Biosynthesis of Metallic Nanoparticles and Their Applications

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Biosynthesis of Metallic Nanoparticles and Their Applications

Adam Schröfel and Gabriela Kratošová

Abstract Biosynthesis and biofabrication of the metallic NPs have became an important approach to NP preparation. They are not only equal with the chemical or physical methods, but also offer quite a few assets compared to classical tacks. In this review, we present comprehensive overview of existing published records, which include clear and realistic application of biosynthesized metallic NPs. Our survey covers NP utilization from biosorption and catalysis to medicinal and sensing applications. Moreover, we add current review references and comparison (or synergy) with chemical and physical methods.

Keywords Biosynthesis • Nanoparticles • Biosorption • Bioremediation • Catalysis • Precious metal

Abbreviations

NP	nanoparticle
AuNP	gold nanoparticle
AgNP	silver nanoparticle
PdNP	palladium nanoparticle
PEI	polyethylenimine
CP	chlorophenol
PCB	polychlorinated bifenyl
PBDE	polybrominated diphenyl ether
TCPP	tris(chloroisopropyl)phosphate
G+	Gram positive
G−	Gram negative

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γ-HCH	γ-hexachlorocyclohexane (lindane)
ICM	iodinated contrast media
TCE	trichloroethylene
bioPd	biofabricated palladium
PEM	polymer electrolyte membrane
4-NP	*p*-nitrophenol; 4-nitrophenol
SERS	surface enhanced Raman scattering
IR	infra red
BM	bacterial magnetosome
PTP	tyrosine phosphatase
PVDF	polyvinylidene fluoride
GCE	glassy carbon electrode
QD	quantum dot

1 Introduction

"Green" approach toward nanotechnology research became widely used in last few years. Inspiration by nature and processes inside the living organisms can produce new opportunities and perspectives for whole wide nanotechnology branch of research and industry. Particularly, synthesis of NPs and nanostructures can easily profit from usage of nature equipment located in cells. Right attitude towards world of biomolecules, function groups, enzymes and other important factors leads to large number of advantages compared to common chemical or mechanical methods.

Scientific interest in the NPs originates from their unique and variable properties. They create connecting link between bulk material and individual atoms or molecules. When the bulk materials have constant physical properties, the same materials have been uncovered to exhibit different interesting properties when studied in the nanoscale. For instance, NPs have a much larger surface compared to bulk materials. Generally, it follows that they have higher reactivity and different chemical properties. Also, the wavelength of NPs is similar to the wavelength of light. This results in unique optical properties (NPs are transparent).

Possible applications of metallic NPs are taking place in varied areas such as electronics, coating technology, packaging, cosmetics, biosensing, medicine and they will be discussed in corresponding sections.

2 Preparation of Nanoparticles

There are three main approaches for the metallic NP synthesis: physical, chemical and biological. From the structural point of view, we can describe methods as "bottom-up" and "top-down". Bottom-up approach is way to assemble the final structure atom by atom, molecule by molecule. The building units are formed at

first and, subsequently, formed into NPs or nanostructures – the final product. Some of the major assets of the bottom-up approach are resulting NPs without structural defects and with homogenous chemical compositions. Using "top-down" techniques, starting bulk material is reduced in size by mean of mechanical or chemical methods. The major drawback of this approach is often imperfection of the obtained structure. The surface defects have significant impact on physical properties and surface chemistry behavior.

2.1 Physical Methods

Commonly utilized are physical (or mechanical) methods such as attrition, evaporation/condensation, laser ablation and pyrolysis. In attrition, particles are ground by a size-reducing mechanism (e.g. ball mill). Resulted NPs are critically affected by the used starting material, time of drilling and medium which is used as atmosphere. This method is typical representative of "top-down" approach. Inert gas condensation can be used in atom creation (e.g. for the preparation small clusters of gold atoms). As a result of interatomic collisions with the gas atoms in the chamber, the evaporated metal atoms lose their kinetic energy and condense in the form of small crystals (Lee et al. 2007). Laser ablation method is used to produce NPs by using the pulsed laser irradiation on the metal target in liquid or gas environment (Yang 2007). In pyrolysis, the precursor solution is atomized into series of droplet "reactors". These very small droplets are introduced by a carrier air gas into a hot-wall region under atmospheric pressure conditions. The solvent in the droplets evaporates inside the furnace, and the remaining solutes cause precipitation, thermal decomposition and intraparticle reactions to form product particles (Widiyastuti et al. 2010).

The advantage of these methods is narrow particle size distribution of the produced NPs, while its limitation is the need for expensive equipment (lasers etc.). Also the production rate is lower compare to chemical methods. Higher energy consumption for maintaining the pressure and temperature conditions used in the aforementioned procedures are an additional handicap.

