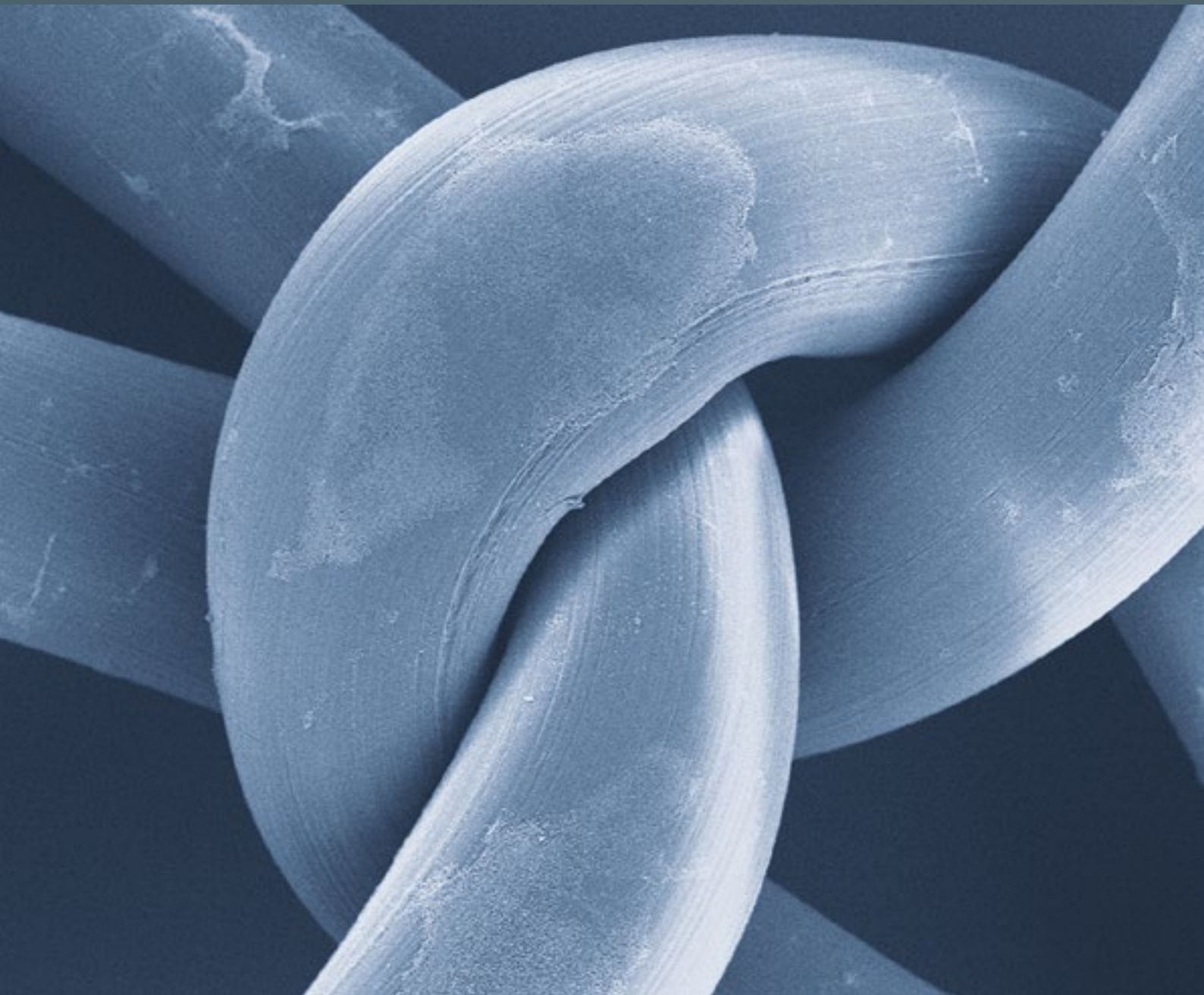
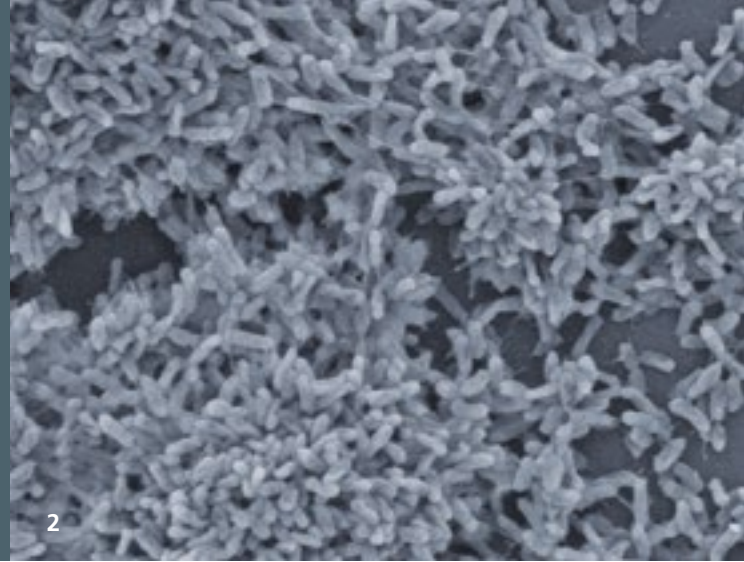


