Partners in the deep

It is truly a feat to create conditions like those in the deep ocean in a research laboratory. **Gunter Wegener** has mastered the art. Together with his team from the **Max Planck Institute for Marine Microbiology** in Bremen, he hopes to discover how microorganisms degrade methane and other hydrocarbons on the seabed.

TEXT **KLAUS WILHELM**

The heap of mud is the starting point for Gunter Wegener's scientific adventure. It originated in the depths of the ocean. In 2009, Wegener dived with a research submarine in the Guaymas Basin in the Gulf of California and sampled the seabed at a depth of 2000 meters.

"I'm in the process of writing for a chapter of one of the standard textbooks in microbiology," says Wegener. "I always dreamed about passing on knowledge by writing a book for students." His chapter deals with how microorganisms degrade natural gas in the absence of oxygen – known to scientists as anaerobic oxidation of methane – and Wegener's own discoveries in the field.

The focus of the research of Gunter Wegener and his team is a biocommunity of so-called archaea and bacteria in the seabed. These organisms really like it hot. Archaea are organisms that look like bacteria, but they are fundamentally different genetically. Scientists, therefore, place them in a separate domain in the tree of life.

At the time of that dive in the Gulf of California, little was known about the microorganisms that degrade natural gas without the help of molecular

In the Guaymas Basin in the Gulf of California, brine flows out of the seabed at a depth of 2000 meters and forms towers of minerals. The yellow microbial mats (in the background) contain communities of bacteria and archaea. Diving in the research submarine Alvin, Gunter Wegener took samples, which he later cultivated in the laboratory.

