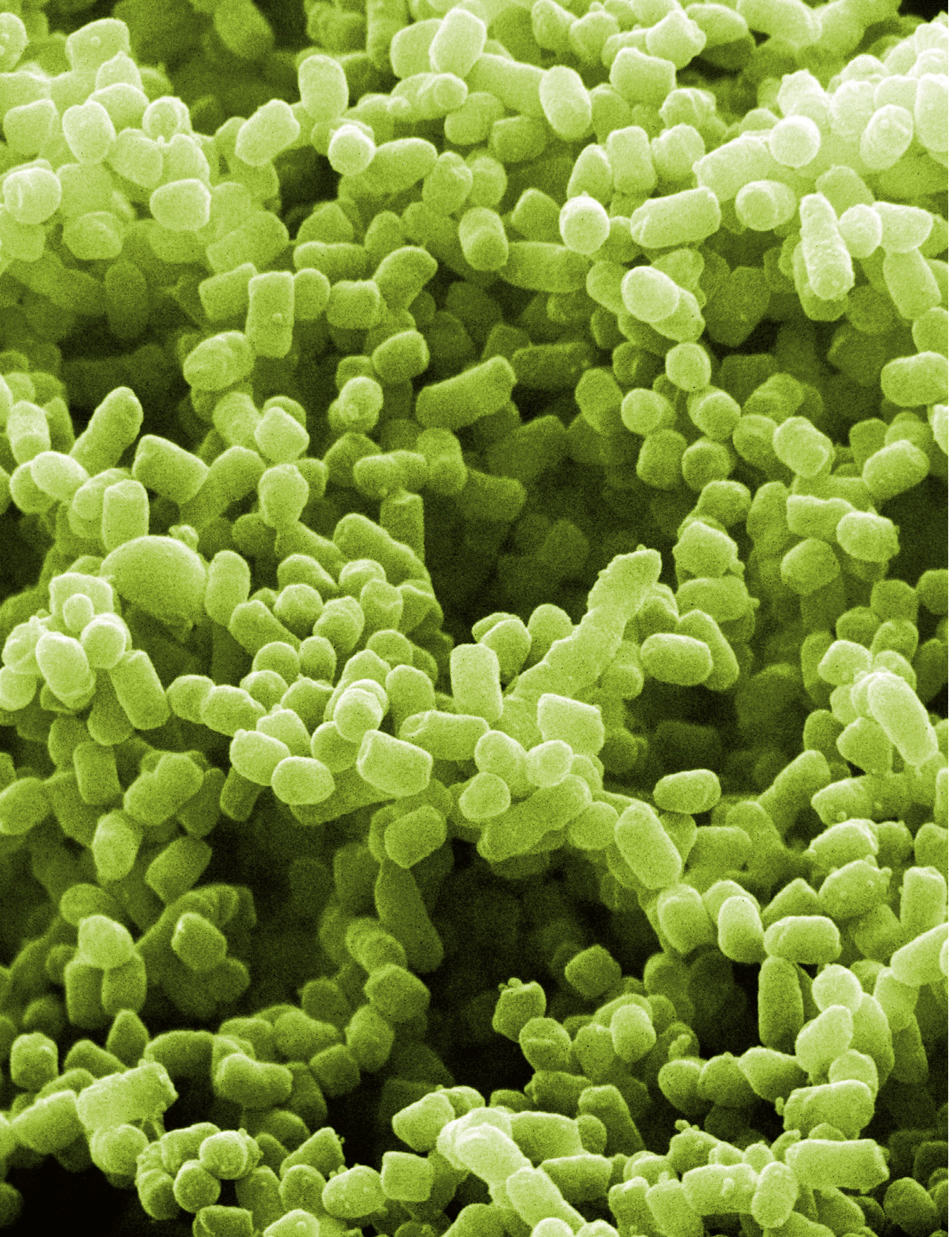


Powerful by their very nature, bacteria can easily synthesize a wide variety of substances

