs, and ensuring safety when working with potentially hazardous materials like GMOs through clear guidelines. It helps in the reduction of development time and cost thereby, increasing accuracy and precision (7).

III. Tools and Techniques- Design-Built-Test-Learn cycle

A. Design -build-test-learn Cycle

Design -build-test-learn cycle forms the cornerstone of synthetic biology. It is widely used for the development of microbial chassis thereby having a wide application in the food industry. In synthetic biology, the more recent approaches involve several mechanisms and strategies capable of suitably reprogramming the cellular machinery towards a better outcome for industrial purposes. The synthetic biology workflow is based on the design-build-test-learn cycle (8).

The DESIGN process involves biological and operational design, specifying desired cellular target functions and determining optimal sample amounts and performance specifications. To implement these functions, appropriate biological parts must be identified and assembled. Standard registries are needed to characterize biological parts under various contexts and conditions. New mathematical and computational tools are needed to solve optimization problems and specify constraints. Design-of-experiment (DoE) approaches can efficiently search for and assemble genetic parts and circuitry, but still require choices and computational methods. The result is DNA sequences with multiple genetic parts that generate desired functions in specific biochemical, cellular, organismal, or biome contexts (9).

The BUILD process involves DNA assembly, incorporating it into a host organism, and verifying the assembled sequence in the expected genetic context. This iterative process combines multiple DNA fragments with molecular biology techniques and robotic automation. The build constructs are verified using DNA sequencing, restriction enzyme digests, and software tools. It also involves delivering the build genetic construct into the host organism as an independent genetic entity or by integration into a host chromosome. The efficiency of transformation and selection of cells is often optimized through automation and high-throughput screening (9).

During the TEST process, researchers assess the performance and usability of the synthetic system throughout the testing phase. This entails running tests to see whether the biological construct was created to perform as expected. Researchers can track the synthesis of specific molecules, monitor how the system reacts to environmental changes, or evaluate its overall effectiveness. This includes functional testing, characterization, and the optimization of performances.

For strain screening and phenotyping profiling, other technologies like the biology phenotype chip, microplate high-throughput screening, microfluidics, fluorescence activated droplet sorting system (FADS), Raman light spectrum, Fourier transform infrared spectroscopy or near-infrared spectroscopy, and advanced spectral sensor have been used (10).

The Learn process of the DBTL cycle involves the data analysis and visualization of omics data and modeling for the beginning of the next DBTL cycle. Analysation of data is done by various bioinformatics and machine learning approaches. Various databases have been established such as BioModels, BiGG Models and Kbase for the understanding of various microbes. The integration of these analysis tools and the result display provides us with web page visualization (11).

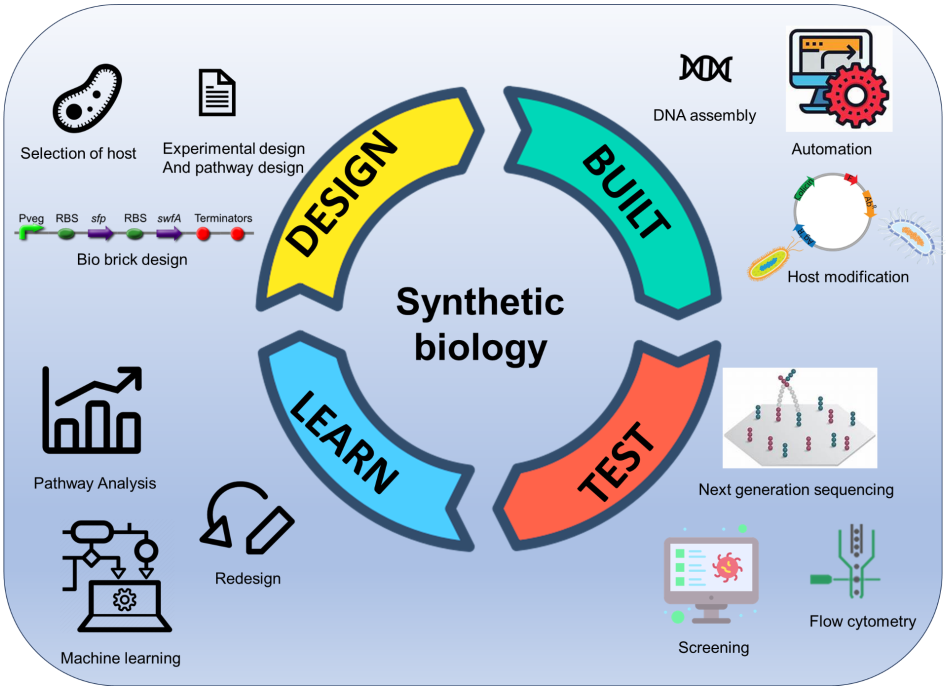

Figure 3 Design-Built-Test-Learn Cycle of Synthetic Biology- The figure represents DBTL cycle of synthetic biology

Synthetic biology is developing the tools and methods that will increase control over regulatory interactions and ones with the surrounding environment, eventually resulting in an integrative synthetic biology that will allow ground-up cellular optimization. Synthetic biology can be contextualized into three tiers i) Engineering the process of central dogma, ii) Transcription engineering and control, iii) Translational engineering and protein regulation. Efforts at each of these three tiers attempt to control cellular systems and take advantage of emerging tools and approaches. Eventually, it will be possible to integrate these approaches and realize the vision of integrative synthetic biology when cells are completely rewired for biotechnological goals (12).

