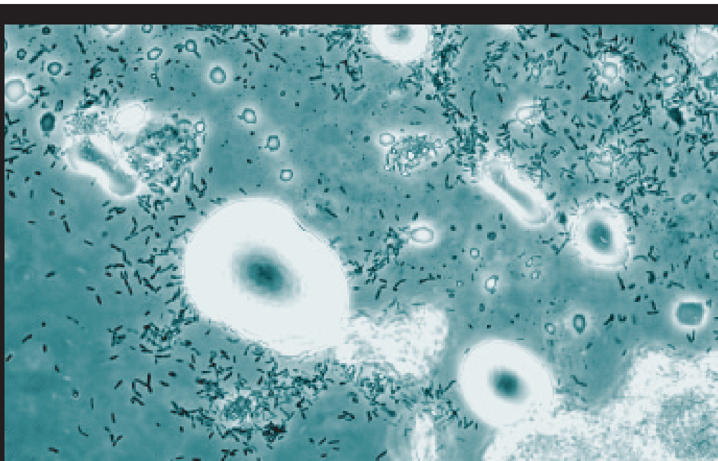
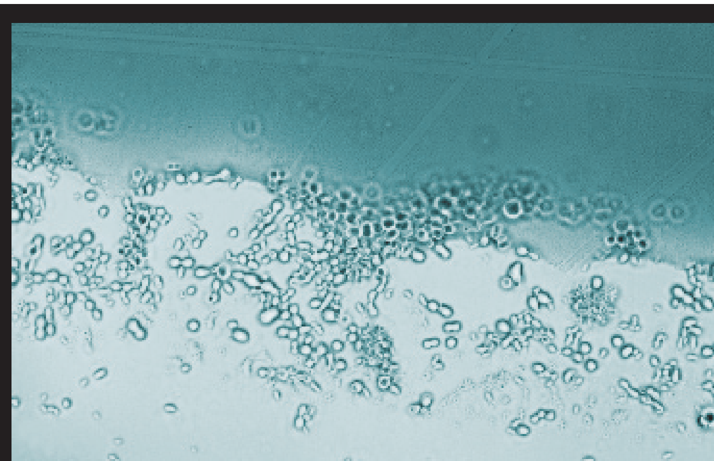
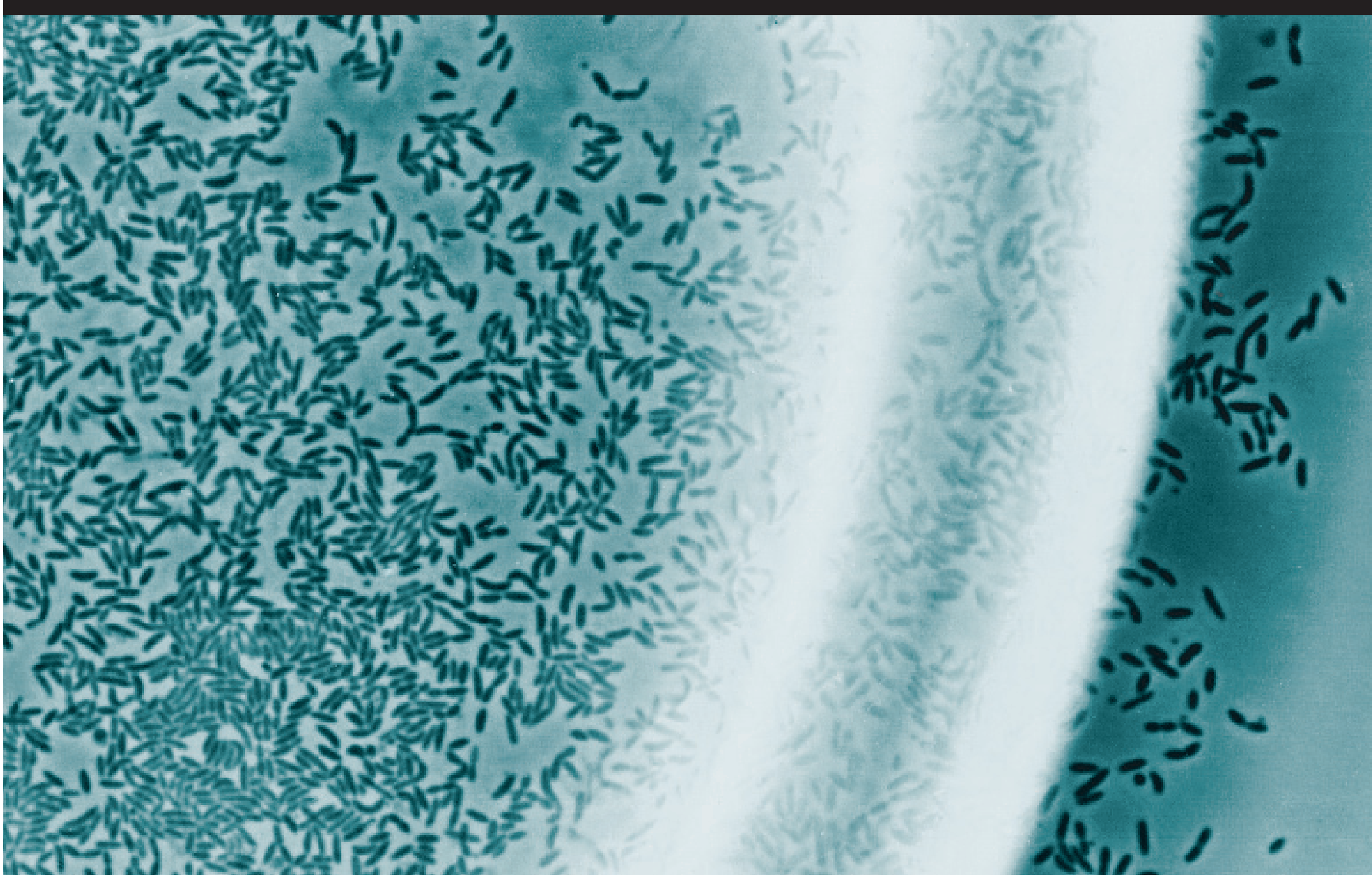




