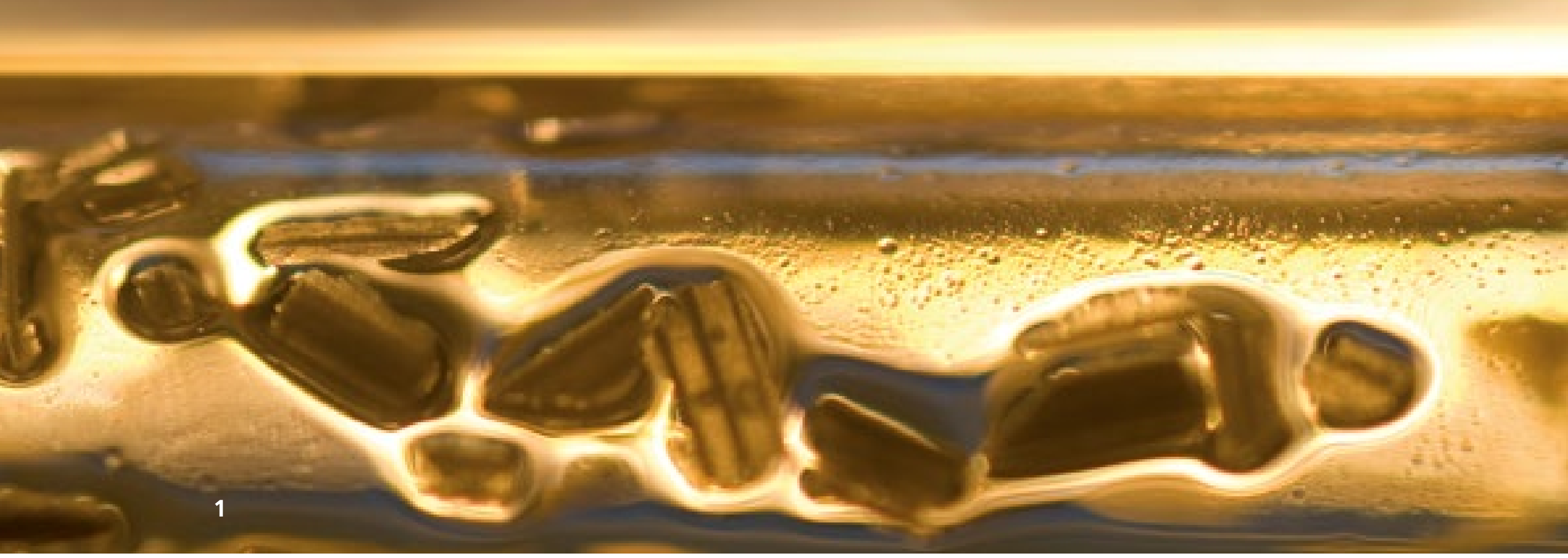
FRAUNHOFER INSTITUTE FOR INTERFACIAL ENGINEERING AND BIOTECHNOLOGY IGB

INDUSTRIAL BIOTECHNOLOGY
NATURE’S OWN CHEMICAL PLANT



INDUSTRIAL BIOTECHNOLOGY – NATURE'S OWN CHEMICAL PLANT

Supplies of fossil resources such as oil, coal and natural gas are limited and are running out. Their use has contributed substantially to an increase of the carbon dioxide in the atmosphere. Coping with climate change and at the same time supplying a growing world population with food, raw materials and energy, is one of the greatest challenges of our times.

Bioeconomics – sustainable economic management

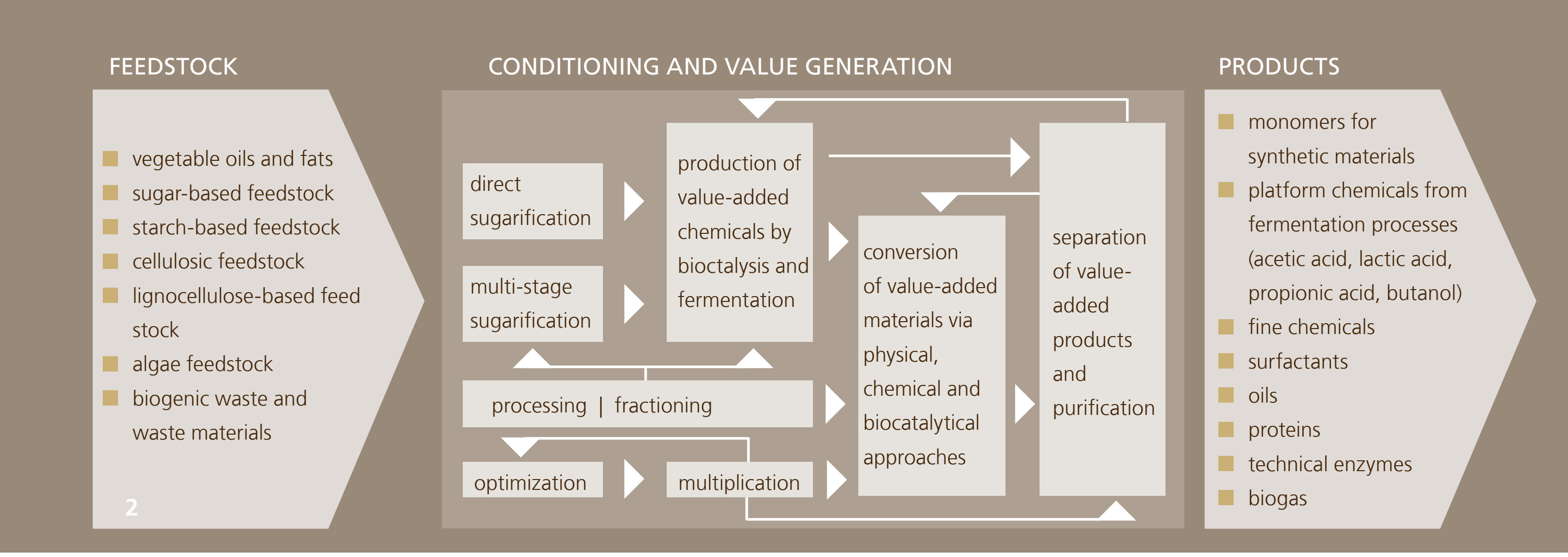
Bio-based economics – bioeconomics for short – sees these challenges as an opportunity. As the only renewable alternative carbon source to fossil raw materials, biomass will provide a new raw material base for the chemical industry. The objective here is to achieve the greatest possible harmony between economics and ecology. That means developing at the same time processes in an environmentally responsible manner and making them economically viable so that they can be used as quickly as possible on an industrial scale. This represents a first important contribution to a "post-growth economy". This no longer assumes that the available quantity of energy is inexhaustible, but is limited by the input of solar energy available. At the Fraunhofer IGB we therefore understand bioeconomics as a direct implementation of the concept of the life sciences in sustainable, environmentally compatible products and processes, realized in as socially a responsible way as possible.

An economically significant field within bioeconomics with enormous potential, but still requiring a great deal of research, is the use of renewable raw materials. If renewable resources are used instead of fossil carbon sources for the production of chemicals, this helps to reduce the emission of greenhouse gases and to protect the climate.

From industrial biotechnology to biorefineries

Industrial or white biotechnology, in which chemicals and chemical feedstocks are manufactured using biotechnological or combined processes, will play a key role in this context. Biotechnological processes are in many cases more energy- and resource-efficient because of the highly specific catalysts involved – and thus more environmentally compatible and more cost-effective than conventional methods, as the production of highly complex compounds such as vitamins and cosmetics or amino acids using microorganisms already shows today.

A sustainable approach to the manufacture of chemical products for which there are no complete synthesis pathways in nature, is utilizing biomass as completely as possible on the refinery principle. The biorefinery is conceived in analogy to the petrochemical refinery, in which a raw material is separated into various fractions and processed to make a wide variety of products of varying complexity and added value using different process steps, some of them consecutive. Accordingly, a biorefinery first of all produces mainly platform chemicals and semi-finished products that are converted into products with higher added value by means of further conversion steps. Typically, the processing and synthesis pathways are subdivided according to the major substance groups of biogenic raw materials.



At the Fraunhofer IGB – using the substance groups cellulose/sugar and lignin (page 14) – we develop utilization pathways for the lignocellulose biorefinery that are based on plants with a more or less large share of lignocellulose. Besides this we have a further option with the use of microalgae. They provide a lignocellulose-free biomass with the substance groups starch, lipids and proteins (page 18). We also establish processes for the production of basic and fine chemicals from starch and vegetable oil (pages 12 and 8).

In order to exhaust the potential wealth of plant biomass materials, biotechnical processes are combined with suitable chemical methods as required. Our objective is to set up the conversion in such a way that the resulting compounds are directly available as platform chemicals for the chemical industry and to develop scalable processes that can be closely linked with already existing production structures. Also, the integrated use of biomass to obtain materials and energy – by means of coupled production or multiple sequential use – is of central importance.

1 *Shredded straw in a solution enriched with enzymes. The enzymes convert cellulose into its basic module glucose. Also pictured on the cover.*

2 *Sustainable value creation from renewable raw materials.*

DEFINITIONS

BIOECONOMICS – COMBINING ECONOMICS AND ECOLOGY

According to the definition of the European Commission, to which the Bio-Economy Council of Germany is affiliated, bioeconomics comprises all industrial and economic sectors and associated services that produce, treat and process biological resources (plants, animals, microorganisms), or use them in any form whatever. These include agriculture and forestry, the food industry, fisheries and aquacultures, but also parts of the chemical, pharmaceutical, cosmetics, paper and textile industries as well as the energy sector. People often speak of the "four F's": food, feed, fiber and fuel.

