Microbe-made jet fuel

The aviation industry readies to embrace aviation fuel produced by fermentation.

By Emily Waltz

Over the next few months, the world's first commercial-scale ethanol-to-jet fuel plant will begin producing its first gallons of aviation fuel. The plant, located in Soperton, Georgia and developed by LanzaTech and its spin-out LanzaJet, represents a major milestone in their nearly 20-year effort to turn waste industrial gases into ethanol, and then to jet fuel.

LanzaTech's aim is to use gas fermentation, in which microbes metabolize waste industrial gases as feedstock and convert them into ethanol and other useful chemical compounds. For now, the company's aviation-focused spin-out, LanzaJet, produces ethanol-to-jet fuel using catalytic steps adopted from the petrochemical industry, but their ultimate goal is to leave much of the catalytic work to microbes through gas fermentation.

Organisms that ferment gas can use industrial carbon dioxide emissions as a feedstock. Such gas fermentation is not nearly as well developed as traditional fermentation, in which microbes are fed sugars or other biomass – including crops. But both – whether biomass or gas-based – are amenable to genetically manipulating selected microbial strains for their metabolism and then growing them for fermentation in large tanks.

Carbon dioxide-based technology may have a long road ahead of it, but the opportunity to use this and other waste emissions as feedstocks makes it a technology worth pursuing. "A bioeconomy without gas fermentation is an incomplete bioeconomy," says Fiona Mischel, director of international outreach at SynBioBeta, an industry organization and network for biological engineers. "Carbon is an amazing resource. Our whole world is built on it. But right now, it's in the wrong form in the wrong spot – it's atmospheric carbon – and we need to be utilizing it through biotech," she says.

LanzaTech is one of several companies globally exploring the use of gas fermentation to make specialty chemicals, fuel precursors and proteins (Table 1). Visolis, for example, is developing different organisms that can utilize gas, biomass or municipal solid waste to produce mevalonic acid as an intermediate, which can be processed into rocket fuel, aviation fuel, synthetic rubber and specialty chemicals through further chemical catalysis.

In gas fermentation, carbon monoxide, carbon dioxide or gasified biomass are

Technologies that capture carbon-rich greenhouse gases from industrial emissions and use them as feedstock have been embraced by the aviation industry.

CREDIT: SCHARFSINN / ALAMY STOCK PHOTO

News in brief

Tome launches CRISPR tool for oversized DNA

Tome Biosciences has debuted with $213 million to bring their genome-editing platform to the clinic. The technology can insert large DNA sequences into the genome in any position to correct genes in vivo. The tool, dubbed PASTE – programmable addition via site-specific targeting elements – was developed by Tome co-founders Omar Abudayyeh and Jonathan Gootenberg from the Massachusetts Institute of Technology. Investors include Andreessen Horowitz, Arch Venture Partners, Polaris and Fujifilm.

PASTE uses a CRISPR–Cas9 nickase fused with two other enzymes: a reverse transcriptase and a serine integrase. The guide RNA-directed reverse transcriptase creates landing sites for the serine integrase, which inserts large sequences of DNA. Tome has shown that its tool can insert sequences of ~36 kb in various dividing and non-dividing cell types. And, unlike other editing tools, it doesn't rely on repair responses to the double-strand breaks that can result in insertions, deletions and off-target effects.

Tome initially plans to develop this technology as gene therapies for monogenic liver diseases and cell therapies for autoimmune diseases. In gene therapies, PASTE could insert a whole wild-type gene without the need to be tailored to specific mutations, while in cell therapies, engineered genes might be added at precise loci. Shortly after debuting, Tome acquired startup Replace Therapeutics for $65 million, adding another genomic integration tool to its repertoire. Replace's technology is like PASTE, except the integrase is replaced by a DNA ligase that inserts short DNA sequences.

Table 1 | Selected companies capturing or converting waste gas for fuel and chemical production using biotechnologies such as gas fermentation and enzymes

