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2022 The 1st Western China symposium on the international frontier of synthetic biomanufacturing

A B S T R A C T

The 1st western China symposium on the international frontier of synthetic biomanufacturing was successfully held on July 8–10 in 2022. The conference is firstly launched by Professor Dan Wang in Chongqing University, and will be organized regularly every year by different universities in western China. The aim of this symposium is to show the cutting-edge knowledge of the synthetic biology developed in China and worldwide, provide a chance to meet international colleagues, and also to promote the academic and economic development of western China. Due to COVID-19, the 2022 symposium was masterfully delivered on the combination of online and offline operation, and the organisers must be commended for a really excellent and interactive meeting.

The content of the conference involves two modules of synthetic biology and green biomanufacturing, covering eight aspects: synthetic biology, metabolic engineering, biological process engineering, industrial microbial breeding, biocatalysis and biotransformation, synthetic bio-materials, bio-medicine and biological separation engineering. More than 400 representatives were invited to gather together to exchange the latest research results and development trends in the field of synthetic biology and biomanufacturing. There was a significant focus on the younger scientists, both in terms of oral reports and posters. There were many excellent invited lectures and sessions beyond the remit of this short summary, including "Pharmaceutical manufacturing by biological methods" by Yuguo Zheng, Academician of the Chinese Academy of Engineering (CAE) Member of China, and a lecture "The third generation of biological manufacturing: preparing chemicals with CO_2 as raw material" by Tianwei Tan, Academician of the CAE Member of China, a lecture on the biotransformation and green separation of natural products by Prof. Huizhou Liu, a lecture of the synthetic biology of Halophilic bacteria by Prof. Guoqiang Chen, a lecture of design principles to engineer yeasts as microbial factories by Ass. Prof. Zengyi Shao in Iowa State University, and a outstanding overview of the development of synthetic biology from basic research to industrialization in China to list just six.

In this article we will cover some pertinent areas of synthetic biology and biomanufacturing amidst the unavoidable spectra of COVID-19.

1. The development trend of synthetic biological and biomanufacturing

2022 is the key year to implement "the 14th five year plan" of China and the strategic goal of "carbon peak and carbon neutrality". As a hot field of the interdisciplinary of Biochemistry, Engineering and Information Technology, the booming synthetic biotechnology focuses on the "cell factory" approach to solve the green manufacturing of bulk chemicals and high-value chemicals. Especially, after China put forward the vision goal of "carbon peaking and carbon neutralization", the importance of synthetic biomanufacturing in CO_2 capture and high-value transformation has attracted more and more attention of researchers. At present, a series of studies have been carried out in the design of new biosynthetic pathways, the creation of microbial chassis cells, multi enzyme catalysis, new extraction and separation, and green biological manufacturing of antifungal antibiotics and other drugs, active natural products such as Ginkgolides, bio based materials and chemicals such as acetaminophen has been achieved. In order to thoroughly implement the spirit of the Sixth Plenary Session of the 19th CPC Central Committee and promote high-quality economic and social development, under the guidance of Chongqing Science and Technology Bureau and the Professional Committee of Biochemical Engineering of International Engineering Sciences Conference (IESC) in China, the 1st western China symposium on the international frontier of synthetic biomanufacturing was successfully held in Chongqing organized by Chongqing University, People's government of Chongqing Shapingba District, and together with Sichuan University and Three Gorges University, from July 8 to 10, 2022 (Fig. 1). The theme of this session is "synthetic biomanufacturing leads low-carbon economy". The communication forms of the conference include: special invitation report, invitation report, oral report, wall newspaper display, and *etc.*.

The conference shows the development trend of synthetic biomanufacturing, and people's willingness to create a new era of low-carbon economy. The research on the synthesis of natural products through metabolic engineering is the most popular, and literature research also shows that China's output is more than the global output. This shows that the metabolic engineering of synthetic biology in China has a good foundation, especially the synthesis of natural products. This can also be attributed to the research of Chinese traditional medicine and the various animals and plants growing on the vast land, which have given Chinese scientists more natural inspiration. Chinese scholars pay more attention to the application of synthetic biology in the field of chemical engineering, energy and environmental protection, and biomass energy electrical energy conversion than scholars from other countries, which may indicate that China has an urgent need to solve energy and environmental problems. This may also be related to the fact that synthetic biology was born in the Department of Chemical Engineering of American universities worldwide, so Chinese scholars in the field of Biochemical Engineering were the first to come into contact with the historical development of synthetic biology. In comparison, China's synthetic biology research is still relatively weak in methodological innovation, genome information analysis, genome network editing,

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Fig. 1. Group photo of the opening ceremony of the conference.

medical application, etc., and needs to be substantially strengthened.

