



Research Highlights

Engineered microbes: Making valuable chemicals from waste gases

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Many daily used products such as clothes, cosmetics, disinfectants, and plastics are predominately derived from fossil resources. Their production processes often generate substantial amounts of carbon dioxide (CO₂), a greenhouse gas that is believed to result in global warming. The Paris Agreement's temperature goals require global CO₂ emissions to halve by 2030 and reach net zero (or carbon neutrality) by 2050 [1]. Hence, feasible strategies and robust technologies are needed to capture and utilize CO₂ to achieve the goals. One of the promising solutions is developing biotechnological and sustainable approaches for CO₂ fixation and utilization. Recently, a notable carbon-negative platform for the production of commodity chemicals was developed on the basis of engineered bacteria by eating CO₂ rather than emission during the whole fermentation process, which is reported in March 2022 in *Nature Biotechnology* [2]. The research teams are located in Chicago area, Illinois, which are led by two synthetic biologists Michael C. Jewett at Northwestern University and Michael Köpke at LanzaTech, respectively. Specifically, in this work Liew et al. used an ethanol-producing bacterium, *Clostridium autoethanogenum*, as a microbial cell factory and selected two useful industrial chemicals acetone and isopropanol (IPA), both of which have a combined global market value of >\$10 billion, as their target products for production. The engineered microorganisms were able to grow on the steel mill's waste gas CO₂ in large-scale loop reactors to synthesize acetone and IPA in a sustainable and environmentally-friendly manner.

First, a non-model, autotrophic microorganism *C. autoethanogenum* was chosen as a starting strain, which is a native ethanol producer that has been used by LanzaTech to produce ethanol at industrial plants [3, 4]. However, this strain lacks the metabolic pathways to produce acetone and IPA. To reprogram the bacterium for acetone and IPA production, the authors initially started to search for enzymes, including thiolase (ThlA), CoA transferase (CtfAB), and acetoacetate decarboxylase (Adc), to reconstitute a heterologous pathway for acetone biosynthesis in *C. autoethanogenum*. By mining a collection of 272 acetone-butanol-ethanol (ABE) strains, 30 of acetone biosynthesis enzymes (ThlA: 4; CtfA: 6 and CtfB: 10; and Adc:10) were finally selected to construct the acetone pathway. Afterward, these enzymes and their variants were combined to assess their performance on acetone production. To maximally accumulate acetone, the gene of a native primary-secondary alcohol dehydrogenase (sAdh) known to reduce acetone to IPA was deleted from the genome of *C. autoethanogenum*.

Based on this sAdh knockout strain, a total of 247 strains were generated by transferring various acetone biosynthesis pathway designs from a combined plasmid library. After screening, the best strain produced up to 100 mM of acetone. To enable IPA synthesis, another library of sAdh enzymes was transformed into the acetone-producing strains to convert acetone to IPA. The results suggested that a nearly full conversion rate (>97%, based on 20 g/L of fed acetone) was achieved by using both the wild-type sAdh and two engineered variants (S199A and A199R).

Next, Liew et al. aimed to improve the production strains for enhanced productivity. Since unwanted byproducts such as 2,3-butanediol (2,3-BDO) and 3-hydroxybutyrate (3-HB) were observed during acetone production, the authors then used a combination strategy of metabolic flux analysis, proteomic measurements, kinetic modeling, and cell-free prototyping to optimize the acetone biosynthetic pathway and minimize the side-products. In particular, the so-called "iPROBE (*in vitro* prototyping and rapid optimization of biosynthetic enzymes)" [5] cell-free technology was employed to rapidly evaluate candidate genes for knockout efficacy within several days, which can streamline an often laborious and time-consuming *in vivo* strain engineering process. Moreover, iPROBE was used to identify critical bottlenecks for acetone production, and the results indicated that CtfAB is a rate-limiting step in the biosynthetic pathway. Consequently, CtfAB was overexpressed to enhance the strain's performance to drive metabolic flux to the target product.

Finally, the authors sought to scale up the fermentation process using optimized strains for acetone and IPA production, respectively. A benchtop continuous stirred-tank reactor (2-L scale) was initially used for gas fermentation. This bioprocess showed about 3 weeks of stable steady state production, which could generate ~2.5 g/L/h of acetone and ~3 g/L/h of IPA, respectively, with >80% gas utilization. In addition, expression of an extra copy of CtfAB (the rate-limiting step) could further increase the performance of acetone and IPA strains, achieving up to ~90% product selectivity in the benchtop continuous fermentation. Afterward, both bioprocesses were scaled up from the benchtop reactor to a 120-L pilot plant using a loop bioreactor. While the strains performed similarly in both reactors, Liew et al. pointed out that bioprocess development can be further carried out to reduce the spatial heterogeneity (e.g., gas substrate zones) of the reactor environment with different scales to achieve even higher productivity. Remarkably, life cycle analysis (LCA) confirmed a negative carbon footprint for both

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Available online 13 April 2022

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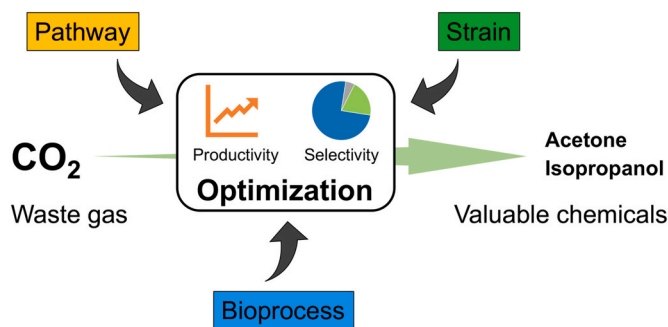


Fig. 1. Development of a carbon-negative production platform through pathway, strain, and bioprocess optimization. Engineered *C. autoethanogenum* strains use the waste gas CO₂ as a substrate for the production of valuable industrial chemicals (acetone and isopropanol) by gas fermentation with high productivity and selectivity.

products. The gas fermentation process consumed 1.78 and 1.17 kg of CO₂ to produce 1 kg of acetone and IPA, respectively. By comparison, traditional petrochemical production of acetone and IPA emits 2.55 and 1.85 kg of CO₂, respectively, per kilogram produced.

In summary, Liew et al. utilizes rational strategies and robust technologies to develop a carbon-negative production platform, including a three-pronged approach of pathway optimization, strain improvement, and bioprocess development (Fig. 1). For example, cell-free biotechnology helps accelerate the process of pathway design and strain optimization, which is particularly important for the hard-to-manipulate bacteria such as the acetogen *C. autoethanogenum* used in this work. In addition to small molecules as shown here, cell-free systems have also been used to produce complex natural products [6], demonstrating that a broad spectrum of valuable products might be manufactured in large scale and high quantity through a combined *in silico*, *in vitro*, and *in vivo*

optimization process [4,5,7]. Looking forward, this study will provide a blueprint for the development of many other high-value chemicals/compounds that can be produced in a similar sustainable, scalable, and carbon-negative way. Just like the authors stated, “carbon from agriculture, industrial and societal waste streams is recycled into a chemical synthesis value chain and displaces manufacture of products from fresh fossil resources”. In this context, a bright future might be coming soon that tiny microorganisms can help solve big climate problems and benefit human society.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant no. 31971348) and the Natural Science Foundation of Shanghai (grant no. 19ZR1477200).

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