2.2 Chemical Methods

The second route is a chemical approach (usually "bottom-up" methods), particularly the wet chemical procedure – the metal ions of dissolved precursor (e.g. $AgNO_3$) is reduced in defined conditions which allows the subsequent formation of small clusters or aggregates of metal atoms (Khomutov and Gubin 2002; Oliveira et al. 2005). Similar procedures can be used also for creating of metallic molecules such as sulfides or oxides (Seoudi et al. 2010). Specifically, methods such as the sonochemical method (Salkar et al. 1999; Shchukin et al. 2010), the polyol process, the solvent-reduction method (Bonet et al. 2000), the template method

(Fukuoka et al. 2002), and the reverse micelles method (Yadav et al. 2003) have been developed for the preparation of NPs.

There are also several modified chemical methods including seed-mediated growth where small particles (produced by other techniques like irradiation) are utilized as seeds and fresh metallic ions are reduced by reducing agent and grow along the surface of the seed particle (Samanta et al. 2010). Reduction agent usage differs depending on the purpose, required properties, types, sizes. For instance, the first reduction of gold salt by the so-called Turkevich method was introduced in 1951, when a sodium citrate reduction of $HAuCl_4$ was used for synthesis of stable gold nanoparticles (AuNPs) (Nguyen et al. 2010). There are also other reducing agents such as $NaBH_4$ (Wagner et al. 2008), methoxypolyethylene glycol (Mallick et al. 2004), stannous chloride (Vaskelis et al. 2007) and ascorbic acid (Wagner and Köhler 2005). Amine or hydroxyl-containing molecules such as branched poly(ethyleneimine) (Note et al. 2006), azacryptand, amino acid (Selvakannan et al. 2004) or chitosan (Shih et al. 2009) were also reported as a suitable reducing agents for metallic NP preparation.

The chemical methods are relatively inexpensive for high volume, usually easy to perform, and very variable. However, their disadvantages include toxic chemicals usage, likely contamination from precursor chemicals or reducing agents, and also formation of dangerous and hazardous reaction byproducts.

2.3 Biological Methods

Because of certain aforementioned limitations of physical and chemical method, there is an increasing need to develop methods, which will be nontoxic, fast, high-yield, energy saving (occur under normal conditions – normal air pressure and temperature), and environmentally benign. Consequently, researchers have turned to biological systems for inspiration. Bioprocesses mediated by living organisms (employing their cells, enzymes, transport chains etc.) therefore became important for metallic NP synthesis. For this purpose, we have a vast variety of organisms in nature such as viruses, bacteria, yeast, fungi, algae, plants and plant products at our disposal.

The ability to form inorganic materials by many organisms either intra- or extracellularly has been well known for almost 30 years (Wilbur and Simkiss 1979; De Stefano et al. 2008). Many biotechnological applications, such as the remediation of toxic metals, employ microorganisms such as bacteria (Pérez-de-Mora et al. 2006). Therefore, many microorganisms (e.g. fungi, bacteria) were found as possible nanofacilities for NP fabrication. These nature-derived processes contributed and led to development of relatively new biosynthesis methods for fabrication of nano- and microscale inorganic materials by microbes and other living organisms (Ahmad et al. 2003). Until now, a large number of both unicellular and multicellular organisms have been known to produce intracellular or extracellular metallic NPs (Thakkar et al. 2010).

Biosynthesis of NPs is becoming an emerging consequence of overlap between nano- and biotechnology. In the last few years it has received attention due to its potential to develop environmentally benign technologies in material science. But in fact, this type of NP synthesis method is also a "chemical" approach. Living cells are extremely complex system with thousands of molecules. These molecules have varied functional groups (such as hydroxyl, amine etc.) which each can possibly facilitate metal reduction. Therefore, it is very difficult to describe a specific place or process responsible directly for NP growth. This can result in certain drawbacks for biosynthesis methods. The resulting matter is usually mixture of cells (cell debris) and NPs, accompanying with thousands of metabolic products and other molecules. Frequently it is very complicated to separate the tiny product particles from the cell debris. Moreover, surrounding matrix and capping proteins contribute to NP stability (Lynch and Dawson 2008) and can influence their properties. Among the other disadvantages of precursors (such as $AgNO_3$) is their toxicity to the target organisms. Therefore, this does not allow the usage of higher concentrations of the salts.

In this article, we provide a brief overview of the current research worldwide on the use of organisms such as bacteria, cyanobacteria and actinomycetes (both prokaryotes), as well as algae, yeast, fungi and plants (eukaryotes) in the biosynthesis of metal NPs with emphasize on their applications.

3 Applications of Metallic Nanoparticles

Although current research results show a wide field for biosynthesized NPs, we can segment these applications into several groups. The following division is based primarily on the purpose of biofabricated NPs (even though the chemical composition, shape and source organisms will be mentioned too).

3.1 Biosorption

Different organisms have ability to change metal oxidation state and concomitantly deposit resulting metal compounds and zero-valent metals on the cell surface or inside their cells. A variety of biomaterials have been known for a long time to bind the precious metals (including algae, fungi, bacteria, actinomycetes, yeast etc.). along with some biopolymers and biowaste materials (Table 1).

