Review

Applications of biosynthesized metallic nanoparticles – A review

Adam Schröfel\textsuperscript{a,b,e}, Gabriela Kratošová\textsuperscript{c}, Ivo Šafařík\textsuperscript{a}, Mirka Šafaříková\textsuperscript{a}, Ivan Raška\textsuperscript{e}, Leslie M. Shor\textsuperscript{b,d}

\textsuperscript{a} Department of Nanobiotechnology, Institute of Nanobiology and Structural Biology of GCRC, Academy of Sciences, Ceske Budejovice, Czech Republic
\textsuperscript{b} Department of Chemical and Biomolecular Engineering, University of Connecticut, Storrs, CT, USA
\textsuperscript{c} Nanotechnology Centre, VSB-Technical University in Ostrava, Ostrava, Czech Republic
\textsuperscript{d} Center for Environmental Sciences & Engineering, University of Connecticut, Storrs, CT, USA
\textsuperscript{e} Charles University, Institute of Cellular Biology and Pathology, Prague, Czech Republic

\textbf{A R T I C L E  I N F O}

Article history:
Received 16 January 2014
Received in revised form 13 April 2014
Accepted 21 May 2014
Available online 9 June 2014

Keywords:
Biosynthesis
Metallic nanoparticles
Nanomedicine
Bioimaging
Sensors

\textbf{A B S T R A C T}

We present a comprehensive review of the applications of biosynthesized metallic nanoparticles (NPs). The biosynthesis of metallic NPs is the subject of a number of recent reviews, which focus on the various “bottom-up” biofabrication methods and characterization of the final products. Numerous applications exploit the advantages of biosynthesis over chemical or physical NP syntheses, including lower capital and operating expenses, reduced environmental impacts, and superior biocompatibility and stability of the NP products. The key applications reviewed here include biomedical applications, especially antimicrobial applications, but also imaging applications, catalytic applications such as reduction of environmental contaminants, and electrochemical applications including sensing. The discussion of each application is augmented with a critical review of the potential for continued development.

© 2014 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

\section{1. Introduction}

The unique properties of nanoscale materials have given rise to tremendous research activity directed towards nanoparticle (NP) fabrication, characterization and applications. In order to reveal ever more favorable NP functionality, some researchers look to nature for methods to produce NPs with novel properties or enhanced function. Many organisms are known to form inorganic materials either intra- or extracellularly. For example, iron-reducing bacteria have been known to reduce labile and/or toxic metals into their zero-valent form \cite{1}. The same or similar metal-reducing capability used as an NP production method is called biosynthesis \cite{2}. As reviewed recently, many different prokaryotic and eukaryotic organisms have been used to produce metallic NPs \cite{3}, and biosynthesis of NPs has attracted increasing attention in the past 10 years \cite{4} (see Fig. 1).

Among the key advantages that the biological approach has over traditional chemical and physical NP synthesis methods is the biological capacity to catalyze reactions in aqueous media at standard temperature and pressure. Production in aqueous media under standard conditions leads to many cost advantages, in terms of both capital equipment and operating expenses, especially in the purchase and disposal of solvents and other consumable reagents. Biosynthesis can be implemented in nearly any setting and at any scale \cite{3}. In addition, extensive investment in biotechnology knowledge for optimized production of food, pharmaceuticals and fuels also informs NP biosynthesis techniques. One important drawback of NP biosynthesis methods is the requirement in some applications to purify the sample or to separate the NPs from the biological material used in their synthesis.

The properties of biosynthesized materials may differ from materials prepared by other methods. Biosynthesis can result in forms that are difficult to make using other techniques, such as alloys and wires. Biosynthesized NPs can also have enhanced stability and biocompatibility and reduced toxicity, mainly due to coating them with biogenic surfactants or capping agents. The potential range of sizes, shapes and compositions of biosynthesized NPs translates into a broad domain of existing and new nano-material applications.

Applications of biosynthesized metal-based NPs range from various biomedical purposes (e.g. antimicrobial coatings, medical imaging and drug delivery) to catalytic water treatment and environmental sensors. We have organized this review according to the applications that use biosynthesized NPs. The chemical composition, form and organism used for the biosynthesis are also described.
2. Biomedical applications

Applications of metallic NPs in the biomedical fields are numerous, and there is strong potential for continued growth in this area. Metallic NPs are widely used for their antimicrobial functionality; for example, silver NPs (AgNPs) have been incorporated into wound dressings, bone cements and implants [5]. Gold NPs (AuNPs) have medically relevant optical and anticancer properties. For example, Alanazi et al. [6] describe how surface plasmon absorption and surface plasmon light scattering can be used for diagnostic and therapeutic applications, and Patra et al. [7] describe the fabrication and application of AuNPs for targeted cancer therapy. As described by Pankhurst et al. [8,9], magnetic NPs appear promising for targeted drug delivery and hyperthermia applications.

2.1. Antimicrobial applications

The ongoing development of antimicrobial agents is important due to the continuous selection of antibiotic resistance traits in bacteria and other pathogens. Different metallic NPs, including titanium, copper, magnesium and particularly silver and gold, are known for their antimicrobial, antiviral and antifungal capabilities [10]. These metallic NPs are actively investigated as disinfectants, in food processing, and for use as additives in clothing and in medical devices [11].

The following sections describe the key antibacterial, antiviral and antifungal properties described in the literature for biosynthesized NPs (Tables 1 and 2). However, due to the large number of papers describing antimicrobial activity using slightly different materials, endpoints or methods, in some cases only representative examples are provided.

2.1.1. Antibacterial activity

AgNP exposure causes toxicity to bacteria, primarily from Ag ions released into aqueous solution following partial oxidation [5]. Ag ions and small NPs interact with the plasma membrane, disturbing cellular functions, including permeability and respiration, and ultimately leading to lysis. AgNP exposure can prevent DNA replication and protein synthesis by binding to DNA or by denaturing ribosomes [5].

The detailed mechanisms of AuNP antibacterial functionality against *Escherichia coli* is described by Cui et al. [12]. AuNPs were shown to collapse membrane potential, strongly inhibiting ATPase activity and resulting in a decrease in cellular ATP levels. Another consequence of AuNP exposure was inhibited binding of tRNA to the ribosome subunit.

Bacterial susceptibility to antimicrobial agents can depend on the cell wall structure. Bacteria are classified into two categories, based on their cell wall structure: Gram-negative (G−) bacteria have a multi-layer cell wall and Gram-positive (G+) bacteria have a single-layer cell wall. Antibacterial activity of biosynthesized NPs has been studied for both types of bacteria.

A variety of biological materials have been used for the biosynthesis of NPs with demonstrable antibacterial effects. These materials include fungal, bacterial and algal biomass, as well as extracts of botanical materials, including leaves, bark, roots and tubers. One of the first reports of antibacterial effects of biosynthesized NPs was published by Vigneshwaran et al. [13]. These authors used the edible mushroom *Pleurotus sajor-caju* for the synthesis of AgNPs and performed successful antimicrobial testing against...
**Staphylococcus aureus** (G+) and **Klebsiella pneumoniae** (G−/C0) bacteria. Raheman et al. [14] reported the extracellular synthesis of AgNPs by means of an endophytic fungus, *Pestalotia* sp., isolated from leaves of *Syzygium cumini* and the antimicrobial properties of AgNPs against *S. aureus* (ATCC-25923) and *Salmonella typhi* (ATCC-51812). Synergistic effects were observed for combined exposure to biosynthesized AgNPs and the commercially available antibiotics gentamycin and sulphamethizole.

Antimicrobial properties of bacteria-biosynthesized NPs were illustrated in two studies by Sadhasivam et al. [15,16]. **Streptomyces hygroscopicus** cells were used for biosynthesis of Ag- and AuNPs with antimicrobial properties against *Bacillus subtilis*, *Enterococcus faecalis*, *E. coli*, *Salmonella typhimurium*, *Staphylococcus epidermidis* and *S. aureus* (see Fig. 2).

An example of algae-based NP biosynthesis is described by Merin et al. [17], where AgNPs with antibacterial properties were synthesized by the microalgal strains *Chaetocerus calcitrans*, *Chlorella salina*, *Isochrysis galbana* and *Tetraselmis gracilis*. These NPs showed antimicrobial properties against *K. pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *E. coli*, as measured using a clearing zone assay. Venkatpurwar and Pokharkar [18] introduced a method for synthesis of AgNPs using sulfated polysaccharides isolated from the marine red alga *Porphyra vietnamensis*. The dose-dependent effect of AgNPs synthesized in this study revealed strong antibacterial activity against *E. coli* (G−) and lesser effectiveness against *S. aureus* (G+). The synthesis of antibacterial AuNPs using powder or ethanolic extract from a marine alga *Galaxaura elongata* was demonstrated by Abdel-Raouf et al. [19]. These authors also performed spectroscopy experiments in order to identify the algal compounds which may play a role as reducing agents or nanoparticle capping agents.

By far the most abundant studies on biosynthesized antibacterial NPs are those using plant tissues and extracts as reducing agents. As previously mentioned, biosynthesized NPs may require purification to remove pathogenic or poisonous compounds, particularly for materials intended for use in vivo. One approach to avoiding toxicity concerns is to use only well-characterized and benign botanical extracts for biosynthesis [20]. For example, green tea extract (prepared from *Camellia sinensis* leaves) is a widely used reducing agent used in the biosynthesis of NPs. Vaseeharan et al. [21] used tea extract to prepare AgNPs with demonstrated antibacterial activity against *Vibrio harveyi*. Other botanical extracts have been used for biosynthesis of antimicrobial AgNPs. Stem callus extract of bitter apple (*Citrullus colocynthis*) was used for AgNPs synthesis and demonstrated activity against biofilm-forming *E. coli*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Listeria monocytogenes* [22]. No activity was observed in the case of *Proteus mirabilis*, *Salmonella enteritidis* or *Staphylococcus aureus*. Kaviya et al. [23] studied biosynthesis of AgNPs using navel orange (*Citrus sinensis*) peel extract and demonstrated antibacterial activity against *E. coli*, *Pseudomonas aeruginosa* and *S. aureus*. Finally, Nagajyothi and Lee [24] used Chinese yam (*Dioscorea batatas*) rhizome extract for the biosynthesis of antibacterial AgNPs.

Several reports have described synergistic antimicrobial effects of phytosynthesized nanoparticles used in combination with antibiotics. Ghosh et al. [25] synthesized AgNPs prepared with tuber extract of *Dioscorea bulbifera*, then measured synergistic antimicrobial potential using 22 types of commercially available antibiotics and seven bacterial strains. For instance, they found an 11.8-fold inhibition efficiency increase when streptomycin and AgNPs are used in combination against *E. coli* compared with the inhibition efficiency using streptomycin only.
Plant extracts have also been used for the biosynthesis of AuNPs with antibacterial activity. Kumar et al. [26] used a deciduous tree (*Terminalia chebula*) extract for the biosynthesis of AuNPs effective against *S. aureus* and *E. coli*. MubarakAli et al. [27] used *Mentha piperita* leaf extract for biosynthesis of AuNPs and AgNPs, and likewise demonstrated antimicrobial effects against *S. aureus* and *E. coli* bacteria.

Biosynthesized metallic NPs that are immobilized on cotton cloth have many important applications as material for wound dressings. For example, Durán et al. [28] reported the extracellular production of AgNPs mediated by the fungus *Fusarium oxysporum*. Here, AgNPs were incorporated into cotton fabrics for inhibition of *S. aureus*. However, one limitation of immobilized AgNPs in fabric is the loss of the Ag ions, and sometimes the AgNPs, with washing. El-Rafie et al. [29,30] demonstrated the use of immobilized biogenic AgNPs on cotton fabric. In these studies, the AgNPs were prepared with *Fusarium solani* and were applied to cotton fabrics using an acrylate based binder. The cotton fabric showed a high antimicrobial efficiency of approximately 90% against *S. aureus* and *E. coli* after 20 washing cycles.

