Research review paper

Metal binding by bacteria from uranium mining waste piles and its technological applications

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Abstract

Uranium mining waste piles, heavily polluted with radionuclides and other toxic metals, are a reservoir for bacteria that have evolved special strategies to survive in these extreme environments. Understanding the mechanisms of bacterial adaptation may enable the development of novel bioremediation strategies and other technological applications.

Cell isolates of Bacillus sphaericus JG-A12 from a uranium mining waste pile in Germany are able to accumulate high amounts of toxic metals such as U, Cu, Pb, Al, and Cd as well as precious metals. Some of these metals, i.e. U, Cu, Pd(II), Pt(II) and Au(III), are also bound by the highly ordered paracrystalline proteinaceous surface layer (S-layer) that envelopes the cells of this strain. These special capabilities of the cells and the S-layer proteins of B. sphaericus JG-A12 are highly interesting for the clean-up of uranium contaminated waste waters, for the recovery of precious metals from electronic wastes, and for the production of metal nanoclusters. The fabricated nanoparticles are promising for the development of novel catalysts. This work reviews the molecular biology of the S-layer of the strain JG-A12 and the S-layer dependent interactions of the bacterial cells with metals. It presents future perspectives for their application in bioremediation and nanotechnology.

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Keywords: Uranium mining waste piles; Bacillus sphaericus; S-layer; Bioremediation; Biocers; Metal nanoclusters

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1. Uranium mining waste piles as a reservoir for novel bacteria

During the last decades significant amounts of radionuclides discharged in process effluents produced by industrial activities allied to the generation of nuclear power (Lloyd and Macaskie, 2000), from mining activity, as a consequence of nuclear weapons, and via accidental releases.

Soils, sediments, and waters of these environments, heavily polluted with radionuclides and other toxic metals, are a reservoir of unusual bacteria well adapted to the toxic conditions. These bacteria possess fascinating mechanisms for interaction with and bio-transformation of radionuclides and other heavy metals, thus regulating the mobility of the metals in the environment. Well known are the enzymatically catalyzed reduction of U(VI) to U(IV) (Lovley et al., 1991; Lovley and Phillips, 1992), resulting in the precipitation and crystallization as the mineral uraninite (UO₂) (Gorby and Lovley, 1992), biotransformation of organic and inorganic uraninite complexes (Francis et al., 1991), biosorption, and bioaccumulation (Hennig et al., 2001; Merroun and Selenska-Pobell, 2001; Merroun et al., 2003a). The understanding of the role of the bacteria in the migration of radionuclides and other heavy metals as well as the study of the underlying mechanisms are of great importance for the development of bioremediation strategies. Conventional methods for remediation of contaminated sites such as precipitation, coagulation, membrane based processes, and ion exchange are expensive and less effective at low metal concentrations. Therefore, the ability of many microorganisms to accumulate and to immobilize various toxic metals and to transform minerals gave rise to growing interest in the use of microorganisms for the decontamination of heavy-metal polluted environments (Barkay and Schaefe, 2001; Iwamoto and Nasu, 2001; Kretschmer et al., 2004). Microorganisms could be used to clean-up metal contamination by removing metals from contaminated water and waste streams (Raff et al., 2002, 2003), sequestering metals from soils and sediments, or dissolving mineral phases to facilitate metal extraction (White et al., 1997).