MICROBIOLOGICAL CHARACTERIZATION OF ANTIMICROBIAL AND PHOTOCATALYTIC ACTIVE SURFACES





MICROORGANISMS ON SURFACES

Microorganisms on surfaces occur widely in nature. Bacteria, fungi and algae have adapted to growth on various surfaces and benefit considerably from this way of life, which is adapted to each particular location, for example on stones in a stream, and also in various kinds of piping. Their growth becomes visible to the human eye when biofilms develop excessively. Frequently the microbial growth causes damage to the material or impairs the functioning of technical equipment. If biofilms occur in a hospital clinic, for example on implants, they can cause health problems in human beings. Initially, the development of biofilms begins in a completely harmless way. Individual cells of microorganisms stick to surfaces and reproduce in the moist environment. Many types of bacteria are able to build up a layer of slime, which on the one hand provides them with protection against environmental influences. On the other hand, they enable the organisms to accumulate nutritive substances even from environments that are very low in nutrients.

Definition and characterization of biofilms

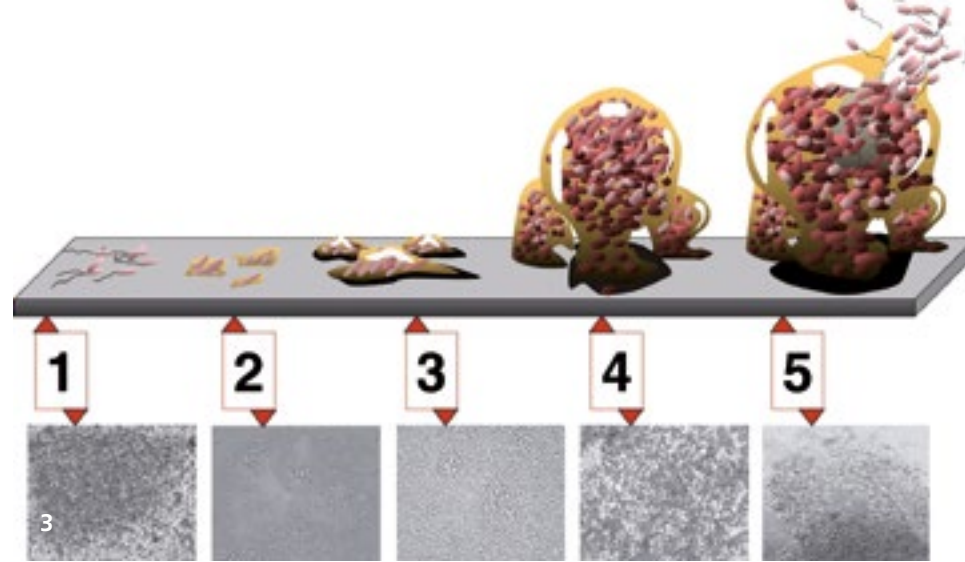
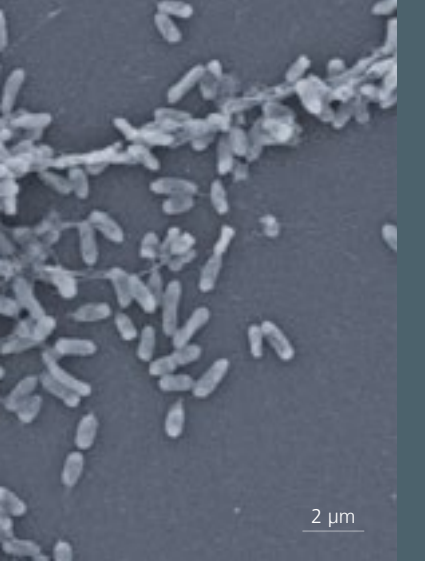
Biofilms are symbiotic communities of bacteria, fungi or algae that adhere to surfaces and grow there. They are adapted to the particular environment in question and have a higher resistance to environmental conditions than free-floating cells. The metabolism of individuals organized in biofilms differs from that of planktonic cells.