Tripathi et al. [31] examined biosynthesis of AgNPs using an aqueous extract of *Azadirachta indica* leaves and their subsequent immobilization on cotton cloth. These authors observed the bactericidal effect against *E. coli* and *S. aureus* with washing in distilled water. Similarly, the biosynthesis of AgNPs in *Eucalyptus citriodora* and *Ficus bengalensis* leaf extracts and their loading into cotton cloth...
fibers was reported by Ravindra et al. [32]. Cotton fibers were immersed in the leaf extract containing AgNPs, kept on a shaker for 24 h and then tested against E. coli using a modified disk diffusion method.

Antibacterial effect against E. coli was also tested in AgNPs that were prepared by means of the extract and powder of Curcuma longa tubers [33], then immobilized on cotton cloth. AgNPs were resuspended in water or in polyvinylidene fluoride (PVDF), then sprayed over pre-sterilized cotton cloth. Cloth treated with NPs in PVDF exhibited lower antibacterial activity but much longer reusability. Recently, antimicrobial applications for biosynthesized AgNPs incorporated into nonwoven fabrics have been demonstrated. Yang and Li [34] prepared AgNPs using mango peel extract and demonstrated the antimicrobial effectiveness of these nanoparticles immobilized on non-woven fabrics against E. coli, S. aureus and B. subtilis.

Sundaramoorthi et al. [35] described a wound-healing application for biosynthesized AgNPs, prepared extracellularly using the fungus Aspergillus niger. The efficiency of the AgNPs was demonstrated following excision in a rat model. The study supported both the antimicrobial effects of Ag and the ability of AgNPs to modulate cytokines involved in wound healing. Similarly, the medicinal plant Indigofera aspalathoides was used for biosynthesis of AgNPs tested in wound-healing applications following excision in animal models [36].

Finally, biogenic AgNPs derived from Chrysanthemum morifolium have been added to clinical ultrasound gel. In the study, the gel was used on an ultrasound probe, and the bactericidal activity and instrument sterility were evaluated [37].

2.1.2. Antifungal activity
Several studies have described the bacteria Aspergillus niger antifungal activity of biosynthesized NPs. Gajbhiye et al. [38] described the antifungal properties of biosynthesized NPs against Phoma glomerata, P. herbarum, Fusarium semitectum, Trichoderma sp. and Candida albicans in combination with fluconazol (a triazole antifungal drug). AgNPs biosynthesized by another fungus, Alternaria alternata, enhanced the antifungal activity of fluconazole against all tested strains except P. herbarum and F. semitectum. In another study, mycelia-free water extracts of the fungal strain Amylomyces rouxii were used for biosynthesis of AgNPs that were effective against the bacteria Shigella dysenteriae type I, Staphylococcus aureus, Citrobacter sp., E. coli, Pseudomonas aeruginosa and Bacillus subtilis, and also against the fungi Candida albicans and Fusarium oxysporum [39].

Antifungal activity of biosynthesized AuNPs has also been described. Das et al. [40] synthesized AuNPs on the surface of the fungus Rhizopus oryzae and demonstrated the growth inhibition of G− and G+ bacterial strains, as well as the fungi Saccharomyces cerevisiae and C. albicans. AuNPs that inhibit the growth of C. albicans have also been obtained following biosynthesis using a banana peel extract [41].

2.1.3. Antiviral activity
Although viruses also represent serious problems in medicine or agriculture, there have been relatively few reports on the antiviral activity of biosynthesized NPs. Vijayakumar and Prasad [42] described the antiviral activity of Ag NPs prepared intracellularly.
Table 1
Antibacterial activity.

<table>
<thead>
<tr>
<th>NP</th>
<th>Organism used</th>
<th>Activity against</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>Pleurotus sazer-caju</td>
<td>Staphylococcus aureus, Klebsiella pneumonia</td>
<td>Vigneshwaran et al. [13]</td>
</tr>
<tr>
<td>Ag</td>
<td>Pestalothia sp.</td>
<td>S. aureus, Salmonella typhi</td>
<td>Rahman et al. [14]</td>
</tr>
<tr>
<td>Ag</td>
<td>Streptomycyes hygroscopicus</td>
<td>Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Salmonella typhimurium</td>
<td>Sadhasivam et al. [15]</td>
</tr>
<tr>
<td>Au</td>
<td>S. hygroscopicus</td>
<td>B. subtilis, E. faecalis, E. coli, S. typhimurium, S. epidermidis, S. aureus</td>
<td>Sadhasivam et al. [16]</td>
</tr>
<tr>
<td>Ag</td>
<td>Chaetomium calcitrans, Chlorella salina,Isochrysis galbana, Tetraelmis gracilis</td>
<td>K. pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, E. coli</td>
<td>Merin et al. [17]</td>
</tr>
<tr>
<td>Ag</td>
<td>Porphyra vietnamensis</td>
<td>S. aureus, E. coli</td>
<td>Venkatpurwar and Pokharkar [18]</td>
</tr>
<tr>
<td>Au</td>
<td>Galaxaura elongata</td>
<td>E. coli, K. pneumonia, S. aureus, P. aeruginosa</td>
<td>Abdel-Raouf et al. [19]</td>
</tr>
<tr>
<td>Ag</td>
<td>Camellia sinensis</td>
<td>Vibrio harvey</td>
<td>Vaseeharan et al. [21]</td>
</tr>
<tr>
<td>Ag</td>
<td>Citrullus colosynthisis</td>
<td>E. coli, Vibrio parahaemolyticus, P. aeruginosa, P. vulgaris, Listeria monocytogenes</td>
<td>Satyavani et al. [56]</td>
</tr>
<tr>
<td>Ag</td>
<td>Citrus sinensis</td>
<td>E. coli, P. aeruginosa, S. aureus</td>
<td>Kaviya et al. [23]</td>
</tr>
<tr>
<td>Ag</td>
<td>Dioscorea batatas</td>
<td>B. subtilis, S. aureus, E. coli</td>
<td>Nagajothy and Lee [24]</td>
</tr>
<tr>
<td>Ag</td>
<td>Dioscorea bulbifera</td>
<td>Acinetobacter baumannii, Enterobacter cloacae, E. coli, Haemophilus influenzae, K. pneumoniae, Neisseria mucosa, Proteus mirabilis</td>
<td>Ghosh et al. [25]</td>
</tr>
<tr>
<td>Au</td>
<td>Terminalia chebula</td>
<td>S. aureus, E. coli</td>
<td>Kumar et al. [20,26]</td>
</tr>
<tr>
<td>Au, Ag</td>
<td>Mentha piperita</td>
<td>S. aureus, E. coli</td>
<td>Mubarakhali et al. [27]</td>
</tr>
<tr>
<td>Ag</td>
<td>Fusarium oxysporum</td>
<td>S. aureus on cotton fabrics</td>
<td>Durán et al. [28]</td>
</tr>
<tr>
<td>Ag</td>
<td>Fusarium solani</td>
<td>S. aureus, E. coli on cotton fabrics</td>
<td>El-Rafe et al. [29,30]</td>
</tr>
<tr>
<td>Ag</td>
<td>Azadirachta indica</td>
<td>S. aureus, E. coli on cotton fabrics</td>
<td>Tripathi et al. [31]</td>
</tr>
<tr>
<td>Ag</td>
<td>Eucalyptus citridora, Ficus bengalensis</td>
<td>E. coli on cotton fabrics</td>
<td>Ravindra et al. [32]</td>
</tr>
<tr>
<td>Ag</td>
<td>Curcuma longa</td>
<td>E. coli on cotton fabrics</td>
<td>Sathishkumar et al. [33]</td>
</tr>
<tr>
<td>Ag</td>
<td>Mangifera indica</td>
<td>E. coli, S. aureus, B. subtilis on non-woven fabrics -</td>
<td>Yang and Li [34]</td>
</tr>
<tr>
<td>Ag</td>
<td>Aspergillus niger</td>
<td>Wound healing activity</td>
<td>Sundaramoorthi et al. [35]</td>
</tr>
<tr>
<td>Ag</td>
<td>Indigofora aspalathoides</td>
<td>Wound healing activity</td>
<td>Arunachalam et al. [36]</td>
</tr>
<tr>
<td>Ag</td>
<td>Chrysanthenum morifolum</td>
<td>Bactericidal ultrasound gel</td>
<td>He et al. [37]</td>
</tr>
</tbody>
</table>

Table 2
Antifungal, antiviral and anti-parasite activity.

<table>
<thead>
<tr>
<th>NP</th>
<th>Organism used</th>
<th>Activity against</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>Alternaria alternata</td>
<td>Phoma glomerata, Phoma herbarum, Fusarium semitectum, Trichoderma sp., C. albicans – combination with flucnazole</td>
<td>Gajbihye et al. [38]</td>
</tr>
<tr>
<td>Ag</td>
<td>Amylomycyes rouxii</td>
<td>Candida albicans, Fusarium oxysporum</td>
<td>Musarrat et al. [39]</td>
</tr>
<tr>
<td>Au</td>
<td>Rhizopus oryzae</td>
<td>Saccharomyces cerevisiae, C. albicans</td>
<td>Das et al. [40]</td>
</tr>
<tr>
<td>Au</td>
<td>Musa sp.</td>
<td>C. albicans</td>
<td>Bankar et al. [41]</td>
</tr>
<tr>
<td>Ag</td>
<td>Aspergillus ochraceus</td>
<td>M13 phage virus</td>
<td>Vijayakumar and Prasad [42]</td>
</tr>
<tr>
<td>Ce</td>
<td>Leptothrix discophora, Pseudomonas putida</td>
<td>Bacteriopehage UZ1</td>
<td>De Gusseme et al. [43]</td>
</tr>
<tr>
<td>Ag</td>
<td>Lactobacillus fermentum</td>
<td>Bacteriophage UZ1, application to NanoCeram filter</td>
<td>De Gusseme et al. [44]</td>
</tr>
<tr>
<td>Ag</td>
<td>Lactobacillus fermentum</td>
<td>Bacteriophage UZ1, application to PVD F membrane</td>
<td>De Gusseme et al. [45]</td>
</tr>
<tr>
<td>Ag</td>
<td>Nelumbo nucifera</td>
<td>Larvae of Anopheles subpictus, Culex quinquefasciatus</td>
<td>Santhoshkumar et al. [46]</td>
</tr>
<tr>
<td>Ag</td>
<td>Rhizophora mucronata</td>
<td>Larvae of Aedes aegypti, Culex quinquefasciatus</td>
<td>Gnanadesigan et al. [47]</td>
</tr>
<tr>
<td>Ag</td>
<td>Lawsonia inermis</td>
<td>Pediculus humanus capitis, Bovicola ovis</td>
<td>Marimuthu et al. [48]</td>
</tr>
<tr>
<td>Ag</td>
<td>Manilkara zapota</td>
<td>Rhipicephalus microplus</td>
<td>Rajakumar and Rahuman [49]</td>
</tr>
</tbody>
</table>

in Aspergillus ochraceus. In the study, AgNPs embedded in a carbo-
naceous matrix were obtained by heat treatment of the cells, and the
effectiveness against M13 phage was determined using the pla-
que count method.

In another study, De Gusseme et al. [43] described virus inhibi-
tion by zero-valent cerium produced by aqueous Ce(III) added to
Leptothrix discophora and Pseudomonas putida cultures. The as-
prepared cerium exhibited efficient antiviral activity against bac-
teriopehage UZ1. In another study by the same researchers [44], Lacto-
 bacillus fermentum bacteria were used both as a reducing agent
and as a scaffold for AgNPs. Their antiviral activity was determined
for murine norovirus 1 and bacteriophage UZ1. In another study,
the continuous antiviral capability of AgNPs embedded in Lactoba-
cillus cells was tested in an aqueous environment by depositing
the cells onto a NanoCeram electropositive filter. The study dem-
strated that the filter with cell-embedded NPs had remarkably
higher antiviral activity compared with the original filter. Similarly,
De Gusseme et al. [45] demonstrated the inactivation of UZ1 bac-
teriophages using a similar approach with cell-embedded NPs
on a PVDF membrane.

2.1.4. Anti-parasite applications

Biosynthesized NPs are effective against various disease-caus-
ing insects or parasites. Santhoshkumar et al. [46] compared larvi-
cidal activity of AgNPs biosynthesized using different lotus leaf
(Nelumbo nucifera) extracts. In the study, antilarval activity was
measured for the fourth instar larvae of Anopheles subpictus and
Culex quinquefasciatus, both well-known vectors for malaria and
lymphatic filariasis. A similar study by Gnanadesigan et al. [47]
evaluated AgNPs biosynthesized with mangrove (Rhizophora mu-
cronata) leaf extract against Aedes aegypti and C. quinquefasci-
tus larva.