2. The opening ceremony

In the opening ceremony, first of all, Ying Tian, deputy director of People's government of Chongqing Shapingba District, pointed out that Shapingba district is the main front of Chongqing Science City, which has a solid foundation for the development of science and technology and comprehensive assistance policies for innovation and entrepreneurship. This meeting is of great significance to the establishment and improvement of the enterprise-oriented, market-oriented, industry-university-research combined technology innovation system and the promotion of the innovation and development of Chongqing industry. Afterwards, Zhipeng Xu, deputy director of Chongqing Science and Technology Bureau, pointed out that Chongqing will firmly grasp the strategic opportunities of the new round of global scientific and technological revolution and industrial reform, take the major strategic needs of the country as the lead, and deepen the supply-side structural reform as the main line. Finally, Zhenyi Wu, the secretary of the Professional Committee of Biochemical Engineering of IESC in China, believes that the development of synthetic biomanufacturing is a major national strategy for all countries in the world to enhance their core competitiveness and seize the commanding heights of future development. He hopes that through this summit forum and academic seminar, the participants in the field can broaden their horizons, exchange ideas, learn from each other and encourage each other. To promote the progress of biochemical engineering technology to make more contributions, to help achieve the dual-carbon strategy and pursue green, low-carbon and high-quality development.

3. Major progress in the field of natural product biomanufacturing

Secondary metabolites from plants and microorganisms are natural active substances. However, the traditional production mode of plant extraction faces environmental pollution problems. The wild plants are lack of resources, grow slowly, suffer from diseases and insect pests, and are difficult to be planted artificially. China is a big country in biological resources. Long term research on biological resources, phytochemistry and microbial metabolic engineering laid a good foundation for the production of the natural products from microbial cell factories through synthetic biology technology.

Through the construction of microbial chassis and reconstruction of gene circuit, a variety of natural active substances could be synthesized as reported by the lectures, including terpenes, such as rare ginsenosides (ginsenoside), steviol glycosides (memory disorder drug), lycopene (antioxidant health food), celangulin V (vegetable crop insecticide), *etc.*; Flavonoids, such as pinocembrin (department anesthesia), breviscapine (expansion of cerebral blood tube, anti coagulation), *etc.*; Plant steroids, such as canosterol (cam-pestrol (plant hormone), 21 eoxycor-Tisol (precursor molecule of medicinal glucocorticoid), diosgenin (basic raw material of steroid hormone drugs), *etc.*; antibiosis elements and drugs, such as streptomycin and tunicamycin, aureomycin, nitroglycerin comycin fragment, carbamoyl polyoxalic acid, salvianic acid A, cannabinoids, hydroxytyrosol, and *etc.*. By studying the mechanism of quorum-sensing sensors, as be told in his presentation, Prof. Qipeng Yuan from Beijing University of Chemical Technology created a library of quorum-sensing sensors, realized multi-target genes regulation, designed gene circuits, and realized the biosynthesis of high value-added compounds such as salicylic acid.[1] The multi-metabolite mutually beneficial symbiosis strategy was used to maintain the multi-cell stable symbiosis and the biosynthesis of natural products from silybin isolength pathway was realized, which is the highest yield of *de novo* synthesis at present.[1] Prof. Yongjin Zhou from Dalian Institute of Chemical Physics, Chinese Academy of Science (CAS) presented that engineering yeast cell factories for efficient biosynthesis of natural products. Engineering cofactor supply and recycling with tailored strategies enabled the highest production of caffeic acid (5.5 g/L) and ferulic acid (3.8 g/L) in *S. cerevisiae*.[2] And global metabolic rewiring the cellular metabolism resulted high-level production of diterpenoid sclareol of 11.4 g/L with enhanced supply of precursor acetyl-CoA and cofactor NADPH in *S. cerevisiae*.[2] These observations shows that yeast has high potential for industrial production of natural products. Prof. Zengyi Shao from Iowa State University and affiliated with the National Science