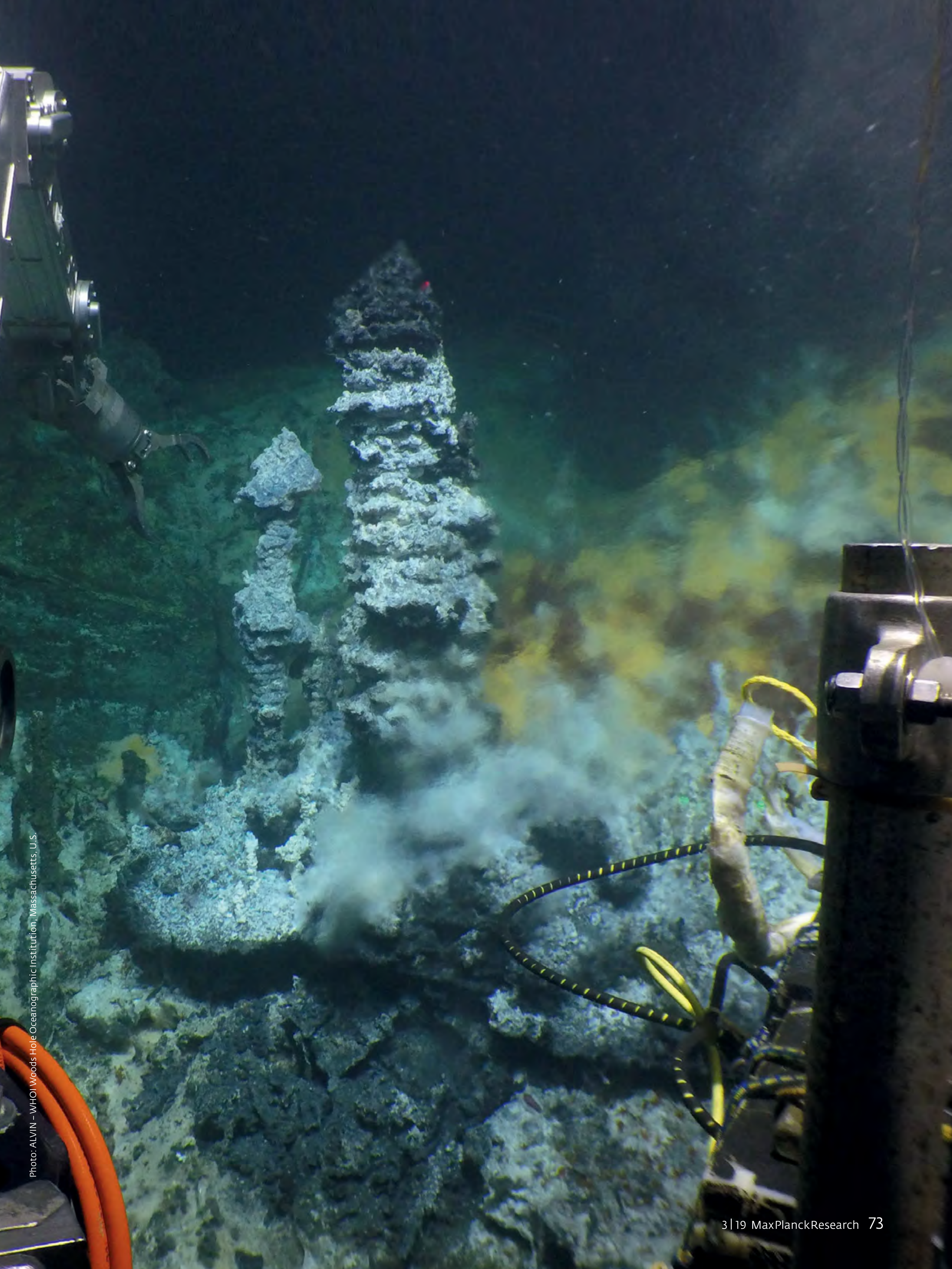


Photo: ALVIN – WHOI Woods Hole Oceanographic Institution, Massachusetts, U.S.

» Archaea make an important contribution to the formation and degradation of hydrocarbons on Earth.

oxygen. In the Guaymas Basin, geological processes in the Earth's crust produce large quantities of methane. Nevertheless, only small amounts of the greenhouse gas ascend and enter the atmosphere. For this reason, scientists suspected for a long time that there must be organisms degrading the methane. It wasn't until 2000 that Antje Boetius from the Max Planck Institute in Bremen was able to prove that it was a combination of archaea and bacteria that convert methane – even without oxygen, which does not exist deep in the seabed.

It seemed that these archaea generate energy from the oxidation of methane to carbonate. Their partner bacteria benefit, because they consume sulfate and an intermediate product, unknown at the time, produced during methane oxidation. "They reduce sulfate to sulfide," Wegener explains, using the correct scientific terminology.

There was a problem however. Boetius and her colleagues only found the aggregates of methane-oxidizing archaea and sulfate-reducing bacteria in cold habitats at minus 1.5 to 20 degrees Celsius. Under these conditions, the organisms reproduce incredibly slowly: once every six months. And at that rate, it's almost impossible to cultivate the organisms in the laboratory and analyze metabolic processes such as methane degradation.

Archaea from hot biotopes such as the Guaymas Basin reproduce much more rapidly, as has been known for some time. Some methanogenic species can also produce methane under pressure at over 100 degrees. And because anaerobic methane degradation is biochemically very similar to methanogenesis, Wegener was crossing his fingers that he might be able to find the methane-consuming species in the pile of mud.

THE ART OF BREEDING MICROBES

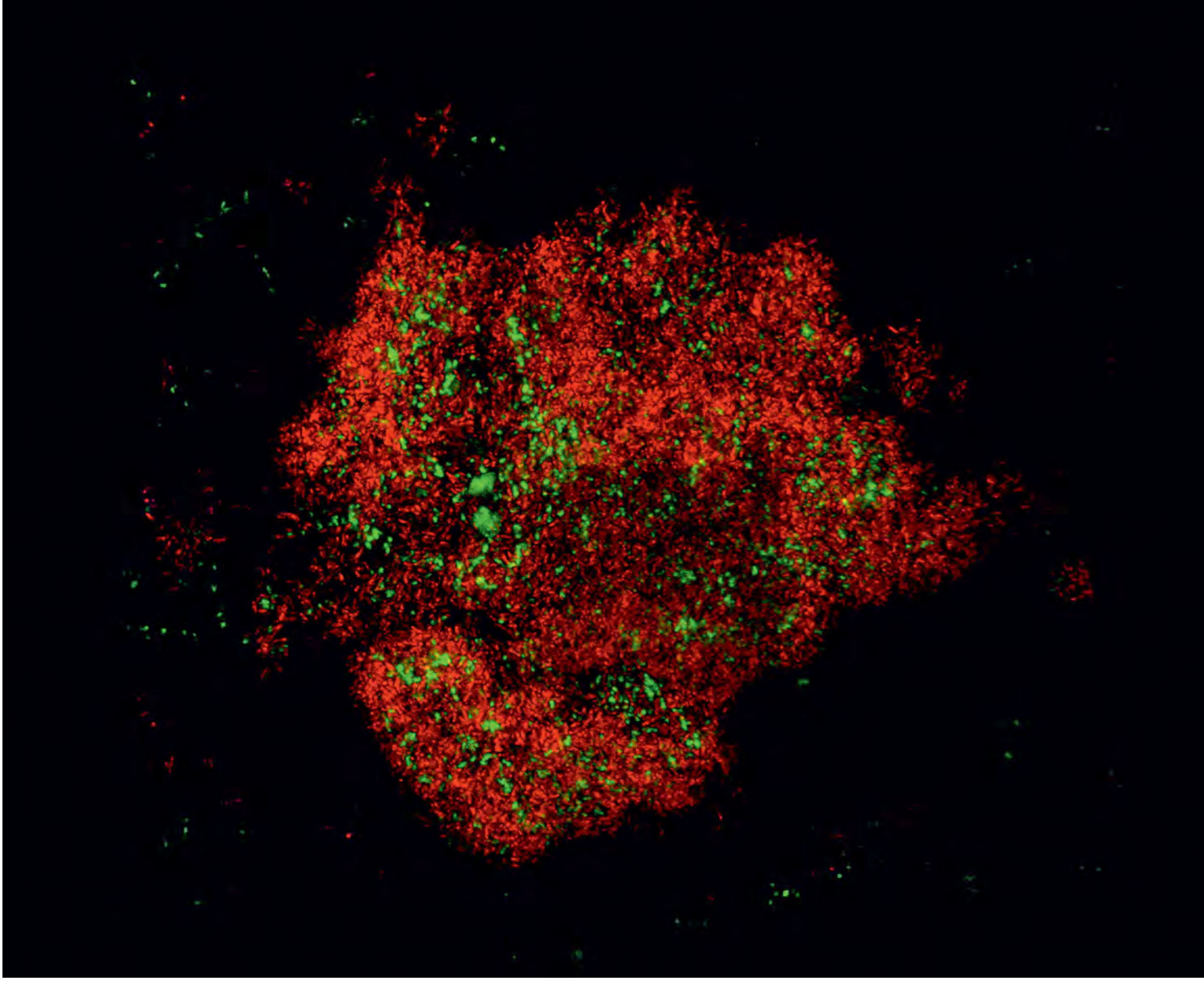
The research team at the Max Planck Institute succeeded in extracting and cultivating the sought-after archaea from the mud. "It actually wasn't that difficult," says Wegener, "you only need two things: time and patience." Because both are in short supply nowadays, Wegener's colleagues have somewhat lost the art of cultivating microorganisms. Instead, researchers have focused on collecting the genetic material of all organisms contained in a water or soil sample, decoding it and attributing it back to individual living organisms. The method, known as metagenomics, provides a quick but rather superficial insight into the abilities of organisms.