In the Southeast part of Germany (Saxony and Thuringia) the intensive uranium mining and milling that was performed between 1952–1989 with a total production of about 231,000 tons have caused significant pollution by uranium and other toxic metals (www.wismut.de). During the last years microbial communities of several sites heavily polluted with radionuclides and heavy metals from different uranium mining waste piles in Saxony, Germany, have been extensively studied (Selenska-Pobell et al., 1999, 2001; Selenska-Pobell, 2002, in press). Many bacterial strains have been cultivated from these sites and their interactions with radionuclides and metals have been analyzed (Panak et al., 2000; Merroun and Selenska-Pobell, 2001; Merroun et al., 2002, 2003a; Raff et al., 2004). The Bacillus sphaericus JG-A12 strain was isolated from a uranium mining waste pile situated near the town of Johanngeorgenstadt in Saxony. The cells of this strain are able to bind selectively and reversibly high amounts of heavy metals such as U, Cu, Pb, Al, and Cd (Selenska-Pobell et al., 1999). Further analyses showed that the cells of this strain are enveloped by a proteinaceous surface-layer (S-layer), which is able to bind high amounts of uranium up to 20 mg U/g protein (Raff, 2002). In addition, the cells and S-layer proteins are able to bind noble metal ions such as Pd(II), Pt(II) and Au(III) from metal salt solutions, thus enabling the synthesis of Pd nanoclusters of a defined size by the addition of a reducing agent. The S-layer proteins and genes of B. sphaericus JG-A12 and its closest relative NCTC 9602 as well as the interactions of the proteins with metals and the formation of metal nanoparticles were studied in detail (Merroun et al., 2003b; Merroun et al., in press; Pollmann et al., 2005, in press). The special metal binding abilities of the bacterial cells and S-layers make them interesting for technical applications in bioremediation and nanotechnology. This article presents an overview of the properties of the uranium mining waste pile isolate B. sphaericus JG-A12 and its S-layer, the
molecular biology of the latter, and possible technological applications of the biological material.

2. S-layer proteins

2.1. Characteristics and applications of the surface layer proteins (S-layers)

The paracrystalline proteinaceous surface layers (S-layers) are one of the most common surface structures present in all major phylogenetic groups of bacteria and in almost all archaea (Sára and Sleytr, 2000). They are composed of protein or glycoprotein monomers of a molecular weight between 40 kDa and 200 kDa with the ability to self-assemble into two-dimensional paracrystalline arrays exhibiting oblique (p1, p2), square (p4), or hexagonal (p3, p6) symmetries or other structures (Kuen and Lubitz, 1996; Sára and Sleytr, 2000). After secretion and self-assembling of the protein subunits, the S-layer envelopes of the bacterial cells serve as an interface between the bacterial cell and the environment. The S-layers form a highly porous protein meshwork (30–70% porosity) at the cell surface with regularly distributed pores of uniform size and morphology. Pores are typically 2 to 8 nm in diameter (Sleytr et al., 1997, 1999). In Gram-positive bacteria and archaea, the S-layer subunits are linked to the peptidoglycan-containing layer or to the pseudomurein. In Gram-negative bacteria, attachment involves components of the outer membrane. In many archaea S-layers are the only cell wall component (Sára and Sleytr, 2000). S-layer proteins share some general features, such as low levels of sulfur, their ability to self-assemble into paracrystalline arrays, and the composition of mostly hydrophobic amino acids. Nevertheless molecular analyses of S-layers from various genera have shown that the primary structures of the protein share not much similarity.

When present, S-layer proteins are one of the most abundant cellular proteins, comprising up to 15% of all proteins of a cell and exhibiting a rate of up to 500 subunits synthesis per second (Sára and Sleytr, 2000). In case of B. sphaericus JG-A12 our analyses indicate an S-layer protein content of even 20%. Although the synthesis of S-layers is highly energy consuming, the function of the S-layers is difficult to identify in most cases. In archaea such as Methanococcus, Sulfolobus and Thermoproteus the S-layers determine the cell shape and can direct cell division (Beveridge et al., 1997; Sleytr and Beveridge, 1999). In bacteria, S-layers function as molecular sieves (Sára and Sleytr, 1987), as virulence factors in several pathogenic bacteria (Ishiguro et al., 1981), as an attachment structure for high-molecular-weight extracellular proteins (Matuschek et al., 1994), and as a protective coat. Due to its ability to accumulate heavy metals, the S-layer of the uranium mining waste pile isolate Bacillus sphaericus JG-A12 was assumed to protect the cells against the toxic metals (Merroun et al., in press; Pollmann et al., in press).