Foundation (NSF) Center for Biorenewable developed a new CRISPR platform, named Lowered Indel Nuclease system Enabling Accurate Repair (LINEAR), which enabled precision editing without NHEJ disruption with efficiencies of 67–100% in four industrially relevant yeasts. Then she used it to rapidly engineer a microbial factory to produce (S)-norcoclaurine, an entrance molecule of the large group of benzylisoquinoline alkaloids with medicinal applications.[3] Prof. Dan Wang utilized a novel decarboxylation-oxidative deamination enzyme PcDHPAAS from *Psilocybe cubensis* to transform L-DOPA to hydroxytyrosol for the first time. She also combined the global regulators and co-culture strategy to achieve a super performance.

4. Major progress in the pharmaceuticals production with synthetic biology

Prof. Yuguo Zheng, academician of Chinese Academy of Engineering (CAE), Dean of School of Bioengineering of Zhejiang University of Technology and president of Zhejiang Bioengineering Society, gave a report on pharmaceuticals production, introduced the significance of biomanufacturing technology in low carbon recovery and green production of medical products, analyzed the status quo and latest progress in China and abroad.[4] In his view, there are five major trends in the development of the global pharmaceutical manufacturing industry: first, the demand for higher production efficiency and greater productivity; second, green technology innovation is making the market more competitive; third, downstream engineering technology is still the bottleneck that restricts sustainable development; fourth, the demand for high-quality products is still growing rapidly; fifth, the market in China and India is maturing. The international market is still growing. Pharmaceutical bio-manufacturing will transform the pharmaceutical production route and process, solve the bottleneck of traditional manufacturing, and promote the high-level sustainable development of the pharmaceutical industry. Prof. Ya-Jun Wang from the same school developed specific directed evolution methods for aldo-keto reductases and carbonyl reductases. And then he used the method to engineer *Km*AKR, *Km*CR, *Ls*CR and constructed robust industrial enzyme preparations, which displayed outstanding performance in the synthesis of *tert*-butyl 6-cyano-(3*R*,5*R*)-dihydroxyhexanoate, *tert*-butyl 6-chloro-(3*R*, 5*S*)-dihydroxyhexanoate, (1*S*)-2-chloro-1-(3,4-difluorophenyl)ethanol, the chiral intermediates of atorvastatin, rosuvastatin, pitavastatin and ticagrelor.[5–7]