THE BIOREFINERY – COMPLETE USE OF RENEWABLE RAW MATERIALS

Complete utilization of biogenic raw materials is especially economical and sustainable: as chemicals to produce substances and as fuels for energy. This is the aim of the integrative concept of the biorefinery. Biomass is first of all converted into basic or intermediate chemicals by means of fermentative or biocatalytic processes, which are further processed via connected product trees in consecutive process steps to obtain fine chemicals or biopolymers. Finally, the biomass is also used for energy – as automotive or heating fuel.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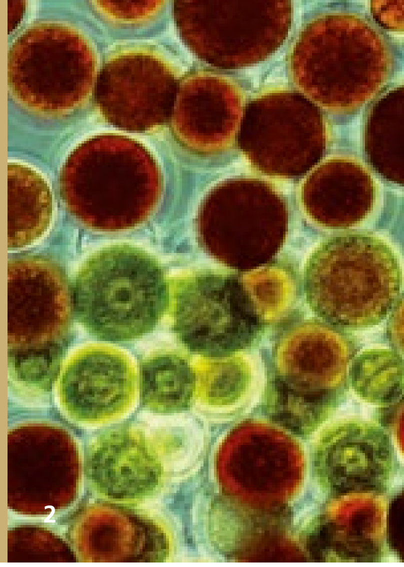
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FROM THE RAW MATERIAL TO THE FINISHED PRODUCT – OUR OFFER IN R&D

The Fraunhofer IGB has a long tradition in the biotechnological processes nowadays collectively called "white biotechnology". These methods are used for the preparation of various industrial products, such as fine chemicals, bulk chemicals, enzymes, active compounds for cosmetics, additives to food and animal feeds, fuels and for the production of pharmaceuticals. Our biologists and biotechnologists work hand in hand with chemists, physicists and process engineers to develop efficient new screening and expression systems, as well as production and processing methods that meet the highest demands for product quality and time-volume yields.

Frequently, the desired conversions only become possible through the combination of biotechnological with chemical processes. New chemical/catalytic processes are developed by the IGB Project Group BioCat and the Fraunhofer Institute for Chemical Technology ICT in Pfinztal, with whom we work in close cooperation. At the Fraunhofer Center for Chemical-Biotechnological Processes CBP, the processes are scaled up to production-relevant dimensions.

Biogenic raw materials

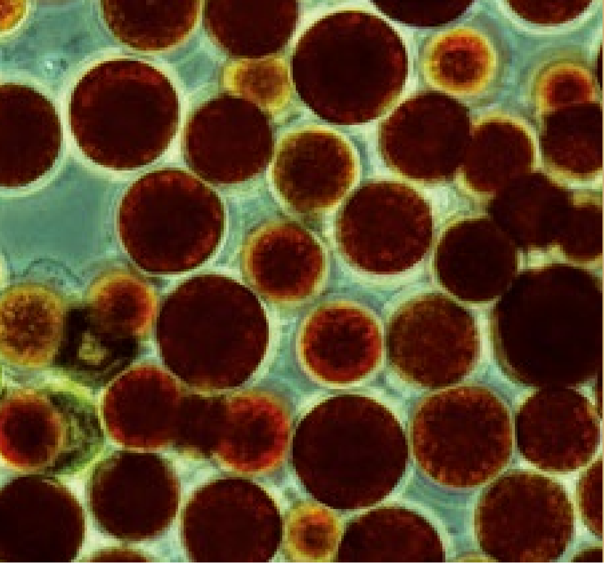
The only renewable alternative to the fossil resources as a source of carbon is the use of biomass – renewable raw materials and organic residual materials. Renewable raw materials are already widely used today to produce biofuels: seeds containing oil such as rape for the production of biodiesel, sugarcane and corn for the production of bioethanol for admixing in petrol. However, the use of agricultural products to manufacture biofuels is controversial.

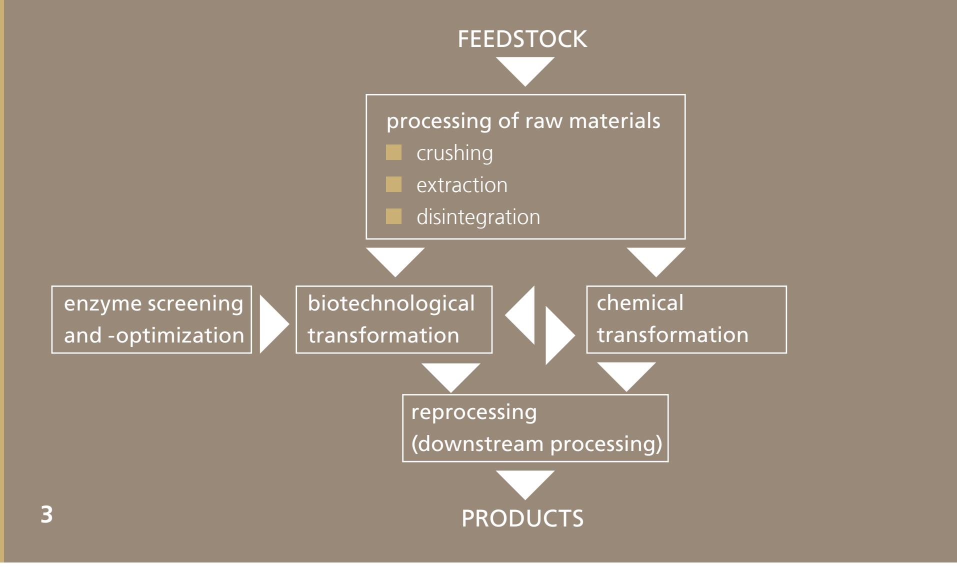
Using organic residues and wastes

With the aim of sustainability we are therefore developing processes to make use of residual biomass from forestry and agriculture (wood, straw) as well as organic residual materials from the foodstuffs industry (acid whey, crab shells) as a source of raw materials. We can solve the problem of waste disposal and at the same time obtain bulk products with the aid of integrated bioprocesses in which microorganisms are used to convert the residues in question. In current projects are treating straw to obtain lignocellulose in order to produce various types of sugar as a chemical or fermentation feedstock.

Aquatic resource microalgae

Also, we are focusing on microalgae, which produce numerous valuable chemical compounds, such as pigments, unsaturated fatty acids, and also starch and lipids that can be used energetically. Similarly to plants, unicellular microalgae living in fresh or sea water use photosynthesis to fix atmospheric carbon dioxide. However, unlike rapeseed and corn, they do not require any agricultural land. In addition, they grow much faster and are more productive than plants on land.





Furthermore, algae biomass is free of lignocellulose and can be fully utilized after the extraction of the high-value target substance by using the residual biomass as animal feed or fermenting it to obtain biogas.

Upstream processing

In order to make the raw material components accessible to the microorganisms or the enzymes, depending on the type of source material, several processing stages are necessary. These may be mechanical, thermal or chemical processes and have to be adapted to the following bioconversion.

Biotransformation: Development of bioprocesses and fermentation methods

The Fraunhofer IGB develops and optimizes fermentation processes, ranging from laboratory to semi-technical scale, using bacterial systems and fungi. This is done with the aid of processes that may be continuous and feature a high cell density, with cell retention involving filtration or immobilization. Several fermentation and processing methods have been established, e.g. for the manufacture of lactic, acetic and itaconic acid, and amino acids as well as for proteins such as thaumatin and bacteriorhodopsin. Using renewable raw materials such as rapeseed oil or algae lipids, production methods were also demonstrated for the biotechnological synthesis of basic materials for plastics production such as long-chain dicarboxylic acids and fatty acid expoxides.

Downstream processing

In the case of biotechnological processes, the product to be prepared is present in dilute form in the fermentation broth and has to be concentrated, isolated and purified from the other constituents of the fermentation medium, such as by-products and cells. The Fraunhofer IGB develops mild and efficient processing methods for chemical building blocks, foodstuff additives or natural vegetable materials, and is planning the corresponding installations.

Since processing determines how economical the bioprocess is, many of our projects involve the use of specific membrane methods, sometimes with specific adsorber nanoparticles, which can simplify the multistage downstream processes. If necessary, the membrane processes are coupled with conventional separation methods, such as centrifugation and extraction or else with chromatographic methods. Thus, proteins – active compounds or enzymes – are prepared in a highly pure state with the aid of ion-exchange, gel or reversed-phase chromatography.

In addition, we have established the extraction of valuable substances using supercritical fluids. These combine the properties of gases and liquids and have a high dissolving power. The products extracted in this way are free of solvents.

1 *In the multifermenter system various parameters can be screened at the same time.*
2 *Sustainable resource microalgae.*
3 *Process chain for the integrated synthesis of platform chemicals for the chemical industry.*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BIOCATALYSTS: NEW ENZYMES AND STRAIN OPTIMIZATION

The Fraunhofer IGB has a more than 10-year tradition of screening for novel enzymes, their optimization and production, and has already successfully conducted numerous projects with major companies in the chemical and pharmaceutical industries. We have experience in the discovery and the refinement of enzymes such as proteases, lipases, amylases, glycosidases, cellulases, phytases, oxygenases, halogenases, dehalogenases, chitinases, chitin deacetylases, formaldehyde-dismutases, cyanidases and ethene-monooxygenases, as well as their cloning and recombinant expression. By means of molecular-evolutive techniques we optimize enzymes for a very wide range of specific applications. We can evaluate and manufacture these in heterologous systems from laboratory up to the 10-m^3 scale.