STRONG STRAINS

Some coveted products cannot be produced by simply using chemical processes. In many cases, microorganisms can help. Through fermentation, microorganisms can easily bring about even complicated reactions—if the right strains are used and the process know-how is correct

TEXT **ANNETTE LOCHER**

The human race has solved many of its problems with the help of chemistry. For example, the invention of ammonia-based fertilizer in the early 20th century made it possible to sufficiently feed the world's rapidly growing population. But there are some challenges for which even the best chemists cannot find technical solutions that can be implemented cost-effectively. In some of these cases, they are assisted by millions of tiny helpers riding to the rescue: bacteria that make it possible to carry out even very complex tasks by means of biotechnical processes. One of the most important such processes is fermentation.

Its effect has been utilized for centuries in areas such as winemaking. But it was not until the 19th century that Louis Pasteur, the French pioneer of microbiology, made the basic principles of fermentation the object of scientific research. Pasteur identified the mechanism by which grape juice ferments to form wine. The crucial drivers of this process are microorganisms that have no need of oxygen. Pasteur coined the term "fermentation" for this process, which takes place in the absence of air. In the field of biotechnology, fermentation is defined more extensively today. It now refers to the conversion of organic molecules by means of bacterial, fungal or cell cultures or through the addition of enzymes.

The full potential of fermentation is only being genuinely realized today. One example of that is lysine. This is an amino acid that human beings and animals need as a building block for proteins. Because their bodies cannot produce lysine on their own, they must take it in with their food. For many farm animals, lysine is indispensable for a balanced diet and optimal feed conversion.

But there's a problem: Only natural lysine, which is known as the L-form, can be used by the body, and producing it chemically is a very complex process. In the 1980s, chemists at Degussa, one of Evonik's predecessor companies, tried in vain for a long time to →

“Even the most complex molecules can be derived from sugar”

DR. TIMO MAY, HEAD OF THE FERMENTATION PROCESSES GROUP AT EVONIK’S BIOTECHNOLOGY RESEARCH PLATFORM

produce the L-form cost-effectively on an industrial scale. That’s because traditional chemical processes always produce a 50-50 mixture of the L-form and the D-form of lysine. These two forms of a molecule are like the left and right hands of a human being: They are not identical, but instead mirror each other.

The breakthrough came with the introduction of a fermentation process, because certain bacteria selectively form the desired biologically active L-form.

“When the organism and the process are right, even the most complex molecules can be derived from a carbon source such as sugar,” says Dr. Timo May, an expert in fermentation processes at Evonik. Today one of the company’s most important technology platforms is derived from the process for producing L-lysine that was discovered back then (see the article starting on page 23).

FROM ANIMAL FEED TO MEDICINE

L-lysine is only one of many products that can be manufactured cost-effectively thanks to fermentation. The areas of application of microbial processes range from animal feed to cosmetics and medicine. As a rule, fermentation processes are also more sustainable than traditional methods of production. They use renewable raw materials, are efficient, and make it possible to produce bioproducts.

One example of the latter is the algae oil that Evonik produces by means of a fermentation process in a joint venture with Veramaris. It contains the omega-3 fatty acids DHA and EPA and also promotes environmentally friendly fish farming. Another example is rhamnolipids, the first biosurfactants in the world to be produced on an industrial scale. Thanks to fermentation processes, they are especially degradable (see the article on page 25). Customized and ultrapure collagens have also recently been produced by bacteria. These structural proteins are needed in areas that include cosmetics and medical technology in order to smooth away wrinkles, heal cartilage defects, and much more. Conventional production processes use starting materials of animal origin. Production processes that use specialized bacteria eliminate the risk of variations in quality, allergic reactions, and the transmission of illnesses. As a result, they increase safety. A brand-new application is the biotechnological production of nanostructured cellulose, which is used as a dressing in modern wound treatment (see the article starting on page 26).