Unicellular Organisms
with an Appetite for Oil

Bacteria can live on almost anything – some even on oil. **Friedrich Widdel**, Director at the **Max Planck Institute for Marine Microbiology**, is studying microbes that break down oil hydrocarbons without oxygen, deep down in the sediment. Could they be useful in oil spills?



left: Appetite for the exotic: Some bacteria (distinguishable as little rods and round shapes) thrive in unusual habitats and feed on oil (larger irregular structures).

TEXT **TIM SCHRÖDER**

"They may not look like much, aesthetically speaking," says Friedrich Widdel, placing some black and white photos on the table. One photo shows a heap of thin black bars, another contains small grey blobs. Physically, the bacteria do not appear to be much more than an abstract, milling mass. To Widdel, however, they are so interesting that he has spent over 30 years studying them. "Their metabolism is truly fascinating. The bacteria can do things that more highly developed life forms are unable to do."

Indeed, some of them have real hidden talents: they can degrade substances that are indigestible for animals and people (such as cellulose), or that are toxic, such as hydrogen sulfide. The organisms absorb nitrogen from the air and make it available to plants.

IT ALL BEGAN WITH HYDROGEN SULFIDE IN AN OIL TANK

No one knows how many types of bacteria there are, and it is quite likely that most of them have yet to be discovered. There is probably no place on Earth where some form of bacteria doesn't feel right at home. The single-celled organisms colonize soils, tombs, waste treatment plants, hot vents at the bottom of the ocean and even the Arctic sea ice. Many billions of these microscopic creatures romp around in and on our bodies, in the digestive system or on our skin. This is a good thing, as microbes improve our defenses and provide us with essential substances.

Friedrich Widdel is interested mainly in the specialized bacteria that survive in the complete absence of oxygen – the "anaerobes," which take in sulfur or nitrogen compounds from their surrounding environment in order to breathe. The scientists from the Max Planck Institute for Marine Microbiology in Bremen are particularly fascinated by the oil-degrading bacterial strains that thrive in exotic habitats: underneath oil slicks on the shoreline or in anoxic oil sediment on the seafloor.

The subject grabbed Widdel's attention years ago, in 1982, when an engineer friend gave him a tour of an oil storage site. There had been problems with a crude oil tank that was used to separate the oil/water mix from the drill holes; in the containers, a toxic hydrogen sulfide, stinking of rotten eggs, had accumulated. This "sour gas" is a corrosive substance that can even damage steel pipes.