The isolated S-layer subunits maintain their ability to recrystallize into two-dimensional regular monomolecular arrays in suspension and on various surfaces (e.g. silicon wafers, metals, and polymers) or at interfaces (e.g. lipid films and liposomes) upon removal of the extracting agent used for their isolation. These special characteristics of the S-layers have attracted attention for their use as biotemplates in biotechnology, molecular nanotechnology and biomimetics. S-layers have been used for the production of isoporous ultrafiltration membranes with sharp molecular weight cutoffs (Sára and Sleytr, 1987; Sára et al., 1996), as patterning structures in molecular nanotechnology (Pum and Sleytr, 1996) as immobilization matrices for binding of a broad spectrum of biologically active proteins (e.g. enzymes, antibodies, ligands) to develop bioanalytical sensors (Neubauer et al., 1996), immunoassays, affinity microparticles, affinity membranes (Sára et al., 1996), and for the development of vaccines by incorporation of functional domains (Umelo-Njaka et al., 2001). Another interesting application in nanotechnology is the use of S-layers as biotemplates for the synthesis of metal and semi-metal nanoclusters. Naturally, S-layers can act as a template for the deposition of minerals such as gypsum and calcite by providing nucleation sites for mineralization as shown for Synecococcus str. GL24 (Schultze-Lam et al., 1992). In addition S-layers have been used successfully as templates for the synthesis of CdS (Shenton et al., 1997), Au (Dieuluweit et al., 1998), Pt, and Pd cluster arrays (Wahl et al., 2001).

2.2. The S-layers of Bacillus sphaericus JG-A12 and NCTC 9602: proteins, genes and evolution

The metal binding S-layer of the uranium mining waste pile isolate Bacillus sphaericus JG-A12 exhibits square (p4) symmetry with a lattice constant of 12.5 nm (Fig. 1). A similar S-layer is formed by the closely related strain B. sphaericus NCTC 9602, exhibiting as well p4-symmetry with a lattice constant of 12.9 nm (Raff, 2002). Recently the S-layers of both strains have been analyzed (Raff, 2002; Merroun et al., 2003b; Raff and Selenska-Pobell, 2004; Merroun et al., in press; Pollmann et al., in press). Chemical analyses of the S-layers showed, that both S-layers are composed of a
single kind of protein with a molecular weight of 126 kDa. Sequence analyses of the S-layer protein gene slfB of *B. sphaericus* JG-A12 showed that the protein consists of 1238 amino acids with a composition typically found for S-layer proteins such as a high content of hydrophobic and acidic amino acids, a high content of lysine and the absence of cysteine. The S-layer protein SlfA of the strain 9602, consisting of 1228 amino acids, possesses similar features. Similar to most S-layer proteins, the proteins of both strains show a high content of serine, threonine, glutamate and aspartate. Remarkable are the serine, threonine, aspartate and glutamate enriched stretches especially occurring in the central and C-terminal regions (Pollmann et al., in press).

S-layer proteins of many archaea and Gram-positive bacteria are reported to possess covalently bound carbohydrate chains. In contrast, the S-layer proteins of the strains JG-A12 and 9602 possess a different, unusual posttranslational modification. ICP-MS analyses as well as colorimetric methods demonstrated that both proteins are phosphorylated (Raff, 2002; Merroun et al., in press). Apart from these *B. sphaericus* strains, a phosphorylation of S-layer proteins was found to be associated with a putative transposase and a putative integrase/recombinase located on a predicted insertion element. It can be suggested that these mobile elements contributed to the S-layer gene evolution (Pollmann et al., in press).

