Current Status and Prospects of Industrial Applications of Synthetic Biology (Postprint)

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Abstract

Synthetic biology enables the biosynthesis of chemicals, offering a potential pathway to reduce reliance on petroleum resources. The advancement of synthetic biology technology has substantially enhanced the capacity of microbial cell factories for chemical production; newly engineered strains and processes can effectively replace traditional counterparts, significantly mitigating pollution, markedly reducing energy consumption, and elevating the technological standards of the conventional fermentation industry. This article reviews the key factors and representative cases in constructing cell factories and multi-enzyme molecular machines for chemical production using synthetic biology technology, and provides an outlook on its future industrial applications.

Full Text

Current Situations and Perspectives of Industrial Applications of Synthetic Biology

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Abstract

Biosynthesis of chemicals through synthetic biology can partially relieve dependence on petroleum resources. With the development of synthetic biology technology, the capabilities of constructing microbial cell factories for chemical production have been greatly improved. The development of new engineered strains and bioprocesses can replace traditional manufacturing processes, decrease energy costs and pollution, and improve the production capabilities of the traditional fermentation industry. This review summarizes key factors and representative cases for constructing microbial cell factories and multi-enzyme molecular machines to produce chemicals, and discusses future perspectives for industrial applications of synthetic biology.

Keywords: synthetic biology, chemicals, cell factories, multi-enzyme molecular machines

Petroleum refining has traditionally been the primary means of obtaining essential material resources for industrial society. However, this process relies on non-renewable petroleum resources and suffers from high energy consumption and severe pollution in many production processes [1]. Achieving green and clean production of chemicals is crucial for sustainable national economic development. Through synthetic biology technology, we can reprogram the biosynthetic capabilities of microorganisms in nature and even create entirely new synthetic pathways. By constructing efficient cell factories that use renewable biomass resources as raw materials to produce various chemicals, the development of a bio-manufacturing industry will effectively promote the transformation of national economic development models and ensure the achievement of energy conservation and emission reduction goals.

Key Factors for Constructing Microbial Cell Factories

Conversion rate, production rate, and yield are the three critical metrics that determine whether a microbial cell factory can achieve industrial application. Through the design of optimal synthetic pathways, optimization of synthetic routes, and improvement of cellular physiological performance, the conversion rate and production rate of microbial cell factories for chemical production can be significantly enhanced, ultimately leading to increased yields.

Improving Conversion Rate Through Optimal Synthetic Pathway Design In the cost structure of chemical bio-manufacturing, raw material expenses account for the largest proportion (typically over 50%). Therefore, improving the conversion rate of microbial cell factories is the most crucial factor for achieving industrial application. Higher conversion rates mean less raw material consumption and lower production costs. When designing optimal synthetic pathways, two key factors are carbon flux distribution and reducing power supply. The metabolic networks of natural microorganisms are highly complex. To achieve maximum conversion rates, the carbon flux from substrates must be redirected to concentrate maximally on target chemical synthesis. Therefore, cell factory construction first requires creating genomic-scale metabolic and regulatory network models of microorganisms, upon which optimal synthetic pathways for target chemicals can be designed to avoid competition from byproducts and ensure thermodynamic feasibility and adequate energy supply [2]. In addition to carbon flux distribution, reducing power supply is also critical—the reducing power generated from substrate metabolism must meet the requirements for chemical synthesis.

Improving Production Rate Through Synthetic Pathway Optimization The production rate of microbial cell factories is another key factor determining bio-manufacturing costs. Higher production rates lead to shorter fermentation cycles and lower costs. To achieve high production rates, synthetic pathways must operate efficiently. Chemical biosynthetic pathways typically consist of a series of enzymatic reactions. In natural microbial synthetic pathways, the catalytic efficiencies of various enzymes are not optimally coordinated —some enzymes have slow catalytic rates that become rate-limiting steps, while others catalyze too quickly, leading to accumulation of intermediate metabolites that can be toxic to cell growth and metabolism. These issues result in low overall pathway efficiency and constrain the production rate of cell factories. Therefore, synthetic pathways must be optimized to achieve a balanced and coordinated state. Currently, three main approaches are employed:

1. **Multi-gene regulation technology**: This approach simultaneously modifies multiple genes on the chromosome, combined with high-throughput screening techniques, to rapidly and efficiently identify optimal regulatory combinations [3]. Standardized regulatory element libraries, such as promoters, ribosome binding sites, and mRNA stability region libraries, provide a solid foundation for genetic modification. Small molecule RNA technologies based on plasmid expression can regulate target gene expression on a large scale, enabling precise control of synthetic pathways [4].