Company (location)	Gas feedstock	Capture or conversion technology	Products
LanzaTech (Chicago)	Carbon monoxide, carbon dioxide	Fermentation of *Clostridium autoethanogenum*	Ethanol for use in jet fuel, textiles, shoe soles, packaging, cleaning products, surfactants and detergents
Visolis (Hayward, California)	Gas, biomass and municipal solid waste	Fermentation of undisclosed microorganisms followed by chemical catalysis	Mevalonic acid as an intermediate for production of rocket fuel, aviation fuel, synthetic rubber and specialty chemicals
Krajete (Pasching, Austria)	Carbon dioxide	Fermentation of undisclosed archaea	Methane for use as natural gas
Electrochaea (Planegg, Germany)	Carbon dioxide	Fermentation of the archaeon *Methanothermobacter thermautotrophicus*	Methane for use as natural gas
Industrial Microbes (Alameda, California)	Methane	Fermentation of engineered *E. coli* to express an enzyme that catalyzes the direct oxidation of methane to methanol at standard temperature and pressure; pyrolysis and distillation are used to convert methanol to desired products such as acrylic acid, methacrylate and acrylonitrile	Acrylic acid, methacrylate and acrylonitrile
Phase Biolabs (Nottingham, UK)	Carbon dioxide	Fermentation of undisclosed microorganisms	Ethanol
Mango Materials (Redwood City, California)	Methane	Fermentation of undisclosed methanotrophs	PHAs for use in biodegradable fibers and goods
Newlight Technologies (Huntington Beach, California)	Carbon dioxide or methane	Fermentation of undisclosed, non-GMO microorganisms	Biodegradable polymer PHB
Circe (Boston)	Carbon dioxide	Fermentation of undisclosed engineered microorganisms	Biodegradable fatty acid polymers such as PHAs
Ucaneo (Berlin)	Carbon dioxide	Carbon capture using carbonic anhydrase enzymes	Captured carbon
Novonesis (Bagsværd, Denmark) and Saipem (Milan)	Carbon dioxide	Carbon capture using carbonic anhydrase enzymes	Captured carbon

PHA, polyhydroxyalkanoate; PHB, polyhydroxybutyrate. Source: SynBioBeta/Fiona Mischel

pumped into fermenters. Some organisms may require other gases for growth or metabolism, such as hydrogen as an energy source. The gases are mixed into a liquid containing a selected or engineered microbe that will metabolize the gases and produce the molecule of choice.

Some products require two-stage fermentation, in which an intermediate chemical such as acetic acid is produced through a round of gas fermentation. The acetic acid then becomes a feedstock for a round of traditional fermentation, where more complex molecules are produced.

The microbes scientists have focused on for gas fermentation include anaerobic acetogens such as *Clostridium* species, which natively use the reductive acetyl-coenzyme A pathway to synthesize acetate, and a hydrogen-oxidizing knallgas bacterium such as *Cupriavidus necator*, which can produce complex molecules for bioplastics and oils.

But developing microorganisms for gas fermentation has been slow to take off, in part because the microbes are not nearly as well characterized as the workhorses of traditional fermentation, such as yeast and *Escherichia coli*, says Hendrik Waegeman, head of business operations at Bio Base Europe in Ghent, Belgium, which serves as a pilot plant and accelerator for companies developing bio-based products. “It takes seconds to engineer *E. coli* or yeast, but with acetogens, it take years to get simple mutations that improve functionality,” he says.

Since acetogens are strictly anaerobic, working with them in the lab can be tricky. “Even a small spoor of oxygen can kill them, which is not very convenient if you want to do manipulations with engineering,” says Waegeman. He estimates that there are fewer than 30 groups worldwide that have attempted to engineer *Clostridium* species for gas fermentation purposes.

Gas fermentation can come with some expensive or energy-intensive technical challenges. The microbes, to do their jobs, need an energy source such as hydrogen pumped into their fermentation tanks. But conventional hydrogen production is carbon intensive, so some groups are creating renewable hydrogen on site by splitting water into oxygen and hydrogen using electrolysis — an expensive endeavor. Sufficiently mixing gas with microbes, which live in water, has also proved expensive and requires sophisticated engineering.

Adding to the challenges, microbe development and engineering has been laborious. LanzaTech put years into characterizing and developing *Clostridium autoethanogenum* for use in its gas fermentation system. This anaerobic bacterium can take in carbon monoxide and, through its native metabolism, produce acetate. Using directed evolution, LanzaTech steered the organism’s metabolism to convert carbon monoxide into ethanol.

LanzaTech now uses this species at six commercial-scale gas fermentation plants. Five of them — four in China and one in Belgium — are located at steel mills or ferroalloy production plants; they convert waste carbon monoxide from those facilities into ethanol. The sixth plant, in Panipat, India, draws carbon dioxide from an oil refinery and converts it to ethanol.

Most of the ethanol that LanzaTech produces from these plants goes into products that would otherwise use virgin fossil carbon, says John Holladay, vice president of government programs at LanzaTech. These products include textiles, shoe soles, packaging, cleaning products, surfactants and detergents. But LanzaTech is also looking to convert the ethanol it produces into jet fuel. “We’re focused on industries that are hard to decarbonize, and aviation is very hard to decarbonize,” says Holladay.

