Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science

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Metal micro-/nano-particles with suitable chemical modification can be organized into new ceramic-metal (cermet) or organic-metal (orgmet) composites or structured materials. These materials are attracting significant attention because of their unique structures and highly optimized properties. However, the synthesis of composite materials with inhomogeneities on the nanometer or submicrometer scale is a continuing challenge in materials science. Many industrial physical and chemical surface-coating processes using conventional techniques are both energy and cost inefficient and require sophisticated instrumentation. In the future, biology might offer a superior option.

> The use of nanostructured materials is becoming more widespread $^{1\mathchar`-3}$; they are capable of giving unique physical and chemical properties and are gaining importance in areas such as biomedical sciences, optics, magnetics, mechanics, catalysis and energy science. However, the preparation of such nanostructured materials poses several unique challenges. Several different manufacturing techniques are in use, that usually employ atomistic, molecular and particulate processing in a vacuum or in a liquid medium. Most of the techniques are capital intensive, as well as inefficient in materials and energy use. However, completely novel materials with unexpected properties might emerge from biomimetics, an interdisciplinary approach that can be defined as 'materials science and engineering through biology'⁴. This article describes the use of bacteria in a 'living factory' for the production of new functional materials.

It is well known that biological systems can provide a number of metal or metal-containing particles in the nanometer size range⁵. For example, many multicellular organisms use inorganic materials (such as calcium carbonate or silica) in combination with an organic matrix (proteins, lipids or polysaccharides) to produce hard materials, such as skeletal units, teeth and bones. Single-celled organisms can also produce mineral structures that form inorganic materials either intracellularly or extracellularly; examples include magnetotactic bacteria, which produce magnetite (Fe_3O_4) or greigite (Fe_3S_4) and diatoms, which produce siliceous materials. These particulate systems can be used as precursors for the manufacturing of functional materials⁶. The examples below deal with optical coatings for solar energy applications⁷ and ion insertion materials for electrical battery applications. The potential of this approach is immense and biomimetic materials will probably be of considerable interest in the future⁸.

Microorganisms and metals

Bacterial cells are constantly exposed to stressful situations and an ability to resist those stresses is essential for their survival. The ability of microorganisms to grow in the presence of high metalconcentrations might result from specific mechanisms of resistance. Such mechanisms include: efflux systems; alteration of solubility and toxicity by changes in the redox state of the metal ions; extracellular complexation or precipitation of metals; and the lack of specific metal transport systems^{9–11}.

Metal-microbe interactions have an important role in several biotechnological applications, including the fields of biomineralization, bioremediation, bioleaching and microbial corrosion, and have gained growing attention in recent years. For example, the understanding of microbial-influenced corrosion processes in terms of localized changes in the surface chemistry of carbon steel or other alloys has improved¹². Bacteria also intervene in mineral precipitation reactions directly as catalysts of aqueous chemical reactions and indirectly as geochemically reactive solids¹³. The bacterial oxidation of minerals is important in the formation of acid mine drainage and in the extraction of gold, copper and uranium from ores^{14,15}. These processes are commercially exploited in bacterial leaching operations, such as the recovery of gold from arsenopyrite-pyrite ores¹⁶. Recent advances in the understanding of the role and application of microorganisms for the remediation of toxic metal and radionuclide-contaminated sites have also been reported¹⁷. These developments include the use of natural and genetically engineered bacterial strains that are capable of mobilization and immobilization of metal ions. Effective techniques will require bacterial bioremediation coupled with effective chemical and engineering strategies¹⁷.

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Sweden. *e-mail: tanja.klaus@ angstrom.uu.se Microbial-based technology presents an economic alternative for today's mining, and mineral and waste water treatment industries, at a time when highgrade mineral resources are being depleted, energy costs are increasing, and adverse environmental effects are becoming more apparent as a result of conventional technologies¹⁸. In addition, the formation of inorganic particles within organisms has a number of broad implications in materials science and might become a central discipline for major advances in biomimetic and bioengineering applications.

