Editorial Corner

Prospect of *in vitro* Metabolic Engineering

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With advances in improving genetic technologies and understanding of metabolic pathways over the past decades, metabolic engineering has evolved as a powerful tool to design and optimize fermentation processes for production of renewable chemicals, biofuels, biomaterials, and biologically active compounds, especially when chemical synthesis is difficult. Nevertheless, fermentation with metabolic engineered microorganisms often suffers low production titer and yield. The primary goal of microorganisms in the fermentation process is their proliferation while the bioconversion is the "byproduct" of their growth. In addition, there are also inherent natural constraints of microorganisms in the production of non-natural products through fermentation, such as flux imbalances caused by unidentified regulation of natural metabolism and cell death caused by the toxicity of the heterologous expressed biomolecule. There are presently very limited successful cases could really reach into the stage of economical biomanufacturing using metabolic engineered microorganisms.

One possible solutions to address the problems associated with the whole cell metabolic engineering is to construct a simplified biosynthetic pathway extracellularly by assembling only required enzymes and coenzymes from different sources. This emerging bioconversion platform is known as *in vitro* metabolic engineering. Since *in vitro* metabolic engineering is on their early stage of development, many different names have been given in the literature, such as cell-free synthetic biology, synthetic metabolic engineering, cascade enzyme factories, Synthetic Pathway Biotransformation (SyPaB), synthetic cascade biomanufacturing, cell-free biosystems or cell-free biomanufacturing, and synthetic biochemistry. The first cell-free bioconversion systems was reported by Welch and Scope in 1985. The system consists enzymes purified from yeast cell lysate that was capable to converting glucose to ethanol with a nearly 100% molar yield. Later in the 20th century, researchers tried to assemble more enzymes in one reactor to accomplish complex cascade reactions that mimic the natural biosynthesis in the microbial cells. In comparison with the cell based bioconversion, the *in vitro* metabolic engineering has advantages including fast reaction without cell membrane limitation, high conversion yield without formation of byproduct and maintenance of cell growth, moderate operation without using sophisticate bioreactor, and flexible reaction conditions with the adjustable enzyme selection and concentration. Over the past years, more and more renewable and valuable biomolecule had been produced through *in vitro* metabolic engineering at lab scale.

Despite many attractive features, the development of *in vitro* metabolic engineering is far behind the microbial fermentation and relatively little attention is paid to the practical application of *in vitro* metabolic engineering because of some economical unfavorable processes such as enzyme purification and the dependence of expensive cofactors. Currently, many techniques were developed to address these challenges. For example, the employments of thermostable enzymes and cell-free protein production could be useful to ease the cost associated with enzyme purification.

In 2017 summer, the U.S. Department of Energy's Bioenergy Technologies Office (BETO) also held a Cell-Free Synthetic Biology and Biocatalysis Listening Day in Denver, Colorado and invited many professionals and related researchers in this field to discuss the future development of Cell-Free Synthetic Biology or *in vitro* metabolic engineering. Briefly, most attendants in the event agreed with that the development of Cell-Free Synthetic Biology and Biocatalysis or *in vitro* metabolic engineering is still the in infancy stage, it has great potential to become an important platform for bioconversion in the future, especially for non-natural biomolecules and biofuels. At the present development stage, the Cell-Free Synthetic Biology or *in vitro* metabolic engineering can be a very useful tool for prototyping of artificial metabolic pathway.

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