Hydrogen sulfide is found in many oil reservoirs. It was generally known that it is generated by chemical reactions at high temperatures deep in the ground. However, why hydrogen sulfide would form in the lukewarm tank was a mystery. It soon became clear to Widdel that anaerobic bacteria must be responsible for the process in the anoxic tank, as hydrogen sulfide is a typical degradation product of anaerobiosis. But what were the bacteria feeding on?

At the beginning of the 1980s, it was still believed that crude oil could be degraded only by "aerobic" bacteria, organisms respiring oxygen. Crude oil consists of hydrocarbons, mainly so-called alkanes: long chains of carbon atoms with which only hydrogen atoms bond. The metabolism of aerobic bacteria, in which the alkanes are decomposed, is similar to the workings of a car engine. The long chains are torn into pieces and then react with oxygen, leaving only carbon dioxide and water.

OIL COMPONENTS DEGRADED EVEN WITHOUT AIR

The amount of energy released in the reaction with oxygen is enormous. This is why a liter of gas will get you pretty far. For aerobic bacteria, utilizing alkanes with the help of oxygen is thus a sumptuous meal. But what about the anaerobes? Many scientists assumed that anaerobic utilization of alkanes was impossible. Otherwise, surely the bacteria would have finished off the oil reservoirs of the planet during the course of the millennia?

As it turned out, this hypothesis was plausible, but incorrect. Friedrich Widdel brought water with oil residue from the tanks to the laboratory and placed it in air-tight containers. Then he waited. Sure enough, slowly but surely, hydrogen sulfide formed in the test tube. The only possible source was the degradation of the crude oil using the sulfur compound sulfate. To this day, scientists have only partly understood what happens in the metabolism of bacteria. Widdel is thus also trying to find the proteins that are involved in degradation.

It looks as if the anaerobes first extend the chains and then pick them

Photos: MPI for Marine Microbiology (3)

» So far, there has been no real explanation for why bacteria have not long since devoured the planet's crude oil reservoirs.

apart, piece by piece, in order to finally respire them. Instead of oxygen, the anaerobes use, for example, sulfate, which is present in much higher concentrations in seawater than oxygen. Even if the oxygen in the oil separator tank has been used up, the anaerobes can still find quite a lot of sulfate. The end product of the reaction of the sulfate with the alkanes, through a complex sequence of metabolic processes in the bacteria, is hydrogen sulfide. To reduce these and other undesirable bacteria in oil production, antibacterial substances are added to the water, which is pumped into the reservoir.

At first, many thought that this theory of anaerobic degradation of alkanes and oil was too far-fetched. Widdel had to substantiate his findings – it was, after all, plausible that the oxygen had been able to slowly penetrate the rubber plugs of the tightly sealed bacterial containers. There is no such thing as a 100-percent-tight rubber or plastic part. "So we put our oil samples in small glass phials, which we sealed," Widdel says, smiling. More air-proof than that isn't possible. In this way, he managed to get the final proof: the alkane chains are degraded anaerobically to carbon dioxide, and the sulfate is converted to hydrogen sulfide!

GROWING BACTERIA TO COMBAT OIL SPILLS?

Each time a large oil spill occurs, experts argue about how such disasters could be avoided in the future and the best ways to tackle the pollution. Time and again, the use of laboratory-grown bacteria is brought up – bacteria that supposedly could devour the oil with lightning speed – much more quickly and efficiently than naturally occurring microbes. From a microbiological perspective, however, such a huge deployment of bacteria is hardly feasible. Even if the bacteria should survive the abrupt transition from the laboratory environment to the ocean and would begin to feed on the oil, the degradation would soon slow down because the microorganisms also consume essential minerals from the surrounding seawater. Another problem is that the aerobic bacteria, which break down oil very efficiently, require a lot of oxygen to do so. This leads to a lack of oxygen, often within a millimeter of the surface of an oil layer on the shore – and this in turn hampers the degradation process. The anaerobic bacteria, on the other hand, work too slowly to be able to eliminate the oil before it becomes a dense, tarry mass. To make matters worse, the bacteria are active only in the boundary layer between water and oil. Hardly any degradation takes place in the middle of the oil layer, so as the oil slicks become thicker, the degradation rate decreases.