Reliable control of gene expression is critical to achieving functional genetic circuits in cells and is the first step in engineering cells with new functions. The field of synthetic biology is inspired by engineering genetic circuits that function either independently within cells, or together as integrated devices with cells' natural biological networks. To establish independent function within cells, genetic circuits are often designed to be orthogonal to the host, where the control elements for regulating genetic circuits do not exist in the host signaling pathways. The underlying Boolean logic gates built into genetic circuits to dictate the timing, duration, and functionality of these circuits can be controlled with defined triggered inputs (12). In the following subsections, we discuss research tools used in molecular biology and genetic engineering. These tools represent the parts, or modules, that synthetic biologists use to engineer genetic circuits for sophisticated control of cell function. These are the tools included in DBTL cycle of synthetic biology:

i. **Genetic circuit** - Early synthetic biology work is focused on constructing genetic circuits by assembling gene regulatory parts to reprogram cells, using proteins like LacI and TetR. These proteins were fused with regulatory domains to control gene expression. Small molecule inducers like IPTG and tetracycline could reverse their effects. These tools enable flexible gene expression control, used for toggle switches, oscillators, and more. Small molecule inducers, however, have limitations in spatial and temporal control. Optogenetics, using light-sensitive proteins to activate genetic pathways, has emerged to address these issues. It provides precise control over gene expression, promising applications in biomedical engineering (13).

ii. **Chassis -** In synthetic biology, a "chassis" refers to the fundamental biological system or organism that serves as the foundational platform for the construction and implementation of engineered genetic circuits and biological components. Much like a car chassis provides the structural framework upon which various automotive parts are assembled, a biological chassis organism, which can range from bacteria to mammalian cells, offers a biological foundation for synthetic biologists to integrate and express engineered genetic elements. The choice of chassis organism depends on multiple factors such as genetic manipulability, compatibility with the desired function, and the specific objectives of the synthetic biology project. Chassis organisms are essential for creating customized biological systems that can be tailored for diverse applications, including the production of biofuels, pharmaceuticals, and environmental sensing (14).

iii. **Transcriptional Engineering:** Synthetic biologists are exploring natural transcription networks within cells to understand and harness them for controlling genetic circuits. They seek to activate genetic circuits using novel triggers found within cells to detect diseases, engineer new cellular functions, and enable autonomous cell decision-making. DNA-targeting proteins like Zinc finger (ZF) proteins and transcription activator-like effectors (TALEs) can be engineered to bind specific DNA sequences, regulating the expression of genes. Furthermore, the CRISPR/Cas9 system, when modified (dCas9), enables precise transcriptional control in bacterial and mammalian cells through the design of guide RNAs (gRNAs) that direct Cas9 to target DNA sequences. This technology holds promise for various applications, including regenerative medicine, due to its sequence-specific and precise control of cellular behavior (15).

IV. Significance in Food Production

Synthetic biology revolutionizes food production and enhances food security. This cutting-edge technology is transforming the way food is grown, processed, and delivered to people around the world (16). There are certain reasons to synthesize products using synthetic biology, some of which are described below:

1. This technology can be used to create new food sources for example, scientists are working to create proteins from plants that could be used to make meat alternatives. This would reduce the global demand for animal-based proteins and reduce the environmental impact of livestock production.
2. Improve the nutritional content of existing crops.
3. Reduce the environmental impacts of food production. For example, scientists are using synthetic biology to engineer drought-resistant crops. This can help farmers produce more food in dry climates and reduce the risk of crop failure due to drought.
4. Increase in crop yields by focusing on modified crops resistant to pests and disease.

5. It can be used to improve food safety. Scientists are developing new ways to detect and eliminate food-borne pathogens, which can help to reduce the risk of food-borne illness.
6. It can also be used to develop food packaging that can provide better protection against spoilage.

V. Major Challenge of synthetic biology - Food affordability

It has been observed that malnutrition and food affordability are interconnected global challenges. Lack of availability of nutrient rich food at an affordable price led to the major challenges of malnutrition crises. Research links food prices and food insecurity, particularly in developing nations. Food prices have been increasing globally, particularly in recent decades. However, there is limited research on the relationship between food prices, affordability, and food insecurity in the United States. Food insecurity is highest in the South, where food prices are among the lowest, but incomes are lower there. Variation in food costs within the region may also play a role. Temporal correlations between food prices, spending, and food insecurity have been identified, such as when U.S. food prices rose at the start of the millennium, leading to decreased food spending among low- and middle-income households (17).The International Network for Research, Monitoring, and Action Support on Diet, Obesity and Non-Communicable Diseases (INFORMAS) is a global network of public interest organizations and researchers aims to track, compare and support public and private sector actions to create healthy diets. environment and reduce obesity, NCDs and related inequalities (18).

A. Role of Technology in Food Affordability

The modern food industry uses a variety of complex technologies, including agricultural machinery, robotics, blockchain, microwaves, evaporators, metabolic engineering, ultrasound, nanotechnology, ozone, enzymes, computer simulations, and computer networks. These technologies have significantly increased productivity and reduce human labor in various manufacturing sectors. Automation has been used to detect delays and waste in the supply chain, while microwave heating and evaporators are widely used in food processing. Metabolic engineering has been used to produce carotenoids using yeast cells, while ultrasound provides hygienic and non-invasive measurements. Nanotechnology has revolutionized agriculture and food systems, while ozone disinfects drinking water and swimming pools. Enzymes, natural catalysts for chemical reactions, are used in industry and the food industry. Computer simulations are used in R&D to solve complex operational problems, and computer networks such as the Internet support the global food movement (19).