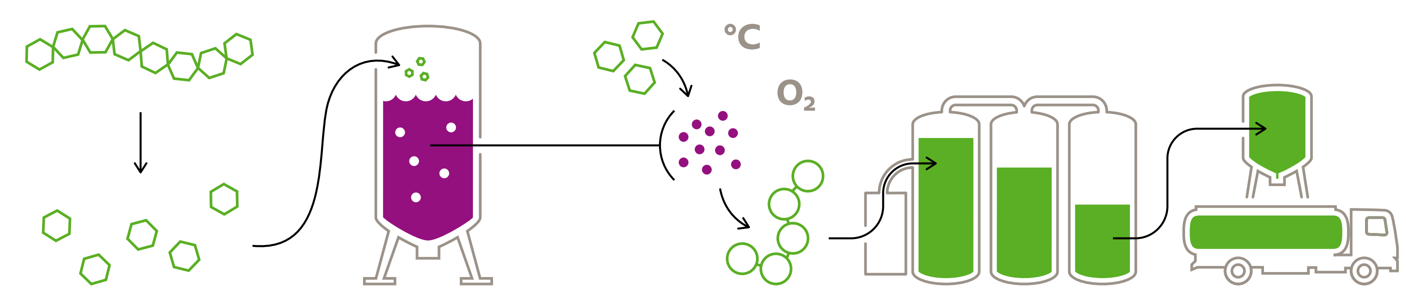
The biologist Timo May and his colleagues are fascinated by the way that microorganisms produce highly complex molecules. “They can conduct several desired reactions simultaneously—reactions that would require consecutive steps in a chemical synthesis and would therefore require very complex equipment,” he says. This capability also makes it possible to conduct processes such as artificial photosynthesis, in which carbon dioxide and water form specialty chemicals when exposed to solar energy. Evonik has joined forces with Siemens to develop this technology. The researchers at Evonik are working to develop a type of fermentation in which bacteria produce specialty chemicals. A pilot plant is now operating at the Evonik location in Marl.

SEARCHING FOR THE BEST STRAIN

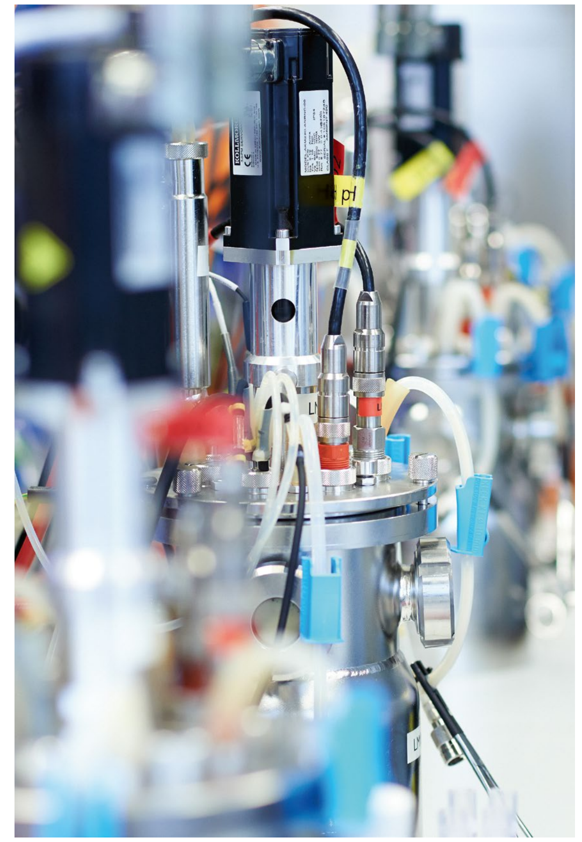
In order to conduct biotechnological production at the top level, expertise in three areas is required: the development of bacterial strains, fermentation, and the refinement of the products. “Biotechnology is much more interactive than chemistry,” says Dr. Wilfried Blümke, the head of the innovation group for the refinement of biotechnologically produced substances. That’s because in biotechnology three components—the organism, the reactor, and the refinement process—all interact strongly with one another. “Only by working as a team can we decide in every individual case on what level we solve a certain problem,” he says. The first task of the scientists is to find a microorganism that can produce the desired product naturally. For example, the bacterial strains *Escherichia coli* and *Corynebacterium glutamicum* have proved to be dependable producers of amino acids. The microorganism is optimized during →