Comparisons with the other known S-layer proteins of different *B. sphaericus* strains showed weak similarities of the N-terminal domains and revealed the unique structures of these parts of SlfA, SlfB, SlIA and SlIB. The monitored strong identity between the latter four proteins was considered as the result of a common origin of these parts (Pollmann et al., in press). In contrast, the central regions of the Slf proteins share a high identity to those of the S-layer proteins of the *B. sphaericus* strains CCM 2177 and P-1. Further, the C-terminal region of SlfB (strain JG-A12) showed a strikingly high identity to that of the S-layer protein of *B. sphaericus* CCM2177, whereas the C-terminal domain of SlfA (strain 9602) showed no similarity to any other known S-layer protein. On the basis of these results it was assumed that horizontal gene transfer and DNA rearrangements were involved in the evolution of the S-layer protein genes (Pollmann et al., in press).

A variation of the S-layer protein, which leads to the expression of different types of S-layer genes or the recombination of partial coding sequences, has been reported for many bacterial strains such as *Campylobacter fetus*, *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Bacillus anthracis* and *Geobacillus stearothermophilus* (Blaser et al., 1994; Boot et al., 1996a,b; Dworkin and Blaser, 1996; Scholz et al., 2001; Cou-
turing-Tosi et al., 2002). In most cases the variation was shown to be based on chromosomal rearrangements. The variation of S-layer gene expression can be induced by the change of environmental conditions, such as oxygen stress or changing temperatures (Ishiguro et al., 1981; Kuen et al., 1997; Scholz et al., 2001; Jakava-Viljanen et al., 2002). The S-layer gene variation monitored in pathogenic bacterial strains has been considered as a kind of antigenic variation responding to the lytic activity of the immune system of the host (Blaser et al., 1994; Dworkin and Blaser, 1996; Sára and Sleytr, 2000; Mesnage et al., 2001; Mignot et al., 2001). In the case of the non-pathogenic S-layer carrying bacteria, it can be assumed that they use the S-layer variations for the adaptation to different stress factors. However, an expression of the silent S-layer protein genes of *B. sphaericus* JG-A12 and NCTC 9602 has never been monitored yet. Thus it remains unclear whether and under what conditions these genes are expressed.

3. *Bacillus sphaericus* JG-A12: interactions with uranium and applications in bioremediation

3.1. The problem: environmental contamination with uranium

Uranium is a long-lived radionuclide that represents ecological and human health hazards. The mining and processing of uranium for nuclear power plants and nuclear weapon production have resulted in the generation of significant amounts of radioactive wastes. It is critical that the uranium in radioactive wastes has to be effectively immobilized in order to prevent groundwater contamination. Microorganisms can mobilise radionuclides through autotrophic and heterotrophic leaching and chelation by microbial metabolites and siderophores. On the other hand immobilization of the radionuclides can result from sorption to cell components or exopolymers (Lueng et al., 2001), intracellular sequestration or precipitation as insoluble organic and inorganic compounds, e.g. oxalates, sulfides or phosphates (Boswell et al., 2001; Renninger et al., 2001). Thus, the use of microorganisms is a promising approach for the clean-up of uranium contaminated soils and waters. Solubilization provides a route for removal from solid matrices such as soils, sediments, dumps and industrial wastes. Alternatively, immobilization processes may enable in situ metal-transformations into insoluble and chemically inert forms and are particularly applicable for removing metals from mobile aqueous phases. In this context the uranium binding capabilities of the uranium mining waste pile isolate *Bacillus sphaericus* JG-A12 and its S-layer are highly interesting. V tentative cells and spores of this strain accumulate selectively high amounts of U (Fig. 2), thus being a good candidate for in situ bioremediation of uranium mining waste pile waters (Raff, 2002; Raff et al., 2002, 2004).

