SYNTHETIC BIOLOGY

Sustainable manufacturing with synthetic biology

Producing commodity chemicals in bacteria that live on industrial air pollution captures more greenhouse gases than it emits.

Corinne D. Scown and Jay D. Keasling

Synthetic biology promises to lead the way to a sustainable manufacturing sector. If the thousands of chemicals derived from petroleum and natural gas — including fuels, plastics and industrial chemicals — could be produced instead with microbes, the annual savings in global greenhouse gas emissions would be substantial. Most manufacturing processes developed by synthetic biology approaches have not been fully carbon neutral, in part because they rely on sugar feedstocks. An important new study in *Nature Biotechnology* by Liew et al.[1] describes a strategy for carbon-negative manufacturing of chemicals at large scale by harnessing a class of autotrophic bacteria called acetogens. These bacteria can live on one-carbon molecules — including the greenhouse gas CO_2 — and convert them into more-complex, organic molecules. But acetogens have proved extremely difficult to genetically engineer for the synthesis of non-native products. Liew et al. describe methods to overcome previous technical hurdles, achieving carbon-negative production of the non-native chemicals acetone and isopropanol (IPA) at industrially relevant efficiency, selectivity and scale. The study provides a roadmap for broadening the range of molecules that can be manufactured sustainably from waste and biomass feedstocks.

Microbial systems are used in industry to create a diverse array of products, most of which are high-value molecules such as flavors and fragrances, cosmetic additives and pharmaceuticals[2]. For commodity chemicals — simple, inexpensive molecules manufactured at large scale — only a small handful are microbially produced (for example, 1,3-propanediol, 1,4-butanediol, isobutanol, farnesene, lactic acid and succinic acid). These processes typically rely on industrial workhorses like *Escherichia coli* and yeast, and they emit substantial amounts of CO_2 waste in order to generate highly reduced products from oxidized starting materials, namely, sugars.

The cost, land, nutrients and energy resources required to produce sugar feedstocks, combined with the loss of carbon to CO_2 during bioconversion, make it particularly challenging to manufacture commodity products and biofuels in microbes while maintaining a low greenhouse gas footprint. Microbial production of commodity chemicals using commonly available starch and sugar feedstocks tends to offer modest emissions savings over conventional petrochemical processes. The degree to which it is carbon-neutral or carbon-negative depends primarily on the emissions intensity of the feedstocks used[3]. Another viable path to carbon-neutral or carbon-negative processes is to capture and then use or sequester CO_2-rich waste gases from fermentation[4]. Autotrophic bacteria can avoid the downsides of sugar feedstocks entirely by growing on simple carbon sources such as CO_2, provided that enough CO or H_2 is present to supply the energy the microbes need to grow.

Autotrophic bacteria live on the thermodynamic edge of life, capable of surviving before Earth's atmosphere contained oxygen by conserving energy and producing biomass using only simple one-carbon feedstocks — in contrast to heterotrophic bacteria, which exploit more energy-rich organic molecules. Acetogens rely on the most ancient carbon fixation pathway, the Wood–Ljungdahl pathway, to convert two molecules of CO_2 to acetate[5]. In earlier work, authors of the Liew et al. study improved production of ethanol, a native product of the acetogen *Clostridium autoethanogenum*, by optimizing the ratio of CO_2, CO and H_2, reaching an ethanol titer of ~9.7 g L^{-1} (ref. [6]). But efforts to engineer acetogens to produce non-native products at industrially relevant titers, rates and yields have not been successful. In the case of acetone, previously reported performance was orders of magnitude below what would be needed for commercialization (for example, 26 mg L^{-1} h^{-1} in *Acetobacterium woodii*[7]).

Liew et al. set out to engineer *C. autoethanogenum* for biosynthesis of the non-native molecules acetone and IPA. Acetone is a commodity chemical used as an industrial solvent and as a precursor to acrylic glass and bisphenol A, among other uses. IPA is widely used in pharmaceuticals, cosmetics and personal care products, and as a solvent and cleaning agent. Both acetone and IPA are manufactured today from petroleum and natural gas using energy-intensive cracking and reforming processes, with emissions of 2.55 kg of CO_2-equivalent in 100-year global warming potential per kilogram of product) (kg CO_{2e} kg^{-1}) and 1.85 kg CO_{2e} kg^{-1}, respectively[8,9]. In previous benchtop studies with autotrophs, production rates have been on the order of milligrams per liter per hour. These rates would have to increase by a factor of a thousand while also increasing in selectivity to establish a microbial process that could be commercially competitive.

