A cleaner way to unlock energy: microbes for biofuel

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Algae and photosynthetic bacteria hold a hidden treasure – fat molecules known as lipids – which can be converted to renewable biofuels. Such microorganisms offer an attractive alternative to the unsustainable use of petroleum-based fossil fuels, as well as biofuel sources requiring arable cropland.

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Graduate researcher Jie Sheng and his colleagues at Arizona State University's Biodesign Institute, have been exploring new methods for performing lipid extraction by less harmful means. Under the guidance of Bruce Rittmann, director of Biodesign's Center for Environmental Biotechnology, the team successfully tested several formulas that recover lipid with high efficiency. The group's results appear in the current issue of Bioresource Technology.

The two best candidates for photosynthetic biofuel production – algae and cyanobacteria – may be readily refined to produce a range of green gasolines, diesels, and other biofuels. But as Sheng notes, cyanobacteria offer several crucial advantages as a lipid source. "Cyanobacteria, particularly the strain we use, (known as Synechocystis) are very simple and have been fully sequenced genetically, so that we can easily modify them." Such genetic re-tooling would allow the quantity and quality of lipid production for biofuel to be optimized.

Further, unlike algae, which must be subjected to conditions of stress to maximize their lipid output, cyanobacteria are most successfully cultured under conditions of optimal growth, so that high-density lipid production is paired with a high rate of biomass production. "When the cell is provided with happy conditions for growth, we are able to get much more lipid out," Sheng says.

But gathering the valuable lipids from cyanobacteria first requires the disruption of a tough, protective membrane. A half-century ago, Jordi Folch, a pioneering neurochemist, developed a method that remains the gold standard for isolating lipids from cells. The Folch method, as it is commonly known, has also been used by researchers to extract lipid from algae and cyanobacteria.

The technique involves the use of methanol and chloroform, which eat away and dissolve the lipids in a cell's protective membrane, so that lipids may be harvested. For biofuel production, the Folch method is not practical, as large quantities of chloroform would wreak havoc on the environment and human health. (Chloroform, once a popular anesthetic, is categorized as a B2 chemical by the U.S. EPA – possibly carcinogenetic.)

Nevertheless, breaking down the durable thylakoid membrane of Synechocystis to get at the valuable lipids is not an easy task. As Sheng explains, alternate, less toxic chemicals have been used with success to extract lipid from algae, including ethanol, isopropanol, butanol, methyl tert-butyl ether (MTBE), acetic acid esters, hexane, and various combinations of these, but their viability for use with cyanobacteria was uncertain. Sheng's team wanted to test such chloroform-free methods, to see if they could be used to extract lipids from Synechocystis.

In addition to the challenge of penetrating the more robust cyanobacterial cell membrane, Sheng notes that the lipids found in cyanobacteria are distinct from the lipids found in algae, vegetable and animal tissue, and the extraction methods may not work.

In a series of experiments, the team first demonstrated that the Folch method, as well as a closely related technique (Bligh & Dyer), were the most efficient means of lipid extraction for Synechocystis. Electron microscopy imaging showed effective penetration of the cell membrane and the ability to extract cyanobacterial lipids with high specificity.

The results closely matched the predictions the group had made through molecular modeling of the process. In contrast, ethanol, isopropanol, butanol, acetic ester, hexane, and combinations of these chemicals were significantly less effective in recovering lipid and were less specific, also recovering more impurities.

Intriguingly, the combination of methanol and MTBE showed high efficiency in cell penetration and lipid recovery, roughly comparable to the Folch and Bligh & Dyer methods. The group believes the combination of methanol and MTBE, which allows for the reduction of methanol and eliminates chloroform, may lower the toxicity and environmental impact of the extraction process.

The research was carried out through the collaborative efforts of biologists and engineers, and continuing work in conjunction with other teams at the Biodesign Institute will explore mutant strains of Synechosystis boasting much higher lipid yields than the wild variety used in these experiments.

Further studies, sponsored by Department of Energy, involve genetically modifying Synechosystis so that the portion of the lipid refined into biofuel – the fatty acids – may be directly secreted through the cell wall. These and other ongoing efforts are helping to advance biofuel production from benchtop to eventual commercialization.

Provided by Arizona State University