Cyanobacteria, previously known as blue-green algae, were one of the earliest inhabitants of planet Earth. The huge potential of these simple organisms is only gradually coming to light. They started emerging in the Earth’s oceans and soils around three billion years ago, but have only been the focus of research for the past few decades. Researchers are constantly discovering new, unexpected features and capabilities of cyanobacteria. Prof. Dr. Annegret Wilde and Prof. Dr. Wolfgang Hess from the Institute of Biology III at the University of Freiburg have been using the versatile cyanobacteria for quite some time. The two researchers are part of the project "Cyanosys - Systems biology of cyanobacterial biofuel production", which aims to use cyanobacteria for the large-scale production of biofuels from sunlight and carbon dioxide.

They have basically been around forever and can be found in almost every habitat: lakes, oceans, deserts, polar regions, on the walls of buildings and in symbiosis with other organisms. Since their emergence around three billion years ago, these phototrophic bacteria have developed exceptional morphological and physiological heterogeneity. Their most outstanding feature is without a doubt their ability to carry out oxygenic photosynthesis. They use sunlight to convert water and carbon dioxide into organic substances. They have a broad range of different metabolic pathways, and can produce all kinds of organic compounds.

Cyanobacteria can be used to produce colour pigments, essential amino acids, oils and vitamins for application in the food industry. They give high-quality extracts, and their entire biomass can also be used as raw material for different industries. "They still have a great deal of potential in many areas," says Prof. Dr. Annegret Wilde from the Department of Molecular Genetics at the University of Freiburg, "including for the production of toxins and bioactive substances." Prof. Dr. Wolfgang Hess from the Department of Genetics and Experimental Bioinformatics at Freiburg University adds: "They also produce antibiotics and have repeatedly led to the discovery of new types of antibiotics. They are therefore an excellent resource for all kinds of things." The ancient – in evolutionary terms – bacteria are also easy to cultivate; their requirements are relatively modest. In fact, they only need sunlight, water and a few inorganic nutrients as they are able to fix atmospheric carbon, and in some cases even atmospheric nitrogen.

**Cyanobacteria as fuel factories**

Many funding programmes are focused on the biotechnological application of cyanobacteria, notably for producing energy-rich compounds that can be converted into fuels. At present, biofuel production
usually involves the cultivation of sugar cane and rapeseed at the expense of farmland for food production, which affects poor countries the most. That said, using cyanobacteria could potentially resolve the conflict between the use of agricultural crop as food or fuel. Wilde and Hess are strongly committed to finding ways to use cyanobacteria for the economical production of ethanol. The algal carbohydrates can be turned into ethanol by way of alcoholic fermentation. The resulting bioethanol can then be used as fuel for vehicles in its pure form or as a fossil fuel additive.

"The procedure is already well established, and it is already cheaper to produce bioethanol from cyanobacteria than from sugar cane," says Hess. Producing biodiesel is not rocket science either. Oil produced from a variety of algae can be converted into a diesel-like fuel by way of transesterification.
The EU-funded project "DIRECTFUEL" with Hess as principal investigator and nine partners from seven countries ended last year. The goal of the project was to equip organisms with new metabolic abilities to convert light and carbon dioxide into engine- and infrastructure-ready transport fuels. Propane was chosen as a key target as it is volatile at room temperature and can easily be liquefied at moderate pressure. There is another reason why gaseous hydrocarbons such as butane and ethylene are generating such interest: they are energy-rich intermediary products that can be combusted and used as raw materials for bioplastics production. These fuels are therefore smart alternatives to fossil fuel-based polymer production.

**Coupling photosynthesis and fuel production**

In "Cyanosys", Wilde and Hess are working to improve the cyanobacterial carbon metabolism to enable the bacteria to produce larger quantities of ethanol. The two researchers from Freiburg are working with a company called Algenol Biofuels that already produces bioethanol in Florida, USA. The company's German subsidiary, Algenol Biofuels Germany, mainly contributes the scientific background required for the production process. The production strains are normally incapable of alcoholic fermentation, which is why researchers have to help the process along: they introduce the required genes into the bacteria, which are then able to ferment glycolysis end products into ethanol. The unicellular model organism Synechocystis used by the Berlin-based company is naturally competent. "This means that this particular cyanobacterial strain can be easily genetically modified, as taking up DNA comes completely naturally to it," says Hess.

Some cyanobacteria are also able to synthesise alkanes thanks to the presence of two special enzymes. "This is currently the only known natural biosynthesis pathway that leads to alkanes," says Hess. "Nobody had ever previously considered the idea that cyanobacteria might be able to do this biologically." In the chemical industry, alkanes can only be produced at high pressure and high temperatures. It would be interesting to find out whether the catalytic centre of the alkane enzymes can be modified to enable them to produce short-chain alkanes for fuel production.

Algenol expects to be able to produce 75,000 litres of ethanol per hectare per year. The ethanol-producing bacteria are grown in hanging plastic bags, so-called photobioreactors, that let in an optimum quantity of light. Each bacterial cell is a small ethanol factory that releases ethanol into the medium. The water-ethanol mixture is separated from the algal biomass at regular intervals by centrifugation. The amount of cyanobacterial biomass should not be underestimated. However, the advantage is that it does not need to be thrown away; it can also be used as raw material for other applications.

Cyanobacterial production of ethanol is currently not yet profitable, but it is better than producing ethanol from sugar cane. However, if the bacteria are also allowed to form hydrocarbons (butane, propane, ethylene), which remain in the biomass, then the hydrocarbons can be further processed into biodiesel and bioplastics. "Producing ethanol at the same time as using the algal biomass is a
dual process that makes economic sense," says Wilde. "And this could well be the key to making the process interesting."

Protein hunger and the greenhouse effect

Bill Gates is one of the investors behind the funding of algae research. Many investors have realised that our economic system needs to be changed in view of the nine billion people that will need feeding in the not-too-distant future. Climate change also needs to be addressed. Wilde estimates that cyanobacteria have an even greater potential for the food industry than for fuel production.

For example, Spirulina produces the semi-essential amino acid L-arginine, which is known as a dietary supplement. "L-arginine is normally produced from animal protein, and can now be produced with cyanobacteria," says Hess. "Cyanobacteria could represent an environmentally friendly source of protein for developing countries and vegans. This would then be one way of satisfying protein hunger in the world. Carbon dioxide emissions need to be reduced dramatically in order to slow down the greenhouse effect in the atmosphere. "Cyanobacteria would be perfect for this as they love absorbing CO₂ and producing something from it," concludes Hess.

Spirulina – grown biologically and pressed into tablets. © Stephanie Heyl

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- Bioeconomy: a new model for industry and the economy