As in their previous work on ethanol[10], Liew et al. used *C. autoethanogenum* grown on steel mill waste gas containing approximately 50% CO by mass, 20–30% CO_2, 10% H_2 and 10–20% N_2. Starting in a 2-liter continuous stirred-tank reactor, they document the step-by-step process by which they achieved continuous production of acetone and IPA at a rate of ~3 g L^{-1} h^{-1} with up to 90% selectivity, ultimately achieving 12 days of steady-state production, and subsequently scaling up to industrial pilot scale in a 120-liter loop reactor (Fig. 1). This would be impressive for an easily engineered organism but is even more impressive for an organism that is as difficult to engineer as a *Clostridium*.

Prototyping biosynthetic pathways can be extremely time consuming. It is particularly challenging with clostridia, which grow slowly, are hard to transform, and offer fewer genetic tools than *E. coli* and yeast. To identify enzymes that would enable high-rate production of acetone and IPA with minimal side-products, the authors used a combination of metabolic flux analysis, kinetic models of metabolism, proteomics and iPROBE (in vitro prototyping and rapid optimization of biosynthetic enzymes)[11]. The iPROBE approach proved particularly useful in

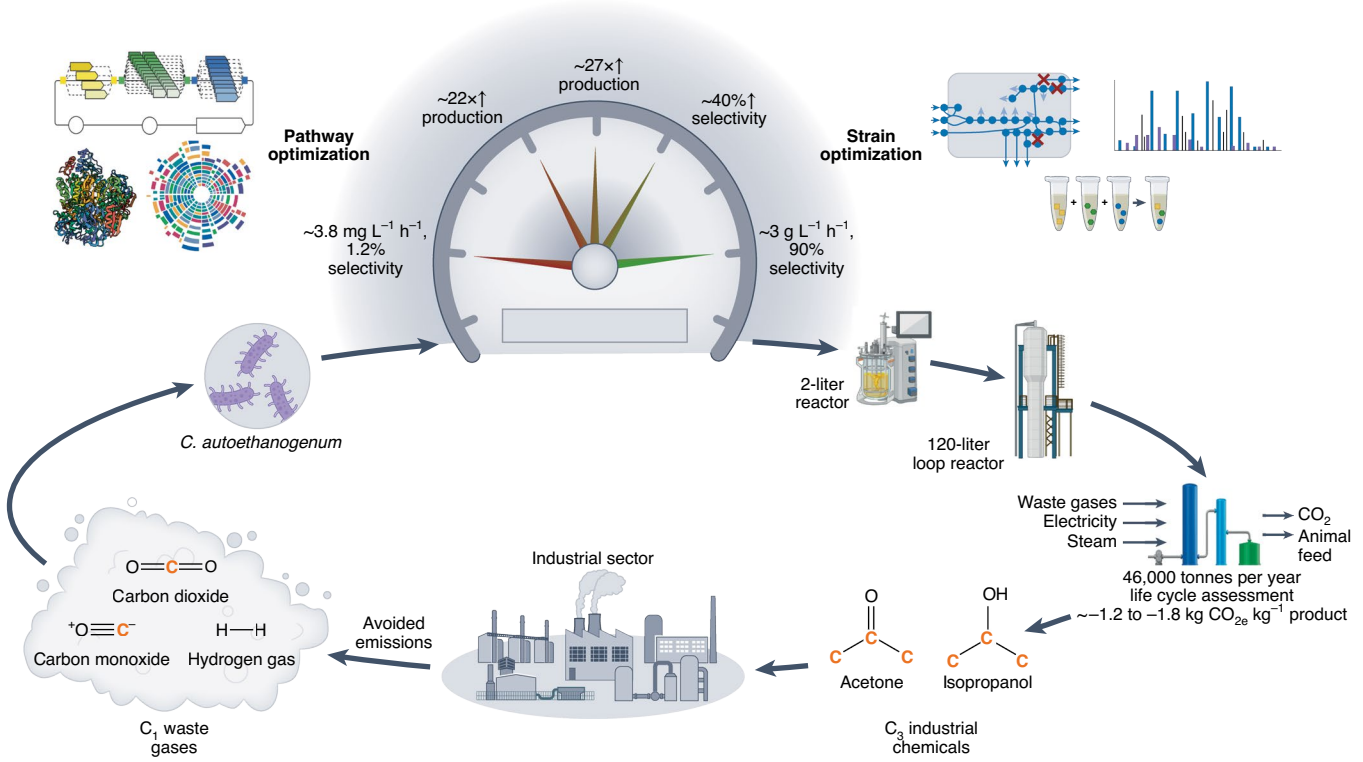

Fig. 1 | Synthetic biology promotes a circular carbon economy. Liew et al. outline methods for engineering the acetogen *Clostridium autoethanogenum* to convert industrial waste gases into useful industrial chemicals at commercially relevant efficiency, selectivity and scale.

screening candidate enzymes that produce unwanted 3-hydroxybutyrate, reducing the number of candidates from 13 to 3 and saving months of work to knock out all the genes. The identification of these genes was enabled by in vitro reconstitution of acetone production from glucose using native *E. coli* catabolism. iPROBE was also valuable in sorting through candidate enzymes to find those that would most efficiently produce acetone and IPA, saving effort to test all the enzymes in vivo.