Screening for new enzymes

Both conventional methods of enriching microorganisms – with the aim of identifying and isolating new enzymes – play a role at the Fraunhofer IGB as well as molecular methods such as the complete screening of the genes of microbial communities, e.g. via metagenomic libraries. With the help of molecular methods we can also examine the genome of isolated microorganisms and their proteins in detail. We offer a platform for the rapid identification and optimization of new enzymes for our clients. Here we make use of extensive experience in high-throughput screening and heterologous production of recombinant proteins. This experience is supported by the systematic use of the information that is available from the plethora genome sequencing projects.

Non-cultivatable microorganisms: Genetic libraries directly from the environment

More than 90 percent of the microorganisms occurring in nature may not be cultivatable in the lab. In order to utilize the metabolic potential of these organisms too, we use a screening strategy at the Fraunhofer IGB that circumvents this problem. We have directly isolated the DNA of microbial communities of various habitats and using optimized expression vectors introduced them into host strains. These libraries can be examined easily for enzymatic activities. With these metagenomic libraries we offer interested partners access to new, as yet unknown enzymes and methods to optimize them according their requirements.

Resource production by means of strain optimization

In the field of industrial biotechnology we use enzymes or microorganisms in order to produce organic basic and fine chemicals. For this purpose, we develop new production strains with the help of classical and biotechnological methods. For example, by means of molecular methods, we can examine in detail the genome of isolated microorganisms (by means of parallel sequencing methods) and their proteins (by proteome analyses), if required optimize it and produce high-performance expression strains. Microorganisms can be provided with new properties using metabolic engineering. This makes it possible to implement new products from complex biogenic materials.

Expression systems for technical enzymes and their industrial-scale production

Together with research and industry partners the Fraunhofer IGB is developing further efficient methods for the recombinant production and purification of technical enzymes (hydrolases, e.g. phospholipase C, oxidoreductases, cellulases, xylanases, lipases) on an industrial scale. In a first step, prokaryotic and eukaryotic expression systems are being evaluated and new expression strains and vectors are being developed. For variants that lead to higher yields at laboratory scale, we are pursuing process development and process optimization up to pilot-plant scale (10 m^3).

Currently we are working on wild-type and protease-deficient strains of *Kluyveromyces lactis* and *Pichia pastoris* as eukaryotic expression strains. *Kluyveromyces lactis*, a non-methylotrophic yeast, can be cultivated and induced with various sugars, such as lactose from whey waste material. With *Pichia pastoris*, a methylotrophic yeast is employed. Methanol is more economical than many conventional culture media and inductors, and the strong promoter of the alcohol oxidase I allows a product yield on recombinant enzymes of up to 30 percent of the cell protein. The strains used are well suited to this application due to their effective secretory pathways, in particular for the production of enzymes that are emitted into the medium. As a result, both production control and downstream processing of the enzymes are significantly simplified.

ADVANTAGES OF BIOCATALYSTS

ENVIRONMENT-FRIENDLY PROCESSES

The chemical-catalytic production of substances is generally carried out with a high time-volume yield, but frequently requires high pressures, high temperatures or the use of organic solvents. On the other hand, substrate-specific biosyntheses take place under mild conditions in a watery solution, however normally with a low time-volume yield. The optimum combination of chemical and biotechnological processes, compared with purely catalytic chemistry such as for example in the production of vitamin B2, often manages with a lower consumption of raw materials and energy as well as lower disposal costs.

HIGH SPECIFICITY

The focus in industrial biotechnology is on bioconversion, in which raw materials are transformed into usable products either with microorganisms (fermentation) or enzymes (biocatalysis). Biocatalysts, in particular enzyme preparations, permit highly specific implementations and can also be used for the production of compounds that are chemically difficult to access. Biocatalytically manufactured products are generally of high purity, normally there are no toxic by-products and catalyst residues.

NEW PRODUCT PROPERTIES

Besides advantages of an economic and ecological nature, biocatalytic processes also offer possibilities for the development of completely new products, for example biodegradable polymers.

1 *Automated screening for dehalogenases*

2 *Automated mass screening for new enzymes.*

3 *The DNA of microorganisms extracted from soil samples is expressed in laboratory strains.*

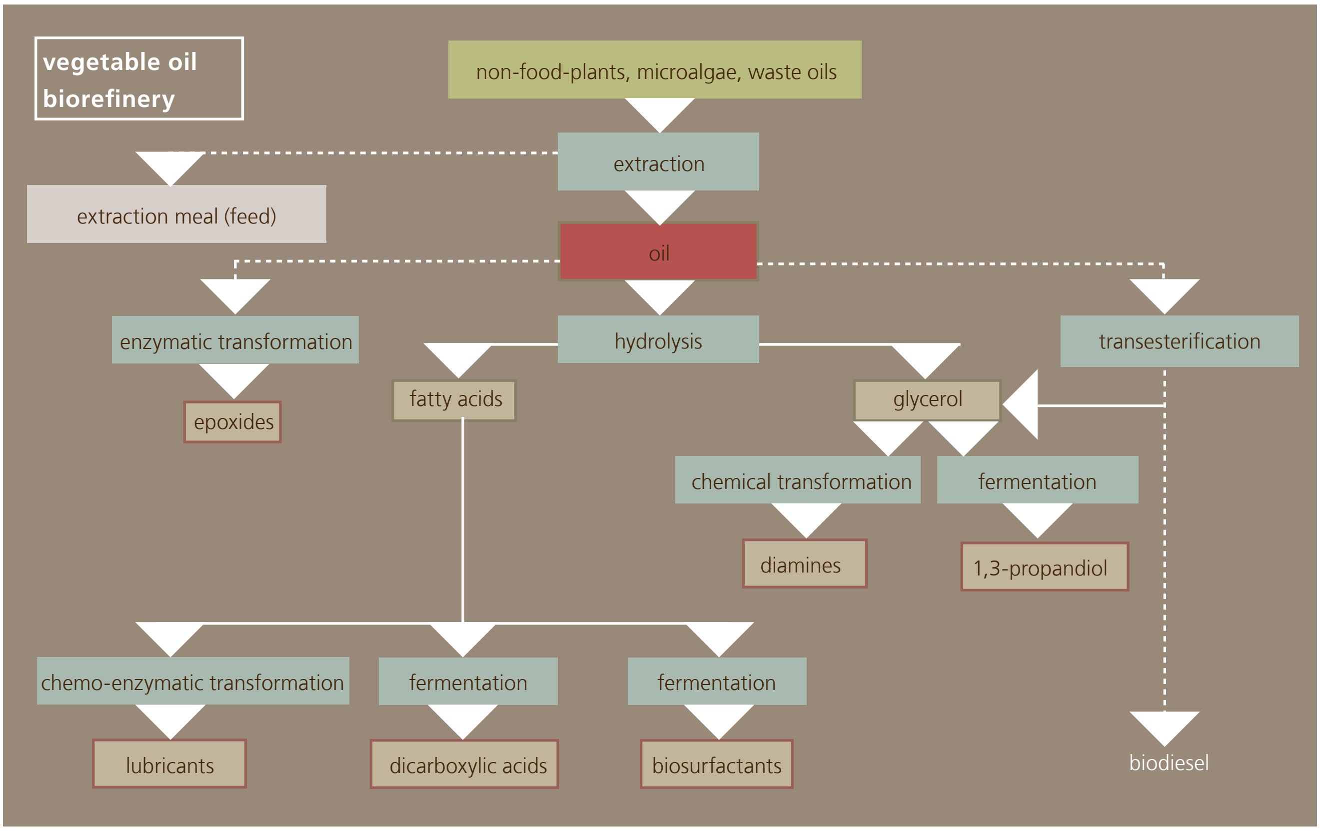
4 *Soil – source of new biocatalysts.*

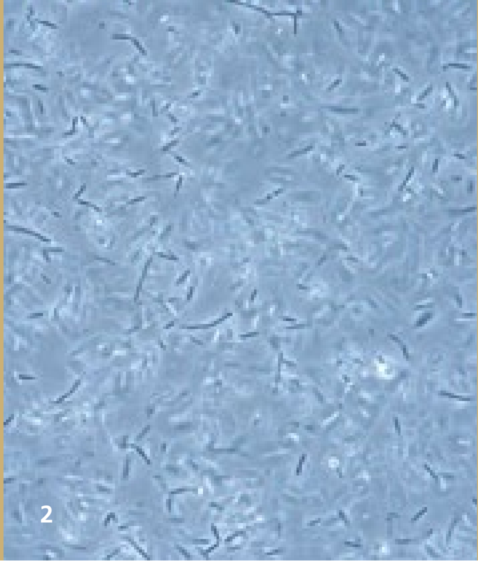
1

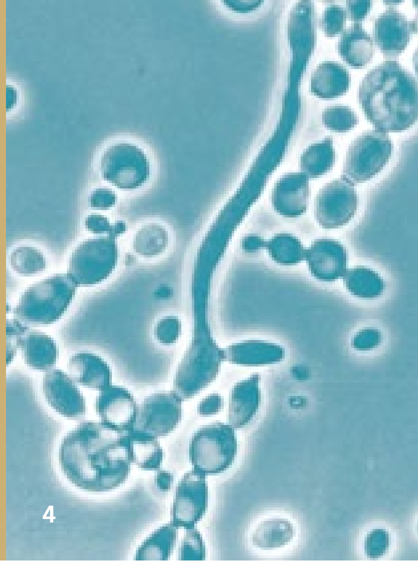
BIO-BASED RESOURCES – LIPIDS AND OILS

Vegetable oils consist of triglycerides, glycerol esters, which differ in the composition of the fatty acids. Because of the variable chain distribution, different physical properties, which lead to different application fields, result for the basic products obtained from vegetable oils, such as fatty acids, fatty alcohols, and esters.

