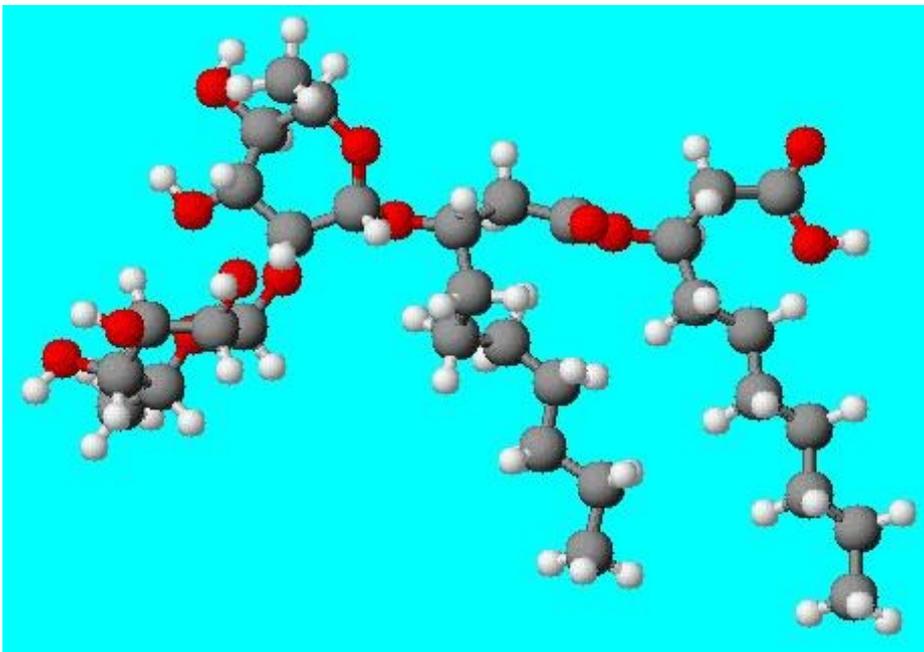


## Foam from bacteria

**All washing agents and household detergents contain surface-active agents that bind and dissolve dirt. Up until now, these agents have been produced from organic compounds extracted from mineral oil. Due to the ongoing debate on sustainability, more and more manufacturers are focusing on biological alternatives. The research group led by Dr. Rudolf Hausmann at the Karlsruhe Institute of Technology (KIT) is investigating the conditions under which microorganisms produce so-called biological surfactants. These substances are as effective as their synthetic counterparts and they are also biologically degradable. How can bacteria be induced to produce such compounds in large quantities and at low cost? And how can the pharmaceutical, food and cosmetics industries benefit from such developments?**



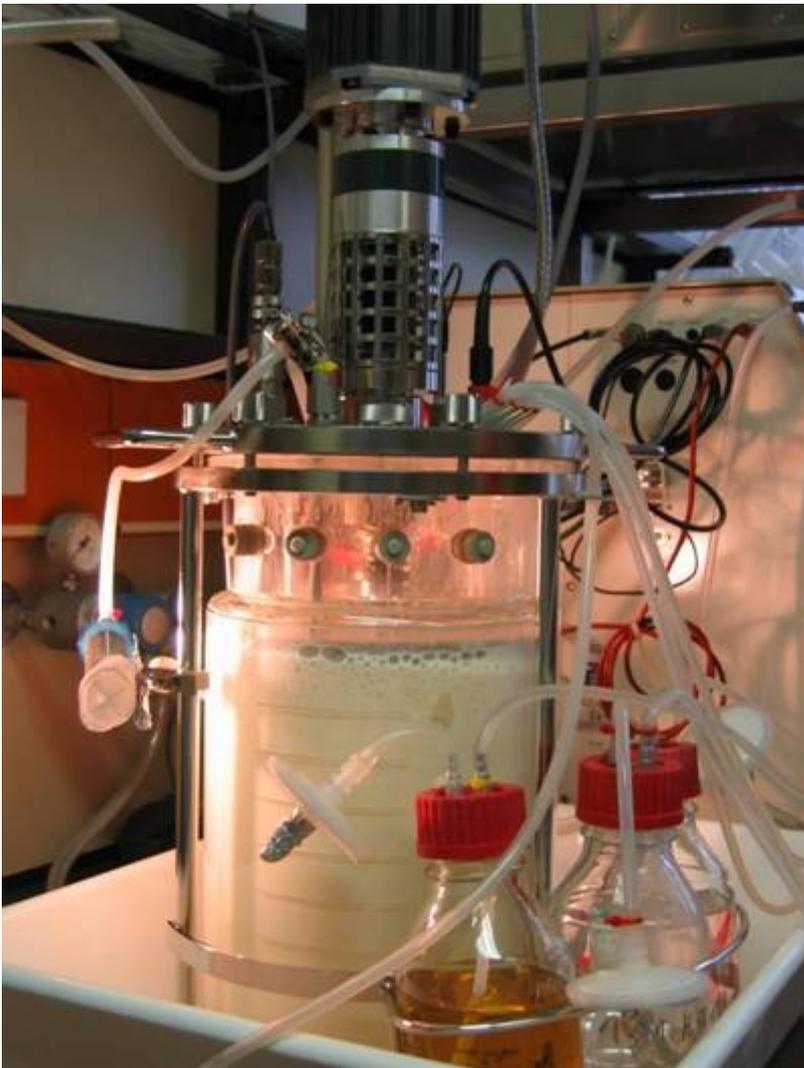
An example of the chemical structure of a biological surfactant.  
© Dr. Rudolf Hausmann

