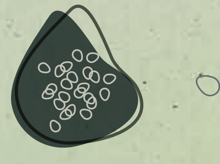
The Search for Efficient Processes to Generate **Green Crude**

Crude oil

400 dollars
per ton of fossil kerosene



1.4 percent of global CO_2 emissions

The production of “green aviation fuels” from algae is not yet commercially viable because of the extensive costs and amount of space required. Researchers from the Industrial Biocatalysis Group at TUM are looking to change that.

Algae

2,000 dollars
per ton of biokerosene

No additional CO_2 emissions

Estimates of the area needed to produce enough algae to cover the EU's total fuel requirements:
92 km²–580,000 km²

Global aviation industry

1.7

1.7 billion liters
of kerosene per year

1.7 cubic hectometers or volume of water in the Ammersee near Munich

Graphics: ediundsepp

TUM has built a globally unique AlgaeTec Center in cooperation with Airbus Group. This high-tech facility located at the Ludwig Bölkow Campus in Ottobrunn/Taufkirchen is a new innovation hub for the development of sustainable biokerosene production processes based on algae biomass.

Picture credit: Heddergott

Bernhard Epping

Kerosin aus Algen – die Suche nach der Billigproduktion

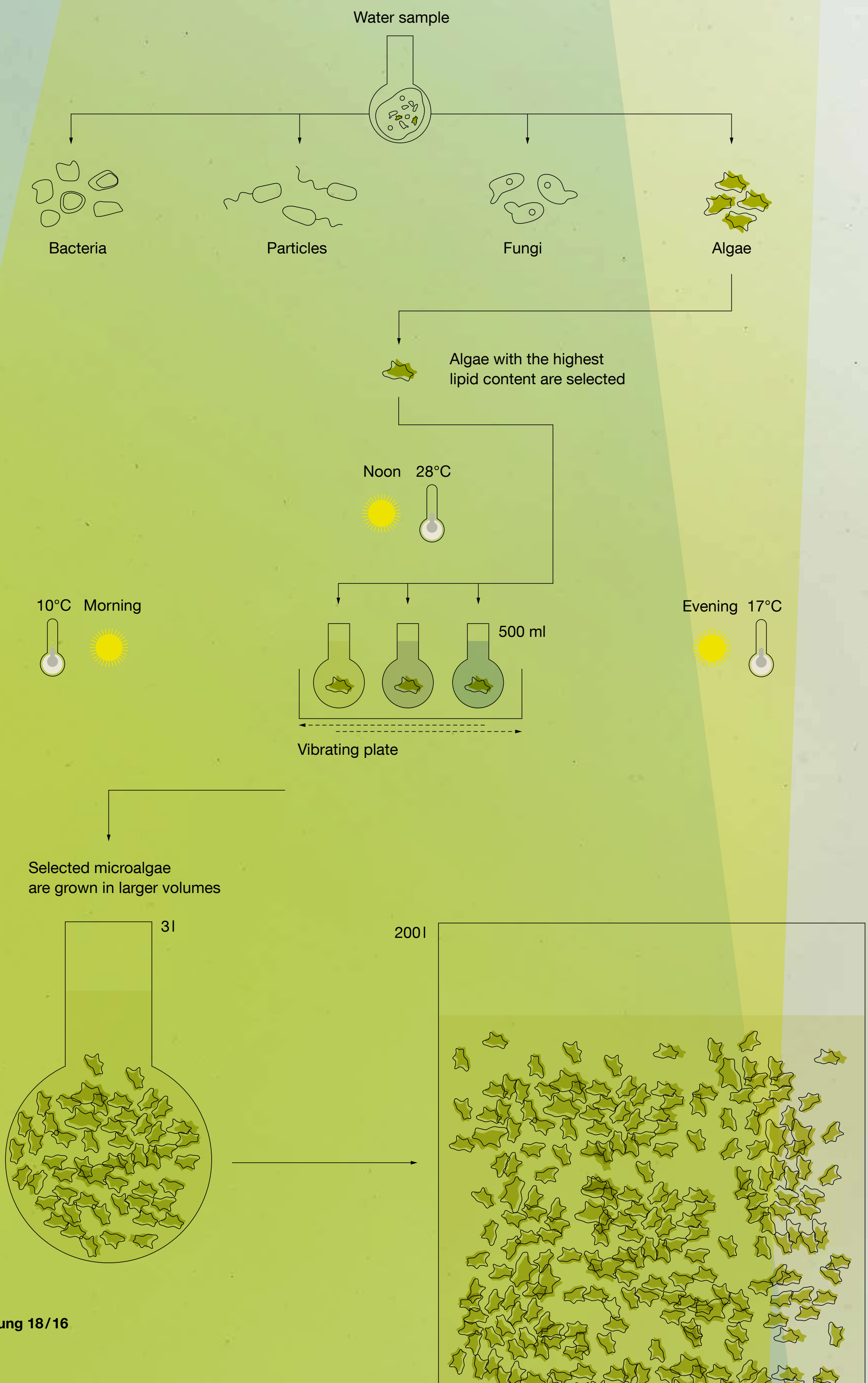
Das Fachgebiet für Industrielle Biokatalyse der TUM in Garching unter der Leitung von Prof. Thomas Brück koordiniert mehrere Großprojekte für die Entwicklung einer neuen Rohstoffbasis für Kerosin aus Algen.