Fatty alcohols, for example, are used as raw materials for different tensides, primarily fatty alcohol ethoxylates (non-ionic tensides) and fatty alcohol sulfates (anionic tensides). Branched fatty acid derivates can be synthesized by means of an oligomerization of unsaturated fatty acids with petrochemicals, e.g. short-chain alkenes. In the process, products are obtained that exhibit a higher thermal stability, a lower solidifying point, and a relatively low viscosity compared to the majority of linear parent substances; they are therefore suitable for use in lubricants.







1,3-propanediol from raw glycerol

In the production of biodiesel from rapeseed oil, raw glycerol is formed as a by-product of the transesterification of the vegetable oils. It is produced as an 80-percent viscous fluid with a pH of 11 and contains some fatty acids and salts. 1,3-propanediol is a basic chemical material used, for example, in the production of polyesters or paints. Up to now it has been manufactured by means of chemical synthesis. However, there are also microorganisms that are able to transform glycerol into 1,3-propanediol.

In a biotransformation process developed at Fraunhofer IGB, *Clostridium diolis*, a strictly anaerobic spore-forming bacterium is used to produce propanediol from raw glycerol. For a successful fermentation the fatty acids remaining after the transesterification first of all have to be separated from the raw glycerol. Laboratory experiments showed that at high concentrations both the substrate glycerol and also the product 1,3-propandiol inhibit the growth of the bacteria. Continuous operation of the bioreactor proved to be successful, as there was no longer any inhibitive effect at conditions approaching full conversion of the glycerin.

In this way we were able to achieve a stable process with high product concentrations of 42–60 g/L. The yield of 1,3-propandiol in relation to the substrate used amounts to about 50 percent (w/w). Further possibilities for optimization resulted from high cell density fermentations with integrated biomass retention in the bioreactor and by using microorganisms specially selected for high product concentration.

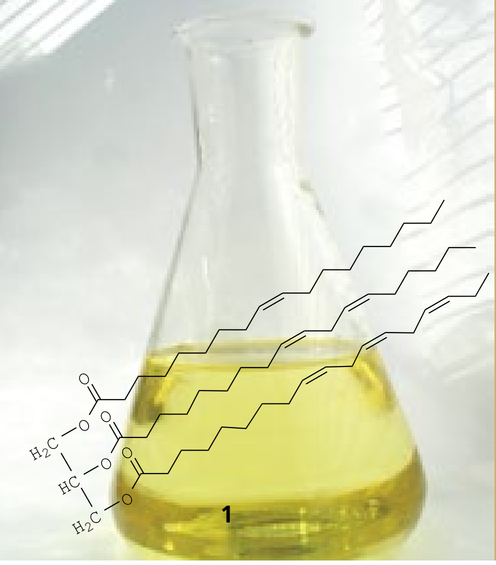
Dicarboxylic acids from rapeseed oil

Long-chain dicarboxylic acids (C>12) are of great interest as additives in the synthesis of polymers (e.g. polyamides and polyesters) with novel properties. However, chemical synthesis of long-chain dicarboxylic acids is not easy.

The metabolic pathway for the synthesis of dicarboxylic acids in microorganisms is known as ω-oxidation and comprises three successive enzymatic steps involving the oxidation of fatty acids (monocarboxylic acids). This metabolic pathway is common in well-known yeast strains such as *Candida tropicalis* and *Yarrowia lipolytica*. At the Fraunhofer IGB we are therefore pursuing various approaches to the fermentative production of dicarboxylic acids from vegetable fatty acids. The development of the method follows the example of rapeseed oil, but is also applicable to oils that are not used in the foodstuffs sector.

The development of various fed-batch processes with organisms of the genus *Candida* has already made it possible to obtain dicarboxylic acid concentrations of up to 100 g/L from oleic acid. At present we are examining a series of other organisms for the preparation of new, easy-to-handle production strains that permit the highest possible yield of dicarboxylic acid. In the second approach, selected genes are genetically modified. These recombinant strains are also currently being examined as regards their suitability as production strains.

1 *In the production of biodiesel from rapeseed oil, raw glycerol is formed as a by-product.*
2 *Clostridium diolis produces 1,3-propanediol from raw glycerol.*
3 *Cultivation of yeast strains in a lab-scale bioreactor.*
4 *Yeasts are able to synthesize dicarboxylic acids from fatty acids.*



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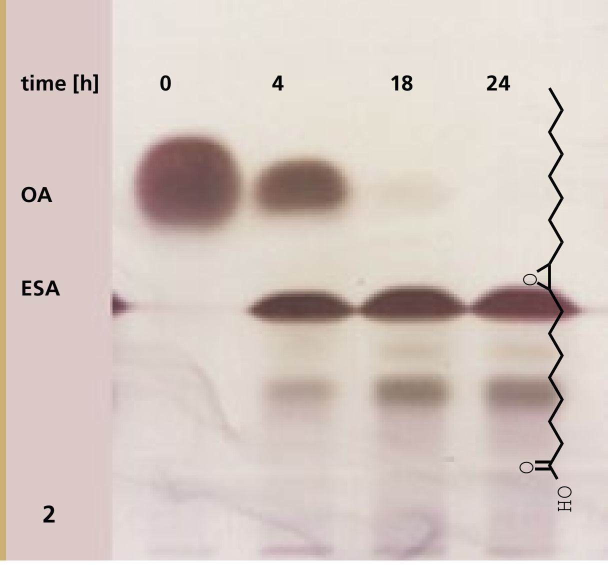

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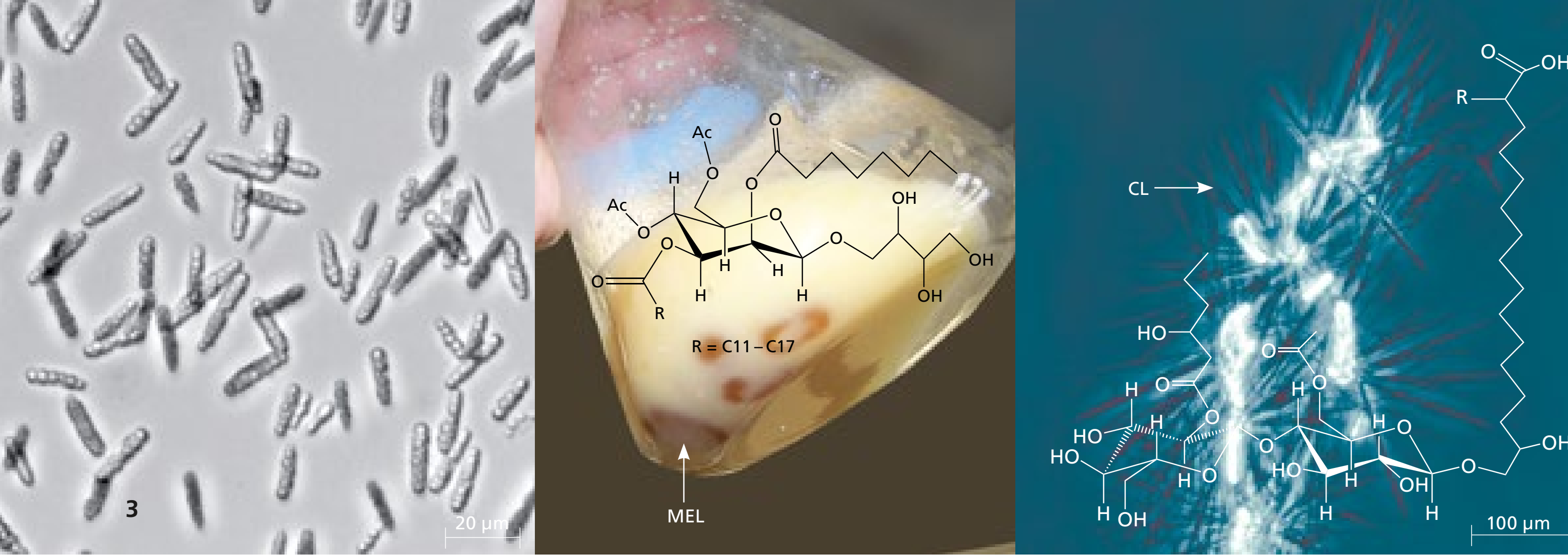
Epoxides from plant oils

During epoxidation of unsaturated fatty acids and triglycerols, polar compounds with increased reactivity are generated. These epoxides can therefore be used as PVC stabilizers, plasticizers, crosslinkers in powder coatings, in epoxy resins, or as additives in lubricating oils. Up till now, epoxides have been manufactured from petrochemical base substances. Recently, plant-based epoxides have been obtained on an industrial scale, primarily from soy oil. In this context, the so-called Prilezhaev reaction is used, in which the olefinic double bonds of the unsaturated fatty acids are oxidized by peracid to epoxides (oxiranes). The peracid formation takes place via a chemical process, frequently in situ, based on reaction of hydrogen peroxide with acetic or formic acid using stronger mineral acids or ion exchange resins as the catalyst.