3.2. Interactions of the cells and S-layers of the strain JG-A12 with uranium

For the characterization of metal-microbial interactions, a variety of physical techniques have been used such as Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy (Hennig et al., 2001; Merroun et al., 2003a), Time-Resolved Laser-Induced Fluorescence Spectroscopy (TRLFS) (Panak et al., 2000), Infrared Spectroscopy (IRS) (Yun et al., 2001), etc. The interactions of the cells and the S-layer of *B. sphaericus* JG-A12 with uranium were studied in detail by using TEM, EDX and EXAFS-methods (Raff et al., 2004; Merroun et al., in press). EXAFS measurements provide element-specific short-range structural and chemical information about the U(VI) coordination environments, including identities of, coordination numbers of, and bond distances to the neighbor atoms. The EXAFS data indicate that in the whole-cell sample U is coordinated by phosphate groups in monodentate bonding mode and by carboxyl groups in a bidentate fashion (Merroun et al., in press).

![TEM micrograph of uranium accumulated on the cell surface of *Bacillus sphaericus* JG-A12.](image-url)
These groups arise from the biocomponents of the cell surface, as demonstrated by the TEM analysis. EXAFS analysis of the uranium bound to the S-layer demonstrated that U is coordinated to phosphate and carboxyl groups of the protein (Merroun et al., in press). Analyses of posttranslational modifications confirmed the phosphorylation of the protein. Compared to the S-layer protein of the closely related strain *B. sphaericus* NCTC 9602, the S-layer protein of the uranium mining waste pile isolate *B. sphaericus* JG-A12 contains about six times more phosphorus (Merroun et al., in press). This difference may reflect an adaptation of *B. sphaericus* JG-A12 to its highly with uranium contaminated environment. The high metal-affinity of phosphate groups on the surface of the cell may allow *B. sphaericus* JG-A12 to bind selectively large amounts of uranium before getting damaged by this toxic radionuclide. Analyses of the amino acid composition of the S-layer proteins demonstrated a high content of glutamic acid and aspartic acid (both residues with carboxyl groups) as well as other amino acids like serine and threonine (both residues with hydroxyl groups), the latter are potential phosphorylation sites (Pollmann et al., in press). As stretches of these amino acids are preferentially found in the central and C-terminal parts, it can be assumed, that these regions are involved in the binding of uranium.

3.3. Perspectives in bioremediation

To use biocomponents as effective parts of filter materials in bioremediation processes for cleaning radionuclide and heavy metal contaminated drainage in different environments, the pH-stability of the biological components, the possibility of a repeated use of the filter material and the immobilization of the biocomponents are of special importance. Sol–gel technology allows the immobilization of various biomolecules or microorganisms without losing their activity and structure (Carturan et al., 1989; Braun et al., 1992; Böttcher, 2000; Livage et al., 2001). Other advantages of sol–gel ceramics are a high mechanical, thermal and photochemical stability, biological and toxical inertness and controlled matrix porosity. This technology was applied to produce a filter material capable of binding metals selectively for bioremediation processes. Cells, spores and S-layers of *B. sphaericus* JG-A12 were embedded in silica gels using an aqueous sol–gel process to produce a porous filter matrix (bioceramic, biocer) with a homogeneous structure and completely immobilized biocomponents (Raff et al., 2002, 2003; Selenska-Pobell et al., 2003). After the encapsulation of the different JG-A12 biocomponents, the biomaterial still retained its metal accumulating capacities. The produced bioceramics were successfully used to remove copper and uranium from contaminated water (Fig. 3). The binding of the metals is reversible and both metals can be completely removed by using aqueous citric acid. Embedded cells, spores and EDC-stabilized S-layers of JG-A12 were found to be stable in acidic drain water at pH 4 and below (Raff, 2002; Raff et al., 2002). Due to the high stability of the biocers, the safe immobilization of the biocomponents, the high metal binding capacity and the simple and complete removal of the bound metals, the biocers are well suited for the reversible usage for bioremediation purposes.