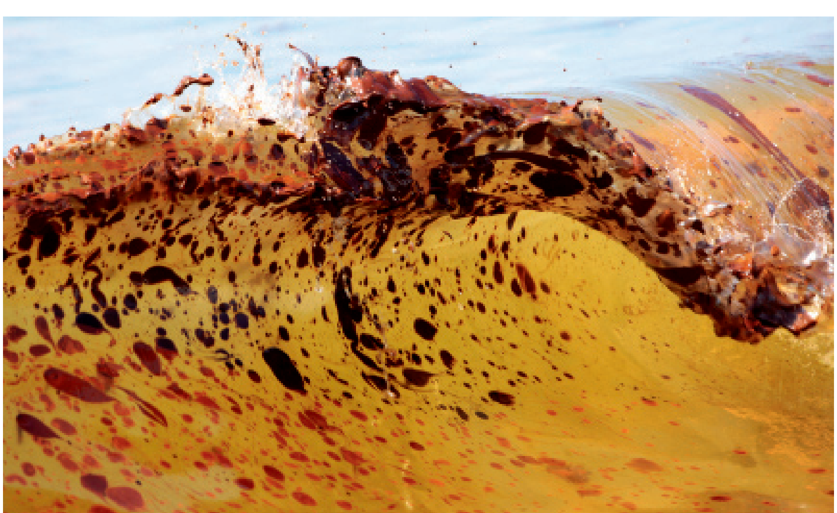
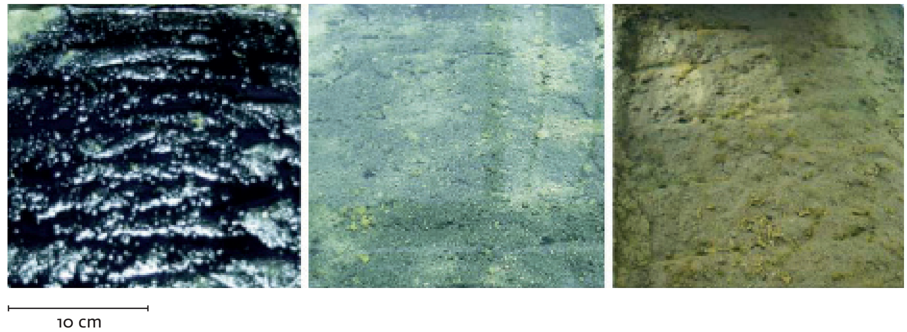


Photo: AP

MOST ANAEROBES WORK SLOWLY

So far, there has been no real explanation for why bacteria have not devoured the oil reservoirs over the millennia. It is possible that some oil reservoirs were very hot and therefore remain almost free of bacteria to this day. In other cases, it is clear that bacteria have been at work. The tiny things are gourmets. From the hundreds of different alkanes present in crude oil, they look for the ones that are easier to digest, such as hexadecane, a molecule comprising 16 carbon atoms.

If such an oil sample is analyzed using a gas chromatograph, a device that



Natural cleanup: In a laboratory experiment (left – day 1, middle – day 6, right – day 26), microorganisms clean a mudflat covered in oil – but only on the surface. Beneath the sand, a tenacious oil layer remains.

can trace specific substances, it becomes clear that certain alkanes are missing. The varying properties and qualities of crude oil from different reservoirs may, in part, be due to the "feeding habits" of the anaerobes.

People who work with anaerobes need a lot of patience. Many anaerobes belong to the "sloths" of the microorganisms. They grow and reproduce in slow motion. Nobody is quite sure why this is. In contrast, the famous laboratory bacteria *Escherichia coli*, the biotechnologists' workhorse, is a true sprinter. It stays alive and functions using oxygen. When in good shape, it splits once every 20 minutes. In 10 hours, one such bacterium creates billions of successors.

In that period, Widdel's bacteria have not even started growing. They may take days or weeks to split. "They live and die slowly," says the scientist. This makes growing such microbes a tedious task. Microbiologists aim to create pure bacteriological cultures of bacteria to understand their function. However, a sample of water or sediment contains a whole host of different bacterial strains. In order to find the exact bacterial strain that degrades the oil in this microscopic soup, the sample is diluted over and over until, finally, statistically speaking, only one bacterium is left floating in the laboratory receptacle.