Small organisms with a big effect

How marketable products are produced by the fermentation of starch



1. Starch from a source of carbon is processed into simple sugars.
2. Special microorganisms are grown in a fermenter and fed with sugar either in the presence of oxygen (aerobically) or without oxygen (anaerobically).
3. The microorganisms convert the sugar. Depending on their environment (temperature, oxygen etc.), they orient their metabolic processes toward the desired product.
4. Separation of cells and byproducts. In some processes (e.g. for components of animal feed) the bacterial biomass is also a component of the product.
5. Storage, filling, and transportation.



Small-scale fermenters play an important role in the development of fermentation processes

Industrial production takes place in multilevel fermenters such as these at the Evonik plant in Slovenská Ľupča

Before the bacteria are used in production, they are tested in the laboratory and their properties are optimized for the desired application

the process of strain development. The goal is to get the microorganism to convert as much as possible of its carbon source, usually sugar, into the desired product. If the organism that has been found is not suited for industrial production, for example because it is pathogenic to human beings, molecular biologists transfer the relevant genes into a harmless microorganism that has proved its dependability.

FROM A BROTH TO A PRODUCT

During the first phase inside the fermenter, the microorganisms are still allowed to multiply and form biomass. Then, at a certain point in time, they are expected to direct their metabolic processes toward creating the desired product. The biotechnologists control this change of direction by adding or removing certain substances, for example. After a period that may last anywhere between a few hours and several days, the microorganisms are inactivated and the fermentation broth is drained off. Finally the desired product can be extracted from this broth.

"The refinement process varies, depending on the type of product," says Blümke. That's why the bioengineers confer with the marketing experts at an early stage in order to precisely customize the process. In many cases, several consecutive separation processes, such as filtration, centrifugation, and extraction, are needed. For example, in the production of a probiotic—in other words, living bacteria in spore form—a gentle drying process is very important. As a component of a dietary supplement or feed additive, the bacteria of course need to become active once again inside the guts of people or animals so that they can deliver their valuable metabolic products.

"We are looking for the most robust and most cost-effective of all the possible solutions," says Blümke. In this case, "robust" means that the refinement process delivers a product that precisely fulfills the quality requirements even if the results of the fermentation process vary. Between approximately 20 and 30 tried and tested refinement processes are available to the biochemical engineers at Evonik. All of the steps are modeled and simulated in the laboratory, then tested and improved in a pilot plant, and finally—if everything works well and the product is successful on the market—implemented on an industrial scale.

But at that point, the experts are still not satisfied. Even in a large-scale technical installation, biotechnological processes are constantly being optimized. For example, a higher-performing microorganism makes it possible to adapt the production and refinement process. Conversely, the bacterial strains are developed further in order to make even more cost-effective processes possible. The ultimate goal is to enable the ancient cultural technique of fermentation to help solve mankind's present-day problems sustainably with the help of cutting-edge scientific efficiency.

TOWARD CLIMATE NEUTRALITY

The use of fermentation methods at Evonik began with the production of BIOLYS® more than 30 years ago. Bioengineers are continuously improving the productivity and reducing the carbon footprint of this production process

TEXT **ANNETTE LOCHER**

The new head of process development in Blair, Nebraska, had an ambitious goal. "We can boost the plant's productivity by 50 percent," said Dr. Henning Kaemmerer in the fall of 2018, shortly after he had moved to the USA from Hanau. The plant, which had been producing the amino acid BIOLYS® for almost two decades, had been repeatedly optimized after its initial commissioning. But…50 percent? A productivity boost of this magnitude was hard to imagine.