The characteristic feature of biofilms is that the cells are surrounded by a microbially induced matrix, generally consisting of polysaccharides, which are flushed by an aqueous solution. They may consist of individuals of a single species or of mixed populations of various types of organisms (Fig. 1). The microorganisms organized in biofilms make use of the metabolic properties of the other species or their protective mechanisms or metabolize cells or cell parts of other microorganisms. The formation of the biofilm depends on and is regulated by genetic factors. Here the formation and the exchange of so-called signal molecules are decisive for the microbial communication.

The example of a biofilm of the species *Pseudomonas aeruginosa* is represented in a scanning electron microscope image in Fig. 2. The three-dimensional structure of the bacteria cells colonized on a polycarbonate surface is clearly visible.

Economic significance

In Germany alone the costs caused by fungal damage in old buildings are estimated to be 210 million euros per year. In addition, there is roughly 1.5 billion euros per year resulting from deterioration in the efficiency of heat exchangers and 12.5 billion euros per year for corrosion damage – of these, approx. 20 percent is damage caused by microbes. In the health sector too enormous efforts are being made to prevent



the growth of biofilms on natural surfaces such as dental material, and also on synthetic materials such as implants, catheters or medical equipment – also because of the enormous follow-up costs if a person’s health is affected.

Preventing and combating biofilms

If biofilms have once established themselves on a surface, it is generally difficult to deal with the problem. In this life form they are distinctly more resistant than individual cells and in many cases withstand even high doses of disinfectants and cleaning agents. In order to hinder the initial formation of biofilms, the use of suitable antimicrobial surface modifications, for example by means of the bonding of biocidal substances or photocatalytic finishes, is therefore indicated. An appropriate surface finish can prevent the microbial adhesion on the surface of the material or the reproduction of the cells from the outset. At the Fraunhofer IGB interfacial engineers – in cooperation with microbiologists and cell biologists – have already developed various surface finishes. They can be characterized with the assistance of physical and chemical methods, however their biological efficacy can only be demonstrated using living systems.

Technical solutions for preventing and combating microbial growth on surfaces

Physical measures

- Changing the ambient conditions (e.g. cooling or heating)
- Influencing by changing the flow conditions or mechanical removal
- Radiation
- Surface properties