Nanostructures in biology

Biological systems provide many examples of specifically tailored, nanostructured molecules with highly optimized properties and characteristics. These biological materials can be used in their native form directly extracted from the living systems, or they can be processed after extraction and modified to their desired form. Thus, the biological material can be seen as a nanophase system in its own right and as the starting point for producing other novel nanophase systems. The formation of these materials in vivo is often owing to a biologically controlled mineralization process that produces materials with well-defined characteristics. Many multicellular organisms produce hard materials, such as bones, shells and spicules using inorganic materials to build complex structures¹⁹. These biominerals are composite materials and consist of an inorganic component and a special organic matrix; the organic matrix has a vital influence on the morphology of the inorganic compound.

Single-celled organisms also use inorganic materials to produce cermet or orgmet composites, formed either intracellularly or extracellularly. An example of biologically controlled mineralization in single-celled organisms are the magnetotactic bacteria, which represent a heterogeneous group of procaryotes with a variety of morphological types. The vast majority of magnetotactic bacteria, including cocci and rods, as well as all cultivable vibrios and spirilla, are members of the α-Proteobacteria^{20,21}. They all intracellularly synthesize magnetic nanocrystals in magnetosomes. The particles consist of Fe_3O_4 or Fe_3S_4 have a specific size range (35-120 nm) and are surrounded by a membrane. The morphologies of the magnetic-iron particles include cubo-octahedral, elongated hexagonal prismatic and bullet-shaped²¹. The formation of magnetic particles within biological membranes is of great interest, and small highly uniform synthetic particles are grown under closely controlled conditions. Numerous uses of these small magnetic crystals have been considered. For example, it has been suggested that magnetotactic bacteria could be employed for the removal of heavy metals and radionuclides from wastewater²². Another more esoteric application regards the use of magnetotactic

bacteria to locate magnetic poles on meteoritic magnetic grains²³. Isolated magnetosome particles are used as carriers for the immobilization of bioactive substances such as enzymes, antibodies, DNA and RNA (Ref. 24). Future applications might include the incorporation of magnetite conjugates in medical diagnostics, as well as new memory devices for technological applications.

Further examples of nanostructures in biology include diatoms (which produce siliceous materials), bacteria that play an active role in the precipitation of carbonates^{25,26} or in the formation of calcium pyrophosphate crystals²⁷, and S-layer-producing bacteria²⁸. Crystalline bacterial cell surface layers (S-layers) are the outermost cell envelope component of many eubacteria and archaea and are composed of 2D crystalline arrays of identical protein or glycoprotein subunits. The potential biotechnological applications of these monomolecular arrays lies in the production of ultrafiltration membranes, their usage as stabilizing structures for lipidfilms and liposomes and as matrices for the controlled immobilization of biologically active macromolecules^{29,30}.

Bacteria are capable of forming a diverse array of minerals. Several investigations have shown that cadmium sulphide particles can be microbially produced in *Klebsiella aerogenes*³¹ and the yeasts Candida glabrata and Schizosaccharomyces pombe^{32,33}. S. pombe detoxifies cadmium in the environment using the active intracellular uptake of the metal followed by its sequestration within small iso-peptides. The metal-peptide complexes form cadmium sulphide microcrystallites, which can function as quantum semiconductor crystallites and are ~1.8 nm in diameter. Such metal sulphide crystallites have optical absorption, photosynthetic and electron transfer properties that are sizetuneable. The formation of biominerals by several bacteria has been reported. Crystalline, needlelike deposits are formed around the cells of Pseudomonas aeruginosa in the presence of La(NO₃)₂ (Ref. 34). The cells of *E. coli* sp. form electron-dense La deposits in the periplasm³⁵. A *Citrobacter* sp. accumulates heavy metals as metal phosphates derived from enzymatically liberated phosphate. The tolerance of bacterial cells to metal ions and the formation of the mineral particles is very much dependent on the composition of the growth environment. The absorption capacity of cells for metal ions can be relatively high. Uranyl phosphate accumulates as polycrystalline HUO₂PO₄ at the cell surface with a maximum uptake of ~900% of the cellular dry weight³⁶. A maximum uptake of ~20% of the total biomass has been reported for Klebsiella aerogenes cells grown in the presence of 2mM Cd(NO₂)₂ (Ref. 31). Silver concentrations up to 25% of the bacterial dry mass were reached in a mixed culture of Thiobacillus ferrooxidans and Thiobacillus *thiooxidans* after leaching of a sulphide mineral³⁷. Other biomineralization phenomena, such as the