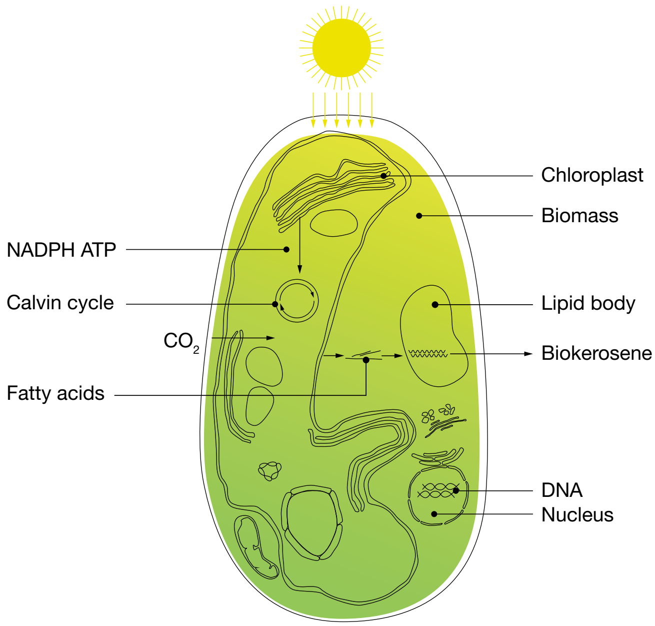
Mikroalgen gelten zwar schon seit einigen Jahren als Kandidaten für die Produktion von Lipiden, die sich danach chemisch-katalytisch zu Kerosin reduzieren lassen. Weltweit fehlt allerdings ein Durchbruch zu einer auch wirtschaftlich konkurrenzfähigen Produktion.

Ein Teil des Problems ist die bislang oft zu unsystematisch betriebene Forschung auf diesem Gebiet. Die TUM-Projekte setzen daher auf eine systematischere Optimierungsarbeit auf allen Ebenen der Prozesskette. Nach drei Jahren erster Forschungsarbeit deuten sich bereits Verbesserungen an.

Ein Screeningprogramm fahndet nach Algenarten, die von Natur aus besonders hohe Mengen an Lipiden synthetisieren. Im Fokus stehen Algen, die von Natur aus im Meerwasser oder noch salzhaltigeren Gewässern vorkommen. Das erlaubt in Sachen Sterilität und CO_2-Sättigung der Nährlösungen eine besonders stabile und damit preiswerte Prozessführung. Gute Lipidproduzenten sind Vertreter aus zwei Gattungen von Grünalgen, genannt *Scenedesmus* und *Picochlorum.* Ein neu entwickeltes Anzuchtprotokoll lässt unter Stickstoffmangel-Bedingungen einige Arten bis zur Hälfte ihrer Biomasse als Lipide synthetisieren, ohne dass die Organismen ihre Teilung, alias ihr Wachstum, einstellen. Bislang hörten Algen unter solchen Nährstoffbedingungen mit dem Wachstum auf. In einem 2015 neu errichteten Algentechnikum in Ottobrunn, Landkreis München, ist auch die Optimierung von Anzuchtbedingungen im bis zu 200-Liter-Maßstab möglich. Es ist die letzte Stufe, der größte Versuchsmaßstab vor dem möglichen Start einer industriellen Produktion. Das Forschungskonsortium um Prof. Brück schätzt den Zeitbedarf für weitere großtechnische Erprobungen auf sechs bis zehn Jahre. Danach könnte eine erste industrielle Produktion in Kooperation mit Firmen starten. Eine Algenproduktion wird es, wenn überhaupt, in tropischen Regionen oder im Mittelmeerraum geben. Die TUM könnte sich aber sehr wohl auf Dauer als Forschungs- und Entwicklungszentrum etablieren. □