V. Application of synthetic biology in food production in agriculture

Affected by the explosive growth of the world's population, climate change, war and especially the COVID-19 epidemic that has swept the world in the past two years, global food security is currently facing severe threats. In 2022, the Food and Agriculture Organization of the United Nations (FAO) released the latest research report, The State of Food Security and Nutrition in the World 20221. According to this report, about one-third of the world's population still faces food insecurity. Meanwhile, the yield growth rates for significant crops have plateaued over the past few years, with limited possibilities for increasing arable land. Therefore, there is an urgent need to find a fast and sustainable way to produce more agricultural products and improve crop nutrition on limited arable land to ensure food security in the future (20).

Plant synthetic biology has advanced precision breeding through genome editing, significantly reducing the time required for trait selection. Addressing the need to increase crop nutritional value to combat malnutrition, projects like Golden Rice have incorporated genes to boost provitamin A (beta-carotene) production in rice. However, engineering complex traits and pathways for other essential compounds demands synthetic biology approaches. Carotenoids and their derivatives, xanthophylls, offer diverse health benefits, including eye and cardiovascular health, immune support, cognitive function, and antioxidant properties. Astaxanthin, a valuable red keto carotenoid in the fish industry, is primarily synthesized synthetically, but exploring plant-based sources for fish feed is promising. A deeper understanding of regulatory mechanisms controlling astaxanthin and fatty acid biosynthesis is pivotal for future engineering strategies in this domain (21). Synthetic biology in food production has many opportunities, such as:

First, a long-standing target for improvement in plant-based agriculture is nitrogen fixation. Nitrogen in a bioavailable form for crops is incredibly expensive to produce and environmentally costly, using 1% of the total annual world energy expenditure. While plants are unable to fix atmospheric nitrogen, microbes can and do, particularly rhizobia in legumes. A joint venture by Gingko Bioworks and Bayer, Joyn Bio, is targeting the reduction of global fertilizer use by one third. By engineering the plant microbiome, they are seeking to improve nitrogen fixation in crop associated microbial species. Similarly, considerable efforts are underway to introduce direct nitrogen fixation into higher plants and to introduce novel symbiotic associations with nitrogen-fixing bacteria (22). Pivot Bio has developed a biological nitrogen fertilizer for corn named Proven. Pivot Bio has created the first biological fertilizer for corn based on a γ-proteobacterium (KV137) that associates with corn roots and has the necessary genes to fix nitrogen. However, the genes are off when most needed, so synthetic biology was used to turn the genes on, which guided the remodeling of the KV137 genome. This bacterium is the active ingredient of the liquid fertilizer PROVEN, that reduces the need for chemical fertilizer by 25 lbs/acre while increasing yields by 5.8 bushels. Unlike chemical fertilizer, rain does not leach nitrogen into groundwater, a major source of pollution, or get released into the atmosphere as the powerful greenhouse gas N_2O. In 2020, PROVEN was used on 250,000 acres, to be expanded to millions in 2021 (23).

Second, improvement includes nutritional value of food by biofortification. Biofortification is a process in which the nutritional quality of food crops is improved through agricultural practices or biotechnology to increase the concentration of essential vitamins and minerals in the edible parts of those crops. The goal of biofortification is to enhance the nutritional value of staple crops, such as rice, wheat, maize, and cassava, to address specific nutrient deficiencies in populations, particularly in regions where people rely heavily on these crops for their diets. Plant synthetic biology holds great potential for addressing malnutrition, particularly micronutrient deficiencies known as "hidden hunger." This includes deficiencies in vitamins A, iodine, zinc, and iron, which affect a significant portion of the global population. To combat this issue, researchers are exploring the biofortification of crops to make them more nutrient-rich and adaptable to various environments. Traditionally, crop improvement involved time-consuming breeding practices, but the advent of gene-editing technologies like CRISPR-Cas9 allows scientists to directly modify plant DNA within a single generation, bypassing lengthy breeding processes (24). Examples of this technology include enhancing ground cherries and domesticating wild tomatoes to improve their nutritional content while maintaining disease resistance. These advancements could lead to the cultivation of previously challenging plant species as crops, expanding food choices and increasing nutrient availability worldwide. Additionally, incorporating nutraceutical compounds like resveratrol into our diets can promote better health. Resveratrol, found in plants like wine and chocolate, offers cardiovascular and diabetes-related health benefits. However, its low concentration in native plants and high metabolism when consumed in isolation pose challenges. To address this, researchers are exploring ways to add resveratrol to foods, making their delivery more efficient. Most plants contain precursor molecules for resveratrol, and modifying a single gene can enable its production in various plants. Optimization of precursor pathways is essential to increasing nutraceutical levels in specific foods.

Third, enhance photosynthesis. It is the only known biological process capable of using the energy derived from light to produce chemical energy for the synthesis of complex carbon compounds. Photosynthesis drives agriculture and is the sole defining and unifying feature of green lineage organisms. It is, however, inherently inefficient, with a theoretical maximum efficiency of ~11% but typically not exceeding a few percent, thus providing many potential targets for synthetic biology to improve outcomes. For instance, the introduction of cyanobacterial carboxysomes into the chloroplast could potentially overcome the inherent suboptimal activity of RuBisCO, the CO_2-fixing enzyme in photosynthesis. The feasibility of this approach has been successfully demonstrated by the localization of β-carboxysomal proteins that self-assemble into empty carboxysomal microcompartments in Nicotiana benthamiana chloroplasts. This potential to increase the capacity and efficiency of plants to fix atmospheric carbon has clear implications for agricultural productivity and natural resource management (23).