4. Interactions of *B. sphaericus* JG-A12 with palladium and applications in nanotechnology

4.1. Interactions of bacterial cells with palladium

The metals belonging to the platinum group such as platinum, palladium, rhodium, are used worldwide on an increasing scale in catalysis and electronics. As a result, there is growing interest in the recovery of precious metals from industrial waste (Yong et al., 2002b). Currently chemical recovery by complex solution chemistries is difficult, and recovery by chelating ion exchange or electrochemical methods is not efficient and is too expensive. A promising alternative for removing Pd from solutions is the use of bacteria. The bioreductive deposition of Pd(0) particles onto biomass of *Desulfovibrio desulfuricans* has been studied in detail (Lloyd et al., 1998; Yong et al., 2002b,a) and recovery of Pd from real processing waste was demonstrated (Yong et al., 2002b). Several studies showed that the small Pd-nanoparticles produced by the cells and deposited onto the cell surfaces are able to catalyse various chemical reductions such as dehalogenation of polychlorinated biphenyls (Baxter-Plant et al., 2003, 2004; De Windt et al., 2005) or reduction of Cr(VI) (Mabbet et al., 2004). Thus, the ability of biomass such as bacterial cells or proteins to bind noble metals as well as the production of metal nanoparticles has attracted an interest.

The cells of the uranium mining pile isolate *Bacillus sphaericus* JG-A12 are able to complex Pd(II) from a solution of Na₂PdCl₄. The bound Pd(II)-complexes can be reduced to Pd(0)-nanoclusters by the addition of H₂ as an electron donor. TEM-analyses demonstrated that the fabricated metal clusters are...
Fig. 3. Sorption (A) and desorption (B) of uranium by 200 mg dw, of biocer types, 36 mg dw cells, and 164 mg dw xerogel (dw=dry weight).
deposited on the surface, most probably on the S-layer, of the bacterial cells (Fig. 4). The interaction of the bacterial cells with Pd(II) as well as the formed Pd-nanoclusters were investigated by using EXAFS-spectroscopy. Due to the coordination of Pd(II) to oxygen as shown by the EXAFS data, it can be assumed that carboxyl groups located at the cell surface are involved in the binding of Pd(II). The formation of Pd(0)-nanoclusters after addition of H2 as reducing agent was confirmed by the EXAFS-analyses. The fabricated nanoparticles consisted of 19–43 atoms with a diameter ranging between 0.85–1 nm formed at a layer of nearest neighbors around a central atom (Merroun et al., 2003b). The produced nanoclusters are promising for the development of novel catalysts. First analyses showed that the B. sphaericus Pd nanoparticles possess catalytic activity in the reduction of Cr(VI) and in organic synthesis reactions of industrial interest (Creamer et al., personal communication).

4.2. Perspectives of S-layers in nanotechnology

Generally, noble metals are employed as highly functional materials with properties such as wavelength selective plasmon absorption resonances, conductivity, and catalytic activity. As the properties of nanoparticles usually differ significantly from those of the material from which they are formed (Seifert, 2004), the synthesis and applicability of noble metal nanoclusters has attracted much attention. The development of cluster-assembled materials with discrete, size-selected nanoparticles is of great interest to enable the fine-tuning of the properties of the nanoparticles (Andres et al., 1996; Seifert, 2004). A promising approach to produce such nanoparticles of controlled size is the use of self-assembling organic templates which allow the synthesis of a wide range of inorganic nanocrystal lattices (Braun et al., 1998; Dieluweit et al., 1998; Mertig et al., 1998; Richter et al., 2001; McMillan et al., 2002; Patolsky et al., 2004). So far, several natural materials have been successfully used for the fabrication of ordered nanoparticle arrays. The self-assembling chaperonins have been used for the organization of gold or CdSe-ZnS semiconductor nanoparticle quantum dots into ordered arrays (McMillan et al., 2002). Silver, gold, palladium, platinum or copper nanoparticles have been deposited on DNA templates by the reduction of metal ions or metal complexes associated with the DNA (Braun et al., 1998; Mertig et al., 1999; Richter et al., 2001), and gold nanowires were synthesized using actin as a template (Patolsky et al., 2004).