In another study, the larvicidal properties of AgNPs biosyn-
thesized with henna (Lawsonia inermis) leaf extract was determined
for the human head louse Pediculus humanus capitis and the sheep
body louse Bovicola ovis [48]. The study determined lousicidal
activity using both a direct contact method (P. humanus) and
an impregnated filter paper method (B. ovis). Finally, the acaridical
activity of an evergreen tree (Manilkara zapota) aqueous extract
and AgNPs synthesized by means of this extract were determined
against the cattle tick (Rhipicephalus microplus) [49].
2.2. Drug delivery and cancer treatment

Biosynthesized NPs can interact with and alter the function of certain mammalian tissues. For example, metallic NPs can interfere with the antioxidant defense mechanism, leading to accumulation of reactive oxygen species, destruction of mitochondria and cell apoptosis [50]. This effect can be targeted, because the in vivo effects of metal NP exposure strongly depend on the capping agent: the same AgNPs with different capping agents have been reported to be both cytotoxic [50] and non-cytotoxic [51]. The use of stable noble metal NPs as carriers may minimize the side effect of conventional chemotherapeutic agents by the selective delivery of anticancer agents to malignant cells without affecting the normal cells. For instance, gold can be readily modified due to its ability to bind strongly to thiols (–SH) and amines (–NH₂). Thus, covering the NP surface with biomolecules acting as chemotherapeutic and targeting agents is relatively straightforward [7]. With the selection of different capping agents, the cytotoxic activity of metallic NPs can be used for both drug delivery and cancer cell targeting (Table 3). Whether the targeted delivery of NPs is therapeutic or the NPs act as carriers for some other agent, biosynthesized NPs are becoming increasingly important in nanomedicine.

2.2.1. Biocompatibility

Whenever NPs are used for in vivo applications, biocompatibility with normal tissue is an important consideration. Moulton et al. [51] described the biosynthesis of AgNPs using tea leaf extract as a reducing and capping agent. These authors exposed these NPs to human keratinocytes and evaluated mitochondrial function to assess cell viability and membrane integrity. The results showed that AgNPs biosynthesized with tea leaf extract were nontoxic, suggesting this method may be promising for future in vivo applications. The biocompatibility of tea-extract-biosynthesized AgNPs may be attributed to the antioxidant effect of polyphenol and flavonoid surfactants.

Kumar et al. [20] described the blood compatibility of AuNPs synthesized with ginger (Zingiber officinale) extract. Upon contact with human blood, these AuNPs were shown to be non-platelet activating, and did not bring about the aggregation of other blood cells. Moreover, they were highly stable under normal physiological conditions compared to chemically synthesized NPs (citrate capped), which tended to aggregate blood cells.

2.2.2. Anticancer NPs

Cytotoxicology studies against various cancer cell lines have been described for biosynthesized NPs. Amarnath et al. [52] published experiments using AuNPs synthesized using phytochemicals present in grapes (Vitis vinifera). These AuNPs exhibited remarkable affinity towards HBL-100 (human breast cancer cells), and AuNP exposure resulted in HBL-100 apoptosis. Mishra et al. [53] described AuNP biosynthesis by the supernatant, live cell filtrate and biomass of the fungus Penicillium brevicompactum. In this work, the cytotoxic properties of biosynthesized NPs were analyzed using mouse myoblast cancer C2C12 cells. In a different study, guava and clove extracts were used to reduce Au and Ag salts [54] (see Fig. 3). Although biosynthesized AuNPs exhibited an anticancer effect on HEK-293, HeLa and HT-29 cancer cell lines, AgNPs did not show any anticancer activity in this study.

In contrast, other reports have demonstrated anticancer effects from AgNPs. Biosynthesis of anti-tumor AgNPs using Piper longum leaf as a reducing and capping agent was reported by Jacob et al. [55]. These NPs showed an excellent cytotoxic effect on Hep-2 cancer cell lines. Similarly, callus extract of Citrullus colocynthis was used to form AgNPs which were effective against Hep-2 cells [56]. Similar efficiency against HeLa cells and a lymphoma mouse model was observed for AgNPs biosynthesized using Melia azedarach, a species of deciduous tree from the mahogany family [57]. A sophisticated evaluation of AgNPs effect on cancerous cell lines was given by Jeyaraj et al. [58]. The cytotoxicity effect of biogenic silver nanoparticles against human breast cancer cells in vitro was studied by means of the MTT, acridine orange/ethidium bromide and Hoechst methods and the COMET assay. Extracellular synthesis of copper NPs was performed using stem latex of Euphoria nivulia [50]. This study concluded that copper NPs are toxic to A549 (human lung carcinoma) cells in a dose-dependent manner.

Recently, the anti-metastatic activity of biologically synthesized AuNPs was reported for the human fibrosarcoma cell line HT-1080 [59]. Although the biosynthesized AuNPs had no toxic effects on HT-1080 cells in terms of cell viability, the study showed that AuNPs inhibit cell migration of HT-1080 cells by interfering with the actin polymerization pathway.

2.2.3. NP drug carriers

Magnetosomes are naturally occurring metallic nanoparticles found in some species of magnetotactic bacteria. These chains are membranous prokaryotic structures, and are composed of approximately 20 magnetite crystals surrounded by a lipid bilayer. Sun et al. [60] demonstrated the use of bacterial magnetosomes as carriers for drug delivery applications. In the study, isolated and cleaned bacterial magnetosomes from the magnetotactic bacterium Magnetospirillum gyrophilus were loaded with the chemotherapy drug doxorubicin (DOX), attached via amino groups. The anticancer effect of DOX-loaded bacterial magnetosomes was demonstrated using a cytotoxicity assay with HL60 and EMT-6 carcinoma cells. Inhibition of cancer cell proliferation and suppression

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Drug delivery and cancer treatment applications.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP</td>
<td>Organism used</td>
</tr>
<tr>
<td>Ag</td>
<td>Camellia sinensis</td>
</tr>
<tr>
<td>Au</td>
<td>Zingiber officinale</td>
</tr>
<tr>
<td>Au</td>
<td>Vitis vinifera</td>
</tr>
<tr>
<td>Au</td>
<td>Penicillium brevicompactum</td>
</tr>
<tr>
<td>Au</td>
<td>Guava</td>
</tr>
<tr>
<td>Ag</td>
<td>Piper longum</td>
</tr>
<tr>
<td>Ag</td>
<td>Citrullus colocynthis (L.) Schrad</td>
</tr>
<tr>
<td>Ag</td>
<td>Melia azedarach</td>
</tr>
<tr>
<td>Cu</td>
<td>Euphoria nivulia</td>
</tr>
<tr>
<td>Ag</td>
<td>Sessbania grandiflora</td>
</tr>
<tr>
<td>Au</td>
<td>Dysosma pleiantha</td>
</tr>
<tr>
<td>Fe₂O₄</td>
<td>Magnetospirillum gyrophilus</td>
</tr>
<tr>
<td>Fe₂O₄</td>
<td>Magnetospirillum gyrophilus</td>
</tr>
<tr>
<td>Au</td>
<td>Porphyra vietnamensis</td>
</tr>
</tbody>
</table>
of mRNA levels of the important oncogene c-myc suggest DOX-loaded magnetosomes are promising for cancer therapy. Bacterial magnetosomes are able to carry a large amount of drug, are easy to prepare, and are more stable and more uniform compared with artificial magnetic NPs. They are also biocompatible. Li et al. [61] demonstrated the biocompatibility of purified and sterilized magnetosomes by using mouse fibroblasts in vitro. Another drug delivery study employed porphyran from the marine alga Porphyra vietnamensis as a reducing and capping agent for the biosynthesis of AuNPs [62]. These NPs were used as a carrier for DOX. The results demonstrated that AuNP-bound DOX showed higher cytotoxicity to LN-229 (human glioma) cells than unbound DOX. Spectroscopy revealed that hydrogen bonds are involved in the DOX–AuNP conjugation.

2.3. Medical diagnostics and sensors

Biosynthesized NPs are also making important contributions to medicine in the sensing and diagnostics areas (Table 4). These materials have been successfully incorporated into chemical sensors that can detect medically relevant compounds, including peroxides and glucose. For example, eggshell membrane was used as a reducing agent and scaffold in the biosynthesis of AuNPs [63]. The sensor showed a linear response to different glucose concentrations, with a detection limit of 17 μM. The same material was used to measure the glucose content of human blood serum samples, and analysis showed agreement with a standard routine medical spectrophotometric test [64].

Hydrogen peroxide has been acknowledged as a diagnostic marker of oxidative stress, playing an important role in asthma or chronic obstructive pulmonary disease (COPD). Wang et al. [65] constructed a hydrogen peroxide sensor based on biosynthesized selenium NPs (SeNPs) prepared using the bacterial strain Bacillus subtilis. The resulting SeNPs were spherical and could be converted into one-dimensional trigonal wires (proteins excreted from B. subtilis cells act as a template). A clean glassy carbon electrode (GCE) was amended with a drop of a colloidal suspension of SeNPs and a drop of the enzyme horseradish peroxidase. Cyclic voltammetry studies confirmed that SeNPs enhanced the detection of H₂O₂ resulting in an 80 nM detection limit. This material can therefore be used, for instance, for the detection of hydrogen peroxide in the exhaled breath condensate of patients affected with COPD (see Table 5).

Other authors have also used a GCE modified with biosynthesized nanoparticles for electrochemical sensing. Zheng et al. [66] synthesized Au–Ag alloy NPs using yeast cells and demonstrated an enhanced electrochemical response for vanillin (from vanilla beans and vanilla tea). With a linear range of 0.2–50 μM and a detection limit of 40 nM, this sensor can be a simple and cheap alternative to analytical instruments involving gas or liquid chromatography, and can be used to check the quality of food products.

Another possible medical application of biogenic AuNPs was proposed and tested by MubarakAli et al. [67], who suggested that the conjugation of DNA with biosynthesized nanoparticles can be used for diagnosis of genetic disease.

2.4. Medical imaging applications

The optical properties of metallic nanocrystals have been of interest for centuries. The incorporation of biosynthesis methods has made possible the preparation of metal NPs with a range of sizes, shapes and dielectric properties. Optical properties associated with metallic NPs include a high- or low-refractive index, high transparency, novel photoluminescence properties, photonic crystals and plasmon resonance [68]. Nanophotonics studies in the field, where the light interacts with particles smaller than its wavelength, lead to novel phenomena, such as localized surface plasmon resonance and a size-dependent semiconductor band gap [69].

The biosynthesis AuNPs by silica-encapsulated Klebsormidium flaccidum microalgae leads to the formation of a “living” biohybrid material [70]. Researchers have used Raman spectroscopy for in situ imaging of entrapped cells and have investigated the influence of the AuNPs on the photosynthetic system of the algae. The coupling of Raman imaging and sol–gel encapsulation might allow...