An alternative to the chemical process is chemo-enzymatic epoxidation, in which the enzyme lipase catalyzes the peracid formation from fatty acids and hydrogen peroxide. Substantial advantages of the chemo-enzymatic methods are thus the milder process conditions and a higher selectivity of the conversion. In this context, the oil of the annual, herbaceous Iberian dragon's head was used. With an immobilized lipase from *Candida antarctica* (Novozym® 435), we improved the turnover of different fatty acids and lipids in terms of substrate concentration, hydrogen peroxide addition, the quantity of lipase used, and the temperature. As a result of the optimization of these process parameters, the substrates were converted into the corresponding epoxides with an efficiency of 100 percent. Beyond this, we have screened for appropriate new commercially not available enzymes, which catalyze peracid formation and thus in a subsequent step the expoxidation of unsaturated fatty acids. The identified enzymes are to be characterized and then produced in large quantities.



Biosurfactants – production and optimization

Most tensides in washing powder, detergents and shampoos are produced chemically – from mineral oil or vegetable oils, so their structural variety is limited. Microorganisms form a great variety of surface-active substances, so-called biosurfactants, comprising a wide range of chemical structures such as glycolipids, lipopeptides, lipoproteins and heteropolysaccharides.

The Fraunhofer IGB investigates the production of cellobiose lipids (CL) and mannosylerythritol lipids (MEL), two classes of biosurfactants suitable for industrial use, with the help of smut fungi of the *Pseudozyma* and *Ustilago* species. In order to increase the time-volume yield of biosurfactant and to lower the production costs, the production process is optimized. As a result of optimized cultivation (oxygen supply, temperature, nutrient solution, substrate concentration) and process control, product concentrations of 20–30 g/L for cellobiose lipids and 100 g/L for mannosylerythritol lipids are currently being achieved.

The biosurfactants are biodegradable and less toxic to the environment than synthetic tensides, their properties regarding the surfactant effect are comparable or superior. In addition, we have established various extraction methods and are at present investigating possible applications of the fermentatively produced biosurfactants. The surfactant structure and thus the surfactant properties can be modified with genetic engineering, enzymatic or bioprocess-technical methods. For example, we have succeeded in increasing the water solubility of a biosurfactant.

1 *Vegetable oils as renewable raw materials for the production of epoxides.*

2 *Thin-layer chromatographic analysis of the lipase-catalyzed conversion of oleic acid (OA) to epoxy stearic acid (ESA).*

3 *Cells of the smut fungus Ustilago maydis in its haploid, vegetative single-cell stage (left). At high product concentrations, mannosylerythritol lipids are deposited as oily pearls (center, with structural formula), cellobiose lipids as needle-shaped crystals (right, with structural formula).*

1

BIO-BASED RAW MATERIALS – STARCH/SUGAR

Starch is an important storage compound in plant cells and is valuable in human nutrition, for example, as the main component of cereals and potatoes. As polysaccharide composed of glucose units, starch can also be used as a renewable raw material for biotechnological processes. For this, starch is broken down and hydrolized to obtain glucose or enzymatically cleaved to form glucose.

Glucose can be converted fermentatively to obtain a large number of platform chemicals that can be used directly in the chemical industry. Lactic acid is an important commodity chemical that can be chemically processed to yield various end products, for example, acrylic acid, 1,2-propanediol or biodegradable polymers (polyactide, PLA).

Lactic acid production from starch

Currently, this process is carried out industrially in two stages. The first involves the digestion of the starch by technical enzymes to form glucose; in the second, the glucose is fermented to lactic acid by microorganisms. We have now developed an alternative one-step process involving the simultaneous hydrolysis of starch and fermentation of the resulting glucose to lactic acid by starch-hydrolyzing bacteria.

A screening for starch-fermenting lactic acid bacteria revealed a strain which is able to convert complex starch like wheat or cornstarch to lactic acid by homofermentative fermentation with high yields. Using this strain, a lactate concentration of 115 g/L was obtained from cornstarch. Such high concentrations were best achieved in a co-culture with a glucose fermenting lactic acid bacterium, because the starch-digesting organism had problems converting glucose at high lactate concentrations.

For further use, the lactic acid from the fermentation matrix has to be purified; here, part of the lactic acid must be available as undissociated acid. The fermentation should therefore be carried out at low pH values. During the chemical conversion experiments it was established that processing of the fermentation broth into reaction products requires a certain proportion of free undissociated lactic acid. This requires in turn that fermentation be carried out at the lower pH values necessary for the undissociated form of the lactic acid.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However, because the growth of the microorganisms is strongly inhibited by low pH and high free lactic acid concentrations, a biphasic fermentation process was developed. In the first phase the pH conditions are adjusted to give optimal pH for growth, and in the second phase the pH was allowed to drop to about pH 4, resulting in a maximum lactic acid concentration at minimum pH value.

Lactic acid from whey

Whey is a high-volume by-product in milk processing, and especially the acid whey is seen as a waste product. After separation of the valuable whey proteins a residue remains, which is costly to dispose of due to its high COD (chemical oxygen demand). Besides inorganic salts the main component is milk sugar (lactose), which is only of little importance in food production because of its poor sweetening power.

But lactose can be converted by lactic acid bacteria to lactic acid (lactate), which is used as a preservative and acidulant in the food industry and as a raw material in the chemical industry – e.g. in the production of polylactides, biologically degradable polymers.

At the Fraunhofer IGB an integrated high-performance process has been developed in which combining membrane processing methods with an efficient biological system (organism, cultivation medium, bioreactor) results in cost-efficient production of lactic acid. In the first process step, the valuable whey proteins are separated from the acid whey. The lactose of the remaining whey permeate is converted in an unaerated bioreactor by a special strain of lactic acid bacteria, which does not need any further supplements, to lactate (salt of lactic acid). High concentrations of biomass and thus high and economic lactic acid productivities are achieved by cell retention using an integrated cross-flow filtration unit. The product recovery is carried out by bipolar electrodialysis, converting lactate directly into the free acid.

1 *Starch is a storage substance of plant cells.*

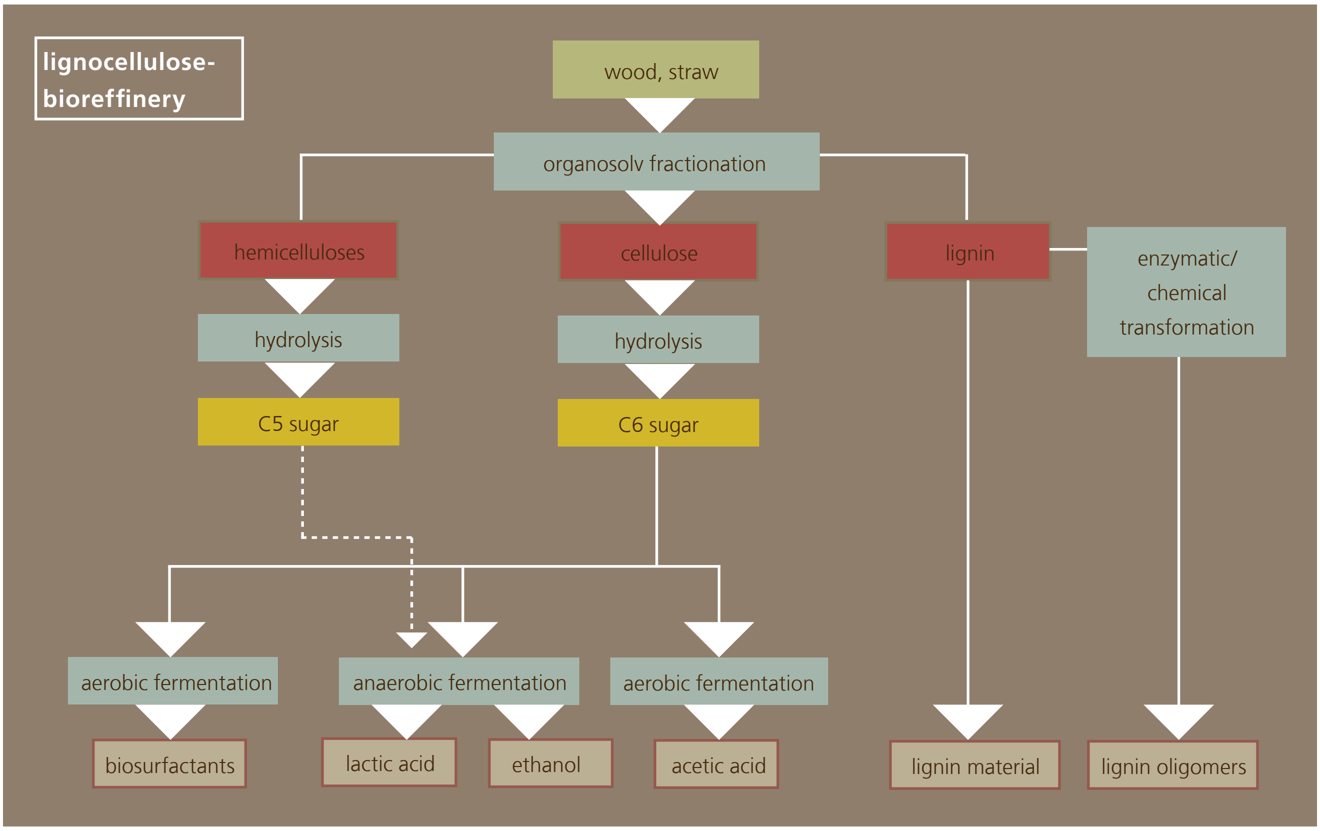
2 *Whey is a liquid residue of cheese production.*

