Fraunhofer
LIFE SCIENCES

FRAUNHOFER GROUP FOR LIFE SCIENCES

BLUE BIOTECHNOLOGY

Editorial notes

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CONTENT

"BLUE", THE NEW COLOR IN BIO

Biotechnological methods have always been part of human culture. Discovered by chance and empirically developed further, they led to regional specialties such as bread, beer, cheese, and wine. Today, biotechnology is present as an independent field at universities. Furthermore, depending on the origin of the organisms and resources, a separate classification has developed that is organized according to colors and easy to remember.

Today, results from research and development in all established areas of biotechnology are present in everyday life. Plants with special characteristics for farming are the main area of green biotechnology. This branch of biotechnology is especially affected by controversy between vision and acceptance. Products of white industrial biotechnology can be found on our shelves as cosmetics or detergent ingredients. Human medicine and pharmacology cannot be imagined without red biotechnology with its possiblities for diagnostics and therapy. In addition, a yellow branch which deals with insects and their products is currently growing.

An additional color is now joining this green-white-red spectrum, which is a little more intensive than the "yellow". It is the "blue". Also in this area of biotechnology, individual procedures such as fish farming in the cloisters of the Middle Ages or the algae harvest in Japan have long been established. Today, the definition of "blue biotechnology" includes all biotechnological procedures that exploit aquatic (marine and limnic) organisms or are aimed at doing so. With this definition "blue biotechnology" is a comprehensive term that spans

TECHNOLOGY

beyond the area of marine biotechnology. Thus, "blue" and "marine biotechnology" are by no means synonymous, even if most of the organisms studied so far originate from the ocean.

In contrast to the color coding of the other three original biotechnology areas – red, green, white – blue biotechnology does not derive its color from its application. It stems from the origin of the organisms and resources used, the water.

Many analyses, reports, or market studies refer solely to marine biotechnology and, thus, exclude all inland water organisms and applications as well as their potential. For example, the Global Industry Analysts Inc. predict a turnover of 4.6 trillion US dollars for the global market of marine biotechnology for 2017. Furthermore, the position paper no. 15 of the European Science Foundation (Marine Biotechnology – A new vision and strategy for Europe) estimates the cumulative annual growth rate at 4 to 5 percent. This developmental trend would increase even more if the potential of inland waters were to be included.

If one considers that our world's oceans and many inland waters are the last three-dimensional habitats which still have to be discovered, we can imagine how many new products, resources, and biologically active substances lie dormant in the water's depths. Therefore, blue biotechnology will play an important role in solving the worldwide socio-ecologic and socio-economic challenges such as supply of sustainable food and energy sources.

The Fraunhofer Group for Life Sciences offers various possibilities for the sustainable use of inland waters and oceans. In this brochure you will find specific examples of the approaches that the Group would like to pursue with you.

Yours

Prof. Dr. Rainer Fischer
Chairman of the Fraunhofer Group for Life Sciences
Director of the Fraunhofer Institute for Molecular Biology and Applied Ecology

SUSTAINABILITY
BIODIVERSITY
ECOTOXICOLOGY

PRESERVING THE ENVIRONMENT AND BIODIVERSITY THROUGH SUSTAINABLE AND MODERN ECOTOXICOLOGY

SUSTAINABILITY

The uncontrolled, massive use of new resources has often led to irreversible changes in the affected ecosystem and even sometimes its death. In the initial euphoria, negative consequences were not taken seriously enough. Often they did not appear until much later. The waters worldwide are also gravely in danger because of over-fishing, waste, and industrial sewage. The Fraunhofer Group for Life Sciences has felt committed from the beginning to the idea of sustainability in the young field of biotechnology. Existing experience and technology of the participating institutes are available to carefully and responsibly exploit the potentials of blue biotechnology.

Safety through binding standards

According to the European Water Framework Directive, all EU member states are obligated to make sure that their natural waters are in an "ecologically good condition" or to keep them in such.

Aside from the structure of the water body and the species spectrum, the chemical condition is also part of the evaluation. Binding quality standards have already been established for priority and priority hazardous substances for all water bodies. For so-called river basin-specific substances, the member states are responsible for their identification and also to establish quality standards.

The experts of the Group were significantly involved in defining quality standards for priority and priority hazardous substances. Furthermore, they are continuously involved in establishing such standards in various projects conducted on behalf of the public sector or the chemical industry. This comprises not only the collection and evaluation of relevant studies and the establishment of quality standards according to current guidelines, but also the direct performance of studies. These studies will help to reduce the uncertainties that still exist in deriving quality standards.

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1 *Conventional aquaculture.*

Shore leave for sea dwellers – land-based integrated multitrophic aquaculture (IMTA)

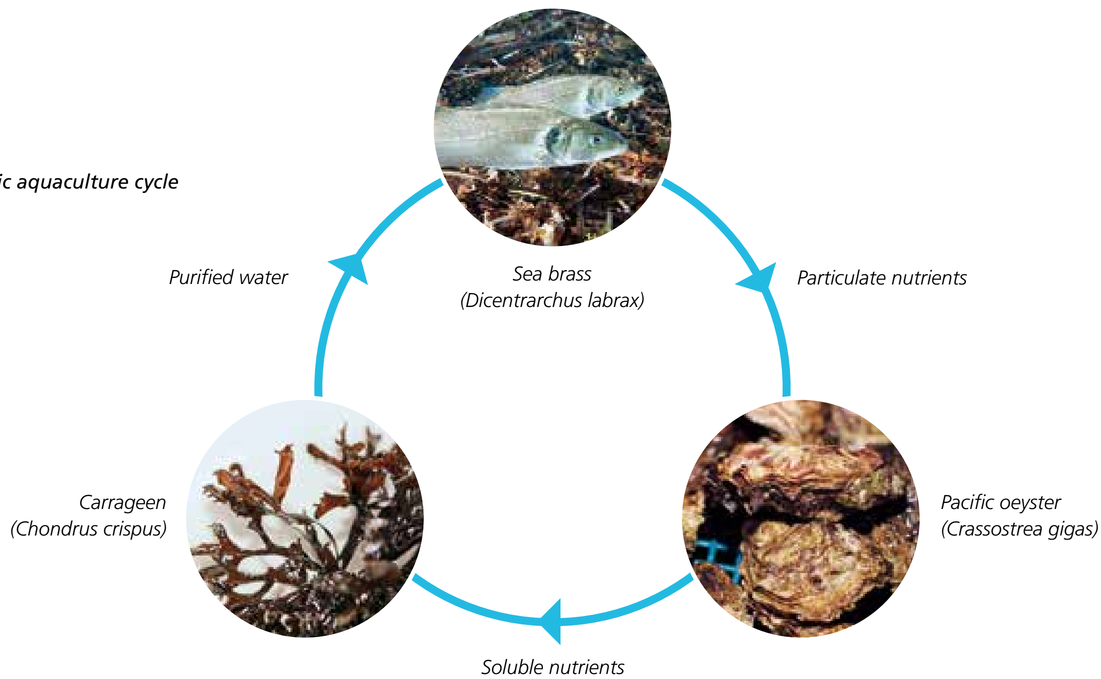
Fish is traditionally a major basic food resource in coastal regions. Worldwide in industrial countries today, more and more meat dishes are being replaced by fish. This is due to its nutrition-physiological characteristics. According to the "The State of the World Fisheries and Aquaculture", FAO report 2012, aquaculture is the fastest growing area of production in the animal food industry. The total amount of fish from catching and farming is already larger than the production amount of beef, poultry, and pork.

The continuously growing demand for fresh fish and seafood is met with the help of aquaculture. The fish usually comes from fish farms in Asia and from conventional fish cages in the ocean, for example in Norway, Canada, or Chile. Meanwhile, it has become evident that both cultivation types have grave disadvantages. Traditional fish farming cannot meet the rising demand for seafood. In crowded fish cages frequent diseases and the consequences of treatment are difficult to control.

To prevent these diseases the fish's sensitive gills and skin must be protected from bacteria and parasites from the beginning. This in turn requires effective sewage treatment.

The intriguing approach of the Group's scientists to transfer the seafood farms onshore sounds paradox at first. However, in reality, many problems are much easier to deal with onshore. There are many possibilities for locations such as a cowshed that is no longer being used or an empty warehouse.

Multitrophic aquaculture cycle



The greatest challenge remains the sewage treatment, because similar to conventional aquaculture facilities the fish are fed with fish feed whose remnants and degradation products pollute the water. Until now, complex filter systems such as biological filters served the purification of water, converting and degrading above all nitrogen compounds with the help of bacterial communities.

The Fraunhofer Group for Life Sciences has also found a convincing alternative to these systems: the integrated multi-trophic aquaculture adds other organisms such as muscles that simply take up and use the energy-rich waste particles, food remnants, as well as fish excrements in their metabolism. This is used to crudely filter the water. Furthermore, the muscles can then be marketed as food.

Soluble waste serves as nutrients for photoautotrophic algae to produce biomass under light. Other chapters of this brochure also deal with the variable use of algae and their products (e.g. p. 12, 18, 22, and 26ff).

Two facilities of this type are already operating at the Fraunhofer Group for Life Sciences. Interested visitors are welcome to get an impression of this innovative system directly on site.

There is a facility that is heated with the waste heat of a biogas plant especially for the culture of the warm temperature-loving African catfish.

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Survival by cryopreservation

According to current information from the IUCN (International Union for Conservation of Nature, effective 2013), more than 1900 fish species are endangered. Cryopreservation is an important tool to preserve species diversity.