Identification of the most process-relevant microalgae from environmental samples. Algae with the capacity to generate a high intracellular lipid content are initially grown in a 500 ml shake flask utilizing specific climate and light conditions.





Algae consume CO_2 for growth and release the same amount when burned as fuel. Algae which produce lipids as energy stores form the basis for biofuels.

Green, roundish in shape and not particularly exciting to look at as they float around individually or sometimes in groups. Such is the life of *Scenedesmus* sp. algae. “Oh look, one of them is swimming.” “Not exactly,” says Ph. D. student Johannes Schmidt. “They can’t move by themselves – they are propelled by gentle flows in the nutrient solution.” The tiny algae, each one measuring only a hundredth of a millimeter, are lying on a slide which Schmidt has just positioned under a microscope. They belong to a species known as green algae. Since they contain chlorophyll, they are able to photosynthesize like land plants; in other words, produce all the compounds they need – fats, sugars and proteins – from water, carbon dioxide (CO_2) and the sun’s light energy. “We gathered this *Scenedesmus* strain from the silty shores of the Baltic Sea – from a sort of lagoon known as a bodden,” explains Schmidt. These little unicellular organisms live for just a few weeks, reproduce by division and, sad but true, often end up being devoured by small crustaceans or fish.

High volume production is the key

The exact species is strictly classified information. Understandably so – in just a few years, this microalgae could produce large volumes of crude for biokerosene, a sustainable fuel for jet engines. First though, the process chain has to be developed, in complete secrecy, although a number of patent applications are in the pipeline. “Progress in this field will require an interdisciplinary approach and cooperation with industry,” maintains Prof. Thomas Brück, head of the Industrial Biocatalysis Group at TUM. For the time being, the researchers are keeping their cards close to their chest.
Biokerosene is yet another biofuel with the potential to help relieve rising climate mitigation pressures. The global aviation industry currently consumes 1.7 billion liters of kerosene per annum, thereby contributing 1.4 percent to global CO_2 emissions. So far, the industry has escaped regulations to cap emissions. In 2013, however, the International Air Transport Association (IATA) set its members the target of halving CO_2 emissions by 2050 – with 2005 as the base year. More efficient jet engines are already being produced, but that measure by itself will not be enough in the face of rising passenger numbers. “A sustainable raw material will be needed as well,” explains Brück. ▷

Link
www.ibc.ch.tum.de

Picture credit: Bauer, Graphics: ediundsepp (source: TUM)

Certain microalgae are able to synthesize oils and fats naturally, using these lipids as energy stores. Scientists have the technology to isolate these lipids and reduce them to kerosene by means of a chemical reaction called hydrodeoxygenation. The end product is just as effective as the crude oil equivalent. Kerosene, composed of straight-chain alkanes and hydrocarbons with 10 to 16 carbon atoms per molecule, has the highest energy density of all fuels.

So the process is viable in principle. The problem is that it is much too expensive. The current price for a ton of biokerosene is USD 2,000, whereas the conventional fossil version was selling at less than USD 400 at the start of the year. Added to this, with the current state of the technology, the EU alone would need to cover an area at least the size of Portugal with algae tanks in order to meet its total fuel requirements. This calculation was undertaken by Maria Barbosa and René Wijffels from Wageningen University and Research Center. “That is optimistic, under current process conditions. It would more likely be the whole Iberian peninsula,” comments Brück. The reason for this huge footprint is that since they require light, algae live on the surface of the water, descending only a few millimeters or maybe a few centimeters at most.