At last, plant-based agriculture lies with land use. Global land use is limited by availability, suitability for exploitation by commercial agriculture, and contamination by industrial processes that render potentially arable land contaminated. Two potential strategies to address this issue are bioremediation using microbes and engineering plants to grow in non-arable land. The first, bioremediation, is the use of biological systems to change an environment. Wild isolates of organohalide-respiring bacteria (ORB) such as *Desulfmonile tiedjei* have been used for the bioremediation of organohalides with some success. Extension of this concept to rational engineering approaches pertinent to agriculture are also being undertaken by researchers to convert microbes such as *S. cerevisiae* and *Escherichia coli* into potential bioremediation agents. These are capable of bioremediation of heavy metal contamination, degradation of toxic aromatic compounds, and biomass-based sugars. The second strategy, engineering new cultivars suitable for non-conventional environments, is increasingly possible due to newly available genome sequences of different cultivars and novel or obscure organisms Using the sequences of hardy cultivars, plants can be reverse engineered to tolerate abiotic stresses. Reverse engineering of traits such as halotolerance from candidate plants like the aquatic and halophilic angiosperm *Zostea marina* into crop plants will potentially play a transformative role in biological remediation areas of the world affected by salinity and will potentially aid in the regeneration of non-arable land. Similar reverse engineering strategies could be used to employ proven microbial solutions to problems directly in higher plants (23).

VI. Challenges of Synthetic Biology in Agriculture

Agricultural yield is mostly influenced by three major components: the efficiency of light energy capture and of light conversion into biomass, and the harvest index — fraction of total energy in plant biomass contained in the harvestable organs. Whereas, the efficiency of light energy capture and the harvest index have reached their biological limits, the conversion efficiency represents only 20% of its theoretical maximum, constituting therefore a potential engineering target (24). However, manipulation of such multicomponent traits is still cumbersome.

The main challenge in implementing synthetic biology in agriculture is the time and cost involved in propagating, transforming, and screening higher plants. Despite advancements such as CRISPR/Cas9-mediated gene editing, speed breeding techniques, genome sequencing of key plants, and the growth of synthetic biology as a field, significant obstacles remain. Plant genomes are often large and polyploid, limiting the effectiveness of precise genetic manipulation. Moreover, compared to microbes, plants typically have less efficient homology-directed recombination mechanisms. Additionally, it is essential for primary industries to consider consumer attitudes toward genetic manipulation, especially in the context of food production (25).

VII. Safety and Regulation

Potential hazards identified by the European Food Safety Authority (EFSA) include exacerbation of weed problems, displacement, and even extinction of native plant species, resulting in a reasonable concern that might escape environmental risk assessment (ERA) because EFSA considers only the characteristics of the original events, leaving aside unexpected or unintended next-generation effects emerging from spontaneous propagation and gene flow (26).

FSSAI and FDA approvals are currently in progress for the approval of the products developed by synthetic biology thereby ensuring food safety. Several research centers in synthetic biology were established, including eight members of the Global Biomolding Alliance, in the Asia region. An inter-regional organization called Asian Synthetic Biology Association (ASBA) was also established to promote its scholarly communication, collaboration and technology commercialization (27).

VIII. Bioeconomy and Environmental Impact

During the later half of the twentieth century, genomic advancements primarily centered around healthcare applications. A report by the OECD revealed that, as of 2003 when the Human Genome Project was published, only a minimal 2% of biotechnology investments were directed toward industrial biotechnology. This figure increased modestly to just 6% by 2010, in stark contrast to the dominant 80% allocated to the health sector (28). Since then, the biotechnology market has witnessed substantial growth. According to a recent study by Grand View Research, Inc., the global biotechnology market, valued at approximately USD 370 billion in 2016, is projected to reach USD 727 billion by 2025, reflecting an impressive annual growth rate of 7.4% . This report highlights key factors fueling this financial expansion, such as regenerative medicine and genetics in diagnostics, which incorporates artificial intelligence to enhance patient-specific diagnoses through a deeper analysis of population data.

Additionally, the report identifies supportive government policies related to synthetic biology as a major driver of growth in the biotechnology sector. It acknowledges that developed economies like the UK and the USA are closely monitoring and funding synthetic biology research and development initiatives. The recent introduction of the UK Bioeconomy Strategy reaffirms the UK's ongoing commitment to supporting this technology.

The evolution of synthetic biology as a distinct approach can be traced back to the 2004 SB 1.0 conference, which initiated a 'parallel track' of developments. These developments were not solely focused on healthcare delivery but aimed at establishing a foundational platform for technology rooted in engineering principles. This approach has led to the development of efficient bio-design and bio-manufacturing capabilities, opening a much broader range of potential applications across the entire bioeconomy. In the years since, numerous synthetic biology SMEs and start-ups have emerged, offering valuable resources for exploring and developing innovative solutions and providing essential training for future skilled workforces (28).