2. **Gene dynamic regulation technology**: Based on the interaction between transcription factors and key metabolic intermediates, dynamic sensor-regulator systems can be developed to control gene expression. Compared with traditional regulation systems, dynamic regulation systems are intelligent—they can automatically adjust the expression of specific genes according to selected intracellular metabolic signals, thereby maintaining dynamic balance of metabolic pathways and achieving the goal of improving product synthesis capacity [5].

3. **Protein scaffold technology**: In intracellular metabolic pathways, the spatial positioning of participating enzymes is an important factor affecting pathway efficiency. Through artificial protein scaffolds, enzymes can be attached at specific positions and sequences, controlling their spatial arrangement. This brings adjacent enzymes in a synthetic pathway into close physical proximity, reducing the distance between substrates and enzymes and increasing biochemical reaction rates [6]. Additionally, protein scaffolds can modulate enzyme catalytic efficiency, and this technology can be used to obtain optimal combinations that ultimately improve cell factory performance [7].

Increasing Yield Through Cellular Physiological Performance Optimization After synthetic pathway optimization, a preliminary cell factory can be obtained. However, to achieve industrial application, further optimization of cellular physiological performance is necessary. To obtain high yields, cells must be able to adapt to high osmotic pressure. To ferment organic acids under acidic conditions—avoiding the need for neutralizing agents and simplifying downstream separation and extraction processes—cells must tolerate acidic environments. Simultaneously, to prevent contamination and save energy, cells must adapt to high temperatures. The development of evolutionary metabolism and global perturbation technologies can effectively improve cellular physiological performance [8-11]. On this basis, various high-throughput omics analysis techniques can be used to analyze the genetic mechanisms underlying improved cellular performance, which can then be applied to the next round of cell factory construction.

Representative Cases of Industrial Application of Microbial Cell Factories

Using the above technical approaches, researchers have created a series of microbial cell factories that enable bio-manufacturing of chemicals previously only accessible through petroleum refining. These advances have also improved the technical level of many traditional fermentation products, with significant effects on energy conservation and consumption reduction.

Bio-manufacturing of Novel Chemicals 1,3-Propanediol: 1,3-Propanediol is an important chemical raw material widely used in the synthesis of polyesters, polyethers, polyurethanes, coatings, detergents, and adhesives. Polytrimethylene terephthalate (PTT), formed by the condensation of 1,3-propanediol with terephthalic acid, is not only biodegradable but also possesses the softness and elasticity of nylon, the bulkiness of acrylic fibers, and the stain resistance of polyester, making it an excellent new material. In the early 20th century, as petroleum prices became increasingly volatile and rising, some international petrochemical giants began exploring bio-manufacturing technologies to partially replace petrochemical routes and enrich their technology and industrial chains. The most representative example is DuPont, whose flagship biotechnology product is 1,3-propanediol. Some microorganisms in nature can efficiently convert glycerol to 1,3-propanediol. However, glycerol raw material costs are relatively high, and due to reducing power constraints, the theoretical maximum conversion rate using glycerol as the sole carbon source is only 0.75 mol/mol, making this production route costly. DuPont was the first to design and create a biosynthetic pathway for producing 1,3-propanediol from glucose. By importing the glycerol synthesis pathway from *Saccharomyces cerevisiae* and the 1,3-propanediol synthesis pathway from *Klebsiella pneumoniae* into *E. coli*, knocking out the phosphoenolpyruvate-carbohydrate phosphotransferase system (PTS system), and downregulating glyceraldehyde-3-phosphate dehydrogenase to reduce carbon flux into the tricarboxylic acid

(TCA) cycle, glucose metabolism toward glycerol was enhanced, significantly improving the conversion rate from glucose to 1,3-propanediol. The final engineered cell factory achieved a 1,3-propanediol titer of 135 g/L, a production rate of 3.5 g/(L • h), and a glucose conversion rate of 0.83 mol/mol [12]. Based on this technology, DuPont established an industrial production line with an annual capacity of 45,000 tons of 1,3-propanediol and developed a series of PTT-derived material products. Compared with traditional petrochemical routes, the bio-based 1,3-propanediol technology reduces energy consumption by 40% and CO_2 emissions by 40%. Tsinghua University, Beijing University of Chemical Technology, and East China University of Science and Technology have also conducted research on producing 1,3-propanediol from glycerol. Following the great success of bio-based 1,3-propanediol technology, DuPont adjusted its strategic investment, continuously increasing R&D investment in biotechnology and promoting the bio-manufacturing industry. Currently, 20% of DuPont' s output value comes from bio-based industries.

