

Engineering direct conversion of CO₂ to biofuel

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Genetically engineered cyanobacteria harvest light energy to directly produce isobutyraldehyde and isobutanol.

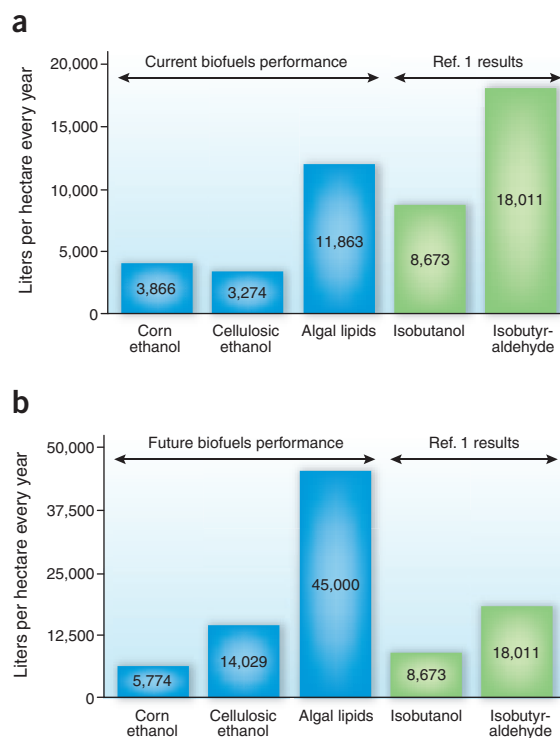
In the wake of recent surges in the price of petroleum and growing concern about climate change, interest in new ways of producing biofuels is rapidly increasing. In this issue, Atsumi *et al.*¹ engineer a photosynthetic bacterium to use sunlight to capture the greenhouse gas CO₂ and turn it directly into a biofuel, isobutanol. Isobutanol, a four-carbon alcohol, is especially promising as a biofuel because it would fit nicely into today's vehicle and fuel-delivery infrastructure.

Recent biofuel research is turning to approaches for replacing petroleum that go beyond ethanol made from corn or sugarcane and beyond biodiesel made from animal fats and vegetable oils. Much effort has been devoted to commercializing cellulosic biofuels made from energy crops, such as fast-growing hybrid poplar and switchgrass, and from wastes, such as corn stover, wheat straw and municipal solid waste. Trees and grasses are far more abundant than waste materials, but cultivating them for biofuels may compete with agriculture intended to supply food, feed and fiber to an expanding world population with rising incomes and appetites. This competition for land has taken on real significance as policy makers try to promote sustainable biofuels that meet the twin objectives of reducing petroleum dependence and reducing greenhouse gas emissions^{2,3}. Of particular concern are recent studies that point to possible unintended release of large amounts of CO₂ when forests and grasslands are cleared^{4,5}.

Enter algae and their photosynthetic cousins in the bacterial world. These waterborne organisms often yield biomass faster than their terrestrial relatives. In addition, because many algae and bacteria thrive in highly

saline water, they can be grown in areas that cannot support agriculture. Thus, they have been proposed as a way of resolving the potential conflict between using land for biofuel or for food. In 1996, the US Department of Energy ended over two decades of support for research on algal biofuels to focus on cellulosic ethanol. The algal program sought to develop high-oil-content algae that grow at very fast rates. In our report summarizing this work⁶, we concluded that algae might be a viable source of biofuels if—among other things—the biomass productivity of these systems could be raised from the achieved levels of 15 g per square meter per day of algae containing 10–20% lipids to 50 g per

square meter per day of algae containing 50% lipids. Research on algal biofuels since then has continued to focus on producing natural oils (lipids) for conversion to some form of renewable diesel fuel. A disadvantage of using biopolymers, such as triglycerides from algae or cellulosic biomass from higher plants, as feedstocks for biofuel production is that considerable energy is expended both in their synthesis and their destruction. Direct conversion of CO₂ to biofuel by photosynthesis would avoid the unnecessary expenditure of energy to create and destroy biopolymers normally used for cell structure or energy storage. Building on earlier work



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in which they engineered the heterotrophic bacterium *Escherichia coli* to produce isobutanol, Atsumi *et al.*¹ have now generated a cyanobacterium that produces isobutanol and isobutyraldehyde at very high rates.

Compared with ethanol, isobutanol has a higher energy content, is more amenable to use in internal combustion engines, is easier to purify from aqueous fermentation broths and is less corrosive to pipelines used to transport fuel. Isobutyraldehyde can be converted to isobutanol and other chemicals, and Atsumi *et al.*¹ suggest that it is relatively easy to purify from production cultures.