1

BIO-BASED RAW MATERIAL – LIGNOCELLULOSE

Lignocellulose – bulk material of the cell walls of all ligneous plants – is the most commonly occurring renewable raw material. For this reason alone it is certain to play an essential role in the supply of both renewable raw materials and energy in the future. In addition to this, waste material such as straw and wood can be used as a basic raw material for industrial biotechnology as it does not conflict with the manufacture of foodstuffs. By means of fermentation or chemical processes it is possible to manufacture the most important starting chemicals – apart from biofuels – of the chemical industry from materials containing lignocellulose, which mainly consist of polymeric C6 and C5 sugars (cellulose, hemicellulose) and the biopolymer lignin.

However, these materials are highly resistant to enzymatic degradation, chiefly due to their compact structure and lignin content. On the other hand, harsh physical/chemical treatment methods result in the loss or a reduced quality of individual fractions. New methods and combinations of methods, therefore, are necessary to obtain technically usable building blocks for chemical reaction products.



Complete utilization of lignocellulose

The resistant structure of the lignocellulose prevents an efficient cleavage of the biomass, so the sugars bound in the lignocellulose and the lignin cannot be recovered direct from plant materials containing lignocellulose. For this reason the plant material generally has to be pretreated using physical, chemical or biological methods.

Three material streams are generated in the fractionation of the lignocellulose using the Organosolv process. First the cellulosic fiber, which can be hydrolized by cellulolytic enzymes to obtain glucose. Then the digestion solution, which contains the dissolved hemicellulose sugars as well as dissolved lignin. After precipitation of the lignin and enzymatic cleavage of the sugar oligomers, the digestion solution can also be used for the fermentation of microorganisms. The fermentability of the digestion solution can be substantially increased here by enzymatic detoxification with laccase.

Within the scope of the project "Lignocellulose Biorefinery" funded by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) the Fraunhofer IGB and Fraunhofer CBP, together with partners from research and industry, scale up the process steps to 1 m^3 in order to implement a biorefinery concept with an integrated process approach from the raw material lignocellulose through to product recovery.

1 *Making raw materials containing lignocellulose available to the chemical industry poses a new challenge for scientists.*

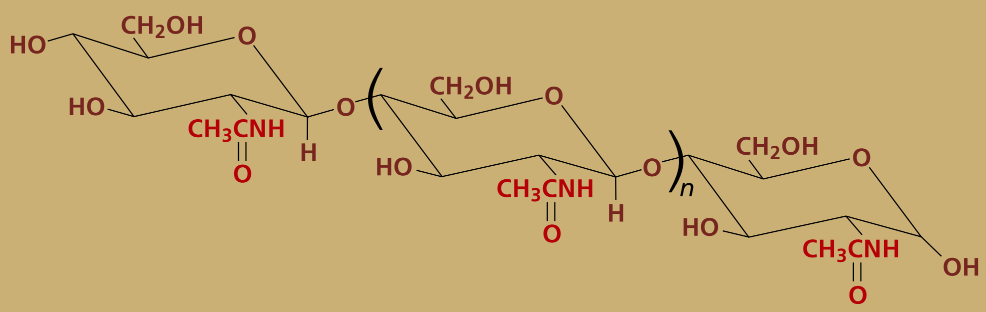
2 *Straw is decomposed in a ball mill before its components can be further processed.*

Extraction of aromatic lignin monomers

An essential precondition for the use of biotechnological processes for the efficient degradation of lignin is the availability of efficient and stable biocatalysts. In the joint project "Innozym" funded by the Federal Ministry of Education and Research (BMBF) the Fraunhofer IGB characterizes and identifies ligninolytic enzymes from white rot fungi and bacteria.

Suitable strains and combinations of strains (co-cultures) were identified and their expression performance optimized by varying the media composition and inductors. Thus lignin-degrading enzymes such as etherases or laccases and peroxidases can be produced in large quantities. Secreted enzymes are purified by means of two-dimensional gel electrophoresis, and characterized biochemically and using mass spectrometry. Characteristic key enzymes are produced selectively in submerged or emerged culture systems and employed free of cells for the enzymatic breakdown of lignin.

Apart from white rot fungi and teredos, some xylophagous insects are able to use lignified plant material as a source of food. Termites and the larvae of some species of beetles and butterflies are able to ingest wood because of their symbiotic way of life with bacteria and fungi. In order to extend the range of the available biocatalysts for lignin degradation, we isolate and characterize symbiotic organisms from the digestive tract of larvae of xylophagous insects. Also, we use methods that are independent of the culture (metagenomic screening, see page x) in order to identify ligninolytic enzymes of symbiotic origin. Additionally we investigate commercially available bacteria strains with regard to their suitability for fermentative lignin degradation.



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BIO-BASED RAW MATERIALS – CHITIN

After cellulose, chitin is the most abundant biopolymer on the earth. The linear, insoluble homopolymer consisting of beta-1,4-linked N-acetylglucosamine (NAG) units occurs in large quantities in crustacean shells, insects and fungi. Every year more than 750,000 tonnes of shells of these crustaceans land on the waste in the EU alone. This waste could be recycled.

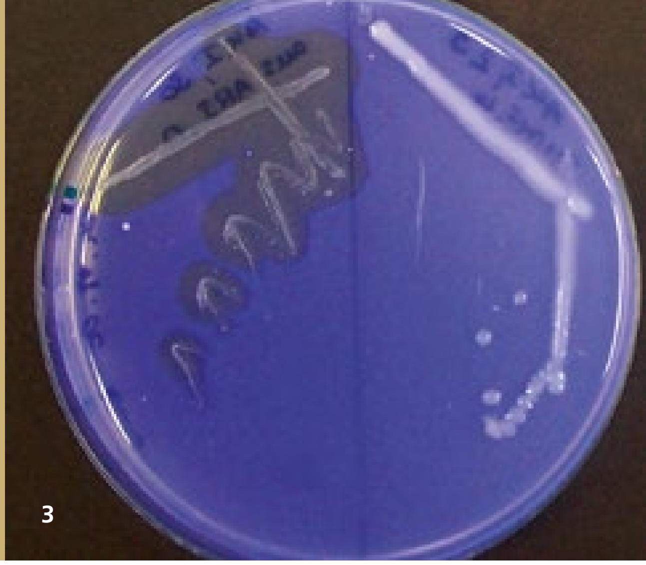
Chitin can be decomposed by many bacteria by means of chitinases. These chitinases split the biopolymer into oligomers or monomers. In Asia, for example, the polymer chitosan is already produced from shrimp shells. This is used to make filters or foils, and also wound dressings. However, the shells of the European crustaceans contain more lime, so processing them to obtain chitosan is not economical at present.

Chitinases for obtaining monomeric building blocks

At the Fraunhofer IGB we are developing an enzymatic process, where chitin is decomposed to monomers which can then be converted hydrothermally to easily modified basic building blocks for use in polymer chemistry. During an enrichment screening process, a set of unknown chitinases still unprotected by patents have been isolated. Lab-scale experiments with the isolates showed that chitinase production was linked to organism growth on chitin and that the enzymes are secreted into the medium. During a two-step process, firstly the enzymes are produced, and after separation of the biomass they are used for biotransformation of chitin. In this way we have succeeded in converting a chitin suspension completely into NAG.

Gene libraries of each organism have been created in *E. coli* in order to characterize the new chitinases both with regard to their characterization at molecular level and to create a recombinant strain for enzyme production. By means of genomic walking and expression-assisted screening we have already identified several new chitinases that are currently being introduced into *E. coli*. One chitinase has been expressed functionally in *E. coli* and its range of products is being examined.

1 *Chemical structure of chitin.*
2 *Crab shells as chitin suppliers. © Biotech Surindo*
3 *Chitinolytic bacteria on agar plate containing chitin.*



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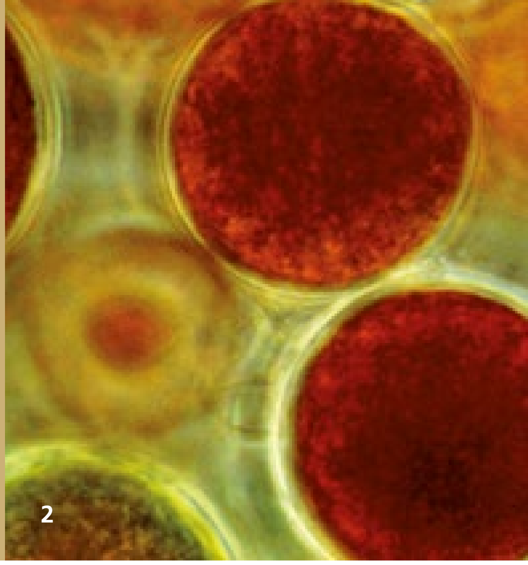
Crustacean shells as a raw material for chemicals

In the EU-funded ChiBio Project the Fraunhofer IGB is continuing these activities. Coordinated by the Straubing Project Group BioCat new methods of utilizing the shells that result in large quantities of waste have been developed in order to use them here in Europe as a raw material for chemicals and new materials. The consortium comprising research and industrial partners from Norway, Austria, the Czech Republic, Ireland as well as Tunisia and Indonesia is focusing on an integrated approach. In the manner of a biorefinery, various material and energetic uses are being developed and optimized for the crustacean shells, in order to utilize the residual material as efficiently and completely as possible.