Of the 27 staff employed at IBC, eight work in the field of algae biotechnology together with half a dozen project partners, including Airbus Group. With this powerhouse of knowledge, Brück plans to accelerate the momentum in this particular research field. “What we need is a more systematic approach to the research in order to identify improvements all along the process chain. This is the only way to bring the cost down.” The IBC has been working on two major projects to this end since 2013. The first is “AlgenFlugKraft” (AFK; Algae Powered Aviation), financed by the Bavarian Ministries of Economic Affairs and Science. The goal is to produce lower-cost biokerosene jet fuel from algae. Then there is the Advanced Biomass Value (ABV) project, which is being funded by Germany’s Federal Ministry of Education and Research. Here, the researchers will be working on an intermediate step – certain algae will initially be used to produce special greases and oils as industrial lubricants. The biomass remaining after extraction of the lipids will then be transformed into kerosene with the help of special yeasts and novel chemical processes.

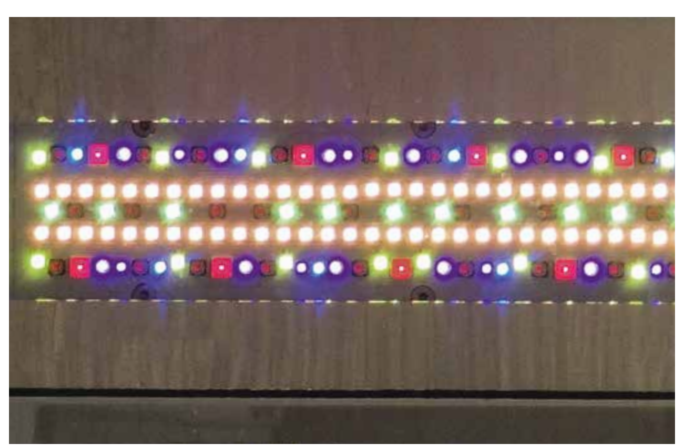
Finding suitable algae candidates

This first step involves painstaking and systematic screening of suitable varieties of algae capable of producing lipids. There are between 40,000 and 150,000 species of microalgae, microscopic single-cell organisms like *Scenedesmus*. The huge span of this estimate shows just how little scientists actually know about these organisms. A mere dozen species have been exploited commercially to date as food supplements as they have been found to be good sources of protein, starch and pigments. When it comes to “lipid specialists”, however, science seems to have drawn a complete blank thus far.