Terpenoids: Terpenoids are the largest class of plant natural products and constitute the main components of liquid fuels, rubber, and fragrances. Terpenoids are not only important industrial raw materials but also have extensive applications in the medical field. Professor Jay Keasling from the University of California, Berkeley, and Amyris Company have made important contributions to the development of terpenoid cell factories, creating the first representative product in synthetic biology history—artemisinin. Amyris constructed a *Saccharomyces cerevisiae* cell factory to produce artemisinic acid, a key precursor to the antimalarial drug artemisinin, which is then converted to artemisinin [13]. In May 2013, the World Health Organization approved artemisinin produced by cell factories for use as a clinical drug, freeing artemisinin production from the limitations of traditional plant extraction methods. It is no longer constrained by land, climate, or time required for plant growth and can be industrially produced through fermentation tanks, providing the possibility of eliminating malaria worldwide.

L-Alanine: L-Alanine is an important platform chemical used to produce surfactants, vitamin B6, and amino acid injections, with a global market demand of 50,000 tons per year. Current L-alanine production technology uses petroleum-based maleic anhydride as raw material to produce aspartic acid, which is then decarboxylated to generate L-alanine. This technology uses non-renewable raw materials and emits large amounts of CO_2 during production. Although natural microorganisms have biosynthetic pathways to convert glucose to L-alanine, the yield and conversion rate are very low, making them uncompetitive compared with enzymatic routes. The Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, constructed a cell factory that efficiently converts glucose to L-alanine through optimal pathway design, synthetic pathway reconstruction, precise pathway regulation, and cellular performance optimization [14]. Currently, this technology has reached the world' s highest level, and an industrial production line with an annual capacity of 30,000 tons of L-alanine has been established using this technology, representing the first international industrialization of fermentative L-alanine production. Production costs are reduced by 52% compared with traditional technology. With the mass production of fermentative L-alanine, world chemical giants led by BASF have begun using it as a raw material to manufacture novel environmentally friendly phosphorus-free detergent methylglycine diacetic acid (MGDA) to replace traditional phosphorus-containing detergents, which is of great significance for protecting aquatic ecosystems.

Industrial Upgrading of Traditional Products Ethanol: One of the main bottlenecks in cellulosic ethanol industrialization is the lack of yeast strains that can simultaneously metabolize xylose and glucose. The Shanghai Industrial Biotechnology Research Center, affiliated with the Center for Excellence in Molecular Plant Sciences of the Chinese Academy of Sciences, with assistance from Novozymes and Shandong University, imported xylose isomerase genes into *Saccharomyces cerevisiae* and adapted them through domestication, achieving rapid fermentation capabilities for corn stover and steam-exploded sugarcane bagasse [15]. This xylose-utilizing yeast has applied for patents in the United States and other countries and has signed yeast supply agreements with multiple cellulosic ethanol projects. In 2017, this yeast was used to produce 28 million liters of cellulosic ethanol, estimated to account for more than half of the global second-generation ethanol market share. This achievement marks that China' s alcohol fermentation yeast design and construction technology has entered the international advanced ranks.

n-Butanol: n-Butanol is a bulk chemical raw material with multiple uses and a high-quality fuel with performance superior to ethanol. The biological synthesis of n-butanol using solvent-producing clostridia as production strains was once the world' s second-largest bio-manufacturing technology industry after ethanol. The Center for Excellence in Molecular Plant Sciences of the Chinese Academy of Sciences was the first in China to systematically conduct research on clostridial n-butanol fermentation. By establishing new genetic editing tools, discovering new regulatory elements and mechanisms, constructing efficient and universal chassis, and reconstructing new product pathways, a new generation of engineered strains capable of utilizing various non-food raw materials was constructed, expanding the substrate spectrum of clostridial fermentation (sugar-based carbon sources, lignocellulose, and gaseous carbon sources) and achieving higher n-butanol synthesis capabilities, demonstrating excellent tolerance and co-fermentation of five- and six-carbon sugars [16]. The team recently achieved the goal of synthesizing n-butanol from one-carbon gases and has initiated technical cooperation with enterprises to promote the development and application of new technologies.

Lysine: Lysine is widely used in animal feed, medicine and health products, and chemical new materials. China is a major producer of lysine, with output reaching 1.57 million tons in 2017, accounting for 56.5% of the global share. As a traditional bulk fermentation product, its production strains have been improved for nearly half a century, and the fermentation level is already very high, making further improvement extremely difficult. However, China' s lysine industry urgently needs upgrading to improve economic benefits, promote energy conservation and emission reduction, and increase international competitiveness, making the development of new-generation lysine fermentation strains imperative. By establishing a complete core strain R&D system including computer-aided strain design, rational design of key enzymes, metabolic pathway reconstruction, and intelligent evolutionary screening of strains, the Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, successfully developed a new-generation lysine synthesis strain with a conversion rate leading the world. Core patents have been granted in China, Japan, the United States, and other countries [17].