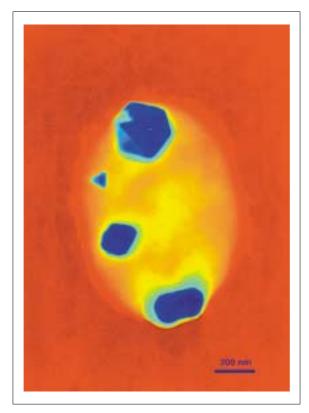


Fig. 1. Transmission electron micrograph of a *Pseudomonas stutzeri* AG259 cell grown on a 50 mM Ag⁴ containing agar substrate. Silver-based single crystals with different size and morphology are associated with the cell.

formation of tellurium in *E. coli* K12 (Ref. 38), the direct enzymatic reduction of Tc(VII) by resting cells of *Shewanella putrefaciens* and *Geobacter metallireducens*³⁹ and the reduction of selenite to selenium by *Enterobacter cloacea*, *Desulfovibrio* desulfuricans and Rhodospirillum rubrum⁴⁰, have been reported. Elemental selenium that is deposited inside or outside cells is found in the form of spherical, fibrillar and granular structures or amorphous aggregates. However, the metal ions involved are less favourable in materials science. Fragments of the wall of *Bacillus subtilis* that had been treated with gold chloride (AuCl₃) revealed dense granules of 5–25 nm within the cell wall substance and exhibited definite geometrical shapes⁴¹.

Advanced materials, naturally

One example of a metal-accumulating bacterium (Pseudomonas stutzeri AG259) that is capable of producing silver-based single crystals in the size range of a few nm up to 200 nm is shown in Fig. 1. These biologically fabricated nanostructures show well-defined shapes and composition. To protect the cells from the toxic consequences of silver, this bacterial strain takes advantage of a detoxification mechanism by the precipitation of silver in the periplasmic space and its reduction to elemental silver. The capability of producing crystalline silver particles in a nanometer size range and with controlled morphology is the basis of using this biological method in the field of materials science. Transmission electron microscopy and quantitative energy dispersive X-ray analysis, together with electron diffraction, reveal a variety of crystal typologies (Fig. 2), such as hexagons and equilateral triangles, as well as three different types of particles: elemental crystalline silver, monoclinic silver sulfide acanthite (Ag₂S), and a further undetermined structure⁴². The thickness of the periplasm constrains the thickness of the crystals, but not their width,

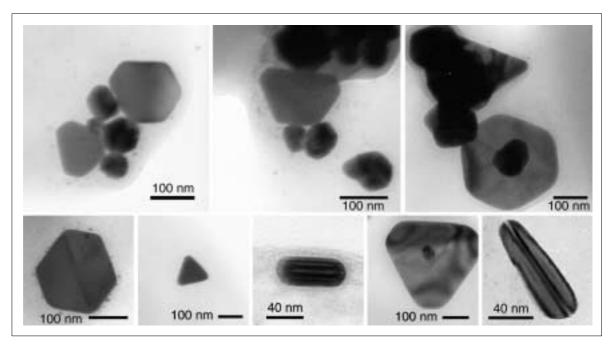


Fig. 2. A variety of silver (Ag) crystal morphologies. The particles are a selection of different crystal morphologies that are found in whole cell preparations and thin sections.