A. Environmental Impact

It’s no secret that current agricultural practices increase greenhouse gas emissions and are one of the key drivers of climate change. Recognizing these effects, the White House’s Inflation Reduction Act in 2022 recommends several greener agricultural options in a bid to help reduce emissions. For example, the plan recommends offering financial incentives to move toward these greener options and driving climate-smart land management. This is an important step toward reducing the impact of agriculture, but there is room to do much more. The problem of traditional methods of agriculture is that they release tons of gases, which causes global warming, but synthetic biology may reduce the environmental footprint, reducing waste, as waste generated by agriculture can be used as feed to be used in bioreactors that generate other bioproducts, such as bioactive compounds. The contribution of current farming practices to ongoing climate challenges is substantial. In 2020 alone, agriculture in the United States emitted approximately 669.5 million metric tons of gases equivalent to carbon dioxide. Of note, 50.5% of that was nitrous oxide from fertilizers. Methane from cattle and livestock accounted for 37.5% of greenhouse gases in 2020. Added to this is the carbon footprint from gas and oil needed to run agricultural equipment (29).

B. Reducing Environmental Footprint

Synthetic biology has the potential to significantly reduce the environmental footprint of food production. By optimizing metabolic pathways in microorganisms, it can lead to more efficient production processes. This means fewer resources are required for the same output, resulting in reduced land use, water consumption, and energy usage. Additionally, the controlled cultivation of engineered microorganisms in bioreactors can minimize habitat disruption and soil degradation associated with traditional agriculture (30).

C. Waste Reduction and Circular Economy

One of the key principles of synthetic biology is the ability to engineer microorganisms to utilize waste materials as feedstock. In the context of food production, this means converting agricultural and food processing waste into valuable products. This not only reduces waste disposal problems but also creates a circular economy where waste becomes a resource for producing food and other valuable compounds. This approach aligns with sustainable practices by minimizing waste generation and promoting resource efficiency (1).

D. Carbon and Resource Efficiency in Food Production

Synthetic biology can lead to greater carbon and resource efficiency in food production. Engineered microorganisms can be designed to produce specific food ingredients with minimal resource inputs, reducing greenhouse gas emissions associated with traditional agricultural practices. In summary, synthetic biology offers innovative solutions to address the environmental challenges associated with food production. It can help reduce the environmental footprint, promote a circular economy, and enhance carbon and resource efficiency in the quest for sustainable and environmentally friendly food systems (1).

IX. Global Market Trends Overview of Synthetic Biology

Synthetic Food Market size reached USD 16 billion in 2022 and is anticipated to exhibit a CAGR of 7% between 2023 and 2032, because of the increased interest in artificial foods as meat replacements. The prominent players are BASF, Bayer, Precigen, Amyris, Gingko Bioworks, Pivot Bio, Mosa Meat, and Twist Bioscience. Artificial food is an increasingly popular trend, with advances in synthetic biology supporting the development of a new line of ultra- processed, synthetically produced foods. These new products seek to replace and imitate animal proteins, food additives, and other rare ingredients. The growing attraction toward alternative proteins will provide an edge to synthetic biology food developers, considering their ability to replicate the taste, appearance, and texture of meat. Consumers are also gaining awareness of the limitations of meat and traditional protein, enabling synthetic food to emerge as a viable alternative (30).

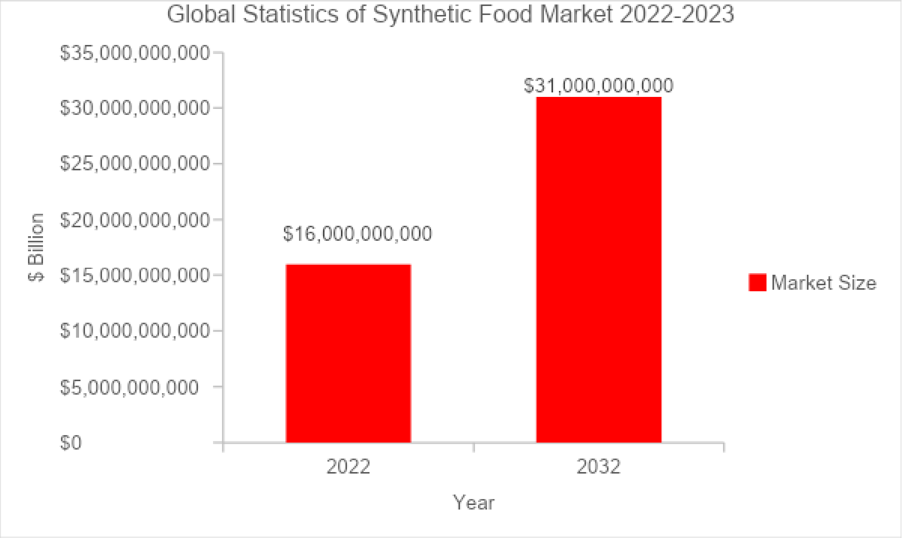

Figure 4 Global Statics of Synthetic Food Market- The diagram illustrates worldwide statistics pertaining to the synthetic food market for the period spanning 2022 to 2023. **(Source: https://www.gminsights.com/industry-analysis/synthetic-food-market)**