---

**Table 4**

<table>
<thead>
<tr>
<th>NP</th>
<th>Organism used</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au</td>
<td>Eggshell membrane</td>
<td>Glucose sensor</td>
<td>Zheng et al. [63]</td>
</tr>
<tr>
<td>Au</td>
<td>Eggshell membrane</td>
<td>Glucose sensor in human blood serum</td>
<td>Zheng et al. [64]</td>
</tr>
<tr>
<td>Se</td>
<td>Bacillus subtilis</td>
<td>H₂O₂ sensor</td>
<td>Wang et al. [65]</td>
</tr>
<tr>
<td>Au–Ag</td>
<td>Saccharomyces cerevisiae</td>
<td>Vanillin sensor</td>
<td>Zheng et al. [66]</td>
</tr>
<tr>
<td>Au</td>
<td>Different microalgae</td>
<td>DNA conjugation</td>
<td>MubarakAli et al. [67]</td>
</tr>
</tbody>
</table>

**Table 5**

<table>
<thead>
<tr>
<th>NP</th>
<th>Organism used</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au</td>
<td>Klebsormidium flaccidum</td>
<td>Photosynthesis-based environmental biosensor</td>
<td>Sicard et al. [70]</td>
</tr>
<tr>
<td>Ag</td>
<td>Trichoderma viride</td>
<td>NP photoluminescence</td>
<td>Fayaz et al. [71]</td>
</tr>
<tr>
<td>Ag</td>
<td>Parthenium hysterophorus</td>
<td>NP photoluminescence</td>
<td>Sarkar et al. [72]</td>
</tr>
<tr>
<td>Ag</td>
<td>Coriandrum sativum</td>
<td>Optical limiting</td>
<td>Sathyavathi et al. [74]</td>
</tr>
<tr>
<td>Te</td>
<td>Bacillus selenitireducens</td>
<td>Optical limiting</td>
<td>Liao et al. [75]</td>
</tr>
<tr>
<td>Au</td>
<td>Maduca longifolia</td>
<td>Efficiency in absorbing infrared radiation</td>
<td>Fayaz et al. [76]</td>
</tr>
<tr>
<td>Au</td>
<td>Cymbopogon citratus</td>
<td>Infrared absorbing optical coatings</td>
<td>Shankar et al. [77]</td>
</tr>
<tr>
<td>CdTe</td>
<td>Saccharomyces cerevisiae</td>
<td>Bialabeling and bioimaging</td>
<td>Bao et al. [78]</td>
</tr>
<tr>
<td>CdTe</td>
<td>Escherichia coli</td>
<td>Bialabeling and bioimaging</td>
<td>Bao et al. [79]</td>
</tr>
<tr>
<td>CdS</td>
<td>Brevibacterium casei</td>
<td>Fluorescence emission</td>
<td>Pandian et al. [80]</td>
</tr>
<tr>
<td>Au</td>
<td>Pseudomonas aeruginosa</td>
<td>Diabetes treatment - tyrosine phosphatase type PTP 1B inhibition</td>
<td>Basha et al. [81]</td>
</tr>
<tr>
<td>Au, Ag</td>
<td>Brevibacterium casei</td>
<td>Anticoagulation activity</td>
<td>Kalishwaralal et al. [82]</td>
</tr>
<tr>
<td>Au, Ag</td>
<td>Solanum nigrum</td>
<td>Free radical scavenging effect</td>
<td>Muthuvel et al. [83]</td>
</tr>
<tr>
<td>Ag</td>
<td>Trichoderma reesi</td>
<td>HIV treatment</td>
<td>Vahals and Dorchel [86]</td>
</tr>
</tbody>
</table>
for the development of photosynthesis-based biosensors (see Fig. 4).

Blue orange light emission from biosynthesized AgNPs was reported by Fayaz et al. [71]. Fungal-mediated AgNPs were prepared using a Trichoderma viride filtrate. Photoluminescence measurements after laser excitation showed an emission in the range of 320–520 nm, making such AgNPs promising for future labeling and imaging applications. A similar study was introduced by Sarkar et al. [72] using Parthenium hysterophorus leaf extract for AgNP biosynthesis.

An important problem of modern laser medicine is the extensive exposure of vulnerable tissues outside of the operational field. This problem can be solved by placing dyes that are capable of reversible darkening of the radiation onto the surface of irradiated tissues. This phenomenon is called optical radiation limiting [73]. AgNPs biosynthesized using Coriandrum sativum leaf extract were found to exhibit strong reverse saturable absorption of laser irradiation [74]. The authors measured nonlinear refraction and absorption coefficients using a Z-scan technique with laser pulses. These results suggest AgNPs are capable of nonlinear optics. Similarly, microbiologically formed nanorods prepared using Bacillus selenitireducens and composed of elemental tellurium Te(0) have been described elsewhere [75]. These TeNPs form unusual nanocomposites when combined with an organic chemical host and exhibit excellent broadband optical limiting at 532 and 1064 nm. In this regard, they significantly exceeded the best commercial optical limiters currently available. Their relative ease of biomanufacturing combined with their unique properties makes these Te-based nanocomposites particularly attractive for their immediate employment as coatings in medicine or industry (e.g. to protect eyes from damage caused by exposure to focused beams and lasers).

Fayaz et al. [76] biosynthesized AuNPs using Maduca longifolia extract and showed strong near-infrared absorption. Although some applications are not directly medical in nature, such as energy-saving window coatings, a similar technology can also be applied for cancer hyperthermia coatings [77].

The same research team published two additional studies describing cadmium telluride quantum dots (CdTeQDs) fabricated via extracellular synthesis using Saccharomyces cerevisiae [78] and Escherichia coli [79]. The authors evaluated the NP’s size-dependent optical properties. CdTeQD were relatively small, capped with protein and highly soluble in water. The optical properties were studied in both cases using UV–visible spectrophotometry and spectrofluorimetry with photoluminescence emission from 488 to 551 nm. CdTeQDs functionalized with folic acid were used for in vitro imaging of cancer cells, and were also found to be biocompatible in a cytotoxicity assay [79]. This study clearly shows the capacity of biosynthesized QDs to be used in bioimaging and biolabeling applications.

Finally, a study using Brevibacterium casei for the biosynthesis of CdSNPs [80] showed that the NPs exhibited fluorescence emission even after immobilization within a polyhydroxybutyrate matrix.

2.5. Other medical applications

Several studies have shown biosynthesized NPs to have potential applications for the treatment of other diseases, including diabetes and bleeding disorders. Biosynthesized AuNPs have been shown to inhibit the enzyme tyrosine phosphatase type PTP 1B in vitro [81]. Since PTP 1B has been found to dephosphorylate insulin receptors, reducing its activity can enhance the activity of insulin. AuNPs prepared by means of guavaic acid from a leaf extract of Psidium guajava showed a significant inhibitory effect, with an IC50 of 1.14 µg ml−1. Another study showed that biosynthesized Au- and AgNPs produced and stabilized using Brevibacterium casei exhibited anticogulation activity [82]. Exposure of the particles to blood plasma for 24 h did not show any significant reduction in the anticogulation activity.

Free radical scavenging activity was demonstrated by Muthuvel et al. [83]. Au-NPs biosynthesized by means of Solanum nigrum were tested for the scavenging effect on 1’-diphenyl-2-picrylhydrazyl radical and hydroxyl radicals according to the method of Blois and the deoxyribose method, respectively. The synthetic antioxidant butyl hydroxyl toluene was used as a positive control, and biogenic nanoparticles showed up to 60% inhibition efficiency for both radical types [84,85]. Interestingly, Trichoderma reesei-produced AgNPs prevent HIV-1 particles from binding to host cells [86].

Finally, Gopinath et al. [87] described enhanced mitotic cell division and pollen germination activity caused by AuNPs synthesized using Terminalia arjuna leaf extract. These studies demonstrate the wide range of biomedically relevant effects possible by exposure to biosynthesized NPs. Additional research will offer new discoveries and insights into the use of biosynthesized NPs as promising treatments for human diseases.

3. Environmental remediation applications

3.1. Metal biosorption, bioremediation and biorecovery

Oxidation–reduction processes are universally used for cellular metabolism; therefore biomolecules with the ability to reduce or oxidize other chemical compounds are abundant in any living cell. Many microorganisms are able to bind and concentrate dissolved metals as an active mechanism to detoxify their environment. Other biological processes, and even dead biomass, simply couple metal reduction with oxidation of an electron donor. Since the product of cellular metal reduction is usually nanostructured, bioremediation of metals in solution and biosynthesis of metallic NPs are in fact closely related processes [88]. In this section, we review studies that describe biological metal removal and NP formation via biologically mediated processes (see Table 6).

The biomass of algae, fungi, bacteria and yeasts, along with some biopolymers and biowaste materials, are known to bind and concentrate precious metals [89]. This so-called biosorption process can represent a cost-effective alternative to the common chemical methods for recovery of various dissolved metals from aqueous solution. The mechanisms involved in binding and concentrating metals by microbes have been extensively studied in natural environments [89].

Chakraborty et al. [90] demonstrated the ability of cyanobacteria and algae to bind and concentrate Au and form AuNPs. The NP formation process is specific to a particular genus, and they described NP formation using the cyanobacterial strains Lyngbya majuscula and Spirulina subsalsa, and the freshwater green alga Rhi- zoclonium hieroglyphicum. AuNPs formation during biosorption by the seaweed Fucus vesiculosus was reported by Mata et al. [91]. These authors described the pH dependence on NP formation and discussed the stages of the bioreduction process.

Dead biomass of the macrofungus Pleurotus platypus was used for biosorption of Ag in a study by Das et al. [92]. The fungal biomass exhibited the highest Ag uptake of 46.7 mg g−1 at pH 6.0 in the presence of 200 mg l−1 Ag(I) at 20 °C. These authors also offered detailed kinetic and thermodynamic analysis of the biosorption process. Zhang et al. [93] demonstrated the use of the G− facultative anaerobic bacteria Aeromonas SH10 to treat Ag in wastewater. In this study, biomass was shown to accumulate Ag+ and [Ag(NH3)2]+ ions as AgNPs. The maximum uptake of [Ag(NH3)2]+ was 0.23 g of Ag per g of cell dry weight.

An example of platinum recovery by polyethyleneimine (PEI)-modified biomass was described by Won et al. [94]. In this study, PEI was attached to the surface of E. coli biomass and the resulting
Metal biosorption, bioremediation and biorecovery. Most environmental remediation applications of NPs through biosorption of waste streams containing precious metals are a major public health problem in Southeast Asia. Selvakumar et al. [96] described a promising solution to this problem through the development of an AgNP sorbent for As(V) removal. In the study, the reducing capabilities of a novel yeast strain of Saccharomyces cerevisiae was used to sequester the metal. Finally, Sinha et al. [97] showed that a heavy-metal-resistant strain of Bacillus sp. formed MnO2 NPs simultaneously with the remediation of manganese.

Hexavalent chromium compounds are dangerous toxins, while Cr(III) ions and compounds are relatively non-toxic. Bare living cells of Shewanella algae, Pseudomonas putida and Desulfovibrio vulgaris have been shown to reduce Cr(VI) to Cr(III) [98]. Many other studies rely on biosynthesized PdNPs to catalytically reduce chromium. (See Section 3.4 on catalytic Cr(VI) reduction.)

3.2 Coupling biosorption with catalytic contaminant degradation

Due to their large surface area per weight, metallic NPs are widely used for catalysis, and in particular for heterogeneous catalysis. Metallic NPs offer selectivity, high activity and stability. The major drawback of metallic NP catalysis is cost: the constituent metals, including gold, platinum and palladium, are very expensive, and their fabrication into nanoparticles is energy intensive. Coupled biosynthetic approaches can generate catalytically active NPs through biosorption of waste streams containing precious metals. The coupling of biological metal removal with catalyst formation has been described in recent reviews by De Corte et al. [99] and Hennebel et al. [100]. These biosynthesized metallic NPs can then be used for catalytic dehalogenation, organic oxidation or metal reduction. Most environmental remediation applications of biosynthesized catalyst employ palladium (Pd) NPs (see Table 7).

The following sections describe catalytic treatment of organic compounds and metals using biosynthesized metallic NPs.

3.3 Catalytic degradation of organic pollutants

3.3.1 Catalytic dehalogenation

Palladium-based catalysts are able to dehalogenate aromatic compounds. This reaction is very important for organic synthesis in research and industry, and also for contaminant remediation. Halogenated organic compounds dominate the priority list of persistent, bioaccumulative and toxic (PBT) pollutants as designated by the US Environmental Protection Agency. By definition, PBT compounds do not break down naturally in the environment, so enhanced processes to dehalogenate these compounds are of great interest. Halogenated organic PBTs include several banned pesticides, including chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin and hexachlorobenzene, as well as polychlorinated biphenyls (PCBs), compounds formerly used in electronic devices and as coolants, lubricants and plasticizers.