5. Major progress in the renewable chemicals production and environmental protection

Professor Tianwei Tan, academician of the Chinese Academy of Engineering and president of Beijing University of Chemical Technology, proposed the idea of "the third generation of biological manufacturing: producing chemicals with carbon dioxide as raw material". He used the third generation of biological manufacturing with CO_2 as raw material, and discussed related routes and key technologies of producing high-value chemicals with CO_2. He believes that future will embrace a new mode of green processes by using biomass, CO_2 and other renewable raw materials to produce energy, materials and chemicals, with industrial biotechnology and chemical engineering technology combined.[8] As an important part of the biotechnology industry, biomanufacturing provides the basic platform for the industrialization of biological products, and also the application of basic scientific innovation such as synthetic biology in the specific process. In the future, the related technologies of green biomanufacturing will permeate into the development of many industries including energy, materials, medicine, food, environmental protection and so on. It will also play a very important role in the transformation and upgrading of our traditional industries.[8] Prof. Dan Wang also does some research on the preparation of chemicals from carbon dioxide, such as succinic acid from carbon dioxide and biomass. Coexpression of phosphoenolpyruvate carboxylase and carbonic anhydrase was conducted to enhance the CO_2 immobilization. Novel pathway of succinic acid synthesis has been designed, and the concentration of succinic acid in her laboratory has achieved up to 145.9 g/L with the development and optimization of succinic acid fermentation process.[9,10] Some people tried to enhance the chemcials production from renewable biomass. Prof. Mingjie Jin's group in Nanjing University of Science and Tchnology developed a novel pretreatment technology as Densifying Lignocellulosic biomass with Chemicals (DLC), which provides a biomass feedstock with high bulk density, high enzymatic digestibility and high fermentability. On this feedstock, a lignocellulosic ethanol titer >80 g/L and a lignocellulosic lipid titer >38 g/L were achieved without washing/detoxifying pretreated biomass. Jin group also constructed metabolic pathways in *Rhodococcus opacus* and converted lignin to muconic acid and gallic acid.[11] Prof. Xiang Zou from Southwest University introduced a polymalic acid fermentation platform in this symposium, who developed a novel L-malic acid (L-MA) downstream process through acid hydrolysis of polymalic acid (PMA).[12] This simply process showed the competitive advantages for the production of the chemicals L-MA. Some metabolic engineering strategies to unitize the different renewable biomass and enhance the cell robustness were compared. The recent progress for the production of succinic acid and fucose-based derivatives through metabolic engineering and process innovation were also introduced. Prof. Dachun Gong from China Three Gorges University obtained a mutant strain M8 of *Saccharomyces cerevisiae* with significantly improved tolerance through mutagenesis selection, which showed 38.2% higher ethanol yield and sugar-alcohol conversion under fermentation conditions containing mixed inhibitors compared to the starting strain. Then, based on this strain the *Ura3* was knockout by CRISPR-Cas9.[13] The strain M8-Δura3/pYES2-XYL1-XYL2-XYL3 was obtained by introducing recombinant plasmid pYES2-XYL1-XYL2-XYL3. 96 h of anaerobic fermentation with xylose as the only carbon source gave an ethanol yield of 0.39 g/g, which was 74.51% of the theoretical value. In Prof. Yaoping Zhang's presentation, he firstly gave an overview about the status of biofuel (mainly bioethanol) production and the challenges for second generation of bioethanol production using lignocellulosic biomass. Then he gave a brief introduction about DOE-Great Lakes Bioenergy Research Center and his research, in where he focusd on the construction of isobutanol-producing *Zymomonas mobilis*, and isobutanol production in rich media and biomass hydrolysates. He also talked about the utilization of the conversion residues after fermenting with *Z. mobilis*. Prof. Weiliang Dong from Nanjing Tech University developed a platform for biological depolymerization and upcycling of waste plastics, which used microorganisms/enzymes to degrade plastics into oligomers or monomers for further conversion into high-value added chemicals (rhamnolipid, terepthalic acid, carbon quantum dots and *etc.*). Through the establishment of plastic wastes biodegradation and upcycling platform, it can provide a new theoretical basis and key technology for the recycling of plastic wastes, and provide economic and green technical support for the development of plastic circular economy.[14]

6. Major progress in the universal methods and strategies

There have already some people studied the general techniques and strategies of synthetic biology in China nowadays. Prof. Guoqiang Chen from Tsinghua University introduced the next generation of industrial biotechnology based on halophilic microorganisms as chassis cells under the title of "Halophilic bacteria Synthetic Biology and the next generation of Industrial Synthetic Biology" Biotechnology. Halophilic microorganism is a kind of high salt tolerant microorganism, including bacteria, archaea, algae and so on. The moderate halophilic bacteria can tolerate 30–150 g/L sodium chloride, which has high research value and application prospect.[15] With the continuous development of molecular manipulation tools for halophilic microorganisms in recent years, the modification of halophilic bacteria has been developed rapidly. Engineered halophiles have been used to synthesize several polyhydroxyl

Fig. 2. Group photo of the closing ceremony of the conference.