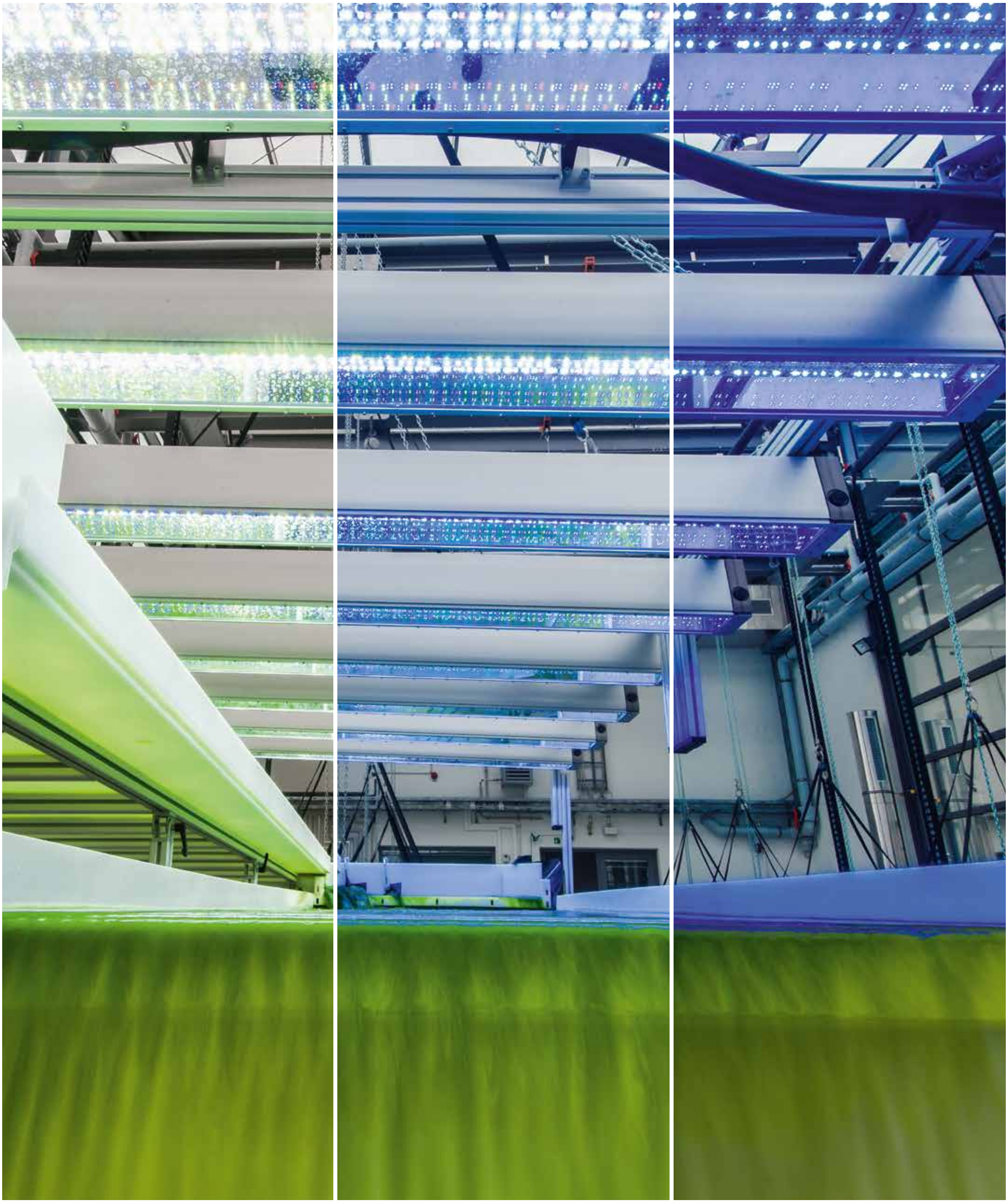
And so the search is on. Researchers at IBC are even on the lookout while on vacation – bringing water and soil samples back from selected locations so that algae can be isolated from them. A FACS (fluorescent activated cell sorter) then gets to work, automatically separating the green-glowing microalgae from the other particles, bacteria and fungi in the samples. “In a second step, we add a pigment which specifically binds to lipids. Our FACS device selects the microalgae with the highest lipid content for us,” says AFK project leader Dr. Daniel Garbe as he pats the large gray box in a corner of the lab. “Then we carry on from this vantage point.”

The IBC has already collected more than 50 proprietary strains of algae. Safely stowed in metal cabinets under lighting and kept in small sterile flasks and test-tubes – all of the painstakingly acquired species are subject to contracts concluded in accordance with the Nagoya Protocol to the Convention on Biological Diversity. This protocol guarantees the country of origin a share of the benefits if a species from their territory proves to be commercially successful.

Iteratively, test after test establishes whether and how these algae can be optimized for lipid synthesis. In practice, this means growing them at different temperatures and light conditions, and using different concentrations of salt, nitrogen and phosphorus in the nutrient solution. In stage 1, the same amount of starter culture is automatically pipetted into the 96 wells of a plastic tray. In accordance with the different protocols developed, widely varying concentrations of sodium chloride and nutrients, primarily nitrogen and phosphorus, are also added. Then it is off to the bioreactor, where the light levels and temperatures change as the day progresses. A few days later, the researchers test the optical density with a photometer, checking how well the algae have grown in each of the experimental conditions – and how much lipid content they are storing. ▷



Proprietary LED based lighting simulates the different radiation intensities and spectral characteristics found in latitudes from the Baltic to the tropics.



The AlgaeTec Center allows simulation of the climate and illumination of any potential production site. The Brück-led research consortium developed an optimized "cascade" cultivation system, which encompasses two small steps that have been incorporated into the 200 liter tanks. Gravity causes the algae cultures to flow down into the next-lowest tank. In the collecting basin at the bottom, the entire solution is re-saturated with CO_2 and then pumped back up to the highest level.