Multi-Enzyme Molecular Machines

In addition to the microbial cell factory route, the multi-enzyme molecular machine route has also attracted widespread attention in recent years. Since many chemicals are highly toxic to microbial cells, it is difficult for microbial cell factories to achieve high yields when producing such chemicals. Multi-enzyme molecular machines do not require microbial cells and have significant advantages in producing toxic chemicals. This technology uses one or more enzymes added to a reactor to efficiently convert biomass raw materials into chemicals. The design of multi-enzyme reactions and the mining and efficient preparation of enzymes are the core aspects of this technical route. The most representative cases of chemical production using multi-enzyme molecular machines are the bio-manufacturing of hydrogen and inositol.

Hydrogen: Hydrogen is the most promising clean energy source for the future and is widely used as a power source for fuel cells. Current hydrogen production mainly uses water-gas shift reactions and water electrolysis, which have high equipment requirements, resulting in high hydrogen production costs, and hydrogen is not easy to store and transport. Researchers from Virginia Tech used multi-enzyme molecular machine technology to adapt 14 enzymes and 1 cofactor, enabling the production of 12 hydrogen molecules from one glucose unit using biomass as raw material [18]. This technology solves the hydrogen storage and transportation problems and provides an energy supply solution for future hydrogen fuel cell vehicles.

Inositol: Inositol is a water-soluble B vitamin and an essential substance for animal and microbial growth, widely used in feed, medicine, food, and other industries. Currently, China mainly uses traditional high-temperature and high-pressure hydrolysis of phytic acid to produce inositol. This process has strict equipment material requirements, high one-time investment, low raw material utilization, complex purification processes, and high production costs. Moreover, this process generates large amounts of phosphate pollutants, causing serious water source and environmental pollution. By adopting multi-enzyme molecular machine technology, researchers from the Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, created an in vitro biosynthetic route from starch to inositol using α-glucan phosphorylase, phosphoglucomutase, inositol-3-phosphate synthase, and inositol monophosphatase [19]. This green catalytic process can replace traditional high-pollution, high-energy-consumption production processes, reducing inositol production costs to half of those of traditional phytic acid hydrolysis while significantly reducing environmental pollution.

Future Perspectives for Industrial Applications of Synthetic Biology

Synthetic biology technology has developed rapidly worldwide in recent years. China has continuously expanded its talent pool and improved its technological capabilities in this field, maintaining overall development levels comparable to international peers. Driven by synthetic biology technology, China' s bio-manufacturing industry has made rapid progress. The technological upgrading of traditional products such as amino acids and vitamins continues to advance, and patent blockades have been partially circumvented for some important products. In new product development, foreign countries have bio-manufacturing technologies for a series of important bulk chemicals such as long-chain alcohols, 1,4-butanediol, and terephthalic acid, while Chinese scientists have achieved world-leading positions in the bio-manufacturing technologies for compounds such as inositol, 3-hydroxypropionic acid, and adipic acid. In research directions that determine future industrial layout, such as new enzyme design and new synthetic pathway design, China has maintained parallel development with international peers.

However, it must be recognized that in the face of international market competition, the high cost of important fermentation raw materials such as corn remains a major disadvantage for China' s bio-manufacturing industry. Therefore, to achieve breakthroughs in the bio-manufacturing industry and build international competitiveness, it is necessary to vigorously develop technologies for utilizing non-food raw materials based on existing synthetic biology industrial applications. On the one hand, lignocellulose utilization technologies should be developed to enable microbial cell factories to efficiently use non-food biomass such as straw and wood chips. On the other hand, biotechnology using CO_2 and other gases as main raw materials can be explored to discover entirely new carbon utilization pathways. As the nation and its people attach increasing importance to ecological and environmental protection, China's bio-manufacturing industry will face higher requirements for wastewater and waste emissions in the future. This demands continuous improvement of cell factory technology to minimize byproduct synthesis and reduce environmental pollution while lowering the costs of separation and extraction technologies.

Most current developments in synthetic biology have achieved bio-manufacturing of chemicals based on existing biosynthetic pathways in nature. However, the vast majority of chemicals lack natural biosynthetic pathways, which poses a great challenge for future development of the bio-manufacturing industry but is also where synthetic biology can truly demonstrate its disruptive value.

Currently, there are very few reports on the de novo creation of entirely new biosynthetic pathways for chemicals, with one of the biggest bottlenecks being new enzyme design capability. To achieve artificial construction of entirely new synthetic pathways, deep interdisciplinary integration of computational biology, synthetic chemistry, chemical biology, protein science, and other fields is essential to achieve breakthroughs in new enzyme design. Therefore, multidisciplinary convergence and the construction of broader cooperation networks will be important characteristics of future synthetic biology development.

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