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which can be rather large (100-200nm). This is also in agreement with the findings published by Brown et al.43 who developed a genetic system in E. coli to study the protein-mediated control of crystal growth using the crystallization of gold as a model system; a flatplate-like morphology then follows. However, the transport mechanism by which silver enters the cell, and the mechanism for the intracellular heavy-metal deposition, remain to be elucidated. The silver ions can be complexed to amine molecules at the cell wall or in the growth medium and the translocation event could be coupled to metabolic energy. Following the interaction of the metal with chemical reactive groups located on the bacterial surface or its translocation into the cell, these sites nucleate the deposition of more metal as a chemical precipitate thus leading to the formation of mineralized crystalline particles. Metal accumulation might therefore, occur in two steps: a rapid reversible and metabolically independent surface binding followed by metabolically dependent, irreversible, intracellular accumulation^{44,45}. It is also possible that *P. stutzeri* AG259 uses the electron-transport system of the cytoplasmic membrane for the reduction of the silver ions to elemental silver. However, the production of the silver-based crystalline particles seems to be connected with the ability of bacterial cells to survive in an environment that would be highly toxic for other bacteria. Such a response results in the detoxification of Ag⁺ because Ag⁰ is a less toxic form.

The interest in bacterial nanophase systems relates to their use as functional materials with specific properties. The possible medical, biotechnological and materials science applications of these nanophase systems depend on several factors. The large-scale cultivation capacity of the biomass and the extraction techniques of the intracellular particles are some of the biological prerequisites for the manufacture of practical materials. On a commercial scale, applications require a fundamental understanding of the biochemical and genetic principles by which the bacterial cell controls the nucleation and growth of the particles. Bacterial metal-ion resistance systems have been found on plasmids, chromosomes and transposons^{10,11}. Heavy metals induce specific responses, including modifications in gene expression that lead to the synthesis of stress proteins. Identification of induced genes, molecular cloning and sequencing and analysis of the gene products will reveal the genetic and molecular basis for silver resistance and crystal growth in *P. stutzeri*. Initial studies that adressed molecular genetics in P. stutzeri AG259 were made using high voltage electrotransformation and intrageneric transfer of silver resistance in *Pseudomonas*^{46,47}. Genetic engineering techniques could potentially be used to improve the particle properties and to control their chemical composition.

The possibility of directly synthesizing metal particles in an organic carbonaceous matrix points

towards new uses of metal-containing bacteria in thin film and surface technology, for which a composite or cermet structure can yield controlled optical, electrical, magnetic and other properties. The following two examples elucidate this in particular.

Spectrally selective coatings for solar energy absorption

Efficient photothermal conversion of solar energy requires spectrally selective surfaces with a high absorption of solar radiation and a low emittance of thermal radiation⁴⁸. The desired optical properties can be achieved using a number of different techniques⁴⁸, with metal–insulator composite films backed by infrared-reflecting surfaces being the most widely used. Compositional grading, antireflection coating, surface texturing can be used to boost the properties.

Several surface coatings are already being used commercially for solar collectors, such as electroplated 'black nickel'⁴⁹, anodically prepared nickel pigmented anodic aluminum oxide⁵⁰ and incompletely oxidized nickel coatings made by reactive magnetron sputtering onto aluminum ribbon in a roll-coating configuration⁵¹. In addition, optically selective paint is used in some systems. An alternative way to produce such coatings using biomimetic techniques is demonstrated in the following example.

A carbonaceous coating containing silver, produced from *P. stutzeri* AG259 and backed by an aluminum substrate shows strong wavelengthselective absorption and has a potential application as a solar absorbing coating⁵². Figure 3 shows a typical film after heat treatment on a glass substrate. Crystalline metallic silver particles, previously synthesized in the bacterial cell, are homogeneously embedded in the film.