In other areas of agriculture such as cattle or pig farming the storage of gametes has long been established. In contrast, the demand for cell banks of aquatic organisms has become apparent only recently, with the growing market size of aquaculture. The diversity of the fish being held in culture today requires an adjustment of the cryopreservation protocols for sperm. This is usually necessary for each individual species. The Group's working group on "Aquatic Cell Technology" explores the conditions under which low-temperature storage of cell material can help preserve aquatic genetic resources.

Cryopreservation of whole fish eggs is not possible so far because of their size. The storage of fish sperm or egg cell nuclei, however, would significantly take the burden off the farmers, because they would no longer have to continuously keep larger parent animal populations. Furthermore, the exchange of genetic material between farmers would be easier, saving populations from disease outbreaks or genetic drift. To be prepared for the focus of aquaculture being placed increasingly on few target populations and, if needed, to preserve new populations, the working group aims to store a genetic reserve of as many farming fish species and breeds as possible.

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ECOTOXICOLOGY

Clean drinking water is becoming scarcer for the broad population in Third World countries. The pollution of inland waters and oceans is increasingly problematic for global environmental protection. New and inexpensive methods are urgently needed to enable quick and efficient determination of water quality. The Fraunhofer Gesellschaft's scientists are consistently developing new detection methods according to the 3-R principle, aimed at reducing, refining or completely replacing animal experiments. The Fraunhofer Group for Life Sciences has modern, experimental animal-free methods that make numerous toxicological tests not only ethically sound, but also more economical.

Predictive risk assessment to protect aquatic ecosystems

Chemical substances and products have to be tested for their risk to aquatic systems. In the EU – different legal regulations are applicable, depending on the application area: REACH for general chemicals and products, EMA guidelines for active pharmaceutical ingredients, and EU Regulation No. 1107/2009 for pesticides. All regulations initially stipulate the use of a tiered approach starting with simple ecotoxicological tests to identify the potential hazard. In addition, a realistic worst-case estimate of potential exposure has to be developed. For risk assessment a ratio of both parameters is calculated. There must be a difference between the lowest toxicity value and the estimated exposure, characterized by a safety factor. This safety factor represents the uncertainty of risk assessment that remains based on the available data.

The experts of the Group develop and improve test and assessment systems that minimize the uncertainty of risk assessment and, thus, allow the use of small safety factors. This includes methods to test the behavior and fate of chemicals in aquatic systems as well as ecotoxicological tests with non-standard organisms (species sensitivity distributions) or tests comprising sensitive life stages and performances such as fish life cycle tests or micro-/mesocosm studies with aquatic communities under realistic exposure conditions. With regard to these higher-tier studies, the Fraunhofer Group for Life Sciences is one of the world's market leaders, also offering models to assess exposure to and effects of substances to predict risks and to trigger tailored experimental studies only if necessary.

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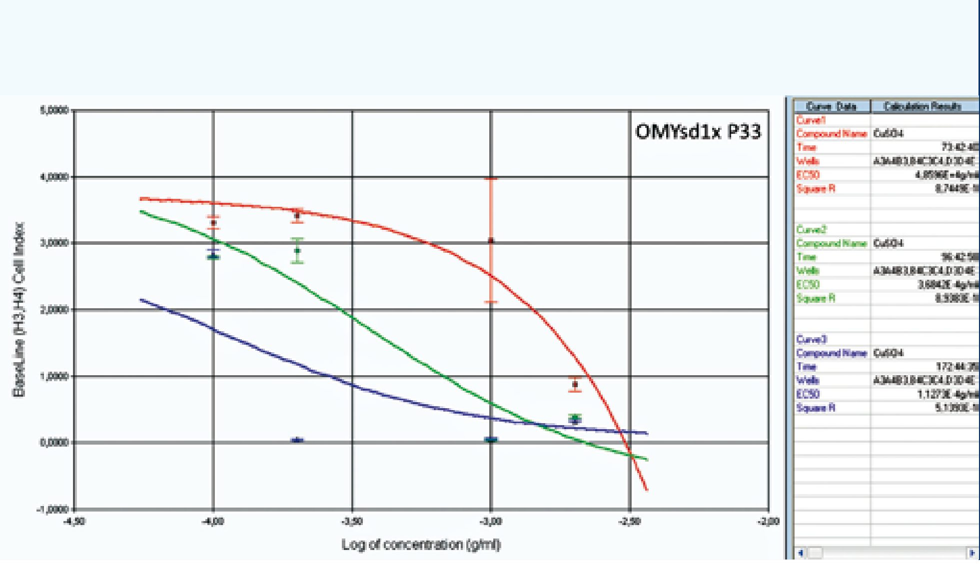
Alternatives to animal tests

The use of vertebrates for toxicological and ecotoxicological testing is being viewed more and more critically in postindustrial societies. Experts of the Group are developing and testing methods that can improve, reduce, or help avoid animal tests. Fish play an important role in ecotoxicological hazard and risk assessments. They are used for toxicity testing of chemicals or to determine the potential of chemicals to accumulate in the food chain.



Dose-response curve after exposure of OMYsd1x cells to $CuSO_4$; impedance-based measurements.

The fish egg (DIN) and fish embryo test (OECD) was developed in cooperation with experts of the Group as a substitute for the acute fish test. This test, which is not rated as an animal experiment, also has a great potential for identifying different mechanisms of action. For example, it can serve as a screening method for unknown pollutants in water samples and also as a method for identifying potentially active substances.

For bioaccumulation testing, methods with primary trout liver cells are being developed in cooperation with experts of the Group, aimed at assessing whether test substances can be metabolized. This generates data that help to realistically model the accumulation in fish through the interaction of uptake and excretion. A more tangible example of fish test replacement is the accumulation test in amphipods. It leads to similar results in a much shorter time and with much smaller test volumes. This test with invertebrates is being developed by experts of the Group as a new OECD guideline.

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Cells from fish skin as an indicator of environmental influences

Skin is the first area of contact with the environment, likewise in fish. It must react in many ways to the soluble substances in the surrounding water. Therefore, stem and progenitor cells from full-thickness skin and from fish scales are the basis for test and sensory systems to measure toxicological effects. The aim is to use these in the future for detection of both potentially damaging substances from inland and flowing waters and potential stressors in aquaculture process water. The tests are being developed mainly to record a rapid and sensitive cell response and to a lesser extent to determine the mechanism of action of toxic or in other ways hazardous substances. However, in addition, these cells can be used for physiological issues. Characterization using immunocytochemical and molecular biological methods shows different cell types that differentiate further in culture. A goal is, therefore, to differentiate the cells in a specific direction so that they can be used in custom-made assays. An application in 3D skin models could then be envisioned.

Aside from the known benefits of cell-based test systems, fish cells have other advantages: due to their cold-blooded metabolism, fish cells in contrast to mammalian cells can be stored at cold temperatures without having to be frozen. If the temperature rises, the metabolism of the fish cells is activated. Additionally, they can be cultured in special media at room temperature without additional CO_2-gas exposure. This is an enormous advantage for tests in the field.

Under these conditions it was possible to establish an assay that uses fish cells in an impedance-based system (xCELLigence® RTCA from ACEA Biosciences) for the fast detection of cell-damaging substances. It became apparent that fish cells react quite similarly to mouse or human cells that are used in established cell tests.

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FROM THE WATER – FOOD FOR ANIMALS AND HUMANS

ALGAE – NATURAL RESOURCE RESERVOIR WITH GREAT POTENTIAL

In Asia, algae are appreciated as healthy food and are part of the traditional diet. Today, it is known that algae produce numerous high-quality substances including vitamins, pigments, essential fatty acids, amino acids, and even pharmaceutically active agents such as antibiotics. Every one of these ingredients makes the application of algae interesting, whether as high-quality food, food additive, or as a substitute for synthetic substances in the cosmetic, chemical, and pharmaceutical industries. Algae are undemanding – sun light, carbon dioxide, as well as nitrogen in the form of ammonium or nitrate, and phosphate ensure rich growth. The composition of the end product can be controlled using specific culture conditions. Harvesting can be done all year long. For downstream processing algae biomass offers further benefits: it is homogenous and does not contain lignocellulose, additionally the water from aquaculture can be recycled, and the waste material contains a lot of nutrients and can thus be further exploited. Special methods have been developed to further process the marine raw material from algae and fish.

High-quality additives for food processing

The positive effect of a targeted diet is undisputed in the context of many health impairments and in certain phases of life. Food additives simplify and increase the choice of suitable foods. Also for these food supplements, consumers prefer natural variants over synthetic additives. Algae, with their high content of nutritionally valuable substances, are an especially promising natural source of raw material.

Antioxidants

Using a simple and inexpensive method scientists from the Group have isolated an extract with antioxidant effects from the macroalgae *Fucus vesiculosus*. One project is aimed at using this extract as a food additive instead of the synthetic antioxidants used to date, for example to prolong a product's shelf life. This would allow these products to carry the "Clean Label". This and other methods for isolating and subsequently analyzing algae extracts are used in the TFAL (Technical Center for Applied Food Research, see p. 37).