fatty acid esters (PHA), as well as for the large-scale production of many polymers, proteins, small molecular compounds, amino acids and cosmetic ingredients.[15] The use of synbiogenic techniques also enables halophilic microorganisms to achieve controlled deformation, which is conducive to the synthesis of more intracellular products and downstream cell separation.[15] Prof. Yifei Zhang from Beijing University of Chemical Technology elaborated on the scale effects of the interplay of reaction and transport in enzymatic catalysis. He first discussed whether the enzymatic reactions could boost the diffusivity of enzymes. He then explained when the substrate channeling-like effect would make a difference in the overall throughput of an enzyme cascade in light of the comparison between the characteristic time scales for diffusion and reaction. At last, he presented a two-enzyme reaction network that would generate spatiotemporal patterns originating from reaction-induced convection. His presentation led to a better understanding of the underlying physicochemical mechanism of some common phenomena in biocatalysis.[16] Prof. Wei Kang from Dalian University of Technology developed a versatile strategy for organizing cascade enzymes on self-assembled protein cages for enhanced biosynthesis. Owning to the enforced proximity between enzymes and improved enzyme kinetics, enzymatic reactions occurring on protein cages exhibited accelerated reaction rate not only in vitro but also inside living cells.[17] As a proof of principle, the authors used engineered strain expressing self-assembled AtoBST-HmgSST-HmgRST-SCMi3 tri-enzyme assemblies to produce lycopene in vivo, which shows an 8.5-fold increase of lycopene production in comparison to the reference strain only expressing freely floating lycopene biosynthetic enzymes.[18,19] This versatile system provides a powerful tool to achieve enzyme spatial organization for broad applications in biocatalysis. Prof. Yinlan Ruan from Guilin University of Electronic Technology has developed the highly sensitive Raman analysers which can be used for online monitoring compositions of the complex fermentation system including energy and nutritional substances, metabolites, and products as well as optical density. Their delicate designs equipping with advanced data analysis tools aim to manage challenging fermentation processes using diversified substrates and have led to a limit of detection down to 1 mg/L for most of process parameters.

7. Process engineering for effective synthetic biomanufacturing

Huizhou Liu, a researcher at the Institute of Process Engineering, Chinese Academy of Sciences, described the process of biotransformation and green separation of natural products with the title of “Biotransformation and green separation of natural products”. As one of the most important frontiers of international science and technology development, synthetic biology will effectively alleviate and solve the bottleneck problems of energy and resource which restrict our economic and social development.[20] In the field of synthetic biology, Qingdao Institute of Bioenergy and Process, Chinese Academy of Sciences has set up the Innovation Center of Synthetic Biotechnology of Shandong Province and the Key Laboratory of Synthetic Biology of Shandong Province, and has made a series of progress in the basic principles and techniques of synthetic biology and the design and synthesis of organisms for industrial applications. The Resource Environment and Green separation team of Qingdao Energy Institute has been focusing on the biotransformation of natural products and the development and application of green separation technology for a long time, and has carried out a series of researches and made relevant progress in the extraction and separation of panax quinsenoside, ginkgo bilobone and emodin. (1) Through the screening of a variety of glycosidases, the biotransformation route of rare ginsenosides CK was realized, and a new green separation system of panax quinsenosides was constructed, laying a foundation for the industrialization of high value utilization of panax quinsenosides.[21] (2) The continuous solid phase extraction technology was used to extract various active products of ginkgo biloba, and the continuous separation of ginkgo biloba a, b and c was realized, which has the prospect of industrial application.[22] (3) A new gas-assisted three-phase extraction technology has been developed to achieve the separation of emodin and rhein.[23] The new technology improves the separation efficiency, reduces the amount of extraction agent, and can separate and enrich low concentration natural products, which

can realize the separation of a variety of natural products. In order to eliminate the inhibition of ethanol on fermentation during bioethanol production, pervaporation membrane bioreactors have been developed with ethanol *in situ* removal by PDMS membrane, as presented in Prof. Shenqing Fan's speech from Sichuan University. Higher ethanol productivity and lower wastewater treatment have been achieved by matching the fermentation unit and membrane separation unit.[24] The energy required for the recovery of permeated steam products can be reduced more than 50% by utilization and management of low-grade heat.[24] Currently, the scale-up design of the whole process and the key equipment of the pilot scale test have been completed, showing a promising future of pervaporation membrane bioreactor for ethanol production.

8. Summary

This successful symposium has gathered the top forces in the field of synthetic biology and reached a consensus on the development of biochemical industry and the implementation of the dual carbon strategy, which will effectively promote the transformation and upgrading of the chemical industry and accelerate the transformation of synthetic biology technology achievements into real industrial application(Fig. 2). Biochemical Engineering technology is becoming an effective means to solve major problems such as resources, environment and human health with its unique advantages. The organizing and scientifific committees as well as the amazing conference secretariat must all be praised for this success. We sincerely hope that we can meet in person next time and look forward to the 2nd Western China Symposium on the international frontier of synthetic biomanufacturing in 2023.

Conflict of interest

The authors declare no competing financial interest.

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