BIOLYS® is added to animal feed—primarily feed for pigs. The animals need L-lysine, an important component of proteins. However, like human beings, they cannot produce this amino acid on their own, so they have to take it in with their feed. The amounts of the amino acid L-lysine in the plant-based components of pig feed are very low. If BIOLYS® is added to the feed as a source of lysine, the volume of the feed can be significantly reduced. That relieves the animals' metabolic processes, conserves natural resources, and reduces feed costs and emissions.

FRUGAL PRODUCERS

BIOLYS® is produced by means of fermentation. In gigantic stainless steel boilers, each with a volume of several hundred cubic meters, bacteria convert dextrose, a sugar, into L-lysine—much more of it than they need for themselves. The BIOLYS® process at Evonik uses a strain of *Corynebacterium*. The process engineers provide the ideal conditions for its growth and production processes: an aqueous medium, the right temperature, a plentiful supply of oxygen, and some mineral substances. After several days in the fermenter, the organisms are inactivated and the fermentation broth is drawn off and then vaporized. The mixture of lysine and bacterial biomass is the basis of the product, which is subsequently processed into a granular form that is easy to handle as animal feed.

Far more than 20 generations of this bacterial strain have been used since 2000. Each strain was a bit more frugal than its predecessor; in other words, it converted more sugar into lysine. But a real production boost resulted from an innovation that was promoted by the ambitious engineer Henning Kaemmerer. Within a few months, the Evonik engineers in Blair succeeded in switching the BIOLYS® process to "semi-continu- →

BIOLYS® helps to make the use of feed in pig fattening more efficient and sustainable

> “We can boost the plant’s productivity by 50 percent”
>
> DR. HENNING KAEMMERER, HEAD OF PROCESS DEVELOPMENT IN BLAIR, NEBRASKA, IN THE FALL OF 2018

ous” operation. Several times during the cultivation phase, part of the fermentation broth was removed and replaced with water, sugar, and the other ingredients. As a result, the product volume per fermentation could immediately be substantially increased—as predicted. The promised 50 percent increase was reached in 2019.

INTERNATIONAL KNOWLEDGE TRANSFER

Kaemmerer feels that his approach has been validated. “This shows what is possible when experts from the areas of research, process development, and production cooperate closely and pool their decades of experience regarding the microorganisms and the processes,” he says. That’s because the bacterial strain and the process must be repeatedly adapted to each other. The only unchanging element is the plant itself. As Kaemmerer explains, “The plant defines the limits within which we work.” This has enabled the product quantity per fermenter to be more than tripled since 2000. Simultaneously, the amino acid content of the initial product has increased significantly from BIOLYS®55 to BIOLYS®77.

Other Evonik locations are making good use of the experiences of their colleagues in the USA. “Of course we’ve benefited from the know-how in Blair,” says Miguel Menezes, who is responsible for BIOLYS® production in Castro, Brazil. The plant in Castro was commissioned in 2015. Since that time, the engineers in Castro have also been tinkering with improvements. Among other things, they have focused on making the production process more sustainable. They have continuously reduced not only the relatively high water requirement of the gigantic fermenters but also the consumption of steam and electricity. Energy is needed to cool the fermenters, operate the stirrers that ensure optimally uniform conditions inside the fermenters, and evaporate the water at the end of the process.

Castro is committed to renewable resources. Hydropower covers a large proportion of the plant’s energy needs, all of the necessary steam is produced with the help of eucalyptus woodchips, and the raw material dextrose is provided by a nearby corn mill. The plant is located in the midst of a corn-producing region that has been used for agriculture for more than 20 years.

Three quarters of the total production is sold in regional markets. That also reduces transportation routes and improves the carbon footprint. A recently conducted life cycle assessment confirms that only 0.1 kilogram of CO_2 equivalent is generated per kilogram of BIOLYS®77. Miguel Menezes is proud of this achievement, and Henning Kaemmerer is equally proud of his productivity increase. “We’re very close to achieving a completely climate-neutral production process,” he says. “In that case, the plant in Castro would be Evonik’s first industrial-scale production plant with a neutral carbon footprint.”