The early laboratory studies by Atsumi *et al.*¹ aimed at fixing CO₂ into hydrocarbons show impressively high productivities: volumetric productivities in small bottle reactors were 6,230 µg L⁻¹ per hour for isobutyraldehyde and 3,000 µg L⁻¹ per hour for isobutanol. These results are orders of magnitude higher than published results for photosynthetic ethanol production in genetically modified cyanobacteria⁷.

Atsumi *et al.*¹ achieved these high productivities by introducing the isobutanol pathway while also overexpressing a key enzyme in the photosynthetic pathway. The genetic modifications were done in stages. In the first stage, a gene expressing a keto acid decarboxylase from the bacterium *Lactococcus lactis* was integrated into the genome of the cyanobacterium *Synechococcus elongatus*. This enzyme catalyzes the conversion of 2-ketoisovalerate to isobutyraldehyde. Next, to increase the flow of carbon to the keto acid precursor 2-ketoisovalerate, three other genes expressing enzymes in the pyruvate-to-keto isovalerate part of the valine biosynthesis pathway were added (one from the bacterium *Bacillus subtilis* and two from *Escherichia coli*). The result was a genetically transformed version of *S. elongatus* that produced 2,500 µg L⁻¹ per hour of isobutyraldehyde.

To further enhance performance, the authors attempted to increase the rate of photosynthesis by overexpressing Rubisco, the heterodimeric enzyme responsible for photosynthetic carbon fixation. Although this did not increase measured rates of photosynthesis, it significantly increased the rate of isobutyraldehyde production in the resulting transformant. Finally, because isobutanol, not isobutyraldehyde, is the product of commercial interest, Atsumi *et al.*¹ added bacterial dehydrogenase genes to convert isobutyraldehyde to isobutanol. Among three genes tested, one from *E. coli* showed the best performance.

But are the productivities achieved by Atsumi *et al.*¹ practically relevant? To answer that question, I have translated their reported productivities into more practical terms (Fig. 1). Volumetric productivity is not the best measure of performance for an algal system. Like conventional plants, algal and bacterial growth systems must be spread out across land so as to capture sunlight for photosynthesis. That is, they must be wide in area and shallow in depth. Large-scale systems for algae production are, in effect, 'algae farms'. We measure their productivity in terms of fuel produced per hectare of land (a hectare is ~2.5 acres). Assuming that light cannot penetrate beyond about 10 cm in a densely growing algae system, we can convert the amount of fuel produced per unit

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volume of algae in water to the amount of fuel produced per hectare.

The numbers reported by Atsumi *et al.*¹ in their proof-of-principle study are impressive, suggesting that bacterial butanol production could greatly reduce the land footprint for biofuels. Their reported isobutyraldehyde productivity is five to six times better than industrially relevant estimates for corn and cellulosic ethanol production and even outperforms current estimates for algal oil productivity. The reported value for isobutanol productivity is less than half that of isobutyraldehyde, but still competitive with current corn and cellulosic ethanol technology.

Future improvements in corn and cellulosic ethanol technology will change this picture (Fig. 1). For the future scenario comparison, I assume that corn yields can reach at least 200 bushels per acre. Monsanto has suggested that their seed development work could raise yields to 300 bushels per acre. Meanwhile, USDA forecasts corn yields of 175 bushels per acre by 2018 (ref. 8). For cellulosic ethanol, I assume that yields of energy crops could rise to 15 tons per acre on average, based on analysis by Sokhansanj *et al.*⁹. Long-term yields of ethanol are assumed to

reach at least 100 gallons per ton for both corn and cellulose, which represents roughly 90% of theoretical yields. Long-term productivity improvements for algal lipids are based on a 'best case' analysis by Weyer *et al.*¹⁰.

As the authors point out, productivity is not the only measure of technical merit. We know, for example, that the high capital cost of algal systems makes these systems unaffordable, even at oil prices of \$100–200 per barrel. It is likely that a genetically modified bacterial system like the one described by Atsumi *et al.*¹ will require closed photobioreactors, which will greatly increase the capital cost compared with the already high cost of open-pond algal systems. Furthermore, one must also estimate the overall energy balance and net reductions in CO₂ associated with bacterial butanol technology, an assessment that cannot be done without more work on the development and scale up of a commercial system. The ease with which isobutyraldehyde can be stripped from water may translate to low energy costs for recovery, but it is still too early to tell. Finally, many other aspects of the technology's sustainability should be considered, including all land, water and air impacts.

Creating a new transportation fuel supply is a daunting task. We therefore need to place our bets on as many technology options as we can. Atsumi *et al.*¹ offer us one more intriguing option on our path to sustainable fuels.

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