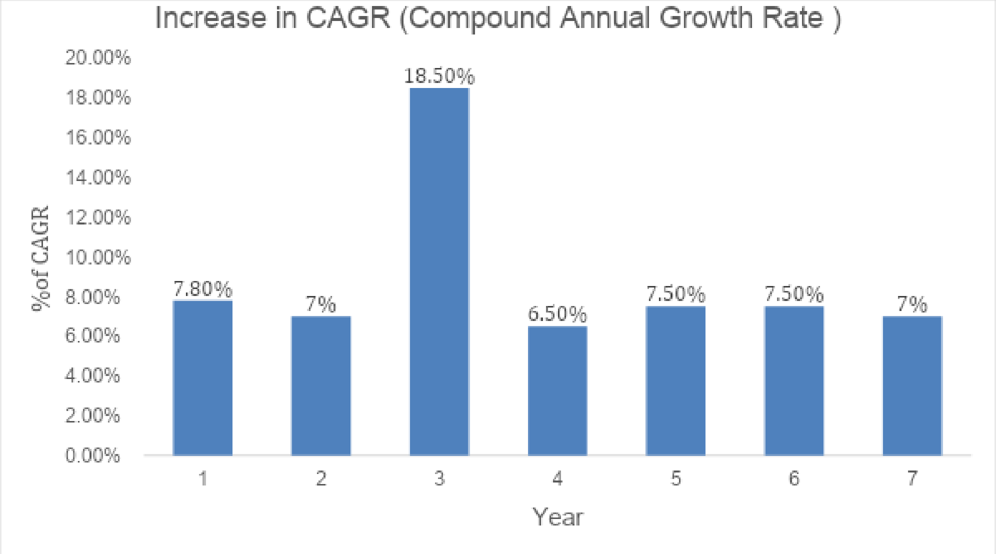

Figure 5 Increase in CAGR (Compound Annual Growth Rate) of different industries- The chart displays the Compound Annual Growth Rate (CAGR) data for various food industries over successive years. **(Source: https://www.gminsights.com/industry-analysis/synthetic-food-market)**

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The global synthetic biology in agriculture and food market holds a prominent share in various countries of North America and Europe. North America is at the forefront of the global synthetic biology in agriculture and food market, with a high market penetration rate in the U.S., and Canada, which are expected to display robust market growth in the coming five years. During the forecast period 2020-2025, the Asia-Pacific and Japan region is expected to flourish as one of the most lucrative markets for synthetic biology in agriculture and food. Asia-Pacific and Japan is expected to exhibit significant growth opportunities for synthetic biology due to increased optimism in the economic conditions of these countries. The countries in this region present immense scope for market development, owing to the increasing urban population size, growing market penetration of advanced technologies, and favorable government investments on the adaptation of innovative farming technologies (30).

X. Social Acceptance and Consumer Perception

Public perceptions of synthetic biology are ambivalent, with enthusiasm for applications addressing societal, medical, and sustainability needs. However, concerns arise about DNA manipulation without sufficient funding for long-term implications. Participants remain skeptical about futuristic technological visions and support funding for applications that meet social and sustainability goals. Oversight priorities include transparency, accountability, and tailored governance with independent checks and balances (31). People tend to have negative attitudes towards the food products developed via synthetic biology. People's attitudes vary toward biotechnology, which affects consumer acceptance or rejection of the technology. Public attitudes toward emergent or controversial scientific issues can lead to rejection of a particular technology. It is the function of a variety of sometimes competing and other times complementary influences, including sociodemographic, personal ideologies, risk–benefit perceptions, prior knowledge, and media framing (32). A cross‐region organization called the Asian Synthetic Biology Association (ASBA) was also created to promote academic communications, collaborations, and technology commercialization (33).

XI. Challenges in Synthetic Biology

The chapter includes definition of synthetic biology, its tools, and techniques. While we are currently some distance away from achieving this, synthetic bsyntheticiology holds the potential to allow us to engineer living organisms much like how we design and create everyday appliances such as dishwashers, cars, computers, or airplanes. Just as engineering has significantly enhanced our quality of life by providing us with countless innovations, it has also led to the development of advanced military technologies like bombers, tanks, and even the atomic bomb (3). In a similar vein, Synthetic Biology has the capacity to be harnessed for both constructive and potentially harmful purposes.

A. Biosecurity

The primary concern within the realm of Biosecurity arises when we consider the potential for rogue states or terrorist organizations to engage in the reengineering of microorganisms or living systems with the intention of causing harm. While this notion can be alarming, the actual process of creating a new pathogenic organism and effectively deploying it is far from straightforward. There are numerous uncertainties surrounding what makes a pathogenic organism virulent in a natural environment as opposed to a controlled laboratory setting. For instance, such an organism would need to outcompete other microorganisms and evade the host's immune response to survive and thrive. These obstacles, along with the complex engineering challenges they pose, currently act as significant barriers. However, it's crucial to acknowledge that as scientific advancements continue to progress, these hurdles may be overcome in the not-so-distant future (34).

B. Ethical Issues

Beyond the safety concerns, it is crucial to address the ethical dilemmas surrounding the complete engineering of novel life forms or the modification of existing species. While these activities might not immediately pose biosafety or biosecurity threats, they can elicit significant ethical apprehensions among certain segments of the population, who perceive them as akin to assuming a God-like role. Although we are not presently at the stage where we can seriously contemplate applying synthetic biology to enhance specific traits in target species, including the potential for human modification, such scenarios require meticulous consideration and the establishment of clear regulatory frameworks, akin to those developed for human cloning. While designing new living systems for scientific purposes may not inherently raise major ethical concerns, the prospect of altering human beings demands a comprehensive ethical assessment. It is worth noting that the perception of "playing God" may be a more contentious issue in the United States compared to Europe, where the redesign of living organisms could be viewed with greater concern. Security threats, in the sense of the intentional release of biological agents, play an important role in the discussion between policy and synthetic biology associated with industry. In this context, the DNA synthesis sector is particularly concerned, since ordered DNA fragments could also bear the danger of being misused, which basically requires a careful screening and checking of all the possible outlets including customers. The security concerns are not new to synthetic biology. Biotechnology and even microbiology also had to deal with these concerns of misuse. Although Martinot and Benner (2004) are confident that artificial genetic systems will not survive outside the lab, research in this field raises profound biosafety questions. Dr. Jonathan King (Pollack,2001), a professor of molecular biology at MIT commented in the New York Times and cautioned that *"It's a powerful technology, and like all powerful technologies, it needs appropriate oversight and regulation"*. One possible scenario he suggested is that proteins with artificial amino acids could elicit allergic reactions if used in drugs or in food. Synthetic biology's promoters are hoping that the promise of a very "green" techno-fix, synthetic microbes that manufacture biofuels cheaply or put a chill on climate change, will prove so seductive that the technology will win public acceptance despite its risks and dangers (35).