Aviation accounts for about 2% of global energy-related carbon dioxide emissions, according to the International Energy Agency, and it has proven difficult to reduce that figure. Battery- and hydrogen-powered aircraft have both been proposed as solutions, but both come with major technical limitations and would require the expensive endeavors of redesigning aircraft and retrofitting airports.

For those reasons, trade groups such as the International Air Transport Association have pushed for sustainable aviation fuel as the most viable option. The International Civil Aviation Organization, a United Nations agency, defines sustainable aviation fuel as renewable or waste-derived aviation fuel that meets sustainability criteria.

Airlines seem to be open to the idea. Delta, Air France, International Airlines Group and Oneworld Alliance have all agreed to a target of 10% sustainable aviation fuel by 2030. In November, Virgin Atlantic and Gulfstream Aerospace separately flew test flights across the Atlantic using 100% sustainable aviation fuel.

Governments are mandating and incentivizing use of sustainable aviation fuel. Europe in October finalized the adoption of the ReFuelEU aviation regulation program, which requires fuel suppliers to blend increasing amounts of sustainable aviation fuel with conventional jet fuel, starting with a 2% minimum blend in 2025 and rising to 70% in 2050. The United States offers tax incentives for renewable fuels and has since 2005 maintained a renewable fuel standard program that requires that a certain volume of renewable transportation fuel replace petroleum-based fuel.

Most sustainable aviation fuel currently on the market is made from cooking oil, vegetable oil, edible animal fats and industrial grease — a product called hydroprocessed esters and fatty acids. But the supply of these fats and oils is limited and fragmented. As a result, trade groups have urged sustainable fuel developers to look more broadly into diverse feedstocks, including biomass and municipal waste.

Converting such feedstocks to jet fuel is likely to require combining different technologies. One option is to pair traditional fermentation, to convert crops or other biomass to an alcohol such as isobutanol or ethanol, with a series of chemical reactions called alcohol-to-jet to convert the alcohol to jet fuel. Another route is to gasify biomass into syngas, which is a mixture of hydrogen and carbon monoxide, and then convert it to jet fuel in the well-established Fischer–Tropsch chemical process.

But these routes require large quantities of biomass and come with land use concerns, particularly when crops are the starting material. This has led some groups to turn to waste gas such as carbon dioxide or carbon monoxide as a feedstock. Gas fermentation is a newer route to converting the gas; thermochemical and electrochemical processes are more established.

It is also possible to capture carbon with enzymes. Novonesis (previously Novozymes) in Bagsværd, Denmark, and its partner Saipem in Milan are developing carbonic anhydrase enzymes that capture carbon dioxide and convert it to bicarbonate. The genes encoding the enzyme came from a thermophilic, deep sea organism. Novozymes transferred the genes to a proprietary host organism that produces the enzyme at large scale.

Saipem built a demonstration plant that captures 30 tons of carbon dioxide per day. Enzymatic carbon capture is more efficient than traditional, solvent-based carbon capture because it requires less energy to operate and less purification of flue gases, says Klaus Skaalum Lassen, Novozymes’ head of carbon capture and storage.

Microbial gas fermentation comes with several advantages over chemical approaches to gas conversion, says Heleen De Wever, biotechnology project manager at VITO in Mol, Belgium. For instance, microbes are better at handling certain impurities and fluctuation in gas composition, she says. And microbes can produce more complex molecules than chemical catalytic approaches, with the production occurring at a higher specificity for the target molecule.

“If you want to make simpler, one-carbon chemicals, like formic acid or methanol, there is no need to go with gas fermentation. There are good chemical catalysts for that,” says De Wever. “But if you want to make more complex molecules with five carbons or more, or even polymers like polyesters, that is exactly what biotechnology is capable of,” she says.

LanzaTech is pressing on with decarbonizing jet fuel, primarily through its partnership with LanzaJet, which converts ethanol