Baxter-Plant et al. [101,100] described the dehalogenation of chlorophenol (CP) and selected PCB congener, including 4-chlorobiphenyl, 2,4,6-trichlorobiphenyl, 2,3,4,5-tetrachlorobiphenyl and 2,2',4,4',6,6'-hexachlorobiphenyl, via palladized cells of G. Desulfovibrio bacteria. Biosynthesized PdNPs were obtained by the reduction of the palladium precursor in the presence of Desulfovibrio desulfuricans, Desulfovibrio vulgaris and Desulfovibrio sp. Oz-7. As described by Bunge et al. [103], reduction in the absence of cells does not lead to the formation of PdNPs; rather, this process requires an electron donor, such as formate (see Tables 8 and 9).

Shewanella oneidensis is another strain of bacteria that has been used to biosynthesize PdNPs for dehalogenation of PCB and other chlorinated organic compounds. De Windt et al. [104] described PdNPs precipitated on the cell wall and inside the periplasmic space of S. oneidensis and demonstrated the effective dehalogenation of PCBs in water and sediment. Their study described in detail bioreduction with different electron donors and varying fractions of PdNPs associated with the biomass. They showed that the

### Table 6
Metal biosorption, bioremediation and biorecovery.

<table>
<thead>
<tr>
<th>NP</th>
<th>Organism used</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au</td>
<td>Lyngbya majuscula, Spirulina subsalsa, Rhizoclonium hieroglyphicum</td>
<td>Bioaccumulation</td>
<td>Chakraborty et al. [90]</td>
</tr>
<tr>
<td>Au</td>
<td>Fucus vesiculosus</td>
<td>Bioaccumulation, biorecovery</td>
<td>Mata et al. [91]</td>
</tr>
<tr>
<td>Ag</td>
<td>Pleurotus platypus</td>
<td>Biosorption</td>
<td>Das et al. [92]</td>
</tr>
<tr>
<td>Ag</td>
<td>Aeromonas sp. SH10</td>
<td>Silver-containing waste water treatment</td>
<td>Zhang et al. [93]</td>
</tr>
<tr>
<td>Pt</td>
<td>Escherichia coli</td>
<td>Biosorption, biorecovery by incineration</td>
<td>Won et al. [94]</td>
</tr>
<tr>
<td>Au</td>
<td>Sargassum sp.</td>
<td>Biosorption, biorecovery by incineration</td>
<td>Sathishkumar et al. [95]</td>
</tr>
<tr>
<td>Ag</td>
<td>Saccharomyces cerevisiae</td>
<td>As(V) bioaccumulation and removal</td>
<td>Selvakumar et al. [96]</td>
</tr>
<tr>
<td>MnO2</td>
<td>Bacillus sp. (MTCC10650)</td>
<td>Mn bioremediation</td>
<td>Sinha et al. [97]</td>
</tr>
</tbody>
</table>

### Table 7
Catalytic dehalogenation.

<table>
<thead>
<tr>
<th>NP</th>
<th>Organism used</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd</td>
<td>Desulfovibrio vulgaris, D. desulfuricans</td>
<td>Dechlorination of CP and PCBs</td>
<td>Baxter-Plant et al. [101]</td>
</tr>
<tr>
<td>Pd</td>
<td>D. desulfuricans</td>
<td>Dechlorination of CP and PCBs</td>
<td>Baxter-Plant et al. [102]</td>
</tr>
<tr>
<td>Pd</td>
<td>Shewanella oneidensis</td>
<td>Dechlorination of chlorophenol and PCBs</td>
<td>De Windt et al. [104]</td>
</tr>
<tr>
<td>Pd</td>
<td>S. oneidensis</td>
<td>Dechlorination of perchlorate and PCBs</td>
<td>De Windt et al. [105]</td>
</tr>
<tr>
<td>Pd</td>
<td>D. desulfuricans, Rhodobacter sphaeroides</td>
<td>Dechlorination of (PCBs) and penta-CP</td>
<td>Macaskie et al. [106]</td>
</tr>
<tr>
<td>Pd</td>
<td>S. oneidensis</td>
<td>Dechlorination of lindane</td>
<td>Metrens et al. [108]</td>
</tr>
<tr>
<td>Pd</td>
<td>S. oneidensis</td>
<td>Dechlorination of TCE, membrane reactor</td>
<td>Hennebel et al. [109]</td>
</tr>
<tr>
<td>Pd</td>
<td>S. oneidensis</td>
<td>Dechlorination of TCE, fixed bed reactor</td>
<td>Hennebel et al. [110]</td>
</tr>
<tr>
<td>Fe/Pd</td>
<td>Camellia sinensis</td>
<td>Dechlorination of TCE</td>
<td>Smuleac et al. [111]</td>
</tr>
<tr>
<td>Pd</td>
<td>D. desulfuricans</td>
<td>Dehalogenation PCBs and polybrominated diphenyl ether</td>
<td>Harraz et al. [112]</td>
</tr>
<tr>
<td>Pd</td>
<td>D. desulfuricans</td>
<td>Dehalogenation of flame retardant materials</td>
<td>Deplanchy et al. [113]</td>
</tr>
<tr>
<td>Pd</td>
<td>S. oneidensis</td>
<td>Deiodination of diatrizoate</td>
<td>Hennebel et al. [114]</td>
</tr>
<tr>
<td>Pd</td>
<td>S. oneidensis</td>
<td>Dehalogenation of diatrizoate in microbial electrolysis cells</td>
<td>De Gusseme et al. [115]</td>
</tr>
</tbody>
</table>
catalytic activity of biosynthesized PdNPs was 100× higher than commercial palladium catalyst powder (see Fig. 5).

In a follow-on report, De Windt et al. [105] performed a detailed assessment of the bioreduction process and its conditions. In particular, they investigated the factors influencing the particle size and catalytic properties of PdNPs formed from a soluble palladium precursor by *S. oneidensis*. Interestingly, the relatively large palladium crystals formed on non-viable biomass exhibited high catalytic efficiency towards hydrophobic molecules such as PCBs, while the smaller PdNPs created on living bacterial cells showed high catalytic activity towards perchlorate.

Macaskie et al. [106] extensively studied the catalytic dechlorination of 2-chlorophenol, pentachlorophenol and various PCB congeners by palladized cells of *Desulfovibrio* and *Escherichia coli*. Redwood et al. [107] compared the efficiency of palladized *D. desulfuricans* with *Rhodobacter sphaeroides* for catalytic dechlorination of PCBs and pentachlorophenol. Mertens et al. [108] demonstrated the efficiency of palladized *S. oneidensis* for the catalytic decomposition of the pesticide lindane. These authors also incorporated the catalytic material into a membrane bioreactor for the continuous treatment of lindane-contaminated water.

Other studies have demonstrated the use of palladized cells for dechlorination of the common groundwater contaminant trichloroethylene (TCE). Hennebel et al. described two reactor geometries for catalytic dechlorination of TCE by biosynthesized PdNPs: a pilot-scale membrane reactor [109] and a fixed bed reactor with PdNPs embedded in a polyurethane foam. These authors described the cumulative removal of 98% of TCE after 22 h, with ethane as the predominant product, and a maximum removal rate of 1.059 mg of TCE per g of biosynthesized PdNPs per day.

Other authors used Fe and Fe/Pd NPs biosynthesized with tea extract (*Camellia sinensis*) then immobilized in polymer membranes for the catalytic degradation of TCE [111]. These authors found that the reaction rate constant was higher for the bimetallic Fe/PdNPs. The biosynthesized NPs exhibited a lower reaction rate than chemically synthesized FeNPs, though the reactivity of chemically synthesized FeNPs diminished to less than 20% within four cycles. By contrast, the reactivity of the green tea extract-synthesized NPs was preserved throughout 3 months of repeated use under conditions simulating a real water treatment system, possibly due to a number of polyphenols that can act as capping agents.

Biosynthesized PdNPs can also catalytically dehalogenate brominated organic compounds. Harrad et al. [112] demonstrated that palladized *D. desulfuricans* is able to catalytically dehalogenate polybrominated diphenyl ether (PBDE). Hydrophobic brominated compounds such as PBDE are used as flame retardants in building materials, textiles and plastics. Deplanche et al. [113] also used palladized cells of *D. desulfuricans* for the catalytic dehalogenation of flame retardants, including PBDE and tris-(chloroisopropyl)-phosphate (TCPF), and compared their performance with chemically reduced palladium powder. The chemically reduced Pd was more effective in debrominating PBDE, but the biosynthesized PdNPs were five times more effective in TCPF dechlorination.

Two studies have demonstrated that biosynthesized NPs can remediate diatrizoic acid (or diatrizoate), a radiointact agent. Hennebel et al. [114] demonstrated that PdNPs encapsulated on PVDF membranes effectively deiodinate diatrizoate in water. Similarly, De Gusseme et al. [115] showed that biosynthesized PdNPs contributed to higher dehalogenation rates of TCE and higher deiodination rates of diatrizoate when applied on the surface of a graphite cathode. The presence of PdNPs on the cathode enhanced the rate of diatrizoate removal by both direct electrochemical reduction and catalytic reduction using the hydrogen produced at the cathode of the microbial electrolysis cell.

### Table 8
Catalytic 4-nitrophenol degradation and catalytic treatment of other aqueous organic compounds.

<table>
<thead>
<tr>
<th>NP</th>
<th>Organism used</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au</td>
<td><em>Sebiuma drummondi</em></td>
<td>Reduction of 4-nitrophenol</td>
<td>Sharma et al. [116]</td>
</tr>
<tr>
<td>Au</td>
<td><em>Caucmen platycladi</em></td>
<td>Reduction of 4-nitrophenol</td>
<td>Huang et al. [117]</td>
</tr>
<tr>
<td>Ag</td>
<td><em>Sepia esculenta</em></td>
<td>Reduction of 4-nitrophenol</td>
<td>Jia et al. [120]</td>
</tr>
<tr>
<td>Ag</td>
<td><em>Brennia rhamnoides</em></td>
<td>Reduction of 4-nitrophenol</td>
<td>Gangula et al. [118]</td>
</tr>
<tr>
<td>Au</td>
<td><em>B. rhamnoides</em></td>
<td>Reduction of 4-nitrophenol</td>
<td>Gangula et al. [118]</td>
</tr>
<tr>
<td>Pd/Au</td>
<td><em>Cupridiavis neator</em></td>
<td>Reduction of 4-nitrophenol</td>
<td>Hosseinkhani et al. [119]</td>
</tr>
<tr>
<td>Ag</td>
<td><em>Rhizopus oryzae</em></td>
<td>Reduction of 4-nitrophenol</td>
<td>Das et al. [121]</td>
</tr>
<tr>
<td>Fe</td>
<td><em>C. sinensis</em></td>
<td>Degradation of aqueous cationic and anionic dyes</td>
<td>Shahwan et al. [122]</td>
</tr>
<tr>
<td>Au</td>
<td><em>C. sinensis</em></td>
<td>Reduction of methylene blue</td>
<td>Gupta et al. [123]</td>
</tr>
<tr>
<td>ZnS</td>
<td><em>Streptococcus thermophilus,</em></td>
<td>Photocatalytic degradation of fuchsine</td>
<td>Zhou et al. [124]</td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus bulgaricus,</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus acidophillus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ag</td>
<td><em>Coccinia grandis</em></td>
<td>Photocatalytic degradation of Coomassie Brilliant Blue G-250</td>
<td>Arunachalam et al. [125]</td>
</tr>
<tr>
<td>Pd</td>
<td><em>Pseudomonas putida</em></td>
<td>Removal of micropollutants (e.g. ibuprofen, diclofenac, mecoprop)</td>
<td>Forrez et al. [126]</td>
</tr>
</tbody>
</table>

### Table 9
Catalytic Cr(VI) reduction.

<table>
<thead>
<tr>
<th>NP</th>
<th>Organism used</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd</td>
<td><em>E. coli</em> mutant strains</td>
<td>Reduction of Cr(VI) to Cr(III)</td>
<td>Deplanche et al. [127]</td>
</tr>
<tr>
<td>Pd</td>
<td><em>D. desulfuricans</em></td>
<td>Reduction of Cr(VI) to Cr(III)</td>
<td>Mabbett and Macaskie [128]</td>
</tr>
<tr>
<td>Pd</td>
<td><em>Desulfovibrio vulgaris, Desulfovibrio desulfuricans</em></td>
<td>Reduction of Cr(VI) to Cr(III)</td>
<td>Humphries et al. [129]</td>
</tr>
<tr>
<td>Pd</td>
<td><em>D. desulfuricans</em></td>
<td>Reduction of Cr(VI) to Cr(III)</td>
<td>Mabbett et al. [130]</td>
</tr>
<tr>
<td>Pd/MM</td>
<td><em>D. desulfuricans, Escherichia coli</em></td>
<td>Reduction of Cr(VI) to Cr(III)</td>
<td>Mabbett et al. [131]</td>
</tr>
<tr>
<td>Pd</td>
<td><em>Serratia sp. (NCIMB)</em></td>
<td>Reduction of Cr(VI) to Cr(III)</td>
<td>Beauregard et al. [132]</td>
</tr>
<tr>
<td>Pd</td>
<td><em>Clostridium pasteuriam</em></td>
<td>Reduction of Cr(VI) to Cr(III) in aquifer environment</td>
<td>Chidambaram et al. [133]</td>
</tr>
<tr>
<td>Pd</td>
<td><em>E. coli, Desulfovibrio spp.</em></td>
<td>Reduction of Cr(VI) to Cr(III)</td>
<td>Macaskie et al. [148]</td>
</tr>
</tbody>
</table>

* Mixed precious metals.
has resulted in a legacy of toxic nitro-aromatic contamination in soil and water. Technologies for catalytic removal of compounds such as 4-nitrophenol (also known as p-nitrophenol or PNP) can help restore impacted environments. Furthermore, the degradation of PNP can be measured easily using widely available and inexpensive optical methods such as UV–visible spectrophotometry. The catalytic degradation of PNP has thus become a common test system to evaluate the catalytic reduction capabilities of biosynthesized NPs.