During this process, the bacteria must keep reproducing so that the scientists can establish whether there are still any oil-degrading bacteria left in the diluted sample. This takes time. So much time that, Widdel says, in the first years, he was rarely able to get even a doctoral student to work on his oil-degrading anaerobes. Thus, for a long time, the dilution series was more of a side job for him and his colleagues.

Some survive without oxygen. In the air-tight bottles, the oil floats over a medium used to cultivate anaerobic bacteria.

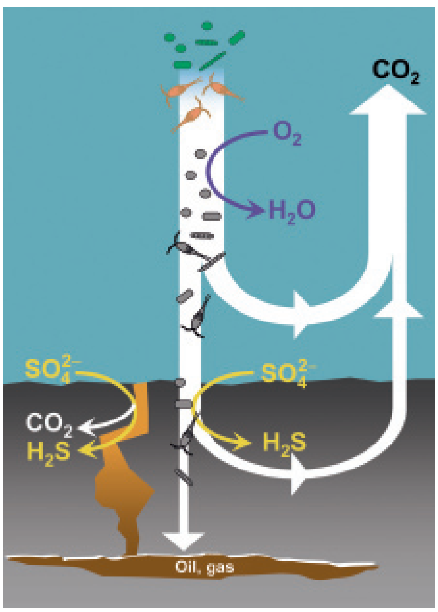
The team in Bremen has now managed to isolate a range of pure strains, but so far they have not found a real winner among the anaerobes. These bacteria are simply not good enough to clean up a major oil spill, according to the scientists. They are much too slow. In cases of oil tanker damage or accidents like the one in the Gulf of Mexico, thousands of tons of crude oil are spilled in a matter of days. Against such volumes, even the quick aerobes, the oxygen-devouring cousins, are powerless. The tide brings the oil and tar masses onto the shore, where often lumps of oily mud and sand form. Widdel's bacteria are active wherever the oxygen becomes scarce, for example underneath the black oil layers on the beach, or in places where oil seeps naturally from the seafloor, or in the oil reservoirs.

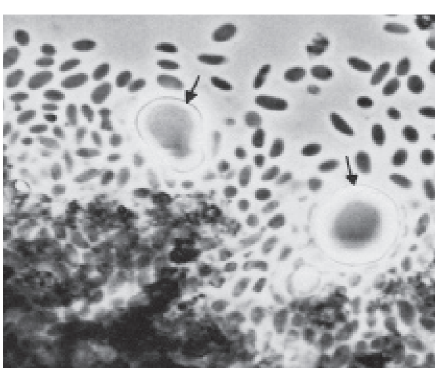
HYDROGEN SULFIDE CAUSES MORE PROBLEMS

Sometimes during an oil spill, anoxic zones will even appear directly in the water, since the aerobic oil-degraders draw the essential oxygen from the seawater. In order to break down one drop of 0.2 millimeters of oil completely, aerobes require the oxygen from up to 80 liters of water. In the case of large amounts of oil in the environment, the aerobes themselves

Photos: MPI for Marine Microbiology – Florin Musat (3, above), MPI for Marine Microbiology – Daniela Lange (below)

» The degradation of thick crude oil masses in anoxic or near-anoxic conditions takes years or decades, and even then the oil is probably incompletely degraded.





top: Many bacteria break down dead plankton into water (H_2O) and carbon dioxide (CO_2) using oxygen (O_2). On the anoxic seafloor, anaerobes decompose a further part of the organic material using sulfate (SO_4^{2-}); creating hydrogen sulfide (H_2S) and CO_2. What is left is converted into oil and gas in the seabed. Around natural oil sources, anaerobes also feed on oil constituents.

bottom: Dinner is served! In the microscope image, anaerobic bacteria dig into tiny oil drops suspended in the segment (arrows).

will deplete their "life elixir." Once the oxygen has been completely consumed, the anaerobes become active. They will go through the oil constituents unhurriedly.

If the oil comes in masses, this may cause an additional problem. Because of the depletion of oxygen and the anaerobic degradation, hydrogen sulfide is formed, which is toxic even in low concentrations. "The more you study the different aspects of oil degradation, the more you realize that we need to handle the valuable resource that is oil with much greater care and responsibility," Widdel says. "An oil spill should concern us all; after all, we all use it."