Another promising biological templates for the production of metal nanoclusters are the regular structured paracrystalline surface layers of bacteria (Dieluweit et al., 1998; Wahl et al., 2001). Due to the crystalline arrangement of the S-layer, functional groups such as carboxyl-, amino- or hydroxyl groups, are found in well-defined position and orientation on the protein meshwork (Dieluweit et al., 1998). S-layers have been used as templates for the fabrication of different inorganic nanocrystal arrays. They work as template for in situ nucleation of ordered two-dimensional arrays of CdS nanocrystals (Shenton et al., 1997). The S-layer lattices of Bacillus sphaericus CCM 2177 were used to produce gold nanoclusters by exposing the S-layer lattices, in which thiol groups had been introduced before, to a tetrachloroauric(III) acid solution groups under electron radiation (Dieluweit et al., 1998) or were used to bind functionalized CdSe and Au nanoparticles to form regular arrays (Györvary et al., 2004). Regular arrays of Pt and Pd nanoparticles were produced also by electron irradiation of the S-layer of the strain Bacillus sphaericus NCTC 9602 after incubating with solutions of K2PdCl4 or K2PtCl4 (Wahl et al., 2001). The nucleation sites of all these nanoclusters were the pores of the S-layers.

In our approach, the deposition of palladium or platinum nanoclusters is performed via a two step process, consisting of biosorption by exposition of the S-layer proteins to a hydrolyzed metal complex solution (I) and of metal reduction (II) by addition of an electron donor. The interaction of Pd(II) with the S-layer of B. sphaericus JG-A12 and the formed Pd-nanoclusters were analyzed by using EXAFS-spectroscopy and dialysis-coupled attenuated total reflectance Fourier-transform-infrared-spectroscopy (ATR-FT-IR-spectroscopy). The EXAFS-spectra demonstrate the coordination of Pd(II) to oxygen after biosorption, thus indicating that most likely carboxyl groups are involved.
in Pd(II)-complexation (Pollmann et al., 2005). The ATR-FT-IR-absorption spectra confirmed this assumption and demonstrated that the S-layer protein is highly stabilized by the complexation with Pd(II), keeping the protein structure stable even in acidic conditions down to a pH of 0.8 (Pollmann et al., 2005). It was proposed previously that the addition of NaN₃ as reducing agent initiates a stable growth of metal clusters (Mertig et al., 1999, Böttcher et al., 2004). However, in the case of the S-layer of B. sphaericus JG-A12, EXAFS-analyses demonstrated that the addition of NaN₃ is not sufficient to enable a complete reduction of the palladium complexes to give Pd metal particles (Merroun et al., 2003b). In contrast, the addition of H₂ as electron donor resulted in the complete reduction of Pd(II) as confirmed by EXAFS-analyses (Pollmann et al., 2005). The size of the formed Pd-nanoclusters was determined by EXAFS-analyses to be smaller than 1 nm. The catalytic properties of the thus fabricated nanoclusters are currently being tested.

5. Conclusions

The study of bacteria from environments heavily polluted with radionuclides and other toxic metals open up new perspectives for applications in bioremediation and nanotechnology. The cells of the uranium mining waste pile isolate Bacillus sphaericus JG-A12 are capable of selective and reversible accumulation of toxic metals. The cells are protected by a proteinaceous surface layer (S-layer) with a high affinity to uranium and noble metals. These special characteristics were used for the development of a filter material (biocer), based on a sol–gel matrix, to clean-up uranium contaminated waters. In addition, the cells and S-layers are able to bind metal ions and complexes from metal salt solutions such as Pd(II), Pt(II) and Au(III) that can be reduced to the corresponding metal nanoparticles by the addition of H₂. Thus, the biomaterial can be used for the recovery of precious metals from industrial waste waters and for the synthesis of ordered nanoparticle arrays, which are promising for the development of novel catalysts.

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