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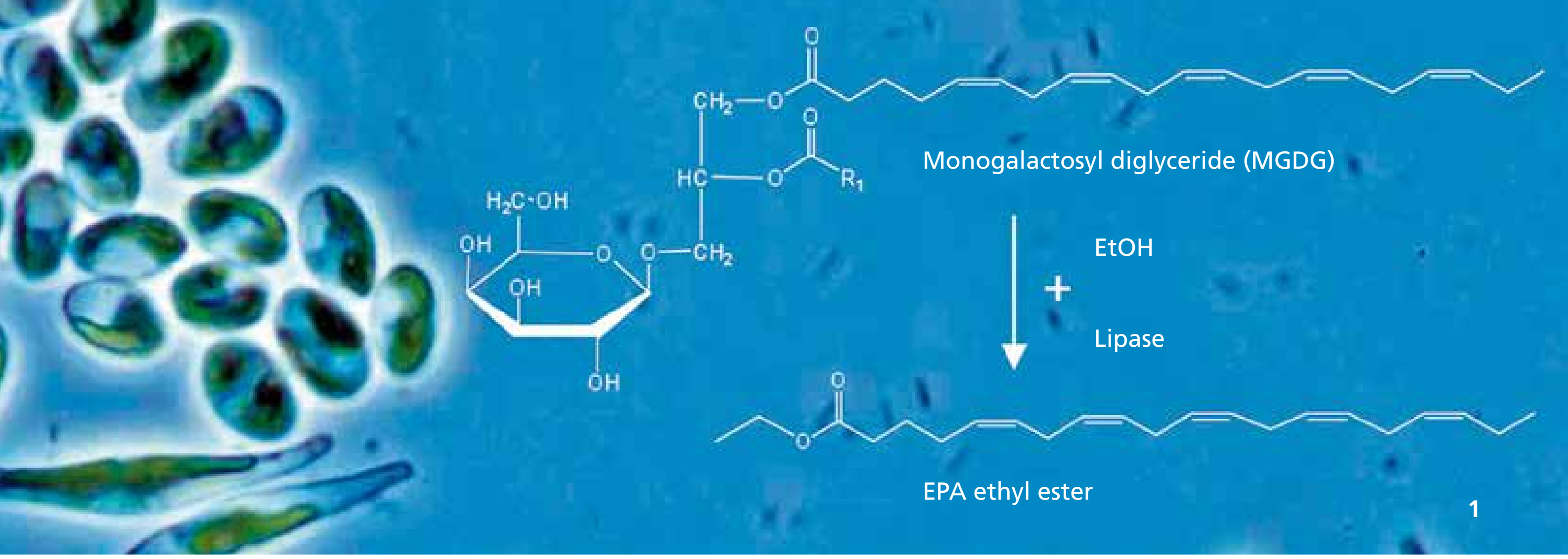

Peak performance of midgets – omega-3 fatty acids from microalgae cultures

The marine microalga *Phaeodactylum tricornutum* produces the highly coveted omega-3 fatty acid. In an ideal combination, it meets all the important criteria of an industrially exploitable EPA (eicosapentaenoic acid) producer: rapid growth and high EPA content, yet a purely photoautotrophic production – with only sunlight as energy source and carbon dioxide as carbon source. The algae are cultured in the flat-panel airlift (FPA) reactor developed by the Fraunhofer Gesellschaft (see p. 33). Under laboratory conditions, all relevant operating parameters and medium components were studied in detail and optimized. By means of this operating mode adapted to the organism, productivity levels of up to 2.0 grams dry material per liter and day can be reached already at medium light intensities. Outdoors, the mean annual yield is between 530 and 1500 mg dry substance per liter and day. The EPA content is between 5.5 and 6.5 percent of the total dry biomass, both in the laboratory and outdoors. This results in outdoor annual average EPA productivity levels in the range of 40 to 65 mg EPA per liter and day.

For further processing, the extraction using supercritical fluids (SCF) is especially beneficial: Since there are no traces of solvent in the extract, it can be directly processed and made available to the market as a food supplement. Furthermore, energetically positive methods are available for further processing of the remaining biomass.

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High-quality products, rich in omega-3 fatty acids due to gentle processing

Whether in algae, fresh fish, or fish oil, omega-3 fatty acids are almost odorless in their natural form. However, if they come in contact with oxygen, they are oxidized. The resulting degradation products not only reduce the quality of the raw material, but furthermore produce a fishy odor.

To allow the healthy marine omega-3 fatty acids to be added also to foods such as meat products, a method protecting the sensitive fatty acids from oxidation is necessary. Scientists from the Fraunhofer Group discovered a special emulsion system that ideally combines the effects of different antioxidants: some of these substances directly protect from oxidation, others support this effect, and still others eliminate compounds that accelerate fatty acid degradation. Thus, oxygen first has to pass through a cascade of shields before it can react with the omega-3 fatty acids. The emulsion can be individually adjusted depending on the product. Only the type and density of the cascade are altered.

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1 *Algae, raw material with high-quality omega-3 fatty acids.*

Plant protein for predatory fish

The popular fish species salmon, rainbow trout, or mackerel require in their diet certain proteins and fatty acids. Currently, this demand is usually met by adding fish meal or fish oil to the feed or by feeding fish offal, bycatch and wild fish catch. Thus, fish farming in aquaculture only partially protects natural fish resources. Within the Fraunhofer Group for Life Sciences two feed alternatives that have no adverse effect on natural fish resources were developed:

- Plant raw material from domestic production such as grain legumes or waste material from oil production contain significant amounts of valuable ingredients such as proteins, lipids, and natural antioxidants. Optimized extrusion and dry fractionation methods substantially increase the nutritional value of the starting product. Protein concentrates thus obtained have shown very good digestibility as a feed ingredient for Atlantic salmon.
- In vitro cultured fish cells could also serve as a suitable feed additive for aquaculture. Furthermore, they could be used to isolate proteins or other bioactive substances. To enable sufficient supply of biomass for such applications, methods for mass production in a bioreactor are currently being developed in the Group.

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Fish to everyone’s taste

Some people just do not like the taste of fish. Thus, they find it difficult to follow the physician’s recommendation to substitute red meat by fish as far as possible. For these people food researchers in the Group’s own technical center developed numerous different processed foods such as fish-based sausages and cold cuts, burgers, or doner kebab. Their basis is meat from African catfish (*Clarias gariepinus*) bred in the Group’s own closed circulation system. The sensory characteristics and texture of the end product are of high quality and the products can be made such that they no longer taste of fish. These products are also a good alternative for people who do not eat pork or beef for religious or ethical reasons.

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WELCOME TO THE GROUP!

The Fraunhofer Institution for Marine Biotechnology EMB in Lübeck has been a full-fledged member of the Fraunhofer Group for Life Sciences since 2013. In a talk with Prof. Charli Kruse, director of the EMB, Prof. Rainer Fischer, chairman of the Fraunhofer Group for Life Sciences and director of the Fraunhofer Institute for Molecular Biology and Applied Ecology in Aachen, discusses the institution in Lübeck, its scientific work to date, and its potential for the future.

Fischer: Your institution has developed to an independent Fraunhofer Research Institution for Marine Biotechnology from the project group "Cell Differentiation and Cell Technology" that was founded in 2004. Could you explain how the development occurred and also how the scientific focus developed?

Kruse: The project group studied the application potential for novel gland tissue stem cells. Multipotent stem cells can be isolated, for example, from pancreas, salivary glands, and sweat glands. They were initially being tested for use in regenerative cell technologies. Later, it was demonstrated that these cells could be isolated from very different species including different mammals and even amphibians and fish. Successful production of sturgeon and rainbow trout cell cultures led to the working group for aquatic cell technology being established, further investigating the biotechnological application potential of fish cell culture. In the past years, other projects in the marine area were initiated with funding from the federal and state governments, so that new groups such as aquaculture and lab device development could be established. I am very happy to personally support the thematic focus of "Marine Biotechnology", because it is a return to my earlier scientific roots. I started my scientific life with the study of marine biology, and I also wrote my dissertation in this area.

Fischer: Where do you see the greatest application potential for blue biotechnology?

Kruse: Society is currently faced with a great challenge. It must change from an economy based on an extensive use of resources to a biobased economy. Biotechnological developments and their economic exploitation should be characterized by sustainability and, thus, rely on renewable resources. To date, however, only one percent of biotech companies are engaged in blue biotechnology. There is thus enormous potential for growth. Therefore, I am sure that the exploitation of aquatic and marine resources will be introduced in various industries. I believe that the potential of using bioactive substances from marine organisms for drugs and cosmetics is especially promising. Even the development of new materials such as glues, paints, or algae-based insulation materials

Prof. Rainer Fischer, Aachen

Prof. Charli Kruse, Lübeck

opens new markets for blue biotechnology. A large application field will be developing in the area of food and feed production. The raising of fish, algae, and other marine organisms in aquaculture facilities will complement traditional farming.

Fischer: In which areas do you see value-adding possibilities for aquaculture in Germany?

Kruse: The EMB focuses on the development and establishment of integrated multitrophic aquaculture (IMTA), which is a co-culture of organisms of different trophic levels (e.g. fish, muscles, algae) in the same system. Our interest is both on land-based facilities and on those located in open waters. Land-based systems have the advantage that the substance flow between the individual compartments is easy to control and regulate. In addition, they offer a good possibility for comprehensive analyses. Another great benefit is that a land-based IMTA has no effect on the existing ecosystems. However, to enable an economic operation, some new technologies still have to be developed to compensate for the high purchasing and operating costs. An IMTA in open waters can only be realized if the technology has been developed to a point where a hazard for the surrounding ecosystem can be excluded.

Fischer: An important division of the EMB focuses on biomedical issues i.e. red biotechnology. How does the link between red and blue biotechnology work?