Picture credits: Bauer

A focus on saltwater species

This project has already managed to establish a number of new basic strategies. The future lipid-producing algae will be at home in briny water. Seawater has a salt content of 3 percent, but some ponds and lakes in tropical regions in particular have salt levels of 10 percent and higher. “We love species from biotopes like these,” underlines Brück. Any organism that is able to survive in such harsh, even hostile, conditions, will be particularly adaptable to large-scale cultivation. There will be little need for disinfection, as the high salt content will take care of most potential competitors like bacteria and fungi. Another advantage from the researchers’ perspective is the fact that CO_2 dissolves particularly well in alkali salt water in the form of bicarbonate ions $((HCO_3)^-)$. This is the form in which algae are able to utilize CO_2. In fresh water, the gas does not dissolve as readily. “The use of saltwater species therefore makes the overall process much more efficient,” explains Brück.

Thanks to the new nutrient solution protocols developed by the Brück group, the algae are for the first time able to form lipids and still keep on growing. Previously, it was not always possible to combine the two. Garden pond owners will know that high levels of nitrogen in the water promote algal growth. In order to yield the maximum amount of lipids, the algae must be deprived of nitrogen. In this particular metabolic state, they can no longer create their favorite product, nitrogenous proteins, so they switch to oils instead. Frustratingly, they usually also stop growing at that stage, too. Until now: *Scenedesmus*, which in its natural form has a lipid content of around 10 percent, has been plumped up to 40 percent lipids thanks to a new cultivation medium devised by the lab researchers in Garching. Interestingly, despite being deprived of nitrogen, the algae are happy to keep on growing. Even more surprisingly, this impressive feature has been outperformed by a microalga of the genus *Picochlorum* which was originally collected from a hypersaline lake in the Bahamas: “*Picochlorum* is our champion. It grows extremely quickly, 15 times faster than *Scenedesmus*, and is currently already forming oil stores of up to 30 percent in each cell,” enthuses Brück. Ramping up the oil production to 50 percent and above is only a question of finding the right media, and the team are already on the case.

Scaling up

The next stage involves scaling up – there is no guarantee that an alga that performs well in the test-tube will also thrive in large tanks. As part of phase two in the lab, the candidates will first be cultivated in 500-milliliter flasks, and after that in three-liter photo-bioreactors. This will reveal whether *Scenedesmus* and *Picochlorum* are on track.

Technical enhancements in the lab will help. It is important for climate conditions to be simulated as realistically as possible; otherwise the entire screening process will produce nothing more than artifacts. Lighting is the key factor. ▷

Prof. Thomas Brück

Exploring marine algae for industrial applications

For Thomas Brück, successful research revolves around interdisciplinary cooperation, ideally also engaging with relevant industry partners.

Born in Cologne in 1972, Brück decided to move to the UK to conduct interdisciplinary studies in natural and social sciences for his undergraduate degrees. After receiving double Bachelor degrees in chemistry and biochemistry with a subsidiary in management science, he went on to complete his Master of Philosophy in molecular medicine at Keele University in Stoke-on-Trent, England. In 2002, he was awarded the title of Doctor of Philosophy at the University of Greenwich (London), for his studies in biochemical reaction mechanisms of the peroxidase enzyme family. Subsequently, Brück moved to Florida Atlantic University, Boca Raton, USA, where he developed an interest in marine algae. For the next few years the biochemist focused on isolating and exploring the structure of pharmacologically relevant compounds derived from marine coral, which are produced in conjunction with symbiotic algae.

In 2006, Brück returned to Germany to take up a position in industry. He started as research manager at Süd-Chemie AG (acquired by Clariant in 2011) before taking up the position of Technology and Patent Portfolio Manager two years later. In 2010, he returned to the world of academia, becoming head of the research group for Industrial Biocatalysis (IBC) at TUM, where he and his team managed to acquire significant funding for projects focusing on biochemical process development for biomass valorization in order to generate sustainable biofuels, platform chemicals, polymeric materials and new pharmaceutical compounds. He is very matter-of-fact about his research. Although he does maintain that research into algae has been grossly neglected around the world. He is convinced that there is a very high chance that algae will provide a sustainable source of biokerosene and also form the basis for new drugs.

Brück is married, with two children aged seven and three. His family is truly international, speaking both German and English at home, since Brück’s wife Diane is British.