The first report of PNP reduction employing biosynthesized NPs was published by Sharma et al. [116]. Seedlings of the plant Sesbania drummondii were grown in a hydrogen tetrachloroaurate solution and stable AuNPs were subsequently formed in plant tissues. This AuNP-rich plant tissue was used as an efficient catalyst for PNP reduction in the presence of sodium borohydride.

Subsequently, others have used plant materials to biosynthesize noble metal NPs for catalytic PNP reduction. Huang et al. [117] carried out an extensive study using 21 species of traditional Chinese herbs and medicinal plants. These plants were divided into four groups, and their leaves, flowers, fruits were used for NP biosynthesis by 30 min incubation in aqueous HAuCl₄. Due to their monodispersity and small size, AuNPs prepared using Cacumen platycladi leaves were used to demonstrate catalytic PNP reduction. Gangula et al. [118] used the stem extract of a medicinal plant Breynia rhamnooides for fast (~7 min) biosynthesis of AgNPs and AuNPs. These authors found the conversion efficiency to be dependent on the NP size, which is possibly controlled by the stem extract concentration used for biosynthesis.

Bacteria have also been used for biosynthesis of bimetallic Pd/AuNPs for PNP reduction. Hosseinikhani et al. [119] formed biosupported Pd(0) and Au(0) NPs on the surface of Cupriavidus necator cells. Bimetallic particles were subsequently formed following the addition of either Au(III) or Pd(II) salt to the biosupported NPs. Although the bimetallic NPs did not have a core–shell structure, they exhibited superior catalytic efficiency in PNP conversion compared with monometallic NPs.

Catalytic PNP reduction has also been done using animal-derived materials for the biosynthesis of NPs. Jia et al. [120] described AgNP biosynthesis and immobilization using a cuttlebone-derived organic matrix from the cuttlefish Sepia esculenta. The calcareous matrix of the cuttlebone functioned as both a reducer and a scaffold for AgNP formation. These researchers also described the reusability of the composite material and the potential for separation of NPs from the matrix. Das et al. [121] showed that the immobilization of the biogenic AgNPs on the nanostructured silica leads to their excellent hydrogenation catalytic efficiency. Protein extract from Rhizopus oryzae was applied on the nanosilica and used for AgNP biosynthesis. The resulting material was subsequently used for catalytic hydrogenation of 4-nitrophenol.

3.3.3. Catalytic treatment of other aqueous organic compounds

Other organic compounds have been effectively reduced using biosynthesized NPs. Iron and gold NPs have been used to catalytically remove dyes from aqueous solutions. In one study, FeNPs biosynthesized by means of green tea extract were used as a Fenton-like catalyst for the decolorization of aqueous solutions of methylene blue and methyl orange dyes [122]. Compared with iron NPs produced by the reduction of sodium borohydride, the biosynthesized iron NPs exhibited faster dye removal and a greater extent of dye removal. Gupta et al. [123] used green tea extract to biosynthesize AuNPs for catalytic reduction of the dye methylene blue in the presence of Sn(II) in both aqueous solution and micelles. The authors showed that a small quantity of AuNPs decreases the activation energy for reduction of methylene blue, and complete removal of dye could be achieved even at low temperature. Zhou et al. [124] described the photocatalytic degradation of the magenta dye fuchsin using PbS and ZnS hollow nanostructures formed on two species of bacteria. Photocatalytic degradation of the dye Coomassie Brilliant Blue G-250 in the presence of UV light has also been shown by AgNPs prepared from the aqueous extract of Coccinia grandis leaves [125].

Finally, Forrez et al. [126] used biosynthesized PdNPs in a membrane reactor to remove micropollutants such as ibuprofen (>95%), diclofenac (86%), mecoprop (81%) and triclosan (>78%) from the secondary effluent of a sewage treatment plant. The authors suggested that the removal mechanisms could include chemical oxidation by PdNPs and/or biological removal by Pseudomonas putida bacteria.

3.4. Catalytic Cr(VI) reduction

Another important environmental application for biosynthesized NPs is the catalytic reduction of the powerful oxidant Cr(VI) to the relatively non-toxic valence state Cr(III). Several recent studies have shown that PdNPs biosynthesized by various species of bacteria, including E. coli, Desulfovibrio, Serratia and Clostridium, can effectively catalyze the reduction of Cr(VI) to Cr(III) under both batch and continuous flow conditions. For example, Deplanche et al. [127] evaluated how three types of hydrogenases encoded by E. coli influence the activity of biosynthesized catalyst for Cr(VI)/Cr(III) reduction. This study also described the hydrogenase involvement in Pd(II) reduction. Macaskie et al. [106] compared E. coli and Desulfovibrio spp. for the biosynthesis of PdNPs for catalytic Cr(VI)/Cr(III) reduction. Mabbett and Macaskie [128] used Desulfovibrio desulfuricans for the biosynthesis of PdNPs for Cr(VI)/Cr(III) reduction in waste water. These authors found that the biosynthesized PdNPs were more efficient reduction catalysts than either bare living D. desulfuricans biomass or chemically reduced palladium. They proposed that the palladium catalyzes the hemolytic cleavage of H₂ and that the H⁺ radicals then donate their electrons to reduce the Cr(VI).

Unlike the previous experiments, all of which were carried out under batch conditions, Humphries et al. [129] reported a continuous flow system for Cr(VI)/Cr(III) reduction. In this study, biosupported PdNPs formed by Desulfovibrio vulgaris were immobilized in agar and the effects of Pd concentration, Cr(VI) concentration and process flow rate were investigated. This work built on a study by Mabbett et al. [130], who had previously demonstrated a continuous flow system for Cr(VI)/Cr(III) reduction by PdNPs biosynthesized by D. desulfuricans. Mabbett et al. [131] subsequently described the formation of a mixed metal catalyst biosynthesized with D. desulfuricans and E. coli from a metal waste stream for Cr(VI)/Cr(III) reduction.

Beauregard et al. [132] used a biofilm-forming bacterial species Serratia sp. to mediate the adherence of bio-PdNPs to porous polyurethane foam. The foam was used as a scaffold for the catalyst and supported the growth of the bacterium. Magnetic resonance imaging was used to directly monitor Cr(VI)/Cr(III) reduction since Cr(VI)aq is non-paramagnetic and Cr(III)aq is paramagnetic. Finally, Chidambaram et al. [133] demonstrated successful Cr(VI) reduction experiments using PdNPs biosynthesized by the bacterium Clostridium pasteurianum. This study is unique because, unlike the previous studies, the C. pasteurianum communities were grown on aquifer material and catalytic reduction experiments were performed in a natural aquifer environment. This study opens up the potential for in situ Cr(VI) remediation via Pd(II) injection into the aquifer.

3.5. Biosynthesized NPs to enhance membrane treatment processes

Technologies to reduce biofouling can greatly enhance the performance of membrane-based water treatment processes. Zhang
et al. [134] showed that different amounts of biogenic AgNPs formed by *Lactobacillus fermentum* prevented biofouling on polyethersulfone membranes. The membranes were used to remove organophosphate pesticides and *E. coli* cells from a waste water model. The authors measured the structure and performance of the membrane and assessed biofouling using *E. coli* and *P. aeruginosa* bacteria both individually and in mixed culture. The nonfunctionalized membrane coating exhibited good antibacterial activity and prevented biofilm formation on the membrane surface during the 9 week test.

4. Industrially important applications

4.1. Catalytic organic synthesis

Cheap specific catalysts for commercially important organic synthesis reactions, including hydrogenation, cross-coupling reactions and epoxidation, are extremely important in industry. Biosynthesized palladium, gold and platinum catalysts have been used successfully in several different organic synthesis pathways (see Table 10).

Creamer et al. [135] used palladized cells of bacterial strains *Desulfovibrio desulfuricans* (G−) and *Bacillus sphaericus* (G+) for catalytic hydrogenation of itaconic (or methylensuccinic) acid. Comparisons performed in a stirred autoclave between biosynthesized PdNPs and commercial graphite-supported catalysts displayed similar activity. Bennett et al. [136] investigated the hydrogenation and isomerization of 2-pentane using palladized *D. desulfuricans* in a stainless steel Büchi reactor. They found that, although the rate of 2-pentane hydrogenation was lower for the biosynthesized Pd catalyst than for the commercial Pd/Al₂O₃ catalyst, the selectivity was similar. Wood et al. [137] used palladized *Rhodobacter capsulatus* and *Arthrobacter oxidans* for the hydrogenation of 2-butyne-1,4-diol. The biosynthesized PdNPs exhibited high selectivity during partial hydrogenation to 2-buten-1,4-diol.

Creamer et al. [138] demonstrated the use of biosynthesized PdNPs to catalyze non-aqueous hydrogenation. *Desulfovibrio desulfuricans* and *B. sphaericus* produced palladium catalyst, which catalyzed the hydrogenation of 4-azidoaniline hydrochloride to 1,4-phenylenediamine and 3-nitrostyrene to 1-ethyl-3-nitrobenzene in methanol.

Unlike the aforementioned studies, Jia et al. [139] published a method for PdNPs biosynthesis that does not require the H₂ donor. Biosynthetic PdNPs were formed by the reduction of palladium chloride in a crude extract of *Gardenia jasminoides*. The authors measured catalytic hydrogenation of p-nitrotoluene and demonstrated a conversion of 100% at 5 MPa and 150 °C for 2 h, with a selectivity of 26.3% for the product p-methyl-cyclohexylamine. Reusability studies demonstrated that the catalyst retained its activity over five cycles.

Biosynthesized NPs have been used to catalyze cross-coupling reactions (i.e., C–C bond formation). Seibjerg et al. [140] showed that palladized bacteria *Cupriavidus necator*, *Pseudomonas putida* and *Staphylococcus sciuri* were effective catalysts for both the Suzuki–Miyaura and Mizoroki–Heck reactions. In a follow-on report, these authors described how biomass/Pd ratio and NPs sizes influenced the hydrogenation of (E)-3-(4-methoxyphenyl)-N-methylacrylamide and the reduction of 4-chloro-nitrobenzene [141]. Finally, Gauthier et al. [142] demonstrated the formation of catalyst for C–C bond formation was coupled to the recovery of Pd from a waste stream using *Cupriavidus necator* cells.