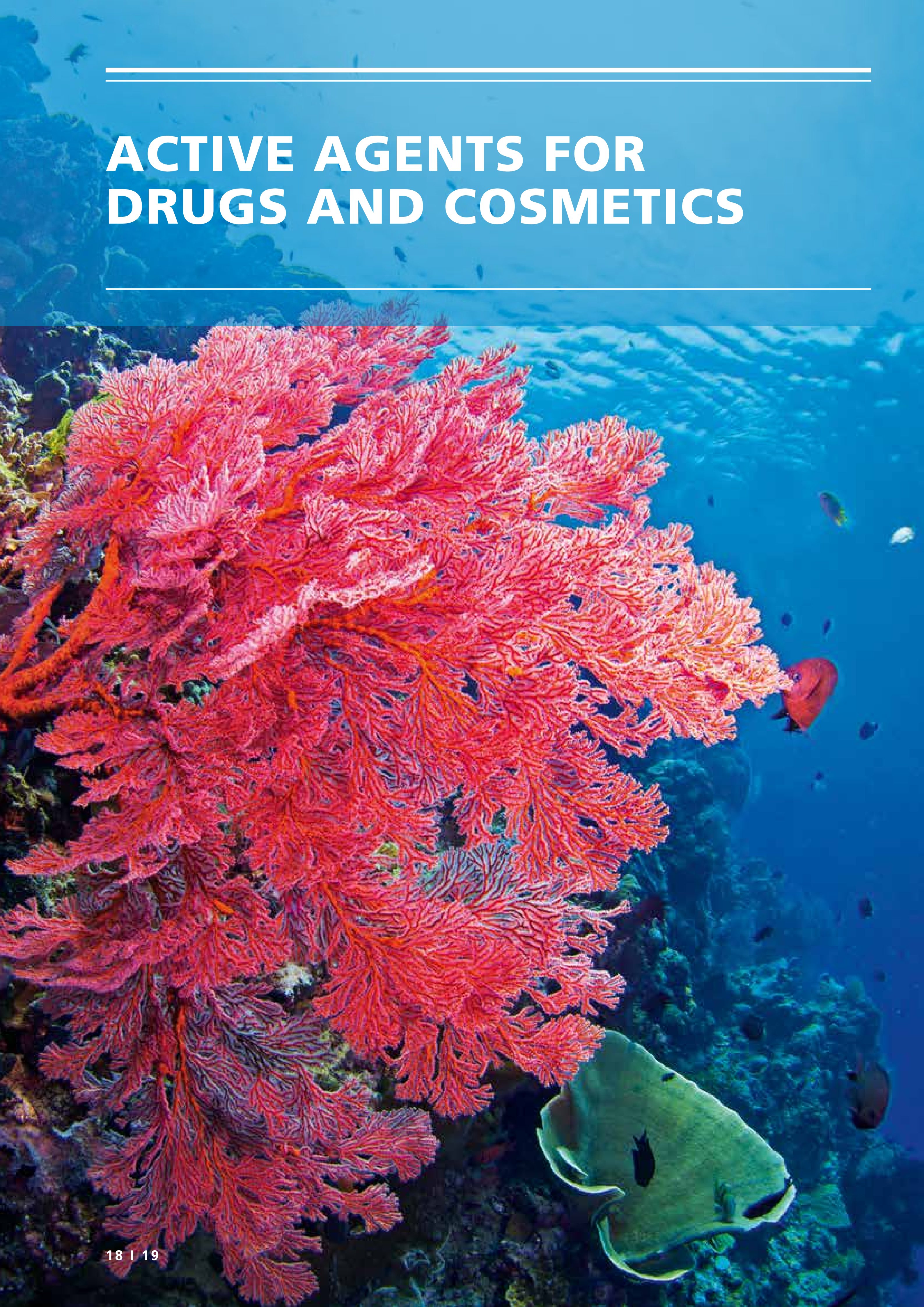
Kruse: The EMB has many years of experience and great expertise in isolating and handling eukaryotic cells. Aside from the traditional mammalian cells of red biotechnology, fish cells are especially suited as test systems for environmentally relevant issues or safety pharmacology studies. But we also isolate different biomolecules from animal cells and also from macroalgae. These can be successfully used in medicine or in the food industry. However, it is necessary to establish new technologies to control their mass production. Especially key technologies such as the expansion of adherent cells in bioreactor systems are being developed at the EMB in interdisciplinary teams.

Fischer: Are you concerned about a lack of academic junior staff and professionals in the expanding area of "blue biotechnology"?

Kruse: Luckily, especially in the past few years, we have found many young, well trained, and dedicated scientists in this area who identify with these project topics. The exchange of scientific information is also very lively in this field. Initiated by the German Society for Marine Research the annual conference YOUMARES offers an exchange platform for young scientists in the marine area. This conference is also supported and co-organized by the EMB.

Fischer: Welcome! We are looking forward to future collaborations.

ACTIVE AGENTS FOR DRUGS AND COSMETICS



SIMPLE ORGANISMS AND COMPLEX COMPOUNDS

Carotenoids and terpenoids are responsible for colors and smells in our natural surroundings. Even today, most of these compounds are isolated from natural resources, because synthesis of their chemical analogues is expensive and complicated. Algae and corals produce a broad diversity of carotenoids and terpenes in different amounts. The Fraunhofer Group for Life Sciences is very active in the development of new sources and processing methods for these valuable substances.

Watermelon snow

At the beginning of the 19th century, there was still speculation about the origin of red snow fields in the polar regions. However, meanwhile it has become clear that specific algal species that have adjusted to the extreme conditions produce special pigments. These offer protection against the high light and UV radiation intensity in these regions of our earth.

Broad variety of products from snow algae:

alpha- and beta-carotene, lutein, neoxanthin, violaxanthin, antheraxanthin, zeaxanthin, echinenon, hydroxyechinenon, adinoxanthin, canthaxanthin, astaxanthin, alpha-tocopherol

Snow and permafrost algae respond to the stresses of high radiation intensity and low nutrient availability in their natural environment with the production of secondary carotenoids, mostly in the form of the dark red pigmented astaxanthin and other antioxidants such as alpha-tocopherol (vitamin E). Especially the strong UV radiation leads to the formation of radicals in algal cells, to which they respond by antioxidant production. As a result of different protection mechanisms, different algal species may show very different pigment patterns.

For large-scale production of carotenoids by means of this mechanism, it is beneficial to use a two-phase process. First, under optimal nutrient and light supply, a large amount of biomass is grown. Then, in the second phase specific stressors induce the synthesis of secondary carotenoids. At the same time, most algae produce large amounts of lipids as an internal storage system for the fat-soluble carotenoids. These lipids are also valuable raw material resources.

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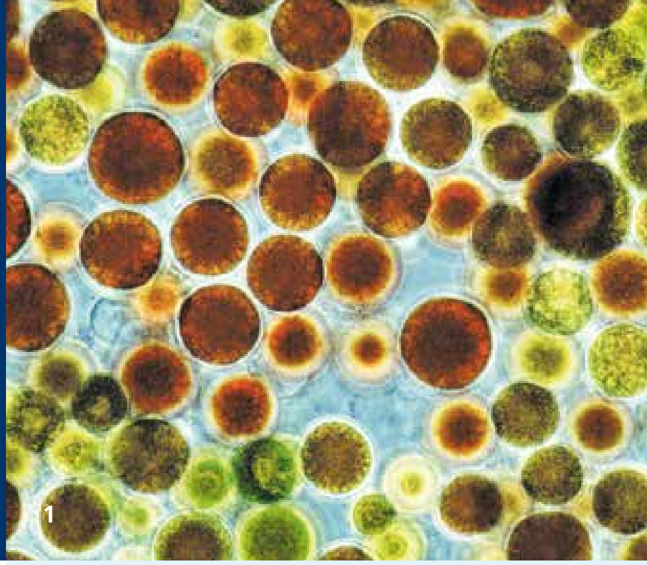
1 *The astaxanthin-producing single-celled alga Haematococcus pluvialis.*



Astaxanthin, the color of salmon and flamingos

Under certain conditions, the single-celled freshwater algae *Haematococcus pluvialis* SAG 192.80 also accumulates the red ketocarotenoid astaxanthin – after all up to five percent of its biomass. This high yield is achieved by optimizing various process parameters such as light intensity as well as CO_2 and nutrient concentration. Economically favorable is a culture in photobioreactors under outdoor conditions. In outdoor panel airlift reactors *Haematococcus pluvialis* reached biomass concentrations of up to 10 grams dry substance per liter. An important precondition for industrial astaxanthin production is met with this high cell density.

The flat panel airlift reactor that was specifically developed to culture *Haematococcus pluvialis* algae is a closed and protected system. The absence of contamination allows direct further processing of the algae products (see p. 34).

According to the holistic approach of the Group, extraction of the raw material of interest is followed by another step, namely fermentation of the remaining biomass for biogas production. After electricity and heat generation in a combined heat and power plant, the resulting CO_2 can be reintroduced into the cycle of algae biomass production cycle.

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Potential of algae products

Because of its strong antioxidant effect, astaxanthin can be used as a food supplement e.g. to prolong the shelf life of food. As a pigment it can be utilized in aquaculture, for example, to impart the natural red color to farmed salmon, and also in cosmetic products.

Lutein and alpha-tocopherol (vitamin E) are used as food supplements and as additives to feed.

Coral enzymes for synthesizing terpenoid drugs

Corals are of special interest to natural products chemists, not only because of the famous beautiful reefs they have formed. In fact, corals are able to produce complex compounds from the group of terpenoids that have become indispensable in the cosmetics, chemical, and pharmaceutical industries. In their metabolism corals produce substances that can be made only by multiple-step chemical synthesis. Fraunhofer scientists are working to decode the natural biosynthesis of these agents to enable development of a sustainable production process that will utilize resources economically and in an environmentally compatible way.

Some crucial enzymes have already been identified. Current activities are aimed at making these available for fermentation production processes. However, the scientists will not content themselves with enabling fermentative production of the known substances. Another goal is to generate new substances with altered characteristics by targeted intervention in the catalytic steps so as to generate novel nature-based active ingredients for the medical challenges of tomorrow.