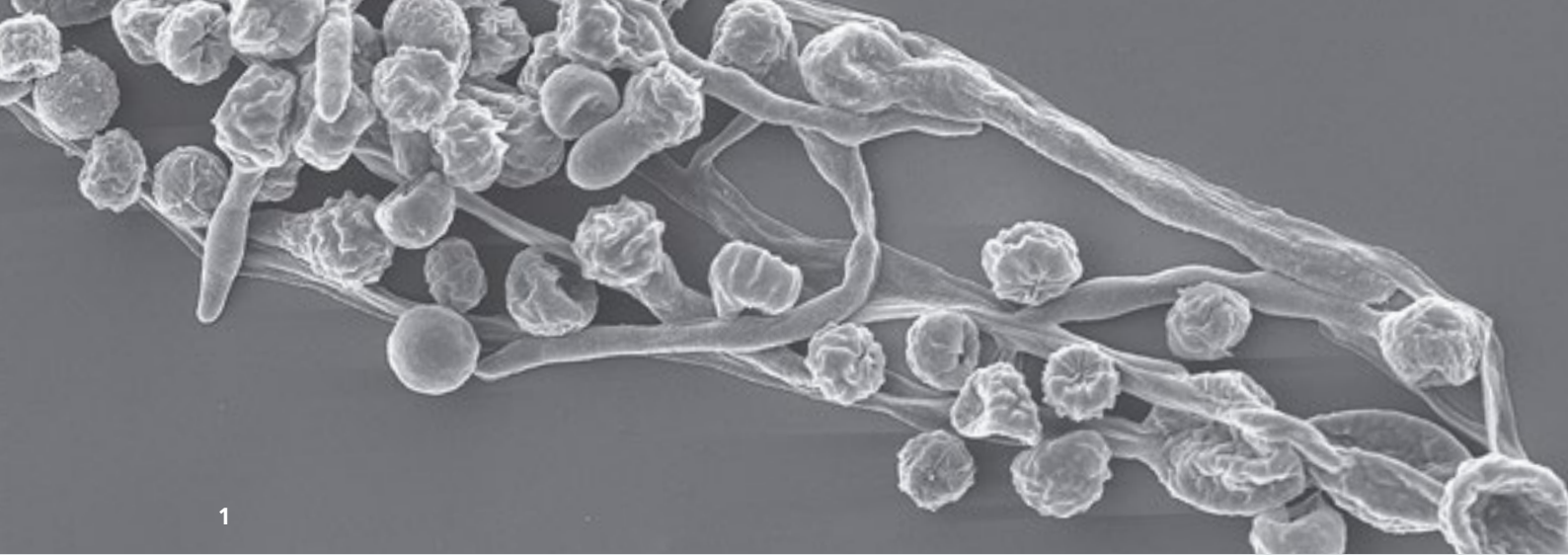
Chemical measures

- Cleaning and/or disinfection
- Changing the pH value
- Properties of surfaces

Biological measures

- Antibiotic substances
- Disturbance of the cell-cell communication

- 1 *Biofilm of a natural mixed population on a porous surface.*
- 2 *Scanning electron microscope image of a biofilm of Pseudomonas aeruginosa. Rod-shaped cells embedded in the matrix, three-dimensional structure visible.*
- 3 *Diagrammatic representation of biofilm formation. © D. Davis*



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FORMATION OF BIOFILMS

The formation of biofilms takes place in several stages. It does not only depend on the type of microorganisms and their properties, but is also influenced by the surface properties of the materials to be colonized as well as the ambient conditions. These include, in addition to the nutrient supply, also the water content, temperature or the flow conditions. In the development of a biofilm (Fig. 3, page 3) the free-floating planktonic cells first of all stick to a surface. The van der Waals forces have a role to play in this still reversible process. In the subsequent development of monolayers and microcolonies, so-called adhesins act as the first adherence to the surface of the material. This process is only reversible to a limited extent. The slime that pervades and surrounds the biofilm is based on polysaccharides, which the microorganisms produce themselves. This structure cannot be removed from the surface by vigorous rinsing. Individual cells are released from the biofilm, and these establish themselves once again and can form a new biofilm.

Microorganisms do not just accumulate nutrients in biofilms. They also offer the population protection from chemical and physical environmental influences such as disinfectant solutions or radiation. If the development of biofilms is to be avoided effectively and lastingly, you have to take action at a very early stage of the biofilm formation or inhibit them at the outset, for example by means of an appropriate antimicrobial finish of the surface.



VERIFICATION PROCEDURE

At the Fraunhofer IGB microorganisms on surfaces have been studied for more than 20 years and used specifically for various biotechnological applications. Based on the experience in dealing with immobilized biomass we have developed various procedures for the qualitative and quantitative verification of biofilms. Today, numerous standard methods as well as application-oriented procedures are available. These are optimized and well established, depending on the specific requirements and objectives of our clients.

Larger experimental series are also one of the services we provide. We are constantly further developing existing methods; within the scope of research projects we establish new test procedures together with industrial partners.