SWEET AND CLEAN

Biosurfactants that are produced by the fermentation of sugar are in demand for the manufacture of cosmetics and detergents

TEXT **CHRISTOPH BAUER**

Five years ago, the laboratory’s first attempts to produce biosurfactants by means of fermentation created huge mounds of foam, recalls Dr. Hans Henning Wenk, the head of Research and Development at Evonik Care Solutions. However, the experts in Slovenská Ľupča (Slovakia) now have the process firmly under control, and are using rhamnolipids produced by bacteria to manufacture biosurfactants.

Surfactants are contained in dishwashing detergents, shower gels, and bath additives, where they ensure that dirt doesn’t collect again on dishes or skin. In the European Union, surfactants have to largely break down during wastewater treatment, which is why the consumer goods industry is increasingly using biosurfactants. However, the demand is also growing in regions where sewage treatment plants are rare but people are becoming increasingly environmentally conscious.

After its start in the cosmetics sector, Evonik and its partner Unilever developed a hand dishwashing liquid based on such biosurfactants. The product is already on the market in Chile and Vietnam, where dishes are generally washed by hand. Thanks to rhamnolipids, hand dishwashing liquids that are based on biosurfactants are on a par with very good products based on synthetic surfactants.

The demand for these sustainable raw materials is rapidly increasing. For example, Unilever wants to stop using fossil carbon in its household and textile care formulations by 2030. Rhamnolipids are now also found in toothpaste, facial cleansers, and shampoos. Evonik is therefore continuing to invest in this production technique in order to strengthen its leading position in this globally growing market.

DOWN THE DRAIN

The biosurfactants that Evonik produces consist of a sugar component and a fatty acid component. For the fermentation process, the company uses a strain of bacteria that produces both components from plant-based sugars. Evonik uses dextrose as a substrate, which is made from plants such as European corn.

Biosurfactants made from rhamnolipids are high-performance materials that are also environmentally friendly and gentle to the skin. They are found in hand dishwashing liquids and in care products

Not only are the raw materials and the production process environmentally friendly, the product itself also has a low environmental impact. Says Wenk: “In the end, it goes down the drain and to the sewage treatment plant. In some regions, it is even released directly into the environment.” However, the environmental impact is vastly reduced because rhamnolipids are much less toxic than conventional surfactants and very easily biodegradable.

The main feature of Evonik’s fermentation process is its scalability. Although many natural bacteria convert fats into rhamnolipids, they only do so in microscopic amounts. At Evonik, this task is performed by *Pseudomonas putida*, a well-researched “safety strain.” “After we gave it the genetic tools to produce rhamnolipids in large amounts, we continuously optimized it further,” says Wenk.

The process ultimately created a strain of bacteria that produces biosurfactants in industrial amounts. “To achieve this, we got bioengineers together with process experts, chemists, and engineers,” reports Wenk. “We benefited here from our experience with the development of surfactants.” The expertise of a physical chemist proved to be crucial because he was able to explain why a surfactant that had some of its parameters modified suddenly behaved completely differently than before. This knowledge enabled the researchers to get the foam under control so that it now only goes into action in sinks and bathtubs.

FROM JENA TO THE WORLD

In the laboratories of JeNaCell, bacteria are producing the material for a dressing that enables wounds to heal quickly and gently. This area of application is scheduled to grow further following the company's acquisition by Evonik

TEXT **NICOLAS GARZ**

The dressings made of biotechnologically produced cellulose can be formed into any desired shape and they optimally protect wounds

Everything in Uwe Beekmann's daily work has to do with a soft and relatively slippery membrane. This gelatinous substance is produced by bacteria in a fermentation process. Beekmann, who has a doctorate in pharmacology, is creating the conditions in which the microbes feel at home as they work. To feed the bacteria, Beekmann pours a glucose solution into a square glass dish. "The bacteria initially use the nutrient solution to survive and multiply," says Beekmann, who works at the research and development department of the biotech company JeNaCell in Jena, Germany. "They then combine glucose components into fine fibers in order to protect themselves against dehydration and environmental factors such as ultraviolet radiation." These spider silk-like objects of nanostructured cellulose eventually form a thin fleece that Beekmann carefully separates from the nutrient solution. It is afterwards cleaned so that all bacteria are removed and only the cellulose and water remain. The material can then be cut into any shape desired.