Picture credit: Bauer

"Being able to simulate all kinds of climate conditions in this one place gives us a huge advantage when it comes to selecting the most suitable candidates for biokerosene production." Thomas Brück

In many labs, the light fed to algae for photosynthesis in previous tests was much gloomier than the daylight they were used to in their natural environment. Brück reveals that microalgae often had to make do with light in wavelengths between 500 and 600 nanometers in past studies. But visible light alone has a wavelength range between 380 and 780 nanometers, and on top of that a certain amount of UV light also seems to play a major role in effective lipid synthesis. Together with the Berlin-based SME FutureLED, the Munich-based researchers have developed proprietary LED-based illumination solutions that mimic a large portion of the sunlight spectrum. With these lights, they can simulate the different radiation intensities found in latitudes from the Baltic to the tropics.

Without the LED lights, *Scenedesmus* would probably have never made it past the screening. Which would have been a mistake. As this species is native to the Baltic Sea, conventional biology theory would contend that it should be optimally adapted to the test location. It should in fact perform best under Baltic conditions. On the contrary, the algae reached lipid values of 40 percent during the preliminary experiments only when the scientists ramped up the light intensity and temperatures to Mediterranean levels. “The species clearly felt more at home in the climate of Almeria in southern Spain,” notes Brück. Which sets the scene for the third and final phase of the experiments at TUM. For this, the 1,500 m^2 glass-roofed

“Progress in developing high-volume production chains for algae will require an interdisciplinary approach and cooperation with industry.” *Thomas Brück*

AlgaeTec Center has been purpose-built on the Ludwig Bölkow Campus in Ottobrunn, south of Munich. The 10 million euros funding for the new facility has been provided by the state of Bavaria and the Airbus Group. Inaugurated in October 2015, it is at the very heart of the AFK project. Every kind of climate from tropical humidity to desert dryness can be simulated in the building, and Brück is keen to point out that no other facility in the world has these climate and light variation capabilities. All that can be heard within is a faint hum, while the eye is drawn to the bright green glow of *Scenedesmus* growing in the large open tanks, each currently with a

The final laboratory-stage algae process scale-up is realized in custom-built bubble column reactors holding 30 liters.

Picture credit: Bauer, Graphics: ediundsepp (source: TUM)

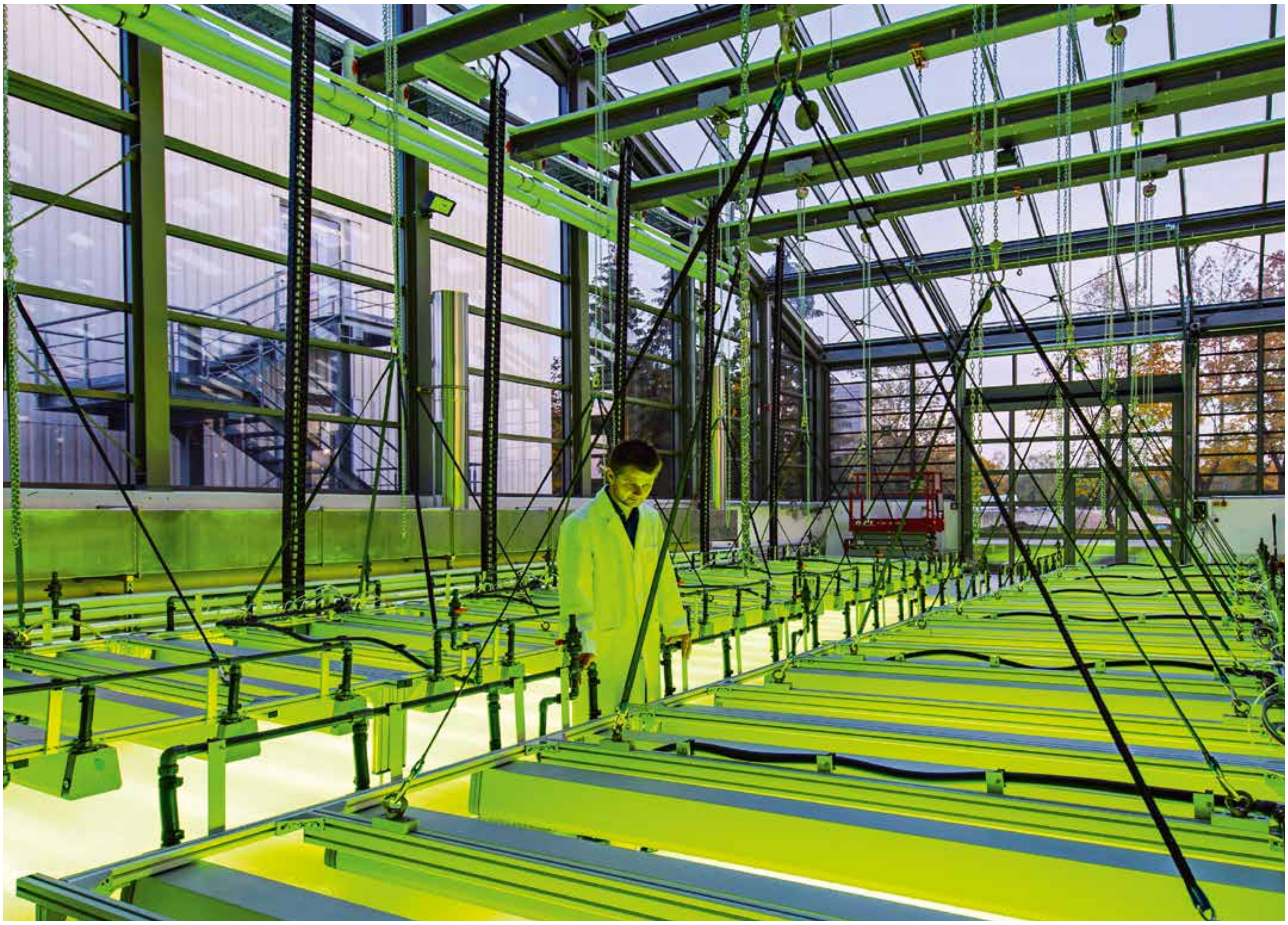
Brück and his team are testing algae from all over the globe with respect to growth efficiency. The new AlgaeTec Center allows them to simulate production conditions at any location. The most promising sites are expected to be situated in the Mediterranean or in the southern hemisphere.