Surfactants are molecules with a water-soluble and a fat-soluble domain. The fat-soluble domains can attach to dirt particles or other fatty substances and form a layer around them. The water-soluble domains extend to the outside and dissolve in aqueous solutions. This is how surfactants help remove dirt from clothes or food residue from plates. Surfactants are the major constituents of washing agents, soap or household detergents. "Industry requires a large amount of these substances," said Dr. Rudolf Hausmann from the Department of Technical Biology led by Prof.

Christoph Syldatk at the Institute of Bio- and Food Technology at the Karlsruhe Institute of Technology (KIT). "At present, the majority of surfactants is produced from mineral oil, but the need for biologically degradable surfactants made from renewable materials has been growing over the last few years.

## A two-step process in stainless steel tanks

Biological surfactants are one of the major interests of Hausmann's research group. "When we started working on this around seven years ago, nobody else was really interested in it," said the researcher. "The situation has now changed completely, and we even have to refuse projects from big companies because we have such a lot of work." The researchers from Karlsruhe are mainly focused on the investigation of rhamnolipids, molecules whose water-soluble (hydrophilic) part consists of different combinations of rhamnose. Fatty acids constitute the fat-soluble (lipophilic) part of the lipids. Rhamnolipids are produced by microorganisms, including *Pseudomonas* bacteria. Bacteria need these substances in times of nutrient deficiency in order to dissolve plant oils in their environment and use them to produce energy. The researchers are trying to achieve an optimal yield of *Pseudomonas* bacteria whilst cultivating them under conditions of scarcity.



One of the bioreactors used by Dr. Rudolf Hausmann and his team at KIT to "cultivate" biological surfactants.  
© Dr. Rudolf Hausmann

"With regard to industrial production, the major problem is that we need to reduce production costs

as much as possible," said Hausmann. "One of the challenges we are faced with is to produce as much biomass as possible in our bioreactors and then use this biomass to produce as many usable substances as possible," said Hausmann. The process is carried out in stainless steel tanks containing green bacterial suspensions, and consists of two steps: The first step is the growth phase and the second the production phase. The yield is already quite high - Hausmann and his team are able to produce around 40 g rhamnolipids from one litre of bacterial suspension, which corresponds to around a thousand-fold of the bacteria's natural production capacity. However, in order to make the process fit for industrial application, the processing time has to be reduced by a factor of ten and the concentration needs to be further increased.

## Focusing on aspects of bacterial metabolism

What prevailing general conditions in bioreactors are necessary in order to reduce production time and increase production yield? Hausmann and his team are currently testing several different parameters and found in experiments that the bacteria need to be exposed to general stress during the production phase in order to produce rhamnolipids. One example of general stress is a lack of iron or copper ions. In addition, the cell density needs to be quite high. The microorganisms will produce the desired substance at a high density. "These conditions can only be achieved with difficulty in normal shake flasks," said Hausmann going on to add, "this is why we need to use bioreactors."

In order to increase bacterial production capacity, the researchers also have to understand how growth-limiting conditions in the media affect the bacteria's physiology. In cooperation with numerous partners, the researchers are therefore also investigating aspects of the bacterial metabolism. Can the metabolism be manipulated using molecular biology methods in order to increase the yield? Modern systems biology methods, such as those used in what is known as metabolic engineering, will be in great demand in the future. This highly promising approach is already being pursued in a project funded by the Agency for Renewable Resources. Another current project, which is funded by the Baden-Württemberg Foundation, uses a computer-assisted approach. "We are focusing on model-assisted process control," explains Hausmann. "We are looking at the molecular level and asking ourselves when certain regulators need to be switched on. We then investigate this using computer models."

## Spick-and-span tanks

In addition to their biological degradability, another advantage of biological surfactants is their high structural variety (around two thousand different molecule species are known). While the washing agent and detergent industry is mainly focused on the general effectiveness of the substances and the cost, the pharmaceutical, food and cosmetics manufacturers are mainly interested in the specific chemical properties of surfactants. For example, the pharmaceutical partners working with Hausmann's team are interested in concrete chemical characteristics that can be used for therapeutic purposes. This is why the researchers from Karlsruhe are attempting to specifically modify the surfactants, for example by attaching different molecule groups (e.g. different sugars) to the fatty acid backbones. "We are investigating possible ways of enzymatic conversion," said Hausmann pointing out that this might also be of interest in future for the food and cosmetics industries.

Hausmann believes that the market introduction of rhamnolipids is just a matter of time. In the meantime, Hausmann and his colleagues are working on the optimisation of the production processes using bioreactors. There is one thing that they do not need to worry about: "We do not

need to flush our bioreactors after the experiments, because the bacteria produce so much detergent that the tanks are meticulously clean once the content has been taken out.”

**Further information:**

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Industrial biotechnology – biological resources for industrial processes