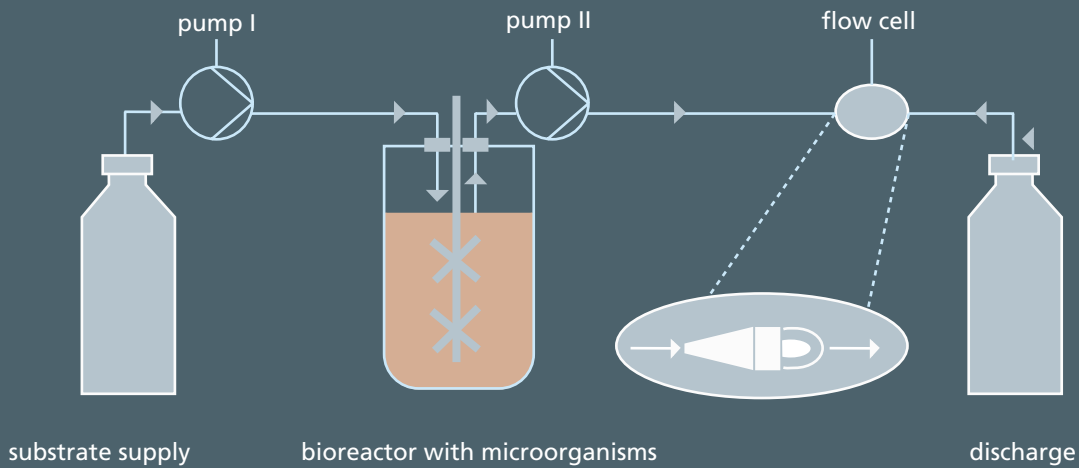
While various parameters that characterize the surface properties of materials can also be demonstrated at the Fraunhofer IGB using established physical or chemical procedures, proving the inactivation of microorganisms depends on biological methods. Inactivation means the loss of the ability to reproduce and is a basic precondition for preventing the further development and proliferation of the microbial cells.

Requirements for biological testing methods for the evaluation of antimicrobial or photocatalytic active surfaces

- Qualitative evaluation
- Quantitative evaluation
- Reproducibility
- Comparability
- Recording the ambient conditions (temperature, water content, radiation, flow conditions)
- Proximity to the application
- Clarifying the action mechanisms

1 *Aspergillus niger.*

2 *Example of an outdoor test facility for the long-term study of photocatalytic surfaces.*



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Established evaluation methods

At the Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB various standard procedures such as the Japanese Industrial Standard JIS 2801, Agar Diffusion Test, ASTM Standards (ASTM 21-09) as well as the American Military Standard Mil-Std-810F are used in order to evaluate surfaces biologically, as these tests are frequently required by clients.

Practice has shown that these standards and norms do not in every case meet the requirements of Research and Development. The existing range of tests has therefore been extended and at present includes: screening tests for active substances that are already used in the selection of components, but also for exploratory studies. An adhesion test was established for the evaluation of the adhesion properties and the biofilm formation under static and dynamic conditions. Various flow cell models (Fig. 1) are available to allow studies of the influence of flow properties.

Further application-oriented as well as outdoor studies (Fig. 2, page 5) were developed and tested in recent years and modified for numerous different applications.

The well-established standard procedures reach their limits when test specimens of varying morphology and composition are to be examined. With the equipment at our disposal we test powders, suspensions or solutions that are to be used as basic materials. Flat substrates of glass, metal, synthetics of various dimensions can also be evaluated. Procedures for the biological characterization of 3D specimens, woven materials, fleeces and lumens such as tubular material or catheters have already been used successfully in the appropriate test procedures.