capacity of 200 liters. The next scale-up level focuses on reaction volumes of 1,000–2,000 liters, which would provide data for industrial process realization. The design and planning for relevant algae cultivation systems is currently ongoing.
Another innovation is the optimized "cascade" cultivation system, which encompasses two small steps that have been incorporated into the tanks. Gravity causes the algae cultures to flow down into the next-lowest tank. In the collecting basin at the bottom, the entire solution is re-saturated with CO_2 and then pumped back up to the highest level. It is important to note that all of this happens at the gentlest pace. Algae cultivation experiments around the world still mostly rely on raceway ponds – large tanks in which the algae solution is vigorously pumped along long rather deep oval ponds. A certain amount of mixing is important to ensure that the cultures receive sufficient light penetration, which is reduced within just a few centimeters of the water column. "We have recently discovered, however, that many species do not cope at all well with mechanical stress," says Brück. Less agitation in the tanks therefore saves energy and reduces the facility's operating costs.

Identifying the best production locations

Brück hopes that his team will be able to offer industry customers the first algae strains for large-scale production of biokerosene in six to ten years. The Ottobrunn based AlgaeTec Center is well set to become a leading international facility for the screening of algae species. "Being able to simulate all kinds of climate conditions in this one place gives us a huge advantage when it comes to selecting the most suitable candidates for biokerosene production," underlines Brück.
After that though, it will be adios to Bavaria! The sunlight levels at this northerly latitude will not be strong enough for future biokerosene production. Brück is planning on moving his algae to more helpful southerly climates, perhaps the Mediterranean, California, Mexico, the coast of Chile or Africa. He has already reached out to potential cooperation partners in various locations. So, how much will the price of biokerosene come down? "It will become cheaper – but everything in its own time," assures Brück. After all, it took 150 years for oil refining to reach its current state of development. "We have a good few years of research and development to go yet," claims Brück. The algae researchers are only just getting into their stride.

Bernhard Epping



The AlgaeTec Center in a Nutshell

1,500 **m²** area

2 **climate zones** can be tested independently

2–6 **weeks** is the duration of an algae production test

up to 4 species can be tested in parallel in two different climate zones

4 **cascade reactors** are currently in place (2 of 200 liters, 2 of 80 liters)

In the future, up to **7 species** can be tested in parallel; additional cascades are being built for that purpose

Picture credit: Heddergott, Graphics: ediundsepp