Agricultural yield is mostly influenced by three major components: the efficiency of light energy capture and of light conversion into biomass, and the harvest index—the fraction of total energy in plant biomass contained in the harvestable organs. Whereas, the efficiency of light energy capture and the harvest index have reached their biological limits, the conversion efficiency represents only 20% of its theoretical maximum, constituting therefore a potential engineering target (35). However, manipulation of such multicomponent traits is still cumbersome.

The main challenge in implementing synthetic biology in agriculture is the time and cost involved in propagating, transforming, and screening higher plants. Despite advancements such as CRISPR/Cas9-mediated gene editing, speed breeding techniques, genome sequencing of key plants, and the growth of synthetic biology as a field, certain obstacles remain. Plant genomes are often large and polyploid, limiting the effectiveness of precise genetic manipulation. Moreover, compared to microbes, plants typically have less efficient homology-directed recombination mechanisms. Additionally, it is essential for primary industries to consider consumer attitudes toward genetic manipulation, especially in the context of food production. An ongoing objective in plant-based agriculture is to enhance nitrogen fixation (35).

XII. Conclusion

In conclusion, the development of economical food products through synthetic biology represents a significant and promising advancement in the field of agriculture and nutrition. Synthetic biology, defined as the interdisciplinary field that combines biology, engineering, and computational science to design and construct biological parts, devices, and systems, has opened new avenues for addressing critical challenges in the food industry.

Synthetic biology employs a wide range of tools and techniques, with the most notable being the CRISPR-Cas9 system. This revolutionary gene-editing technology allows for precise and efficient modification of the DNA of various organisms, including crops, to achieve desirable traits, such as increased nutritional content and disease resistance.

Market trends in synthetic biology indicate a growing interest and investment in this field, with a focus on sustainable and environmentally friendly solutions for food production. The application of synthetic biology in agriculture holds great promise, as it enables the biofortification of essential nutrients in crops, addressing malnutrition concerns worldwide. By enhancing the nutritional content of staple crops and enabling the cultivation of previously challenging plant species, synthetic biology has the potential to diversify available food options and increase the availability of nutrients that are limited in various regions.

In summary, synthetic biology offers a powerful toolbox for the development of economical and nutritionally enriched food products. As this field continues to advance, it holds the key to addressing global food security issues and improving the overall health and well-being of communities around the world. The application of synthetic biology in agriculture is poised to play a vital role in the future of food production and nutrition.

XIII. References

1. Tyagi, Ashish, Ashwani Kumar, S. V. Aparna, Rashmi H. Mallappa, Sunita Grover, and Virender Kumar Batish. "Synthetic biology: applications in the food sector." *Critical Reviews in Food Science and Nutrition* 56, no. 11 (2016): 1777-1789.
2. Ramírez Rojas, Adán Andrés, Razan Swidah, and Daniel Schindler. "Microbes of traditional fermentation processes as synthetic biology chassis to tackle future food challenges." *Frontiers in Bioengineering and Biotechnology* 10 (2022): 982975.
3. Serrano, Luis. "Synthetic biology: promises and challenges." *Molecular systems biology* 3, no. 1 (2007): 158.
4. Li, Jing, Huimiao Zhao, Lanxin Zheng, and Wenlin An. "Advances in synthetic biology and biosafety governance." *Frontiers in bioengineering and biotechnology* 9 (2021): 598087.
5. https://blog.bccresearch.com/the-origin-and-history-of-synthetic-biology
6. Garner, Kathryn L. "Principles of synthetic biology." *Essays in Biochemistry* 65, no. 5 (2021): 791-811.
7. https://kinnu.xyz/kinnuverse/science/synthetic-biology/principles-of-synthetic-biology/
8. da Silva Vale, Alexander, Gilberto Vinícius de Melo Pereira, Leonardo Martins Santana, Dão Pedro de Carvalho Neto, Brigitte Sthepani Orozco Colonia, Vanete Thomaz Soccol, Bruna Leal Maske, and Carlos Ricardo Soccol. "Perspective on the use of synthetic biology in rudimentary food fermentations." *Systems Microbiology and Biomanufacturing* 3, no. 1 (2023): 150-165.
9. https://roadmap.ebrc.org/dbtl/
10. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8617713/
11. Wang, Junyang, Jens Nielsen, and Zihe Liu. "Synthetic biology advanced natural product discovery." *Metabolites* 11, no. 11 (2021): 785.
12. MacDonald, I. Cody, and Tara L. Deans. "Tools and applications in synthetic biology." *Advanced drug delivery reviews* 105 (2016): 20-34.