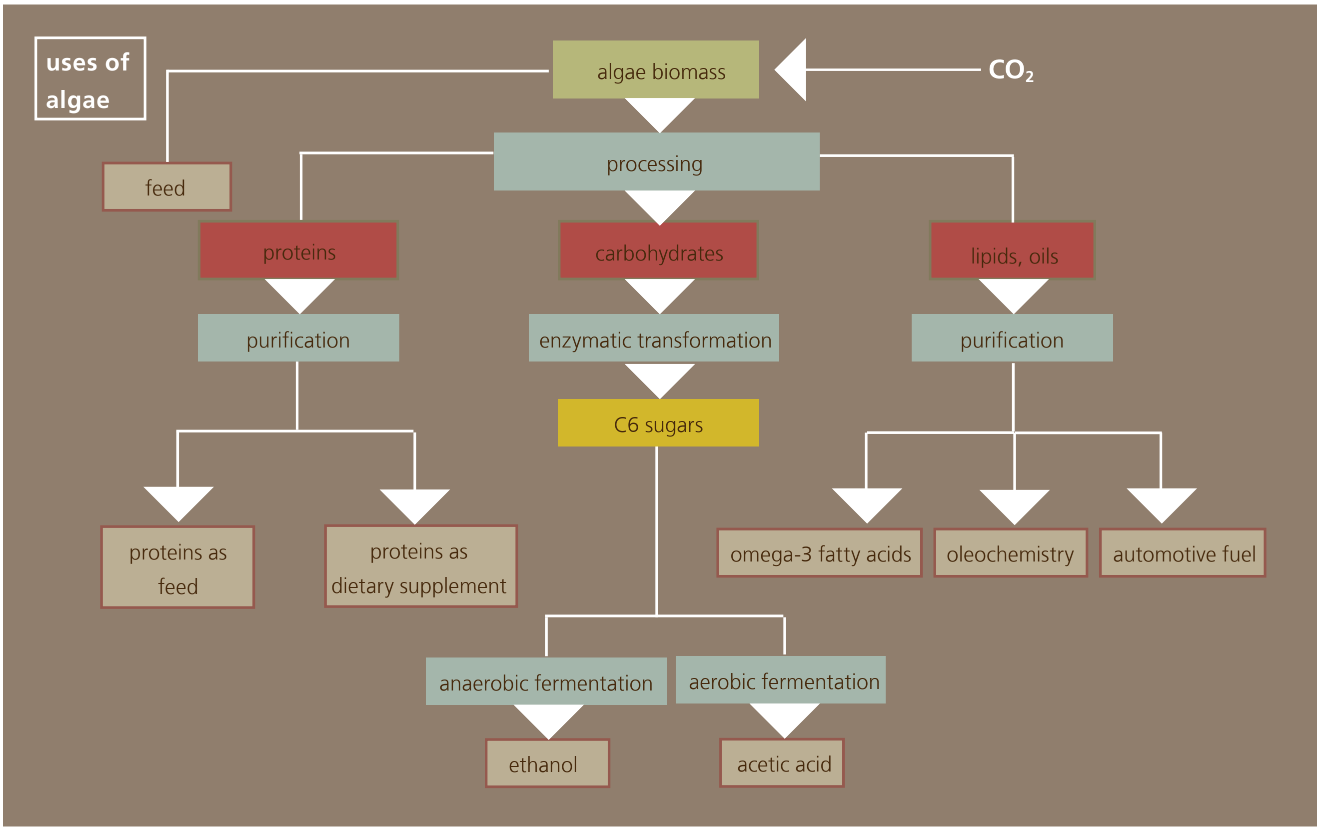
First of all the remaining crab meat has to be removed from the shells. These biomass residues, which consist of proteins and fats, are separated in such a way that they can be directly digested and thus used for energetic purposes. The purified chitin can then be split into its monomeric components, the nitrogenous sugar glucosamine, using the enzymes or microorganisms described above. It will be a great challenge to convert glucosamine into such basic components – or platform chemicals, with at least two functional groups so that they can be combined catalytically to form new bio-based polymers. Here we aim to combine chemical steps with biotechnological processes. The intention is to digest all the bio-based by-products generated in the process chain together with the initially separated proteins and fats to produce biogas as a regenerative energy carrier.

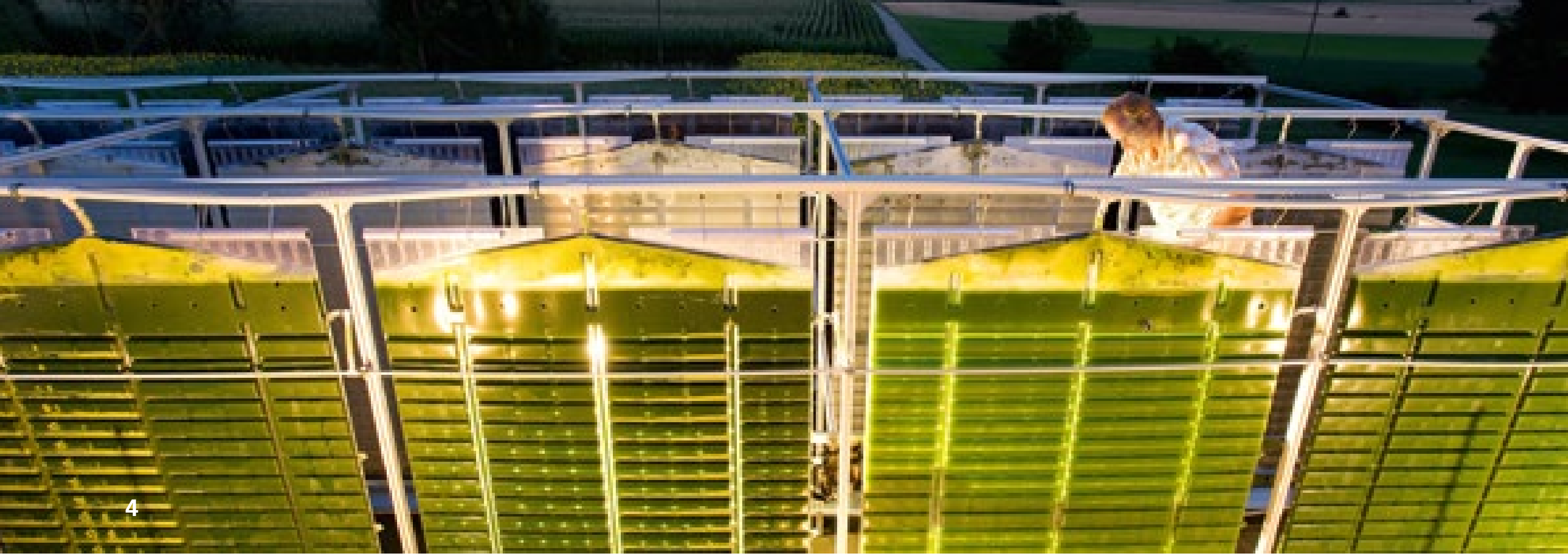


MICROALGAE – HIGH-VALUE PRODUCTS, LIPIDS, BIOMASS FREE OF LIGNOCELLULOSE

Microalgae are a natural source of raw materials that has so far been little used. They produce a wide range of chemical basic materials such as vitamins, fatty acids and carotinoids. These have a high potential for creating value in the pharmaceutical, chemical and foodstuffs industries.

Algae are easily satisfied and – like plants – only need sunlight, carbon dioxide, nitrate and phosphate in order to grow quickly. Continual harvesting over the whole year is possible with algae production; agricultural land is not required. Additionally, the composition of the algae biomass – in itself free of lignocellulose and homogeneous – can be controlled by targeted cultivation conditions. Considerably less water is required for their cultivation, compared with higher plants; in addition, the water can be recycled. Even wastewater streams from the wastewater purification plant containing inorganic nutrients such as nitrogen and phosphorus can be used for their cultivation.





New type of photobioreactor

The production of algae biomass in open ponds is slow and inefficient. The Fraunhofer IGB has therefore developed for the primary production of algae biomass containing raw materials an inexpensive plate reactor, which functions on the principle of an airlift reactor. Unlike reactors developed up till now the FPA reactor (Flat-plate Airlift Reactor) is a fully mixed reactor, in which an improved light and substrate supply of all the algal cells is achieved my means of a thin layer and targeted flow guidance in the reactor by means of static mixers. This results in a high concentration of cells in the reactor, which increases the cost-effectiveness of the production process.