In addition to palladium, biosynthesized gold and platinum NPs have been used to catalyze other organic synthesis reactions. Du et al. [143] used biosynthesized AuNPs to catalyze propylene epoxidation. Negatively charged AuNPs were immobilized on a titanium silicalite support using electrostatic attractive interactions in an extract of *Cucumen platycladi*. These authors achieved a propylene epoxide formation rate higher than previously described in other reports. The authors proposed that the efficiency and selectivity of biosynthesized catalysts could be influenced by residual biomolecules. A more detailed investigation of the same catalyst by Zhan et al. [144] described the optimum reaction parameters, including the Si/Ti molar ratio, Au loading, immobilization pH and reaction temperature, together with possible reaction mechanisms. Similarly, AuNPs immobilized on a titanium silicalite support were calcined and used for industrially important epoxidation of styrene to styrene oxide [145]. This experimental setup enables a styrene conversion of 92.7% and a styrene oxide selectivity of 90.4% under optimal conditions.

Finally, biosynthesized PtNPs have been used to catalyze the synthesis of the organic dye antipyrilquinoneimine from aniline and 4-aminooantipyrine in acidic aqueous solution [146]. In this report, honey-mediated platinum nanostructures were found to be stable in water for more than 4 months.

4.2. Energy-related applications

The ability of different organisms to reduce and absorb precious metal salts and form NPs has been used to enhance several aspects of fuel cell performance. Biogenic NPs have been used to produce H₂ fuel, to catalyze chemical oxidation of the fuel and to improve power recovery (Table 11).

Several investigators have used biosynthesized PdNPs for H₂ production. Bunge et al. [103] used three species of bacteria (*Cupriavidus necator*, *Pseudomonas putida* and *Paracoccus denitrificans*) to biosynthesize PdNPs for catalytic H₂ production from

![Table 10](image)

<table>
<thead>
<tr>
<th>NP</th>
<th>Organism used</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd</td>
<td><em>D. desulfuricans</em>, <em>Bacillus sphaericus</em></td>
<td>Hydrogenation of itaconic acid</td>
<td>Creamer et al. [135]</td>
</tr>
<tr>
<td>Pd</td>
<td><em>D. desulfuricans</em></td>
<td>Hydrogenation of 2-pentane</td>
<td>Bennett et al. [136]</td>
</tr>
<tr>
<td>Pd</td>
<td><em>Rhodobacter capsulatus</em>, <em>Arthrobacter oxidans</em></td>
<td>Hydrogenation of 2-butyne-1,4-diol</td>
<td>Wood et al. [137]</td>
</tr>
<tr>
<td>Pd</td>
<td><em>D. desulfuricans</em>, <em>B. sphaericus</em></td>
<td>Hydrogenation, reduction and selective dehalogenation in non-aqueous solvents</td>
<td>Jia et al. [139]</td>
</tr>
<tr>
<td>Pd</td>
<td><em>Gardenia jasminoides</em></td>
<td>Hydrogenation of p-nitrotoluene</td>
<td>Creamer et al. [138]</td>
</tr>
<tr>
<td>Pd</td>
<td><em>C. necator</em>, <em>Pseudomonas putida</em></td>
<td>Suzuki–Miyaura, hydrogenation of (E)-3-(4-methoxyphenyl)-N-methylacrylamide</td>
<td>Seibjerg et al. [140]</td>
</tr>
<tr>
<td>Pd</td>
<td><em>C. necator</em>, <em>Staphylococcus sciuri</em></td>
<td>4-chloronitrobenzene reduction</td>
<td>Seibjerg et al. [141]</td>
</tr>
<tr>
<td>Pd</td>
<td><em>C. necator</em></td>
<td>Catalysis of C–C bond formation</td>
<td>Gauthier et al. [142]</td>
</tr>
<tr>
<td>Au</td>
<td><em>Cucumen platycladi</em></td>
<td>Propylene epoxidation</td>
<td>Du et al. [143]</td>
</tr>
<tr>
<td>Au</td>
<td><em>C. platycladi</em></td>
<td>Propylene epoxidation</td>
<td>Zhan et al. [144]</td>
</tr>
<tr>
<td>Au</td>
<td><em>C. platycladi</em></td>
<td>Styrene epoxidation</td>
<td>Huang et al. [145]</td>
</tr>
<tr>
<td>Pt</td>
<td>Honey</td>
<td>Preparation of organic dye</td>
<td>Venu et al. [146]</td>
</tr>
</tbody>
</table>
4036

A. Schröfel et al. / Acta Biomaterialia 10 (2014) 4023–4042

hypophosphite. Wu et al. [147] biosynthesized PdNPs with garde-
nia extract on TiO2 supports to enhance photocatalytic H2 evolu-
tion from pure water. Macaskie et al. [148] provided a brief
overview of bacterial hydrogenase activity for several applications,
including waste decontamination, production of precious metal
nanocatalyst and biohydrogen production. These authors also
described how E. coli with modified hydrogenase and dehydroge-
nase regulation could be used to further enhance performance.

Biosynthesized NPs have been incorporated into fuel cells to
catalyze chemical reactions and have been used in electrode con-
struction. Yong et al. [149] bioaccumulated Pt and Pd as NPs using
Desulfovibrio desulfuricans and mixed the dried biocomponent with
activated carbon powder to create carbon paper used in a commer-
cial fuel cell system. Dimitriadis et al. [150] used yeast biomass
immobilized in polyvinyl alcohol (PVA) cryogels for the biorecov-
er of platinum from aqueous solution, then directly incorporated
this PtNP–biomass–PVA material into a fuel cell. Another study
described four different concentrations of biomass-supported pal-
ladium NPs using Shewanella oneidensis as an electrode catalyst in
a polymer electrolyte membrane fuel cell [151].

Orozco et al. [152] studied the ability of PdNPs biosynthesized by
two different strains of E. coli to both produce hydrogen via fer-
mantation and convert the H2 into energy in a fuel cell. Both the parent
strain MC4100 and the mutant strain IC007 were coated with PdNPs
after the bioreduction of palladium precursor, then modified and
used as anodes in a fuel cell. The mutant strain IC007 showed a
threefold greater power conversion compared with the parent
strain, but produced about half of the power of commercial Pd pow-
der or biosynthesized PdNPs made with D. desulfuricans.

Yong et al. [153] described the coupling of waste biorefining with
fuel cell power generation using biosynthesis to recover preci-
ous metals and form nanocatalysts. Palladium was biocovered from
industrial processing waste and transformed into biosupport-
ed PdNPs using D. desulfuricans, E. coli and Cupriavidus metallidu-
rans. The study also described the biosynthesis and performance of
a mixed metal catalyst produced from the waste stream.

4.3. Electrodes and sensors

Fundamental electrochemical phenomena can be better under-
stood through nanoscale science and nanotechnology, and perfor-
mance of electrodes and sensors can be altered or enhanced
using biosynthesized nanoparticles. Several aspects of biosynthe-
sized metallic nanoparticles have been studied in detail, including
their surface chemistry, biological compatibility and electrical con-
ductivity. For nanoelectrochemical applications, special attention
has been paid to AuNPs because they provide a suitable substrate
for the immobilization of biomolecules without altering their
biological activity, and they provide excellent electron transfer
between the biomolecule and the electrode surface [154]. The
presence of capping agents and surfactants in biosynthesized AuN-
Ps can modulate electrode functionality and may enhance the
operating range or selectivity compared with conventional materi-
als (see Table 12).

Several studies have examined the electrical transmission prop-
erties of biosynthesized NPs incorporated into electrodes. In one
study, dried whole plant extract from Scutellaria barbata was used
for the biosynthesis of AuNPs [155]. These AuNPs were applied to
a glassy carbon electrode (GCE), and the authors found enhanced
electrical transmission between the modified electrode and PNP.
Another study also demonstrated enhanced electrical transmission
using a GCE modified with AuNPs prepared from the flower extract
of Rosa damascene [156]. This study employed cyclic voltammetry
in a solution of 0.1 M KCl and 5.0 mM [Fe(CN)6]3-/4- to demonstrate
an increase in the electronic transmission rate for the modified
electrode. Sadhasivam et al. [16] performed cyclic voltammetry
studies using a three-electrode configuration employing AuNPs
biosynthesized by Streptomyces hygroscopicus. Platinum and Ag
were used as counter and reference electrodes, respectively, and
electrochemically coated CuO on a Pt substrate (CuO–Pt–Pt)
with an AuNPs–methylene blue monolayer was employed as the
working electrode.

Various electrochemical sensing applications can make use of
the unique properties of biosynthesized NPs. Jha and Prasad
[157] introduced a biosynthetic method to prepare ferroelectric
BaTiO3 NPs using the bacterium Lactobacillus sp. The resulting
NPs were mixed with a PVD solution then agitated and warmed
until the mixture became viscous. The process resulted in thin
nanocomposite sheets that exhibited significantly enhanced
dielectric properties. Torres-Chavolla et al. [158] biosynthesized
AuNPs using three species of actinomycetes, Thermomonospora
curvata, T. fusca and T. chromogena, and stabilized the NPs using
the cross-linker glutaraldehyde for enhanced biosensing applica-
tions. Shilov et al. [159] investigated different electrophysical char-
acteristics, such as cell zeta potential, surface conductivity,
electrophoretic mobility and the dispersion of cell conductivity,
of Candida albicans yeast cells with Ag precipitate prepared using
the reducer hydrazine.

Du et al. [160] introduced the bioreduction of Au by E. coli cells
and their application for direct electrochemical sensing of hemog-
lin. Biosynthesized AuNPs bound to the surface of the bacterial
cells were incorporated on a GCE. The authors performed cyclic
voltammetry measurements for different electrode configurations
on small samples of hemoglobin placed directly on the electrode
surface. The results showed a pair of redox peaks with a formal
potential of –0.325 V vs. an Ag/AgCl reference electrode. This study
demonstrated that biosynthesized nanocomposites can assist elec-
tron transfer between hemoglobin and the surface of a GCE.

Biosynthesized CdSNPs have also been used to fabricate a het-
erojunction with asymmetric electronic transfer properties [161].
Semiconducting wurzite-type structured NPs were prepared using
Schizosaccharomyces pombe. Tin-doped indium oxide-coated glass
was covered by a thin film of spin-coated polyphenylene vinylene
and washed with S. pombe. While polyphenylene vinylene is a
p-type material, CdSNPs represent an n-type material. With added
Ag contacts, the resulting diode had a forward current of
75 mA cm−2 at 10 V and experienced breakdown at approximately
15 V in reverse mode.

Table 11

<table>
<thead>
<tr>
<th>NP</th>
<th>Organism used</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd</td>
<td>Cupriavidus necator, Pseudomonas putida, Paracoccus denitrificans</td>
<td>Hydrogen production from hypophosphite</td>
<td>Bunge et al. [103]</td>
</tr>
<tr>
<td>Pd/TiO2</td>
<td>Gardenia jasminoides</td>
<td>Photocatalytic H2 evolution from pure water</td>
<td>Wu et al. [147]</td>
</tr>
<tr>
<td>Pd</td>
<td>Escherichia coli</td>
<td>Biohydrogen production</td>
<td>Macaskie et al. [148]</td>
</tr>
<tr>
<td>Pd</td>
<td>Desulfovibrio desulfuricans</td>
<td>Fuel cell electrode</td>
<td>Yong et al. [149]</td>
</tr>
<tr>
<td>Pt</td>
<td>Saccharomyces cerevisiae</td>
<td>Pt biorecovery, fuel cell electrode</td>
<td>Dimitriadis et al. [150]</td>
</tr>
<tr>
<td>Pd</td>
<td>S. oneidensis</td>
<td>Fuel cell electrode</td>
<td>Ogi et al. [151]</td>
</tr>
<tr>
<td>Pd</td>
<td>E. coli</td>
<td>Fuel cell electrode</td>
<td>Orozco et al. [152]</td>
</tr>
<tr>
<td>Pd</td>
<td>D. desulfuricans, E. coli, Cupriavidus metallidurans</td>
<td>Waste biorefining, fuel cell electrode</td>
<td>Yong et al. [153]</td>
</tr>
</tbody>
</table>

Energy-related applications.
Table 12
Electrodes and sensors.