BACTERIA BEAT PLANTS

However, more important than the exterior shape is the material's interior composition. "What's special about the bioengineered cellulose is its close-knit internal web structure," explains Beekmann. It gives the material its great stability and strength. "Added to that are its biocompatibility and good skin tolerance, which make it ideal for use in medical applications and cosmetics." If the material were plant-based, it would not be possible to achieve these properties in this quality. That's why the company is focusing on very productive strains of bacteria.

It all began with a few small Petri dishes full of cellulose that Dana Kralisch discovered in a lab at the Institute for Organic and Macromolecular Chemistry at the Friedrich Schiller University Jena in 2006. Although no concrete application was foreseeable at the time, the material fascinated Kralisch, who had just received a Ph.D. in chemistry, so much that she initiated a research project. In addition to conducting work in the laboratory, she and her team began to look for markets. "We spent months surveying companies from a variety of sectors and investigated the possibility of collaboration," she recalls.

It quickly became clear that the bioengineered cellulose promised to especially benefit the wound healing process. "It completely covers all open nerve ends and continuously cools and moistens the wound," says Kralisch. The dressing particularly reduces the pain of burns because the material doesn't stick to the wound and can therefore be easily changed.

DISCOVERY AND EQUITY INVESTMENT

In 2012 the startup was spun off under the name JeNaCell and its activities really took off. "Although there were already many studies about this topic back then, there wasn't an industrial manufacturing process," relates Kralisch. "We therefore had to develop a biotechnological process that enables the material to be produced in large amounts." The first production facility was soon put into operation so that the material could be supplied to the manufacturers of medical products. Kralisch vividly recalls the moment when she received the first report from a hospital that the biotech cellulose had been successfully employed. "To find out that our product really helps patients to heal faster was the best news we could get," she says.

At this time, the company was also being monitored by Bernhard Mohr. As the head of Evonik's venture capital unit, Mohr is always on the lookout for innovative startups. The decision in which companies Evonik invests is made according to clearly specified criteria: "The technology has to be innovative and attractive for relevant markets," says Mohr. "Our confidence in the company's management team also plays an important role." JeNaCell fulfilled all of these criteria from the very start. "Because Evonik also has developed extensive expertise in fermentation, it was quickly clear that the startup would fit very well into our portfolio."

In 2015 Mohr and his team decided to invest in JenNaCell in order to enable a research and growth offensive. The startup intensified its cooperation with Evonik until it was acquired by the latter in the summer of 2021. Since then, the company has been part of the Health Care business line. "JeNaCell has increasingly evolved from purely a manufacturer of wound dressings into a technology platform," says Dr. Andreas Karau, the head of Medical Device Solutions. The cellulose is now also being used for the dermatological post-treatment of cosmetic surgery and laser treatments. Moreover, the researchers are working on using bacterial nanocellulose in implants.

The material might even carry medication. "It would enable pharmaceutical active ingredients to be transported via the skin precisely to their destination in the body," says Karau. And all of that is thanks to millions upon millions of bacteria in square glass dishes.

> "JeNaCell has evolved into a technology platform"
>
> DR. ANDREAS KARAU, HEAD OF MEDICAL DEVICE SOLUTIONS

Annette Locher has a degree in biology. She has been working at Evonik since 2012. She writes primarily about health, nutrition, and sustainability

Christoph Bauer is a journalist who works at Evonik's Communications department

Nicolas Garz is an editor at the Hamburg-based communications agency Bissinger+. He regularly writes about topics from the areas of research, digital technologies, and sustainability