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Zebrafish can detect neuroactive and pharmaceutical substances

Neuroactive substances can pose a considerable hazard to man and the environment, yet the available information on their neurotoxic potential is often insufficient. Current methods for neurotoxicity testing (performed on rodents and chicken) are not only ethically questionable and expensive, but in addition the results cannot always be transferred to humans. Potential hazards to the environment and the organisms therein are largely disregarded.

A screening system based on fish embryos and fish larvae for testing of neuroactive substances would be a worthwhile alternative. Development, structure, and function of the mammalian nervous system are sufficiently conserved to ensure transferability from fish to mammals/humans. Zebrafish (*Danio rerio*) eggs and embryos, which are used for the tests, are available in large numbers in the laboratory. Due to their small size (egg diameter about 1 mm), good transparency, and rapid embryonic development (about 48 hours after fertilization), they are ideal for screening applications. Morphological, biochemical and molecular test parameters can be measured microscopically, partly in real time in live embryos (live imaging) or in fixed embryos, and behavioral changes can be observed.

The zebrafish embryo model enables testing in a miniature format and promotes image and video-based evaluation methods and thus non-invasive techniques, thereby allowing fast, automatable, substance saving (medium- to high-throughput) testing. In the Fraunhofer Group for Life Sciences, tests with neuroactive insecticides (e.g. inhibitors of the nicotinic acetylcholine receptor) and active pharmaceutical ingredients have already been successfully performed.

Promising is a combination of behavioral tests with so-called transgenic zebrafish embryos. They specifically produce green fluorescent protein (GFP) in certain cell types that can be used as cellular marker. Scientists of the Group are already working with a transgenic fish line that has fluorescent CNS glial cells.

Meanwhile, scientists of the Group are approaching yet another goal, using the zebrafish embryo-based test systems more and more to screen compounds for pharmacological activity. This could be very interesting, not least for the identification of marine-derived active ingredients.

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IMPULSES FOR MEDICINE

ON THE TRAIL OF EVOLUTION

The development and spread of all life started in water. Why then should not simple structures from the aquatic world serve as flexible starting materials and models even for higher organisms? Is there perhaps a rudimentary mutual basis? This basic idea has already been part of different projects of the Fraunhofer Group for Life Sciences and has led to remarkable results. For example, alginate offers a wide application spectrum today. The problem of biofilm development arose for the first time with the progress in transplantation and intensive care medicine. For this issue as well, life-supporting processes have developed in water over the course of evolution, and these are now being exploited by the Group's scientists for modern medicine.

Alginate, a flexible basis for applications

Larger algae such as brown algae constantly have to adjust to their environment – they bend elastically in a stormy, turbulent sea and stand erect and stable in calm weather. This flexibility is provided by the typical structural components of algae, the alginates. Embedded in the cell wall, they are present both in colloidal form dissolved in water and as insoluble polymer gels.

Chemically, alginates are pure carbohydrates. Once purified, they contain only the slightest traces of contaminants such as protein. Only the ratio of its two basic constituents, mannuronic and guluronic acid, determines its characteristics. Given that alginate is not cytotoxic and biocompatible polymerization can be performed, it perfectly fulfills the preconditions for use in medical and biomedical applications. Further modifications, including the formation of microcapsules, result in an extremely broad range of applications, which the experts of the Fraunhofer Group for Life Sciences master and further explore.

Special expertise of the Group includes alginate microcapsules (also with specially coatings), which are used for immunoisolated transplantation of islets of Langerhans, as bioreactors, carriers for controlled-release applications, and to culture human stem cells. Alginate filaments are used for cochlear implants. Alginate foils and coatings can prevent or promote cell colonization (whichever is desired) – as a protection for technical implants or as capsule foil for implantations with local fixation. Laser-structured alginate opens up the possibility to connect immobilized cells, for example, nerve cells.

Already during alginate production, the future application has to be taken into consideration to generate the most suitable material. The Group has profound knowledge with regard to all steps, from harvesting via extraction to the final modification. For process scale-up, interested clients can also benefit from the Group's experience.

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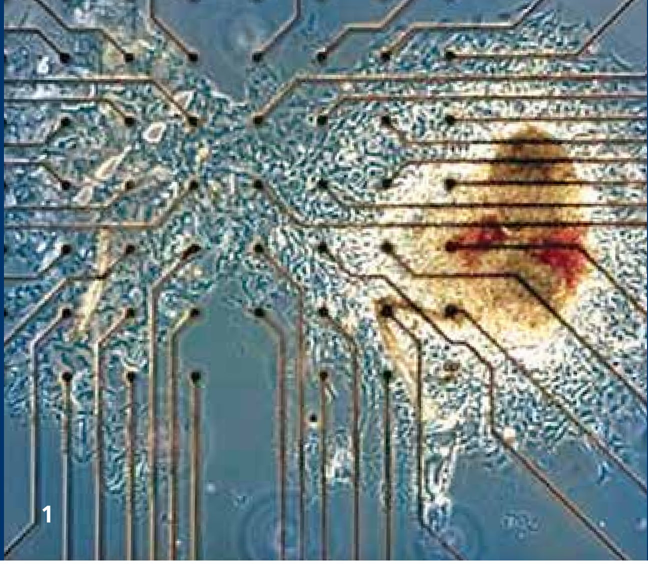

1 *Multielectrode array with aggregated rainbow trout cells.*

Novel model systems for safety pharmacology

New active pharmaceutical ingredients have to go through a long series of testing. Conclusive models in the early phases of drug development have proven successful in many areas of medicine, and have improved the efficacy of drug research. This experience as well as ethical and economic reasons motivate scientists to do intensive research and development in order to establish further models.

In this context, the Group has made another widely acknowledged contribution: a cardiac model system based on spontaneously contracting cell aggregates (SCCs) from rainbow trout (*Oncorynchus mykiss*) larvae has attracted great interest among professionals. A patent for the production method of SCCs has been granted already. Meanwhile, SCCs have been pharmacologically characterized and have been evaluated for their suitability as a model system, taking into account a variety of parameters.

Immunohistochemical staining has demonstated the presence of an ERG potassium channel or the fish homologue in SCCs. Electrophysiological measurements using multielectrode arrays demonstrated the channel's functionality. In these assays, SCCs responded to a blocking of the ERG potassium channel by the ERG blockers dofetilide and terfenadine.

Measurements using the sympathomimetic drug isoproterenol have shown that the model system is suitable to demonstrate effects on cardiac impulse conduction: the positive chronotropic (increased contraction rate), the positive inotropic (increased contraction intensity), as well as the positive dromotropic (accelerated impulse conduction) effects of isoproterenol were detected in the model using this method.

Thus, with SCCs an effective and reproducible cardiac model system is available – ready to be used in safety pharmacology studies on behalf of industrial clients.

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Strategies against biofilms in nature

Macroalgae populate the coastal zones of the oceans where there is strong competition for light, room, and nutrients. This results in the phenomenon termed epibiosis, where algae are used by other organisms as colonization substrate. In the course of evolution, algae have developed mechanical and chemical defense strategies. Among others, they produce protective agents that affect biofilm formation on the surface. These can be very valuable for medical applications, for example, in the fight against the increasing prevalence of nosocomial infections.

This strategy has already been proven for the alga *Delisea pulchra*: *Delisea pulchra* controls biofilm formation on its surface by producing halogenated furanones which interact with the bacterial quorum-sensing system. Such quorum-quenching substances – in this case halogenated furanones – have the potential to become a new generation of antipathogenic substances. They prevent bacterial infections by keeping surfaces free of unwanted colonists.

Nosocomial infections are caused among others by formation of bacterial biofilms on medical products such as implants or central venous catheters. Bacteria imbedded in an exopolysaccharide matrix in a multilayered biofilm are inaccessible by the cells of the immune system and are also protected against antibiotics. This makes these infections hard to treat, also because of the increasing resistances against standard antibiotics. Often the only resort is removal of the implant. The new class of protective agents from algae may be an alternative to prevent biofilm formation on surfaces from the beginning, for example, by using them as coating for medical material and wound dressings.

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Ice-structuring proteins as antifreeze

In their natural environment, snow algae are constantly in danger of freezing. Aside from mechanical destruction of the cell membrane, this can lead to osmotic stress and desiccation. To withstand such unfavorable conditions, some snow algal species can transform into thick-walled resting stages, while others accumulate intracellular substances such as sugars and sugar alcohols to reduce their freezing point or to stabilize their cell structure. Some special psychrophilic snow algae release ice-structuring proteins (ISP), formerly also called antifreeze proteins (AFP), into the near cellular environment to influence ice crystal formation directly.

ISP are highly resistant proteins that irreversibly bind to ice surfaces and actively modify their growth. Instead of uncontrolled, large ice crystals, the interaction of ISP with the lattice structure of the frozen water leads to formation of fine crystals, whose growth in addition is inhibited over time. Such natural antifreeze substances could be applied in the medical sector as alternative cryoprotectants for cells and tissues or as additives for hypothermal perfusion of transplant tissue. Various applications are also conceivable in the food sector.