As Liew et al. point out, mass transfer limitations make scale-up of gas fermentation challenging. To do this, they leveraged their experience with ethanol[12]. In the pilot-scale reactor, the steel mill waste gas must be injected into the fermentation broth and then cycled through the tank, meaning that some zones close to the injection site have very high gas concentrations while others have very little. The microbes themselves also move through the reactor differently than the broth or gas does. While productivity did decrease to <2 g L^{-1} h^{-1} at pilot scale, selectivity remained as high as or even higher than seen at lab scale. Achieving productivity near 3 g L^{-1} h^{-1} and higher at pilot scale will require further process optimization and advances in reactor design. More runs will also be important to ensure genetic stability of this *C. autoethanogenum* strain in continuous large-scale production, which may be more challenging than with the ethanol-producing process[12].

On the basis of the final optimized performance, Liew et al. calculated net negative carbon footprints totaling −1.17 kg CO_{2e} kg^{-1} for IPA and −1.78 kg CO_{2e} kg^{-1} for acetone. They used what is called a cradle-to-gate life-cycle assessment method for quantifying greenhouse gas emissions, which means that emissions are tracked from raw materials through final chemical manufacturing, stopping at the production facility gate. The net carbon emissions are negative, even after factoring in the electricity and heat inputs needed to run the reactor, because the process captures carbon in steel mill waste gases that would otherwise be flared and emitted to the atmosphere as CO_2.

A minor caveat is that common practices in steel mills for using and treating waste gases vary. In Western Europe and the United States, for example, the majority of steel mill waste gases are combusted to generate heat or electricity at the facility, and diverting these gases would require the use of supplemental natural gas or another fuel[13]. The assumptions made by Liew et al. align best with facilities in China, where a large fraction of waste gases from steel mills is flared[13]. Additionally, the degree to which converting waste gases to chemicals can serve as a carbon removal strategy depends on the fate of chemicals at their end of life (disposal). A so-called cradle-to-grave life-cycle assessment would incorporate the fate of products after they are used. Chemicals that end up in plastics or other stable products will likely be sequestered in landfills, thus storing their carbon underground for the foreseeable future. Conversely, industrial solvents are more likely to be burned or evaporated when they are no longer useful, meaning that their carbon ultimately returns to the atmosphere as CO_2.

Steel mill waste gases are not the only underutilized fossil carbon source that acetogens can sequester into products. Approximately 10 gigatonnes of carbon are emitted in the form of waste gases globally each year, about two-thirds of which comes from large stationary sources such as power plants and manufacturing facilities[14]. Post-combustion streams contain mostly CO_2 and N_2, which provide no useful energy on their own. However, post-combustion CO_2 can serve as a carbon source for acetogens if electron donors —

namely, H_2 and CO — are added to supply the energy the microbes need. There are also waste gas resources produced by petroleum refineries and other industrial facilities that already contain varying quantities of CO, H_2, CH_4 and other light hydrocarbons with energetic value. These gases, which are usually flared or emitted directly, could be recovered and used by acetogens with limited or no preprocessing.

As Liew et al. note, potential feedstocks for acetogens extend beyond fossil carbon sources. Gasification of forest and agricultural residues also produces a mixture of CO, H_2 and CO_2 that could be directly used by *C. autoethanogenum*. But biogas and landfill gas are primarily composed of CH_4 and CO_2, so an extra steam reforming step would be needed to convert the CH_4 to CO, H_2 and CO_2. These gases can also contain contaminants, such as siloxanes, H_2S and NH_3, that shorten catalyst and equipment lifetimes. Such a two-step process would need to compete with alternatives in which raw biogas is converted to products by methanotrophs or is purified to produce biomethane as a natural gas replacement. Another option is to convert CO_2-rich streams like those produced from ammonia production, cement manufacturing or ethanol biorefineries. As with post-combustion CO_2 feedstocks, an external H_2 source would be required. Renewable H_2 could be produced via electrolysis paired with wind or solar electricity to supply the necessary energy to the microbial host, although the authors' previous work on ethanol in *C. autoethanogenum* suggests that the presence of CO may play a role in maximizing host performance[6].

Aside from the flexibility in one-carbon feedstocks, an intriguing advantage highlighted by Liew et al. is the potential for flexibility in the final product. Using the same gaseous feedstock, plant operators could theoretically shift between producing ethanol, acetone or IPA by switching to a *C. autoethanogenum* strain that has been optimized for the product of choice. Product switching would require some changes in methods for downstream product recovery because of the chemicals' varying properties and their tendency to form azeotropes with water, but the benefits of flexibility may outweigh any additional capital costs if market prices fluctuate substantially.