---

News in brief

Merck enlists trispecific killers

Merck will pay $680 million to acquire Harpoon Therapeutics, a clinical-stage immuno-oncology company developing a new class of off-the-shelf T cell engager therapies for solid tumors. The acquisition gives the big pharma access to Harpoon’s portfolio of T cell engager agents, and to the biotech’s platforms to manufacture trispecific T cell engager therapies. T cell engagers are engineered proteins with two or more binding domains designed to physically redirect T cells to attack and lyse tumor cells. In the case of bispecific T cell engagers, one arm targets an antigen on a cancer cell and another acts as a trigger molecule on T cells, targeting CD3. The approved T cell engagers, such as Blincyto (blinatumomab), Columvi (glofitamab), Lunsumio (mosunetuzumab) and Talvey (talquetamab), are all bispecific. Harpoon’s so-called trispecific T-cell-activating construct (TriTACs) are designed with three binding domains, with the third added to bind human serum albumin to improve half-life. TriTACs, at around 50 kDa, are smaller than monoclonal antibodies, which may boost tumor penetration. Some TriTACs are engineered as prodrugs; they only become activated once they reach the tumor. The company’s lead molecule is HPN328, which targets delta-like ligand 3 (DLL3), an inhibitory Notch ligand highly expressed in cancers such as small cell lung cancer (SCLC). Preliminary clinical data showed that the trispecific molecule shrank tumors in 48% of patients with SCLC and 54% of patients with other tumor types. DLL3 is also the target of Amgen’s bispecific T cell engager tarlatamab, which is in a phase 3 trial for SCLC. Merck also gets clinical-stage HPN217, which targets B cell maturation antigen, and preclinical HPN601, which targets epithelial cell adhesion molecule. The pharma’s deal with Harpoon builds on a 2020 partnership with Janux Therapeutics that gave Merck access to another T cell engager platform, dubbed TRACTr, to identify tumor-activated T cell engagers.

to jet fuel using a chemical catalyst. The new ethanol-to-jet fuel facility in Georgia, built by LanzaJet, will be the largest alcohol-to-jet plant in the world, with a capacity of 10 million gallons of sustainable aviation fuel per year. Production at this plant alone will account for two-thirds of sustainable aviation fuel in the United States, the company estimates.

But here's the rub: LanzaJet's plant doesn't source its ethanol from LanzaTech's gas fermentation process. For now, LanzaJet's ethanol comes from Brazilian sugar cane. That's because ethanol derived from the waste gas of steel or ferroalloy plants is not eligible for incentives under the US Renewable Fuel Standard program. "Policy is lagging innovation," says Holladay. So LanzaJet is buying its ethanol from a source that qualifies.

While it awaits policy change, LanzaTech is pursuing a flurry of projects that source different kinds of feedstock from non-metal alloy industrial sites. These include syngas, derived from reforming biogas or by gasifying forestry or agricultural residues, and municipal solid waste, which the company will convert to ethanol using gas fermentation and then to jet fuel using LanzaJet's catalytic alcohol-to-jet process. The hope is that these feedstocks will qualify the company's sustainable aviation fuel for incentives, but the projects are still in early stages.

Such is the life of a biotech company on the front wave of a new technology.

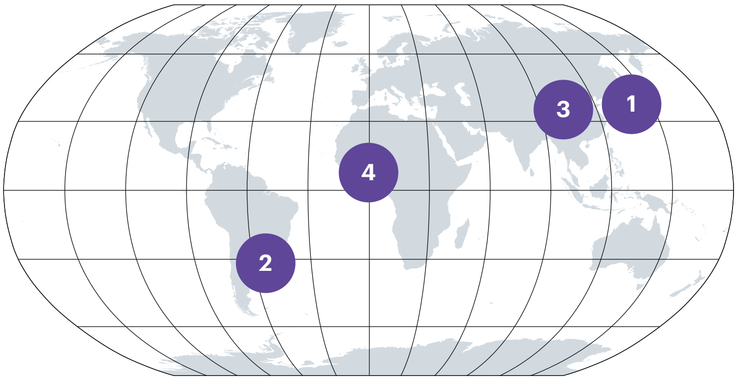
Emily Waltz
Nashville, TN, USA

Biotech news from around the world

1. JAPAN
Japanese banks increase funding for late-stage startups to address a lack of capital that has inhibited growth and contributed to Japan's lack of unicorns. Sumitomo Mitsui Trust Bank will invest $350 million between 2023 and 2025 in pre-IPO startups, and Mizuho Financial Group launches a $69 million fund to assess the earning power of startups using AI to cut the time needed for investment decisions from a month to a week.

2. URUGUAY
The Uruguay Innovation Hub launches a biotech company-building program to support and promote startup creation. The program's aim is to help Uruguay attract international investment, increase the economic growth rate, and generate high-quality jobs for the country's residents.

3. CHINA
China's National Medical Products Administration approves Eisai and Biogen's Leqembi (lecanemab), the first drug shown to slow progression of Alzheimer's disease for people in the earlier stages of the disease. It is the third country to approve the treatment, after the United States and Japan.



4. GHANA
Gavi, the vaccine alliance, establishes the African Vaccine Manufacturing Accelerator, a finance mechanism that will make up to $1 billion available to support sustainable vaccine manufacturing in Africa. AVMA will support at least four African manufacturers to produce 800 million doses over the next 10 years, an initiative aimed at correcting inequities exposed by the COVID-19 pandemic.