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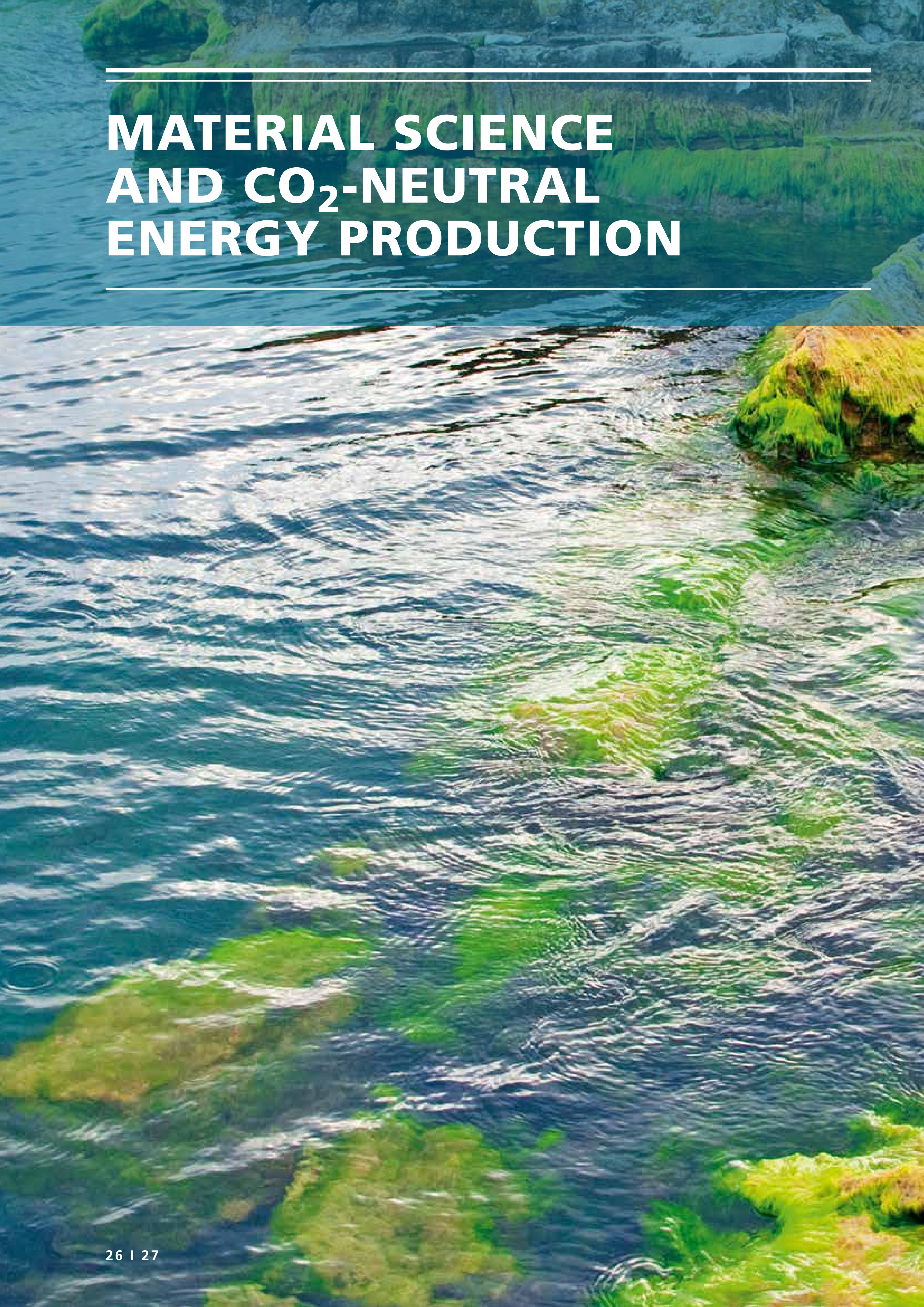

MATERIAL SCIENCE AND CO_2-NEUTRAL ENERGY PRODUCTION

FROM AQUATIC BIOMASS TO CHEMICAL PRODUCTS

Aside from petroleum and natural gas, the focus is now on biomass as a renewable alternative carbon source for chemical products. The requirements on potential biogenic raw materials include sufficient, preferably continuous supply and an efficient, resource-conserving exploitation strategy for these raw materials. The Fraunhofer Group for Life Sciences focuses on material utilization of biogenic residual and waste materials such as crab and shrimp shells to extract chitin and the use of aquatic microalgae. The special advantages of microalgae are that they grow faster than terrestrial plants and that they do not need a land-based crop area.

Chitin – marine biobased raw material

Even for non-professionals, it is easy to see that cellulose is first on the list of the most common biopolymers. Second is chitin, which is produced by some fungi as well; in the first place, however, it is what crustacean shells and insect exoskeletons are made of. Worldwide, the processing of shrimps, crabs, and prawns alone results in more than six million tons of chitinous waste annually. In the EU, more than 100,000 tons of chitinous shrimp shells end up as trash – waste material literally begging for further exploitation.

Chitin can be degraded by many bacteria using chitinases: the biopolymer, a linear, insoluble homopolymer made of beta-1,4-linked N-acetylglucosamine units (NAG), is broken down into oligomers or monomers. In Asia, the polymer chitosan is already being produced from prawn shells. It is the base material for filters and foils and can also be used for wound dressings.

The Fraunhofer Group for Life Sciences is focusing on enzymatic processes that degrade chitin to monomers, which then become the base elements for polymer chemistry. Using novel chitinases that are not yet protected by patent, it was shown that chitinase production is associated with growth and that the enzymes are secreted into the medium. This is the basis for the Group's two-step method: the enzymes are first produced and then, after separation of the biomass, are used for chitin degradation. This method has already been employed to completely degrade a chitin suspension to NAG.

Shrimp shells – from waste to a valuable raw material for chemicals

ChiBio is the name of an EU-funded project which connects ten national and international partners under the leadership of the Fraunhofer Project Group BioCat in Straubing. Their mutual goal is the development of different material and energetic applications for crab shell waste products, similar to a biorefinery.

1 *Starting material for chitin: residual material such as shells from crabs and crustaceans.*

In the first processing step, the shell waste is pretreated to make it stable for storage and transportable. Resulting remnants can be directly fermented and energetically exploited.

Then chitin or chitosan is broken down into its monomer sugar units N-acetyl-glucosamine or glucosamine. The biocatalytic degradation procedures developed under ChiBio use chitin-degrading enzymes from prokaryotic and eukaryotic organisms such as *Trichoderma*, *Aspergillus*, *Bacillus*, and *Aeromonas* strains. This work is complemented by the development of own chitinases and chitin-deacetylases.

Currently, intensive work is being done to optimally tune the enzyme cocktails and to adjust them to industrial processing conditions and production methods on a technical scale. Two functional groups are attached biochemically to the glucosamine that is isolated. Thus, new biobased polymers can be connected. To isolate functionalized N-containing heterocycles, traditional methods of fermentative degradation and modification are used, as are methods of cell-free biotechnology. Most of the enzymes that are needed are now available in recombinant form. They are currently being optimized by enzyme engineering.

Like the initially separated proteins and fats, all other biobased byproducts resulting along the processing chain can also be fermented to biogas as an energy carrier.

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Antifouling – the method of starfish

Organotin compounds, formerly used to prevent the growth of microorganisms on the hulls of ships, represented a substantial environmental hazard in harbors and coastal regions. This prompted the Fraunhofer Group for Life Sciences to search for substitutes in dealing with the fouling problem.

The idea was that sea organisms most likely have also developed chemical defense mechanisms to prevent growth of microorganisms on their surfaces. To find out the University of Rostock screened extracts from different sea organisms with a special test. The extract from the starfish *Asterias rubens* actually showed such an activity. Scientists of the Group tried to identify the chemical compounds responsible for this activity. To this end, the multiple-component mixture of the extract was separated and the structure of the compounds contained in the individual fractions was elucidated. For this task HPLC-NMR-MS coupling was intensively applied. The compounds identified as active belong to the substance class of asterosaponines.

The results of the structural analyses were published in several articles.

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Resource-conserving energy carriers

Aquatic biomass can also be used for energy generation. Various microalgae produce oils that can be implemented as biofuel. Due to the missing lignin fraction, algae residual biomass can be excellently fermented to biogas as energy carrier. Residual material from chitin extraction whose ingredients cannot be used any further can then be utilized to produce biogas according to the principle of a biorefinery as shown in the project ChiBio (see p. 27-28).

Complete utilization in biorefineries

Biorefineries are characterized by a holistic approach to bioconversion. They take into consideration the interaction of resource extraction with processes of material and heat transport, of subsequent product isolation and purification, circulation, as well as coupling and cascade utilization. Parallel or subsequent use of the residual mass for energy production completes the cycle and increases overall efficiency.

Energetic exploitation – fermentation of algae biomass to biogas

Like all plants, microalgae store sun energy in the form of organic carbon compounds using photosynthesis. These are valuable energy stores and also supply carbon elements for chemical exploitation. Fermentation of biomass to biogas, therefore, corresponds to carbon dioxide-neutral energy production. Lignocellulose-free algae biomass, e.g. from *Chlorella vulgaris* or *Phaeodactylum tricornutum,* can be nearly completely converted in a two-step fermentation facility. In an actual process, after extraction of the desired raw materials from algae, the residual material can be converted to biogas in a continuous two-step gaslift loop reactor under mesophile conditions. This process has already been implemented with various types of algae. As expected the composition of the biogases and the yield varied according to cell ingredients, cell wall components, and cell wall stability. Especially the cell protein content played a major role. The biogas yield was between 280 and 400 liters per kilogram dry residue (oTR) depending on the type of algae.

Oil or biodiesel from lipid-rich algae

Different microalgae store carbon and energy in lipid form. Many algae species build these storage lipids if growth is limited by nitrogen or phosphate deficiency. Under optimal conditions the lipid content can reach up to 70 percent. Storage lipids are mainly triacylglycerides with the main fatty acids C16:0, C16:1, and C18:1. These are primarily of interest as fuel – either as oil or after transesterification as biodiesel. Lipid production only occurs if the supply of light and also of carbon dioxide stays sufficiently high. If such algae are specifically selected for their oil content and cultured accordingly, the production of oil and biodiesel from algae lipids could be an alternative to the use of other plant oils as energy carriers. (For details on the culture conditions, see p. 33-34). Due to the missing lignin fraction, the algae residual biomass can be further exploited by fermentation to biogas as energy carrier.