Replicating the successes documented in this study for other products, and even for other non-model hosts, would further expand its potential impact. One can imagine adapting the system to produce a diverse array of chemicals directly in *Clostridium*. Given the limited energy derived from oxidation of H_2 or CO to reduce CO_2, those products will need to be chosen carefully[15]. The range of potential products can also be broadened through chemical upgrading methods. The molecules produced by engineered *Clostridium* can be catalytically upgraded to energy-dense molecules like diesel and jet fuels, as is being done by LanzaJet, a spinout of LanzaTech, where many authors of the Liew et al. study are based. Catalytic upgrading can also turn ethanol into polyesters for plastics and fabrics, and acetone and IPA into products such as plexiglass and polypropylene.

Looking beyond acetogens, the contribution of synthetic biology to creating a sustainable manufacturing sector would be greatly enhanced though diversification of the organic feedstocks that microbial hosts can convert. For example, utilization of pentose sugars derived from biomass remains a challenge in widely used hosts, and lignin-derived aromatics are also difficult to use profitably[16]. Faster design–build–test–learn cycles are key to achieving this goal[17]. The combination of pathway optimization and strain optimization described by Liew et al. provides a detailed roadmap for accomplishing this. The iPROBE approach in particular can save months of work by screening enzymes in vitro rather than testing them in vivo.

If all non-fuel petrochemicals were produced instead through synthetic biology, what would the emissions savings be? Chemical manufacturing, excluding ammonia production, directly emits about half a gigatonne of CO_2 globally each year[18]. Although this amounts to less than 2% of global greenhouse gas emissions, chemical manufacturing is the single largest industrial consumer of oil and gas, and the third-highest CO_2-emitting industrial sector, behind cement production and iron and steel mills[18]. These sectors are notoriously difficult to decarbonize because they rely on high-heat processes that cannot currently be electrified, and they also emit fossil CO_2 as part of the fundamental stoichiometry of the process. Creating a circular or even net-negative industrial sector through novel synthetic biology approaches will not solve the climate crisis on its own, but it can tackle some of the most difficult-to-decarbonize parts of the global economy. The progress described by Liew et al. offers hope that acetogens and other non-model organisms will have a meaningful role in a more sustainable future. ❐

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References

1. Liew, F. E. et al. *Nat. Biotechnol.* https://doi.org/s41587-021-01195-w (2022).
2. Jullesson, D., David, F., Pfleger, B. & Nielsen, J. *Biotechnol. Adv.* **33**, 1395–1402 (2015).
3. Hermann, B. G., Blok, K. & Patel, M. K. *Environ. Sci. Technol.* **41**, 7915–7921 (2007).
4. Yang, M., Baral, N. R., Anastasopoulou, A., Breunig, H. M. & Scown, C. D. *Environ. Sci. Technol.* **54**, 12810–12819 (2020).
5. Schuchmann, K. & Müller, V. *Nat. Rev. Microbiol.* **12**, 809–821 (2014).
6. Heffernan, J. K. et al. *Front. Bioeng. Biotechnol.* **8**, 204 (2020).
7. Hoffmeister, S. et al. *Metab. Eng.* **36**, 37–47 (2016).
8. Wu, M., Wang, M., Liu, J. & Huo, H. *Biotechnol. Prog.* **24**, 1204–1214 (2008).
9. Wernet, G. et al. *Int. J. Life Cycle Assess.* **21**, 1218–1230 (2016).
10. Marcellin, E. et al. *Green Chem.* **18**, 3020–3028 (2016).
11. Karim, A. S. et al. *Nat. Chem. Biol.* **16**, 912–919 (2020).
12. Takors, R. et al. *Microb. Biotechnol.* **11**, 606–625 (2018).
13. Collis, J., Strunge, T., Steubing, B., Zimmermann, A. & Schomäcker, R. *Front. Energy Res.* **9**, 642162 (2021).
14. National Academies of Sciences, Engineering, and Medicine. *Gaseous Carbon Waste Streams Utilization: Status and Research Needs* https://doi.org/10.17226/25232 (National Academies Press, 2019).
15. Katsyv, A. & Müller, V. *Front. Bioeng. Biotechnol.* **8**, 621166 (2020).
16. Baral, N. R. et al. *ACS Sustain. Chem.& Eng.* **7**, 9062–9079 (2019).
17. Carbonell, P. et al. *Commun. Biol.* **1**, 66 (2018).
18. IEA. *Chemicals* (International Energy Agency, 2021).

Competing interests

The authors declare no competing interests.

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