The degradation of thick crude oil masses in anoxic or near-anoxic conditions can take years or even decades, and even then the results will probably be incomplete. That is why, long after a tanker accident, a black, smeary oil residue can still be found deep down in a sandy beach. Nevertheless, the anaerobes are in no hurry. For millions of years now, they have been feeding on the oil that is available in natural reservoirs in the ocean. Indeed, anaerobic oil-degrading bacteria can be found anywhere in ocean sediment containing hydrocarbons, whether in the silt of a Frisian marina or in the Gulf of California, in Western Mexico.

Here, in a sea basin around 2,000 meters deep, hot water rises through cracks in the Earth's crust. The remains of large amounts of dead algae that have made their way down from the surface simmer in the hot water. This is a place where oil forms unusually fast. Normally, crude oil is produced far below the sea, under hundreds of meters of heavy sediment layers, where high temperatures and pressures prevail. In such a geological pressure cooker, sugars or fatty acids, which are rich in oxygen, are boiled down to hydrocarbons from the dead biomass.

SOME MICROORGANISMS BREAK DOWN OIL TO METHANE

However, just a few meters down in the warm seafloor of the Gulf of California, the creation of oil can practically be watched "live." Even before Friedrich Widdel became head of the Department of Microbiology at the Bremen-based Max Planck Institute in 1992, he had been to see the hot vents on an expedition off California's coast in the submersible "Alvin" from the Woods Hole Oceanographic Institution, Massachusetts.

At first, all he could see in the light from the onboard floodlights was gray sediment. Then, all of a sudden, yellowish-white spots appeared: sulfuric deposits the size of pancakes – a certain sign of bacteria. "Using the robotic arm, we pierced the ground and suddenly small yellowish drops of oil rose, like olive oil in a glass of water," Widdel tells us. He found interesting anaerobes there, several of which he still nurses in his bacterial cultures today.

His tiny laboratory inhabitants have sometimes astonished him. Like the time when he was investigating whether something would happen to the long chains of hydrocarbons even if he did not add sulfate. In fact, completely new microorganisms developed that broke the long chains of hydrocarbons down to methane, the main component of natural gas. When he started growing anaerobic microbes in the laboratory, Friedrich Widdel was aware that the formation of gas

Graphic and photo: MPI for Marine Microbiology

What will the microscope tell us? Friedrich Widdel, Edgar Büttner and Romana Appel prepare a sample of anaerobic bacteria that are thriving slowly but surely.

Photo: MPI for Marine Microbiology – Daniela Lange

increases the pressure. Still, he underestimated the unicellular organisms.

One day when he checked the incubator, one of the phials had burst and its contents were spread out everywhere. Widdel still remembers the loss of a productive bacteria colony that he had taken care of for a long time with a bit of sadness. In a complete "microbial alkane gasification," the volume may increase a hundredfold. Such bacteria may have contributed to the gas in the oil reservoirs.

Naturally, oil-degrading bacteria are not Friedrich Widdel's only subject of study. Anaerobes can do so much more. The shelves of his incubator hold tightly sealed phials containing metal strips. The metal is the food source of anaerobes that directly utilize and corrode metallic iron. Widdel wants to use them to understand the problem of biocorrosion in iron pipelines.

However, the area of hydrocarbons is definitely the one that has thus far occupied him for the longest period of time. Friedrich Widdel continues to search for new strains of bacteria with interesting properties in samples that he himself collects from mudflat sediment or in phials brought back by his colleagues from scientific surveys. He is convinced that some bacteria will still display new, surprising properties. There is just one thing that he does not believe in: "Superbacteria that will clean up oil spills in the blink of an eye. Because degradation performance and bacterial metabolism also operate within natural limits." ◂

GLOSSARY

Anaerobes
Organisms whose metabolism works without oxygen, in contrast to that of aerobes. To many of them, oxygen is even toxic.

Gas chromatograph
A device that separates the substances of a mixture. Using a carrier gas, the mixture is sent through a thin, 10- to 50-meter-long column that is wound into a coil. The capillary column is coated with a material to which substances will be absorbed for varying periods of time, depending on their polarity and their vapor pressure. The materials will thus exit the column after varying lengths of time.

Alkanes
Compounds consisting of carbon and hydrogen that contain no double bonds. The carbon atoms thus form a chain that may be branched or joined up in a ring. A hydrogen atom is bonded to any free bonding point. They are obtained primarily from crude oil.