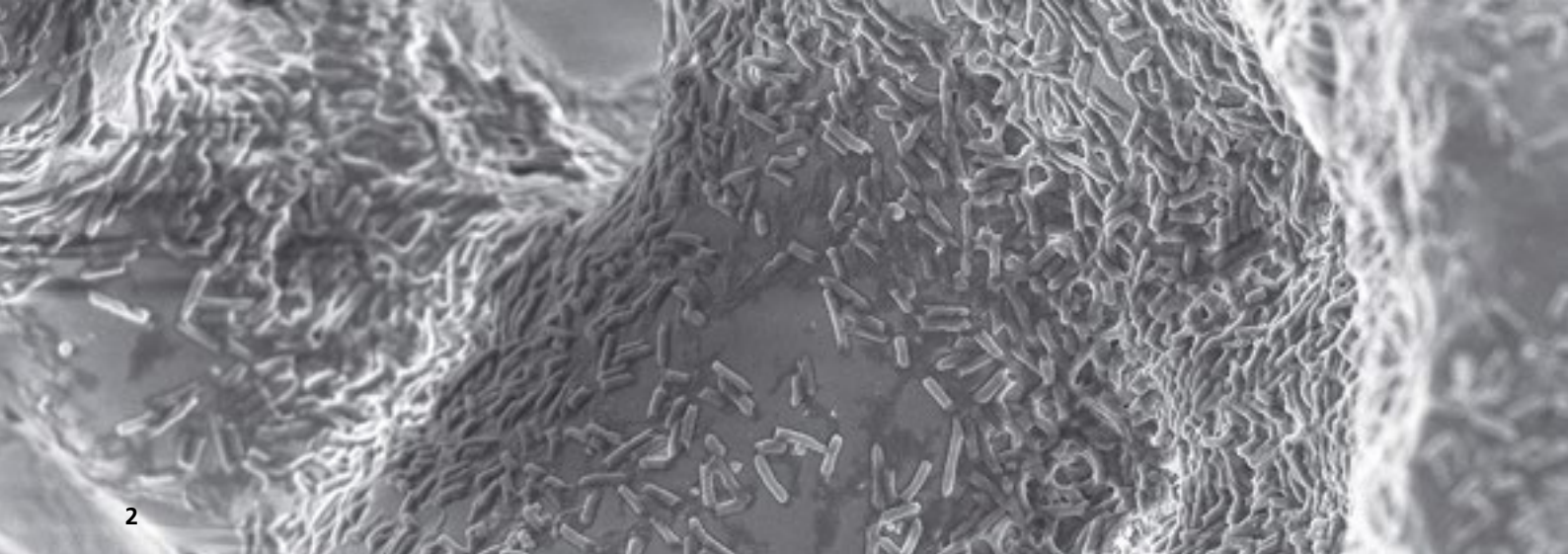
Target branches of industry

We examine surfaces that are coated for a very wide range of applications or are manufactured as compounded products with various microbiological testing procedures appropriate to the application and the problems involved:

- Medical engineering (dental prostheses, implants, catheters)
- Technical applications (filtration units, filters, air conditioning units, ...)
- Consumer goods
- Vehicle construction
- Textiles
- Building industry

1 Flow cell for testing antimicrobial properties.

2 *Escherichia coli*.



Test organisms

Bacteria, fungi and algae are used as test organisms. The range includes studies with defined test strains of axenic cultures (e.g. *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Scenedesmus spec.*) as well as the use of mixed populations typical of the location. The choice of the test organisms is based on the cell wall composition of the organisms, well-known resistances, genetically determined metabolic properties, the fields of application intended for the products as well as the formation of the biofilm.

On the basis of the equipment available and the expert qualifications, the Fraunhofer IGB is able to work with microorganisms of the biological-risk group 1 and 2. In addition, genetically modified strains of relevant organisms were developed that produce fluorescent proteins, which are expressed depending on the stage of the biofilm formation.

Equipment

Microbiological laboratories with modern equipment as well as an appropriately qualified team permit the handling of microorganisms in the risk groups 1 and 2. Activation units are available for the examination of photocatalytically finished surfaces, in which a biological characterization of the specimens can be carried out under defined conditions. Outdoor experiments to study the influence of weather conditions are planned on request.

Range of services

- Evaluation of the microbial contamination on surfaces and in process-contact media (e.g. water, basic materials, products)
- Development of procedures to reduce microbial and chemical contamination in various sectors (e.g. sterilization methods, prolonging the lifetime of cleaning baths, process water treatment, cost-efficient treatment of process media, wastewater treatment)
- Development of innovative materials to reduce the formation of biofilms on packaging materials, piping, medical products and/or for the design of working surfaces
- Evaluation of the antimicrobial properties of specially finished surfaces

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Fraunhofer IGB brief profile

The Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB develops and optimizes processes and products in the fields of health, chemistry and process industry, as well as environment and energy. We combine the highest scientific standards with professional know-how in our competence areas – always with a view to economic efficiency and sustainability. Our strengths are offering complete solutions from the laboratory to the pilot scale. Customers also benefit from the cooperation between our five R&D departments in Stuttgart and the institute branches located in Leuna and Straubing. The constructive interplay of the various disciplines at our institute opens up new approaches in areas such as medical engineering, nanotechnology, industrial biotechnology, and environmental technology. Fraunhofer IGB is one of 69 institutes and independent research units of the Fraunhofer-Gesellschaft, Europe's leading organization for applied research.

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