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ON THE TRAIL OF THE LOFOTEN WITH ARVED FUCHS

First use of the mobile stem cell lab

Fish and also mammalian or human cell cultures have many uses in biotechnology, medical engineering, or pharmaceutics. Fish cells can be used as test systems to detect environmental toxins and thus replace animal experiments. They can be employed to study viral diseases and can, therefore, also be utilized to develop vaccines.

Cell cultures are furthermore the material archived in the German Cell Bank for Wildlife "Alfred Brehm" – CRYOBREHM, one of the most modern bioarchives worldwide. It was founded in cooperation with the Tierpark Hagenbeck and the Zoo Rostock. By collecting living cell cultures of wild animals "CRYOBREHM" preserves comprehensive biological information in live form. Therefore, this cell bank complements the traditional natural history archives by additional options for innovative scientific use.

To establish new cell cultures it is especially important that the starting material be fresh. A mobile laboratory, for example, on a truck, conveniently allows sample collection and processing directly on site, even in remote regions. Furthermore, different samples can be frozen and stored in a nitrogen tank included in the mobile lab.

In January 2013, the off-road-capable lab truck of the Fraunhofer Group for Life Sciences started for the first time for a test run under extreme conditions. This truck includes a completely equipped cell culture laboratory which makes it possible for the scientists to work completely independently and to do their work in the most remote and exceptional places – right there where they collect the fresh samples.

In the middle of winter, this first trip was supposed to go to rough Norway. The aim of the journey was to test the truck with all its functions in a real environment. There were many questions: Would the installed equipment survive the long, arduous journey to the north of Europe? Will it be possible directly on site to take fresh samples, perform complete cell isolation and establish cell cultures? Can the samples be transported over long distances?

Aside from the logistic planning and equipping the truck with all additionally necessary media, solutions, and chemicals, preparation of the expedition also included completion and filing of the customs documents for the temporary import of goods to Norway. On January 5, 2013, the journey started. The truck was driven to Hirtshals (Denmark) and from there transferred by ferry to Bergen, from where it moved on to the Norwegian coast. In a small coastal town, the researchers obtained codfish from a fisherman. They isolated cells from several freshly removed organs and cultured these in the mobile lab. The lab truck was parked directly at the pier next to the fishing boat.

A highlight of the trip was the meeting with the polar explorer Arved Fuchs. He was traveling with his film team along the Norwegian coast at that time. In a documentary the adventurer follows the historical footsteps of the Lofoten fishermen. For centuries, they have been leaving around Christmas for northern Norway to catch the treasured cod there. Like many

FISHERMEN

others, the fishermen in Norway can look back on an eventful history with the rise and fall of modern cod fishing. The Norwegians, however, have succeeded in preserving their stock by strict quota regulations and consistent action. Arved Fuchs is following a route where the cultural and economic importance of historical cod fishing is directly related to today's fishermen, spanning the gamut from traditional "wind-driven fishing" to a trendsetting way of acting.

The further development of molecular and cell biological technologies and establishment of cell cultures of as many important fish species as possible (such as codfish) opens up a large diversity of possibilities for sustainable stock management in the future.

On January 9, 2013, the successful isolation of cells from cod tissue was completed and the lab truck set out for Oslo through the mountainous roads of the Norwegian upcountry to return to Germany from there. Unfortunately, due to national regulations on the management of wild living marine resources, the isolated codfish cells were not allowed to be exported and had to be disposed of in Norway. This was another valuable experience for the planning of future expeditions. On January 11, 2013, the truck and the research team safely arrived back in Lübeck.

After this "baptism of fire" the mobile laboratory is now available for further projects. The Group is offering the use of this truck in cooperation with other research institutes, universities, or industry to support research and development on the road to the future.

Marina Gebert

Expert meeting in the mobile stem cell lab: polar explorer Arved Fuchs and Dr. Marina Gebert.

TECHNOLOGIES
SERVICES
EQUIPMENT

PIONEERS OF INNOVATIONS

Aside from special knowledge and experience, the right equipment is essential for research and development in the area of blue biotechnology. Not only have the Group's experts acquired broad knowledge in a wide range of R&D projects, but they also have at their disposal a large diversity of custom-made technical equipment, and they are always pleased to make use of both on behalf of clients. Besides contract research projects for clients, they offer services such as scale-up, customization to meet special requirements, or cultivation of the client's own cultures on demand.

Photobioreactors – greenhouses of the future

Flat-panel airlift reactor also for outdoor production

Natural algae growth in open ponds is slow and thus inefficient for mass production. Therefore, the Fraunhofer Group for Life Sciences has developed an inexpensive plate reactor for primary production of algae biomass as resource. It works according to the principle of airlift reactors. In contrast to previously developed reactors, the FPA reactor (flat-panel airlift reactor) enables complete intermixing: systematic stream guidance in the reactor induced by static mixers and a small layer thickness ensure ideal supply of light and substrate to all algal cells, resulting in a high concentration in the reactor.

The reactor itself can be manufactured at low cost by a deep-drawing process with plastic foil, creating two half shells including the static mixers. Scale-up to a reactor volume of 180 liters could be achieved for the FPA reactors.

A further scale-up can be reached by linking several reactor modules that are operated together. Intermixing of the individual reactors is achieved by introducing a carbon dioxide/air mixture or combustion gas or fermentation gas from biotechnological fermentation as carbon dioxide source. The modular setup allows each reactor to be controlled separately, so that the process control can be adjusted quickly and efficiently to outdoor conditions. In a pilot facility with 1 to 4 cubic meters of reactor volume, reactor modules are already being used under outdoor conditions for the production of algae biomass, using exhaust gas from combined heat and power stations as carbon dioxide source.

A larger module of these photobioreactors is currently being set up at the Fraunhofer Center for Chemical-Biotechnological Processes CBP in Leuna. With a reactor volume of about 10 cubic meters it is already at pilot scale.

To have a process that is light- and temperature-independent also in the outdoors, an automation concept with measuring techniques that are as simple as possible was developed. An outdoor pilot plant to produce lipid-containing biomass in 30-liter FPA reactors is already operating according to the common industrial standards with a memory-programmable controller (SIMATIC S7-1200, Siemens). All relevant parameters are continuously monitored online and the whole process is visualized on a display. The control software is modularized and, thus, can be easily applied in other production facilities.

1 *Phases of algae growth in a multiLoop photobioreactor.*

Individual program elements can be newly combined to control different production processes of algae biotechnology.

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multiLoop technology for culturing algae under controlled conditions

Microalgae as raw material for high-quality products must grow under sterile and controlled culture conditions. In the medical field in particular, photobioreactors have to fulfill special requirements. Many of the currently available systems cannot be sterilized or are insufficient for sensitive and more demanding algal strains.

The Group's multiLoop photobioreactor is not only extremely adaptable, but its efficient airlift system in combination with an optimal design furthermore prevents fouling and cell sedimentation. Since no mechanical pump and stirring devices are used, there is less cell damage from shear stress. The system can be sterilized in situ by heat or chemicals. The design allows the light source (LEDs or fluorescent tubes) and intensity to be flexibly adjusted to each organism and growth phase.

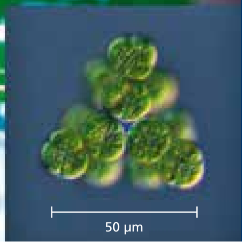
Production of algae biomass under outdoor conditions

Lipids

With Chlorella vulgaris a stable process with an automated control system has been established under outdoor conditions. The process is monitored and controlled exclusively via the reactor temperature and pH. The established process is independent of constant productivity and is, thus, suitable for use in outdoor production with changing light and temperature conditions.
*Maximum lipid productivity outdoors: 0.5 g fatty acids / (L*d); average biomass concentration at the time of harvest: 8.5 g / L.*

Astaxanthine

A two-step outdoor culture process with Haematococcus pluvialis also led to promising results: biomass growth rates of up to 0.25 g dry matter per liter and day, at cell concentrations of up to 2.5 g dry matter per liter.
Astaxanthine production is induced by high light intensity (direct sun light), nutrient deficiency, or inducers such as acetate and NaCl. If these factors are taken into consideration in batch processes, cell weight again increases by a factor of three to four. At the same time, intracellular astaxanthine reaches a level of up to 5 percent of dry cell weight.

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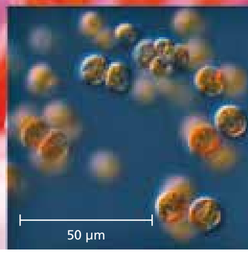

The systems consist of borosilicate glass components, which are flanged according to GMP guidelines and tested according to the European Directive 97/23/EC. Every reactor holds a CE certification for pressure equipment. The special construction according to the "multiLoop principle" allows the biotechnological exploitation of a wide range of phototrophic microorganisms.

Some microalgae need different conditions to build biomass and to produce the desired metabolites. These special requirements suggest the use of a two-step photobioreactor for culturing of such microalgae: the multiLoop technology allows the growth and synthesis phases to be separated in two reactors.

The change is particularly easy to follow in algae of the culture collection CCCryo, which are from permafrost polar and alpine environments. Their cells indicate the different stages by a change in color from green to red.