These biologically prepared coatings exhibit spectral selectivity, although they cannot compete with surface coatings prepared using the more traditional techniques (Fig. 4). However, optimization

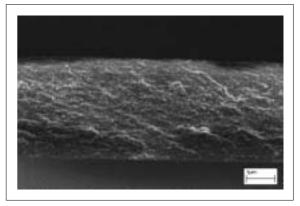


Fig. 3. Scanning electron microscopy image of a cross section of a thin film on a glass substrate. The film is prepared from the biomass of the Ag-accumulating bacterial strain *Pseudomonas stutzeri* AG259 following heat treatment for 1h at 400°C. Small granular silver particles are embedded in the carbonaceous host matrix.

Review

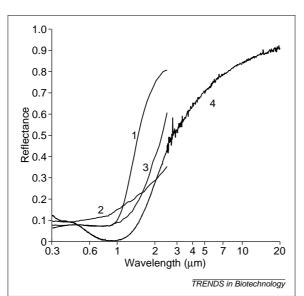


Fig. 4. Spectral reflectance for metal-dielectric composite coatings backed by aluminum for three commercially available solar collector surfaces (plots 2, 3 and 4) and the biomimetic material fabricated with the *Pseudomonas stutzeri* AG259 cells as a carbonaceous host matrix (plot 1). Plot 1, biomimetic; Plot 2, carbon based paint; Plot 3, FeMnCuO_x based paint; Plot 4, sputtered.

of the solar absorption performance is possible by shifting the absorption edge towards longer wavelengths, which is in agreement with theory. This absorption edge can be adjusted by a heat treatment process and by enhancing the metal concentration in the organic material. A broad band antireflecting coating is then possible by a graded index-type structure – one that is achieved by increasing the metal concentration towards the metallic substrate.

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Lithium ion intercalation materials for electrical batteries

Another example of biomimetically produced silver carbon coatings demostrates their possible applications in electrical devices. Porous carbon is an excellent intercalation host of considerable interest in modern battery technology⁵³. Carbon anodes are thus commonly used in efficient lithium ion batteries. High reaction efficiency, high volumetric specific capacity and a long life cycle can be achieved via a high porosity of the intercalation host material and

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through improved contacts by increasing the electronic conductivity as a result of these metal inclusions. New metal–graphite composite anodes with ultrafine silver particles supported on a graphite surface have a higher volumetric specific capacity and longer life cycle than conventional graphite anodes^{54,55}.

Scanning electron microscopy shows that a carbonaceous coating produced from organic material from *P. stutzeri* AG259 has a highly porous structure following heat treatment. Lithium ion intercalation studies show that biomimetic carbonaceous coatings might work well as battery electrodes. Furthermore, a high porosity of carbonaceous films obtained from templating the biological cellular structures increases the active carbon surface and might thus yield superior electrical properties. Thus, it can be expected that, owing to the excellent proton or lithium-conducting properties, biomimetically produced carbonaceous coatings will work well as electrodes for micro-batteries and electrochemical capacitors.

Conclusions and perspectives

An interdiciplinary culture has evolved in nanotechnology that requires the collaboration between physicists, chemists, biologists and engineers. The corresponding disciplines have made remarkable progress in the synthesis, visualization, manipulation, modification and control of materials on the nano- and micrometer scale.

Using metal-accumulating microorganisms as a tool for the production of nanoparticles, and their assembly for the construction of new advanced materials, is a completely new technological approach. The transfer of natural elements of cellular processes into a technical context using a bottom-up strategy can be seen as counterpart to the top-down-structuring procedure in microelectronics. Bacteria that produce mineral phases, in which inorganic particles are grown within or on an organic matrix produced by the organism, exert strict control over size, morphology, composition, and crystallographic orientation of the particles. These unconventional properties have the potential to form new advanced materials in what could be called 'the living factory'.

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