The reactor itself is manufactured at low cost by means of deep-drawing technology from synthetic foil in the form of two half-shells including the static agitators. In the scale-up, the reactor volume of the FPA reactors was increased from 5 liters to first of all 30 liters and then, by Subitec GmbH, a spin-off of the Fraunhofer IGB, to 180 liters. In the pilot plants in Hamburg, Senftenberg and Reutlingen, each with a reactor volume of 1.3 to 4 m³, these reactor modules are used in outdoor conditions and with waste gas from block-type heat and power plants as a CO_2 source for the production of algae biomass.

Products of an integrated process

Production processes for substances that are used as foodstuff additives, animal feed or cosmetic additives, were successfully developed at the Fraunhofer IGB and optimized for production in field conditions.

The microalgae *Haematococcus pluvialis*, for example, produces astaxanthin, a red pigment with properties that are antioxidative and beneficial to the health. The "red salmon pigment" is used both in aquaculture and in the cosmetic industry. The algae *Phaeodactylum tricornutum* produces the polyunsaturated long-chain omega-3 fatty acid EPA (eicosapentaenoic acid). In certain conditions some algae form storage lipids. These are also promising as fuel (see next page).

The approach adopted by the Fraunhofer IGB aims, first of all, to obtain the resources from the algae and then to ferment the residual biomass in a biogas plant. After the production of electric current and heat from the biogas in the block-type heat and power plant, the resulting CO_2 can be returned to the circulation process for algae biomass production.

1 *Individual FPA reactors are interconnected, thus producing algae biomass in kilograms.*

2 *The microalgae Haematococcus pluvialis produces the red pigment astaxanthin.*

3 *Biomass of algae containing lipids after harvesting. © EnBW*

4 *FPA reactors in a pilot plant of Subitec GmbH, which is supplied with waste gas CO_2. © Thomas Ernsting*

ENERGY SOURCES FROM REGENERATIVE RAW MATERIALS

If the utilization of renewable raw materials is to fulfill the requirements of sustainability, the processes have to be considered as a whole. Advantageous processes are those that are not in competition with foodstuff production, do not destroy any forests, do not require too much water and that have the objective of utilizing the raw materials as completely as possible in the sense of a biorefinery.

Biofuels from wood and straw

The use of wood and straw waste provides a sustainable solution. As described above, first of all new methods have to be developed in order to convert the stable natural biopolymers hemicellulose, lignocellulose and lignin into technologically usable monomers. We are working on this at the Fraunhofer IGB. The sugar obtained not only serves as a base chemical; after fermentative conversion it also provides biofuels such as bioethanol
or biobutanol.

Oil or biodiesel from lipid-rich algae

The production of lipids as a carbon and energy store is widespread among microalgae. After a reduction of the growth rate as a result of nutrient deficiency, many types of algae store lipids in the form of oil. Here, the production of lipids depends to a large extent on the light available to the algae and only occurs if the supply of light and also carbon dioxide remains sufficiently high. If such algae are selected specifically for their oil content and they are cultivated correspondingly, the production of oil or biodiesel from algal lipids could provide an alternative to the use of vegetable oils as an energy source. In a current project for the production of bioenergy supported by the Federal Ministry of Education and Research (BMBF) together with FairEnergie Reutlingen and Subitec GmbH, oil is to be obtained from algae and this is to be used to produce energy in a block-type heat and power plant run on vegetable oil.

Methane – end product of the biomass refinery

Renewable raw materials such as microalgae and cultivated plants contain organic carbon compounds, resulting from solar energy that has been used to fix CO_2 by photosynthesis. Therefore, they are not only carbon sources but also energy sources. After extracting the chemicals from the biomass, the residue can be used as a carbon dioxide-neutral energy source. Methane, a constituent of biogas, is an end product of biomass processing that is useful both as a compound and as an energy carrier. Methane is produced during the anaerobic digestion of organic waste such as biological garbage, sludge formed in wastewater purification plants, and residues of renewable raw materials. Biogas can provide heat and electricity, or methane can be converted into methanol for use as liquid automotive fuel. The IGB demonstrates this at two pilot plants. In Stuttgart, waste from wholesale markets and algae biomass are fermented; in Brazil, the biogas resulting from sludge digestion at a sewage treatment plant is used as automotive fuel.

1 *When digesting residual biomass, biogas is produced.*
2 *EtaMax demonstration plant in Stuttgart-Gaisburg.*

R&D SERVICES AT A GLANCE

- Market and technology analyses
- Screening of gene libraries for desired enzymatic activities
- Custom-made gene libraries for special requirements
- Development of new high-throughput enzyme assays
- Subcloning, sequencing, expression and characterization of new enzymes
- Enzyme optimization, further development of new enzymes by evolutive design, in vitro enzyme engineering
- Metabolic engineering of production strains
- Enzyme purification at pilot-plant scale
- Strain and parameter screening in multi-fermenter systems
- Development and optimization of bacterial and fungal fermentation processes from laboratory up to pilot-plant scale
- Combination of biological and chemical processes
- Screening and development of photoautotrophic processes from laboratory up to pilot-plant scale for microalgae and cyanobacteria in flat panel airlift reactors, transfer to open-air production conditions
- High cell density fermentations, also continuous operation, with cell retention by filtration or immobilization
- Processes for the production, isolation/separation and purification of biotechnical products and natural substances (carbohydrates, organic acids, fatty acids, proteins, enzymes, etc.)
- Downstream processing technologies like filtration (micro-, ultra-, nano-), electrodialysis and other membrane processes, extraction, chromatographic methods (ion exchange, size exclusion, reversed-phase chromatography)
- Integration of the use of biomass to obtain substances and energy by means of coupled and multiple sequential use (cascade use)
- Scale-up of biotechnical processes and purification processes
- Fermentation up to 10 m^3

CONTACT

R&D solutions for the use of renewable resources in the field of industrial biotechnology are developed on an interdisciplinary basis by scientists at the Fraunhofer IGB. The specialist skills at the Stuttgart location are supplemented by the Straubing´s branch BioCat. Processes developed at the Fraunhofer IGB can be scaled up for technical or industrial implementation at the Fraunhofer CBP.

Dr.-Ing. Susanne Zibek
Bioprocess Engineering
Phone +49 711 970-4167
susanne.zibek@igb.fraunhofer.de

Dr. Ulrike Schmid-Staiger
Algae Biotechnology
Phone +49 711 970-4111
ulrike.schmid-staiger@igb.fraunhofer.de

Dr.-Ing. Katja Patzsch
Industrial Biotechnology
Phone +49 3461 43-9104
katja.patzsch@igb.fraunhofer.de

LEADING-EDGE CLUSTER BIOECONOMY

The Fraunhofer CBP belongs to the Leading-Edge Cluster BioEconomy funded by the Federal Ministry of Education and Research (BMBF). The objective of the cluster is sustainable value creation from non-food biomass such as wood for the production of materials, chemical products and energy.

Fraunhofer Center for Chemical-Biotechnological Processes CBP, Leuna

The Fraunhofer CBP closes the gap between pilot plant and industrial implementation. By making infrastructure and technical plant available, it makes it possible for partners from research and industry to develop processes so as to utilize renewable resources on an industrial scale. It permits chemical-biotechnological processes ranging from the raw material to the biocatalyst and the scaling of the processes for industrial implementation. The focus is on the use of vegetable oils, the fractionation of lignocelluloses and the production of technical enzymes. With a new building providing floor area of more than 2000 square meters, the center offers modular process capacities up to 10 m^3 plus a wide range of processing, treatment and reconditioning techniques.

Dipl.-Chem. (FH) Gerd Unkelbach
Head of Leuna Site
Phone +49 3461 43-9101
gerd.unkelbach@cbp.fraunhofer.de
www.cbp.fraunhofer.de

Institute section BioCat, Straubing

The Institute section BioCat develops new catalysts and catalytic processes that permit a more comprehensive use of plant biomass in the chemical industry. For this, above all chemical and biotechnological methods are combined in a suitable way. The aim is to use the catalysts, for example, to convert terpenes obtained from plants and residual materials in wood processing, into epoxides and monomers for the polymer industry.

Dr. Michael Hofer
Head of Straubing Site
Phone + 49 172 278-3500
michael.hofer@igb.fraunhofer.de

Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB
(Fraunhofer-Institut für Grenzflächen- und Bioverfahrenstechnik IGB)

Nobelstrasse 12
70569 Stuttgart
Germany
Phone +49 711 970-4401
Fax +49 711 970-4200
info@igb.fraunhofer.de

Director
Prof. Dr. Thomas Hirth
Phone +49 711 970-4400
thomas.hirth@igb.fraunhofer.de

www.igb.fraunhofer.de

Fraunhofer IGB brief profile

The Fraunhofer IGB develops and optimizes processes and products for the business fields of medicine, pharmacy, chemistry, the environment and energy. We combine the highest scientific standards with professional know-how in our competence areas of Interfacial Engineering and Materials Science, Molecular Biotechnology, Physical Process Technology, Environmental Biotechnology and Bioprocess Engineering, as well as Cell and Tissue Engineering – always with a view to economic efficiency and sustainability. Our strengths are to offer complete solutions from laboratory scale to pilot plant. Customers also benefit from the constructive interplay of the various disciplines at our institute, which opens up new approaches in areas such as medical engineering, nanobiotechnology, industrial biotechnology and wastewater purification. The Fraunhofer IGB is one of 60 research institutes of the Fraunhofer-Gesellschaft, Europe’s leading organization for application-oriented research.

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