At the Max Planck Institute in Bremen, the microbes drift in small bottles of artificial seawater completely devoid of oxygen. "The art consists in simply doing nothing and just waiting," explains Wegener, who was born in the Harz region in Germany. "After five weeks at the earliest, I start checking the cultivation success." It's a formula that has remained successful to this day and for Wegener a competitive advantage over scientists under the pressure of short-term contracts.

Wegener and his colleagues had to bide their time for a year and a half before they could reliably reproduce the microbes. Only then did they have a stable culture without sediments that they could study. They discovered that the community of archaea and bacteria in the Guaymas Basin optimally oxidizes methane at 50 degrees, although it can still do so at 70 degrees. At optimal temperature, the microbes double in number about every two months – much faster than their relatives from cold seas.

The microbial communities consist of methane-oxidizing archaea, known as ANME-1 (ANaerobic MEthanotrophs) and *Desulfofervidus auxilii*, the heat-loving sulfate-reducing helper bacterium. The two partners form aggregates of many thousands of cells, or they combine in chain-shaped shells to create a kind of residential community.

Following this first discovery, the discoveries came thick and fast. First, the scientists showed that the methane-oxidizing archaea use the same enzymes as their methane-generating relatives but in the opposite direction. They employ a catalyst called methyl-coenzyme M reductase to activate methane. The archaea produce it in



Microbial community under a fluorescence microscope: the archaea (red) convert butane to carbon dioxide, while their partner bacteria (green) utilize sulfate.

large quantities. The methane molecule is linked to the sulfur compound coenzyme M in this enzyme to form methyl-coenzyme M, and in further reactions it is completely converted to carbon dioxide and finally carbonate.

POWER CABLES CONNECTING THE CELLS

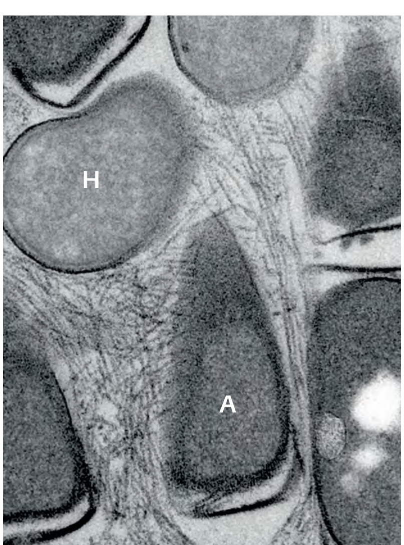
But what holds the partnership of archaea and bacteria together? Wegener has a spectacular answer: "Tiny cables of protein." During methane oxidation, the archaea liberate positively charged protons and negatively charged electrons. The microscopically small power cables allow the electrons to flow into the bacterial cells where they are used for sulfate reduction.