For a two-phase production systems, different versions of multiLoop reactors can be coupled. The production volume per module in the system currently used by the Group is 60 or 20 liters for the reactors covering the first phase and 30 or 10 liters for the second-phase reactors. To further increase the light input in the second stress/synthesis phase, the second-phase reactors, in addition to stronger illumination, have a smaller tube diameter. Transfer of cultures, re-filling of medium and also harvesting can be performed pneumatically. Aside from the temperature, other growth parameters such as pH and O_2-level can be monitored with sensors.

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CCCryo – Culture Collection of Cryophilic Algae

Originally intended as bioresource for their own research, the working group Extremophile Research and Biobank CCCryo set up a comprehensive bank of cryophilic algal strains (Culture Collection of Cryophilic Algae – CCCryo) that is probably unrivaled in diversity. Meanwhile, algal strains may be ordered by public and private research organizations via the working group's CCCryo website (cccryo.fraunhofer.de). The focus of CCCryo is on cryophilic, i.e. cold-loving glacier, snow, and permafrost algae. The algae were collected mainly during the working group's own expeditions to the Arctic (Spitsbergen) and the Antarctic (King George Island), but strains from other polar and alpine regions such as the European Alps, the High Tatras, New Zealand's Alps, the Rocky Mountains, Greenland, North Canada, and Alaska are also found in the biobank CCCryo. Aside from cold-loving algae, different mesophilic and some thermophilic algae are also cultured. Field samples from the last expeditions have extended the collection with eubacteria, cyanobacteria, and also mosses from cold environments. Currently, more than 380 strains are maintained as live cultures at +2 °C, +15 °C, and +30 °C, and some are also cryoconserved.

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New foods – recipes, processing, packaging

The high value of seafood, algae, and fish in the human diet is undisputed among nutritional scientists. Traditionally, this type of food has regional foci. Progress in processing and an extended product range could help win over new devotees to these foods.

The Group has two institutions that specifically work on food from aquatic origins, exploring their benefits to consumers and at the same time offering attractive products. This food research encompasses the development of different food additives and also of ready-to-use products including their packaging.

The "Technical Center for Applied Food Research" (TFAL) serves as a multifunctional research platform both for raw material manufacturers and raw material users of the food industry. For example, algae extracts are produced there from domestic macroalgae. These whole extracts can be used as additives to increase the shelf life of foods in a natural way. It is a special challenge for the scientists to develop right from the start an extraction method that complies with current food legislation.

Another food technical center develops complete products, with a separate group dedicated to fish-based foods. Special products from this technical center are, for example, "meat products" such as burgers based on fish meat (from an African catfish, *Clarias gariepinus*). The products display very high quality with regard to sensory properties and texture and can be produced so that they do not taste like fish.

1 *Deep-sea AUV TIETEK and research vessel ALKOR during a test run in the Baltic Sea.*

The fish comes from the center's own circulation culture facilities that can produce about three tons of fish annually. The center thus has direct access to fresh raw material is for development and sampling purposes. The energy that is necessary to maintain the water temperature at an appropriate level for the African warm-water catfish is provided by waste heat from the biogas plant of a farm. The produced food can be adequately packaged, stored, and evaluated by a trained tasting panel on site.

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Research at a depth of 6000 meters

Commercial exploitation of marine resources requires detailed information on the conditions in this environment. However, little is known as yet about the deep sea with its extreme conditions – less than about Moon and Mars. One reason is that exploration of the deep sea so far has been possible only with highly specialized and very expensive underwater vehicles.

The search for less expensive variants to explore the deep sea has led to a completely new construction which was guided by the following considerations: Automatically executable routine procedures should require minimum technical effort. A modular design offers flexibility for different uses and has proved to be a successful principle in the Fraunhofer-Gesellschaft's research environment. All vehicle parts are constructed such that they can go without a protective shell even at a depth of 6000 meters and an ambient pressure of 600 bar. Light and inexpensive functional units can be built with this so-called pressure-balanced construction method, and these are far superior to conventional units with pressure hull. Examples of these are pressure-balanced multi-frequency side scanners, 3D sonar cameras, obstacle avoidance systems, energy stores (up to 4 kWh), or drive systems. Their fitness for use was then proved in several sea missions with the developed TIETEK-AUV (autonomous underwater vehicle).

The Fraunhofer IBMT offers suitable components and modules for underwater vehicles such as AUVs or ROVs (remotely operated vehicles) for use in the deep sea. These units can be tailored to the client's special requirements and the particular application.

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1 *The "Joseph von Fraunhofer".*

Precise work under high pressure – with the right equipment

Conclusive study results on organisms that develop under extreme pressure can best be obtained under exactly such conditions. Therefore, scientists of the Group have developed tools that are not only suited for the current deep-sea studies, but in addition are proving useful for a broad range of applications.

A miniaturized high-pressure chamber for microscopy is the heart of the device platform. It enables cell biological studies to explore the effect of high pressure of up to 1000 bar (corresponding to 10,000 meters of ocean depth) on cell cultures over several days and weeks. For example, pressure adaptation of cellular processes and components can thus be studied. Studies on food preservation by high-pressure technology are conceivable, as are applications beyond biological issues such as use of the chamber as chemical reaction cell for a wide pressure and temperature range with access for diverse optical analysis methods.

Pressure function generators are available to control and study processes and systems at high pressure levels of up to 2000 bar. Since these processes and reactions take place very fast within seconds, a technical solution was found to create such high sample pressures within a split second. Possible applications of these high-pressure generators are in the areas of materials production and testing and in high-pressure research in a broad range of disciplines (e.g. materials science, geology, chemistry, microbiology).

The development of these devices has progressed to the point that they can be tailored to the client's special requirements. The unique selling point of this system is its ability to adapt the pressure and hold it for a duration anywhere from the sub-second range to several days.

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All aboard! The research vessel “Joseph von Fraunhofer”

The motor yacht type Baltic Trawler 42 was launched in 2011. Made in China of fiberglass reinforced plastic (FRP) and subsequently transformed into a research vessel, the boat has since been underway under the name “Joseph von Fraunhofer”. Important for the scientific work at sea is the boat's great stability, which is due to its deep keel along its whole length. Bow and stern thrusters provide easy maneuverability. An essential requirement for research projects is the exact positioning during sampling, but this poses no difficulty thanks to the state-of-the-art, high-quality navigation system. In addition, sampling can be performed within defined transects. The vessel's comprehensive safety equipment meets high safety standards, and communication with and data transfer to the land base are possible using radio communication and Internet.

The Group uses the trawler for research and development work, for example, to demonstrate technical developments or collect samples for scientific purposes. Advanced scientific training is also offered on board, for example, in the area of marine microbiology. At the same time, scientists learn good seamanship for the next expedition.

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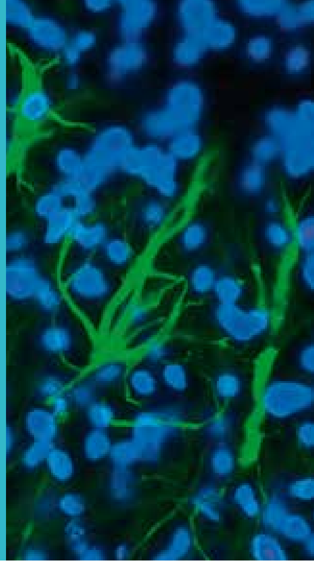


THE FRAUNHOFER GROUP FOR LIFE SCIENCES

The comprehensive and individually tailored services offered by the Fraunhofer Group for Life Sciences for the application of novel technologies require an organization that covers a broad range of disciplines, methods, and equipment. Under the motto "research for human health and the environment", the Fraunhofer Group for Life Sciences offers its clients a rich pool of complementary expertise.

Six Fraunhofer Institutes and a Fraunhofer research institution, each having proven in-depth expertise in different areas within the life sciences, are involved in this Group: the Fraunhofer Institutes for Biomedical Engineering IBMT, Interfacial Engineering and Biotechnology IGB, Molecular Biology and Applied Ecology IME, Toxicology and Experimental Medicine ITEM, Process Engineering and Packaging IVV, Cell Therapy and Immunology IZI, and the Fraunhofer Research Institution for Marine Biotechnology EMB. Their combined knowledge of biology, chemistry, biochemistry, biotechnology, medicine, pharmacology, ecology, and nutritional science is thus pooled and synergized within this Fraunhofer Group. With the Fraunhofer EMB joining the Group in August 2012, marine biotechnology has become an additional focus. In all these Fraunhofer Institutions, the scientists collaborate in interdisciplinary teams, so that tailored know-how concerning information technology, engineering science, and legal requirements is also available. Research and implementation at the client's facilities therefore go hand in hand.

The Fraunhofer-Gesellschaft stands for reliable partnership in applied research. As the largest research organization of its kind in Europe, it develops market-oriented solutions tailored to the specific requirements of each client. A solid basis for this is its own pre-competitive research, geared to the basics and frequently undertaken in close cooperation with universities and other academic institutions.

One of the most important things we have learned: the path from the first idea to the perfect solution is always very exciting – and we will gladly go down this path with you.

Business units of the Fraunhofer Group for Life Sciences:

Medical Translational Research and Biomedical Technology: The Challenge of Innovative Diagnostics and Personalized Therapy

Regenerative Medicine: The Challenge of Qualified Biobanking and Controlled Self-Healing

Healthy Foods: The Challenge of High Consumer Acceptance and Disease Prevention

The New Potential of Biotechnology: The Challenge to Learn from Nature for Industrial Exploitation

Process, Chemical, and Pesticide Safety: The Challenge of Environmental and Consumer Protection


Do you have any questions regarding the Fraunhofer Group for Life Sciences, or any suggestions or requests?

Please contact
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and
Dr. Claus-Dieter Kroggel, Head of the Group's Central Office.
He will be pleased to assist you, so that you can quickly reach your goal.

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