13. Sargent, Demi, Warren C. Conaty, David T. Tissue, and Robert E. Sharwood. "Synthetic biology and opportunities within agricultural crops." *Journal of Sustainable Agriculture and Environment* 1, no. 2 (2022): 89-107.
14. de Lorenzo, V., Krasnogor, N., & Schmidt, M. (2021). For the sake of the Bioeconomy: define what a Synthetic Biology Chassis is! *New Biotechnology*, *60*, 44-51.
15. Benner, Steven A., and A. Michael Sismour. "Synthetic biology." *Nature reviews genetics* 6, no. 7 (2005): 533-543.
16. Roell, Marc-Sven, and Matias D. Zurbriggen. "The impact of synthetic biology for future agriculture and nutrition." *Current Opinion in Biotechnology* 61 (2020): 102-109.
17. Carson, Jess, and Sarah Boege. "The Intersection of Food Availability, Access, & Affordability with Food Security and Health." *University of New Hampshire* (2020).
18. Lee, Amanda, Cliona Ni Mhurchu, Gary Sacks, Boyd Swinburn, Wendy Snowdon, Stefanie Vandevijvere, Corinna Hawkes et al. "Monitoring the price and affordability of foods and diets globally." *obesity reviews* 14 (2013): 82-95.
19. Ramírez Rojas, Adán Andrés, Razan Swidah, and Daniel Schindler. "Microbes of traditional fermentation processes as synthetic biology chassis to tackle future food challenges." *Frontiers in Bioengineering and Biotechnology* 10 (2022): 982975.
20. Wang, Lanteng, Xin Zang, and Jiahai Zhou. "Synthetic biology: A powerful booster for future agriculture." *Advanced Agrochem* 1, no. 1 (2022): 7-11.
21. Goold, Hugh Douglas, Philip Wright, and Deborah Hailstones. "Emerging opportunities for synthetic biology in agriculture." *Genes* 9, no. 7 (2018): 341.
22. Voigt, Christopher A. "Synthetic biology 2020–2030: six commercially-available products that are changing our world." *Nature Communications* 11, no. 1 (2020): 6379.
23. Barnum, Collin R., Benjamin J. Endelman, and Patrick M. Shih. "Utilizing plant synthetic biology to improve human health and wellness." *Frontiers in Plant Science* 12 (2021): 691462.
24. Li, Jing, Huimiao Zhao, Lanxin Zheng, and Wenlin An. "Advances in synthetic biology and biosafety governance." *Frontiers in bioengineering and biotechnology* 9 (2021): 598087.
25. Mao, Ning, Nikhil Aggarwal, Chueh Loo Poh, Byung Kwan Cho, Akihiko Kondo, Chenli Liu, Wen Shan Yew, and Matthew Wook Chang. "Future trends in synthetic biology in Asia." *Advanced Genetics* 2, no. 1 (2021): e10038.
26. Clarke, Lionel J. "Synthetic biology–pathways to commercialisation." *Engineering Biology* 3, no. 1 (2019): 2-5.
27. https://www.forbes.com/sites/forbestechcouncil/2023/03/03/cultivating-sustainable-agricultural-practices-using-synthetic-biology/?sh=3466dff27b3a).
28. Lv, Xueqin, Yaokang Wu, Mengyue Gong, Jieying Deng, Yang Gu, Yanfeng Liu, Jianghua Li et al. "Synthetic biology for future food: research progress and future directions." *Future Foods* 3 (2021): 100025.

29. Pauwels, Eleonore. "Public understanding of synthetic biology." BioScience 63, no. 2 (2013): 79-89.
30. Akin, Heather, Kathleen M. Rose, Dietram A. Scheufele, Molly Simis-Wilkinson, Dominique Brossard, Michael A. Xenos, and Elizabeth A. Corley. "Mapping the landscape of public attitudes on synthetic biology." *BioScience* 67, no. 3 (2017): 290-300.
31. Mao, Ning, Nikhil Aggarwal, Chueh Loo Poh, Byung Kwan Cho, Akihiko Kondo, Chenli Liu, Wen Shan Yew, and Matthew Wook Chang. "Future trends in synthetic biology in Asia." *Advanced Genetics* 2, no. 1 (2021): e10038.
32. Wurtzel, Eleanore T., Claudia E. Vickers, Andrew D. Hanson, A. Harvey Millar, Mark Cooper, Kai P. Voss-Fels, Pablo I. Nikel, and Tobias J. Erb. "Revolutionizing agriculture with synthetic biology." *Nature Plants* 5, no. 12 (2019): 1207-1210.
33. Thavarajah, Walter, Matthew S. Verosloff, Jaeyoung K. Jung, Khalid K. Alam, Joshua D. Miller, Michael C. Jewett, Sera L. Young, and Julius B. Lucks. "A primer on emerging field-deployable synthetic biology tools for global water quality monitoring." *npj Clean Water* 3, no. 1 (2020): 18.
34. Robaey, Zoë, Shannon L. Spruit, and Ibo van de Poel. "The Food Warden: an exploration of issues in distributing responsibilities for safe-by-design synthetic biology applications." *Science and Engineering Ethics* 24 (2018): 1673-1696.
35. Tyagi, Ashish, Ashwani Kumar, S. V. Aparna, Rashmi H. Mallappa, Sunita Grover, and Virender Kumar Batish. "Synthetic biology: applications in the food sector." *Critical Reviews in Food Science and Nutrition* 56, no. 11 (2016): 1777-1789.