The same bacteria can also be found in other microbial communities. They also live together with archaea called Syntrophoarchaeum, which degrade butane rather than methane, as Wegener and his colleagues have discovered. Surprisingly, Syntrophoarchaeum also utilizes methyl-coenzyme M reductases. Until then, experts had assumed that these enzymes were exclusively involved in methane metabolism. However, Wegener and his colleague Rafael Laso-Pérez have discovered new variants of the enzyme that activate molecules with several carbon atoms.

With the help of this metabolic pathway, the newly discovered archaea also degrade propane and ethane – all the short-chain hydrocarbon gases that rise from the seabed in the Guaymas Basin. The discovery has opened up a whole new field of research. Since the findings of Wegener's team, several researchers have detected methyl-coenzyme M reductases in other archaea. "The results suggest that archaea play

Top Rafael Laso-Pérez and Gunter Wegener have had to adapt their microbes to life in the laboratory in order to study their metabolism. It required a great deal of patience, but they were able to develop techniques that allowed the microorganisms to feel comfortable away from the deep-sea sludge.

Below Methane-oxidizing community under the electron microscope. The cable-like structures are thought to allow electrons to flow from the archaea (A) to the bacteria (H). This allows the archaea to convert methane into carbon dioxide and the bacteria to convert sulfate into hydrogen sulfide.



a more important role in the hydrocarbon cycle than previously thought," says Laso-Pérez.

WIDESPREAD GENES

This hunch was borne out by an analysis of genome databases carried out by Wegener and colleagues from the Jiaotong University in Shanghai to identify gene sequences for methyl-coenzyme M reductases. The researchers discovered several previously unknown genes coding for the production of these enzymes. With the help of the new genes, the researchers then reconstructed the genome of the associated organisms in the mass of existing sequences. To the surprise of the researchers, many previously unknown archaea strains possess methane metabolism genes.

Just what the microbes do with the enzymes has not yet been clarified. Some seem to generate methane, others to degrade it. "We have probably discovered the first archaea that can use methane with without sulfate-consuming partner bacteria," explains Gunter Wegener. The electrons released during oxidation are then presumably transferred to receptor substances such as oxidized iron (rust) or other metal oxides.

Wegener now intends to analyze samples from oil wells in northeastern China. The organisms in these samples are apparently capable of converting crude oil into methane – possibly with the aid of methyl-coenzyme M reductases: "We're hoping to test this hypothesis and be the first to cultivate the organisms."

In the future, Wegener intends to concentrate not only on pure research but also on the potential applications of his discoveries. One example is the

Photos: Tom Pingel (top), G. Wegener/MPI for Marine Microbiology (bottom) formation of ethane using archaea. Unlike the widespread and industrially produced gas methane, ethane is not a greenhouse gas and therefore doesn't contribute to global warming.

However, despite the best efforts of researchers, no organisms have yet been found in nature that produce ethane or other short-chain hydrocarbons. The microbes discovered by Wegener in the Guaymas Basin only degrade ethane. "But in the same way that identical enzymes are involved in the degradation and generation of methane, the process that degrades ethane might make it possible to generate it," explains Wegener.

Gunter Wegener and other researchers are, therefore, planning to genetically modify archaea to produce ethane. From the modest beginnings of a small pile of mud, one day a raw material for plastics or climate-friendly fuel for cars could be born. ◄

SUMMARY

- **In the seabed of the deep ocean, bacteria and archaea form a living community. The archaea obtain energy from converting methane, while the bacteria extract it by reducing sulfates.**
- **The archaea and bacterial cells are connected to each other by tiny cell extensions. The electrons generated during methane oxidation can flow through these micro-cables to the bacteria, which need them to reduce sulfate.**
- **A key enzyme in methane degradation is methyl-coenzyme M reductase. Variants of the enzyme that can also activate other hydrocarbons, such as ethane, could potentially be used to generate fuels that are more climate-friendly.**

GLOSSARY

Archaea: Organisms in the Archaea domain are as extensive and diverse as bacteria. Almost every month, a new branch is added to their family tree. At the turn of the millennium, experts had only recognized two principal phyla in the domain: the heat-loving Crenarchaeota and the Euryarchaeota. They were followed by the Nanoarchaeota along with several others that are related to the Crenarchaeota. Contrary to earlier assumptions, archaea don't just live in extreme environments such as hot springs, but also in temperate habitats. However, there they are outnumbered by other organisms and are, therefore, frequently overlooked. Archaea are vital for the natural world, as they are involved in all the key substance cycles.