<table>
<thead>
<tr>
<th>NP</th>
<th>Organism used</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au</td>
<td>Scutellaria barbata</td>
<td>Direct electrochemistry of 4-NP</td>
<td>Wang et al. [155]</td>
</tr>
<tr>
<td>Ag, Au</td>
<td>Rosa damascena</td>
<td>Modified glassy carbon electrode</td>
<td>Ghoreshti et al. [156]</td>
</tr>
<tr>
<td>BaTiO3</td>
<td>Lactobacillus sp.</td>
<td>Nanocomposite with significantly enhanced dielectric properties</td>
<td>Jha and Prasad [157]</td>
</tr>
<tr>
<td>Au</td>
<td>Thermomicrospera curvata, T. fusca, T. chromogena</td>
<td>Applications in enhanced biosensors</td>
<td>Torres-Chavolla et al. [158]</td>
</tr>
<tr>
<td>Au</td>
<td>Escherichia coli</td>
<td>Direct electrochemistry of hemoglobin</td>
<td>Du et al. [160]</td>
</tr>
<tr>
<td>CdS</td>
<td>Schizosaccharomyces pombe</td>
<td>Construction of ideal diode</td>
<td>Kowshik et al. [161]</td>
</tr>
<tr>
<td>Au</td>
<td>Black tea</td>
<td>Capacitors construction</td>
<td>Uddin et al. [162]</td>
</tr>
<tr>
<td>Au, Ag</td>
<td>Solanum lycopersicum</td>
<td>Metallic ion detection</td>
<td>Bindhu and Umadevi [163]</td>
</tr>
</tbody>
</table>

Biogenic AuNPs in polyvinyl alcohol–KH2PO4 films were used by Uddin et al. [162] to ameliorate the percolative behavior of these nanocomposite films and to generate high dielectric permittivity at room temperature. The performance of the material is promising for use in capacitors.

The unique optical properties of metallic nanoparticles can be also used for chemical sensing. AuNPs and AgNPs reduced using extract from the tomato Solanum lycopersicum were used for detection of metallic ions, including Fe3+ and Cu2+, in water based on subtle changes in their surface plasmon resonance spectra [163].

5. Applications for magnetically responsive NPs

Magnetotactic bacteria have a natural ability to synthesize magnetite NPs (MNPs), which are useful for various applications (Table 13). Only certain NP forms are synthesized spontaneously by magnetotactic bacteria [164,163] (see Fig. 6). The biological synthesis of magnetically responsive NPs is well established, and their use in various medical, environmental and industrial applications has been reviewed [166–166].

The yield and properties of MNPs can be manipulated and optimized to suit a particular purpose. Kundu et al. [169] described changes in magnetsome size, number, and alignment with biosynthesis performed in the presence of Zn and Ni salts. Other investigators have described the formation of zinc–ferrite NPs by Thermoanaerobacter strain TOR-39 [170]. Coker et al. [176] described improved magnetic properties of biosynthesized magnetic NPs. In the study, FeCo-oxyhydroxides were created by adding CoCl2.6H2O to FeCl3 precursor and then treating them with Geobacter sulfurreducens culture. The resulting cobalt ferrite (CoFe2O4) NPs exhibited dramatically enhanced magnetic properties compared with simultaneously produced magnetite MNPs. Staniland et al. [171] described controlled cobalt doping of magnetosomtes in vivo, where an estimated cobalt content of between 0.2 and 1.4% resulted in a 36–45% increase in the coercive field. Roh et al. [172] also reported Co, Cr, Mn and Ni doping of magnetite biosynthesized extracellularly by Thermoanaerobacter ethanolicus and a psychrotolerant Shewanella sp. In a follow-up report, these authors described how the process is amenable to large-scale production and can produce magnetic particles at a fraction of the cost of traditional chemical synthesis [173].

Telling et al. [174] employed MNPs to catalyze Cr(VI) reduction (see also Section 3.4). MNPs were biosynthesized by Geobacter sulfurreducens and the reduction of Cr(VI) to Cr(III) at sites within the magnetic spinel surface were studied using X-ray absorption and X-ray magnetic circular dichroism. The same group also investigated the use of biosynthesized magnetites produced by G. sulfurreducens for recovery of metal contaminants from water [175]. MNPs produced by the bacterial reduction of powdered schwertmannite (an iron-oxyhydroxysulfate mineral) were found to be more efficient at reducing Cr(VI) than ferrihydrite (an oxyhydroxide mineral), “gel”-derived biomagnetite and commercial nanoscale Fe3O4.

One key advantage of MNPs is the ease of collection and recovery. Magnetic properties can also ensure that particles remain dispersed and distributed throughout a reaction vessel. Coker et al. [176] employed MNPs synthesized by G. sulfurreducens as a biomagnetic support for palladium nanocatalyst. The Pd-MNPs were used to catalyze the C–C bond-forming Mizoroki–Heck reaction, coupling iodobenzene to ethyl acrylate or styrene. Crean et al. [177] used a similar approach for the continuous removal of Cr(VI).

6. Biosynthesis of unique formulations and geometries

The properties and potential applications of nanoscale materials are determined by their chemical composition, crystal structure, particle size and particle shape. Several studies have described the biosynthesis of NPs with unique compositions and/or physical forms that will modulate performance in a range of applications (Table 14).

NPs with certain chemical compositions have been difficult to synthesize using conventional chemical methods. Ahmad et al. [178] described fungal biosynthesis of transparent p-type conducting oxide CuAlO2 NPs with Humicola sp. In this case, traditional chemical synthesis is challenging because high temperatures are necessary for overcoming reaction barriers, but low temperatures are necessary for maintaining the Cu(1+) valence. Ankamwar et al. [179] introduced a biosynthetic approach using transmetallation.

### Table 13
Application enhancements using magnetically responsive NPs.

<table>
<thead>
<tr>
<th>NP</th>
<th>Organism used</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe3O4</td>
<td>Thermoanaerobacter sp. TOR-39</td>
<td>Magnetite substituted with Zn</td>
<td>Yeary et al. [170]</td>
</tr>
<tr>
<td>CoFe2O4</td>
<td>Geobacter sulfurreducens</td>
<td>Enhanced magnetic properties of biosynthesized composite</td>
<td>Coker et al. [176]</td>
</tr>
<tr>
<td>Fe3O4</td>
<td>Magnetospirillum gryphiswaldense, Magnetospirillum magnetotacticum</td>
<td>Cobalt doping of magnetosomes</td>
<td>Staniland et al. [171]</td>
</tr>
<tr>
<td>Fe2O3</td>
<td>Thermoanaerobacter ethanolicus, Shewanella sp.</td>
<td>Magnetite substituted with Co, Ni, Cr, Mn</td>
<td>Roh et al. [172]</td>
</tr>
<tr>
<td>Fe3O4</td>
<td>Thermoanaerobacter sp. TOR-39</td>
<td>Magnetite substituted with Co, Ni, Cr, Mn, Zn and rare earth metals</td>
<td>Moon et al. [173]</td>
</tr>
<tr>
<td>Fe3O4</td>
<td>G. sulfurreducens</td>
<td>Cr(VI) remediation</td>
<td>Telling et al. [174]</td>
</tr>
<tr>
<td>Fe3O4</td>
<td>G. sulfurreducens</td>
<td>Cr(VI) and Tc(VII) remediation</td>
<td>Cutting et al. [175]</td>
</tr>
<tr>
<td>Fe3O4</td>
<td>G. sulfurreducens</td>
<td>Magnetically recoverable Pd nanocatalyst</td>
<td>Coker et al. [176]</td>
</tr>
<tr>
<td>Fe3O4</td>
<td>G. sulfurreducens</td>
<td>Cr(VI) remediation</td>
<td>Crean et al. [177]</td>
</tr>
</tbody>
</table>
reactions. AgNPs and AuNPs phytosynthesized using *Emiblica officinals* fruit extract were transferred into methanol solution using a cationic surfactant, and transmellation was carried out between AgNPs and chloraurate ions in chloroform.

The creation of metal alloys by casting requires heavy equipment and high temperatures. Bottom-up biosynthesis of Au–Ag alloys by *Brassica juncea* seed was introduced by Haverkamp et al. [180]. Similar studies reported bimetallic Au–Ag alloy biosynthesis employing the fungus *Fusarium* [181,180], Deplanche et al. [183] likewise introduced a hybrid two-step method for the production of core–shell Pd–Au NPs by means of *E. coli* cells.

NP biosynthesis can also result in NPs with various useful shapes. Ankomwar et al. [184] described the phytosynthesis of Au nanotriangles using tamarind (*Tamarindus indica*) leaf extract. The authors measured the electrical conductivity of these triangular NPs in different organic solvents, and suggested a possible chemical vapor sensor application. The biosynthesis of Ag nanplates was described by Xie et al. [185], who employed the green alga *Chlorella vulgaris*. Hollow structures created for various photocatalytic applications were described in a paper by Zhou et al. [124], who coated empty bacterial cells with PbS and ZnS NPs. The authors chose bacteria with spherical (*Streptococcus thermophilus*) and rod-like shapes (*Lactobacillus bulgaricus, Lactobacillus acidophilus*). Although the photocatalytic activity of the resulting hollow nanostructures had been described previously, the authors described additional uses, including as electromagnetic wave absorbers, ultraviolet shielding materials and photocatalysts for solar cells.

Sathish Kumar et al. [186] biosynthesized Au NPs by means of the yeast *Hansenula anomala* and its extract. These AuNPs were further stabilized by the addition of two different types of poly(amide amine) dendrimers. The authors demonstrated the use of these AuNPs as bioink for rubber stamps.

**7. Future outlook of biosynthesized NPs**

This review highlights the key medical, environmental, and industrial applications of biosynthesized NPs. The natural machinery used by all biological systems to execute redox reactions with specificity, in aqueous solution, and under conditions of standard temperature and pressure provides a powerful strategy for producing a wide range of nanosized materials, often at a fraction of the cost of traditional chemical synthesis. Biosynthesized NPs are frequently attached to a biological carrier or scaffold, and many feature biogenic capping materials that enhance the compatibility and stability of the NPs under environmental conditions. Capping agents are specific to the particular organisms used for biosynthesis, so a wide range of interesting properties can be achieved.

Biosynthesized NPs have been used in nearly every field where traditional NPs have been employed. One of the challenges of biogenic NPs is the separation of the NPs from the biological material. In addition, contamination from biological cells can be a problem especially in medical applications, due to the potential introduction of allergens or pathogens.

Considering the volume and growth in NP biosynthesis research in recent years, the field appears to be on the threshold of much more widespread and intensive research into applications. Meanwhile, further research and development of the underlying biosynthesis methods themselves and the creation of novel biosynthetic NP forms is anticipated.

**Acknowledgements**

This book chapter was supported by the Grant Agency of the Czech Republic (grant number P302/12/G157) by the Charles University in Prague (projects UNCE 204022 and Prvouk/11F1). This publication is also supported by the project “BIOCEV – Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University” (CZ.1.05/1.1.00/02.0109), from the European Regional Development Fund, project CZ.1.15/2.1.00/02.0100 from the European Regional Development Fund and grant NSF 1242167.

**Appendix A. Figures with essential colour discrimination**

Certain figures in this article, particularly Figs. 1–4 are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi: [http://dx.doi.org/10.1016/j.actbio.2014.05.022](http://dx.doi.org/10.1016/j.actbio.2014.05.022).

**References**


Vaseeharan B, Ramasamy P, Chen JC. Antibacterial activity of silver nanoparticles (AgNPs) synthesized by tea leaf extracts against pathogenic Vibrio vulnificus and its protective efficacy on juvenile Feneropus indicus. Lett Appl Microbiol 2010;50:352–62


Sathishkumar M, Sreeka K, Yun YS. Immobilization of silver nanoparticles synthesized using Curcuma longa tuber powder and extract on cotton cloth for bacterialic activity. Bioresour Technol 2010;101:7958–65


Li X, Jiang W, Sun J-B, Wang GL, Guan F, Li Y. Purified and sterilized magnetosomes from Magnetoterris gryphiswaldense MSR-1 were not toxic to mouse fibroblasts in vitro. Lett Appl Microbiol 2007;45:75–81


Hennebel T, De Gusseme B, Boon N, Verstraete W. Biogenic metals in bioremediation.


Deplanche K, Caldelari I, Mikheenko IP, Sargent F, Macaskie LE. Involvement of hydrogenases in the formation of highly catalytic Pd(0) nanoparticles by bioreduction of Pd(ii) using *Escherichia coli* mutant strains. Microbiology 2010;156:2630–40.