In particular, recovery of precious metals like gold, silver, palladium, and platinum is interesting due to their increasing market prices and various industrial applications. Conventional technologies (e.g. ion exchange, chemical binding, surface precipitation) which been have been developed for the recovery of such metals are neither efficient nor economically attractive. Biosorption represents a biotechnological innovation as well as a cost effective tool for recovery of precious metals from

Table 1 Biosorption

NP	Organism used	Application	Reference
Au	*Nitzschia obtusa, Navicula minima*	Bioaccumulation	Chakraborty et al. (2006)
Au	*Lyngbya majuscula, Spirulina subsalsa, Rhizoclonium hieroglyphicum*	Bioaccumulation, biorecovery	Chakraborty et al. (2009)
Au	*Fucus vesiculosus*	Bioaccumulation, biorecovery	Mata et al. (2009)
Ag	*Pleurotus platypus*	Biosorption	Das et al. (2010)
Pt	*E. coli*	Biosorption, biorecovery by incineration	Won et al. (2010)
Au	*Sargassum sp.*	Biosorption, biorecovery by incineration	Sathishkumar et al. (2010a)

aqueous solutions. In particular the microbial mechanisms involved in the biosorption and bioaccumulation processes have been extensively studied in natural environments, and researchers have recently gained interest in the applications of microbe–metal interactions in biotechnology, nanotechnology or material engineering. The connection between the recently discovered ability of NP biosynthesis and long-term investigated biosorption is apparent. Since the field of biosorption is wide, there is abundance of suitable and quality literature and reviews (Arief et al. 2008; Das 2010; Gadd 2009, 2010; Hennebel et al. 2009a; Chojnacka 2010; Wang and Chen 2009; Lesmana et al. 2009; Barakat 2010; Kavamura and Esposito 2009; Volesky 2007; Vijayaraghavan and Yun 2008). Furthermore, and without aspiration for more detailed probe, we will discuss some examples and current trends of metallic NPs biosorption application, specifically with regard to their application for metal bioaccumulation and recovery, waste remediation, soil and water treatment. Additionally we will discuss the NPs formation or ion bioreduction process. For more exhaustive analysis of biosorbed and biofabricated palladium and platinum catalysts see also Sect. 3.2.

As instance of noble metals biorecovery, Chakraborty et al. (2006) described experiments of Au bioaccumulation with two diatom strains. These unicellular algae organisms are one of the most abundant amongst the species both in marine and fresh water ecosystems on Earth. Due to low detection limit and also with regard to biosorption of other radioactive heavy metals in previous studies, gold radionuclide ^{198}Au was used. In subsequent study (Chakraborty et al. 2009), AuNP formation process was described and comparison in biorecovery abilities between prokaryotic and eukaryotic algal genera was performed. Gold biosorption and bioreduction with another brown alga *Fucus vesiculosus* was also reported (Mata et al. 2009), describing pH dependence and stages of the bioreduction process. Results of these studies indicate that live algal biomass may be a viable and cost effective for biorecovering of gold.

Bioaccumulation of silver ions Ag(I) from the solution or wastewater is reported e.g. by Das et al. (2010), accompanied also with kinetics studies and thermodynamic calculations on sorption of silver ions on gilled macrofungus *Pleurotus platypus*. This paper represents a modern and innovative approach for the study of interactions between biomass and metal ions.

Concrete example of platinum recovery is presented by Won et al. (2010) by means of biosorption and subsequent incineration of PEI modified biomass (prepared by attaching PEI onto the surface of inactive *E. coli* biomass). Wastewater containing platinum used for the recovery study was obtained from an industrial laboratory for inductively coupled plasma (ICP). Recovery efficiency of platinum in ash after incineration was over 98.7%. Similar study with gold solution and easily accessible biomass of *Sargassum sp.* (Sathishkumar et al. 2010a) confirms recovery efficiency more than 90%.

3.2 Catalysis Applications

Based on the basic knowledge of inorganic catalysis, noble metal NP catalysts are very attractive when compared to bulk catalyst – they have a high surface to volume ratios and their surface atoms are very active. For more knowledge and information about the noble metal nanocatalysists in colloidal solutions or adsorbed on different supports, we recommend numerous review articles, which have been published for many different types of organic and inorganic reactions (Narayanan 2010; Narayanan and El-Sayed 2008; Roucoux et al. 2008; Thibault-Starzyk et al. 2008; Kumar et al. 2004; Shiju and Guliants 2009).

3.2.1 Biosorption and Biosynthesis of Palladium Nanoparticles in Organic and Inorganic Catalysis

The first large group of biosynthesized nanomaterials with catalytic activity is represented by palladium NPs. Baxter-Plant et al. (2003; 2004) reported usage of cell surface of three different species of *Desulfovibrio* (G− sulfate-reducing bacteria) for manufacturing the novel bioinorganic catalyst via reduction process of Pd(II) to Pd(0). Although the presence of reducing agent is necessary (e.g. in form of H_2) for the Pd(0) genesis, reduction process is critically influenced by the bacteria presence and we can indicate this biosorption process as a biosynthesis. On the other hand, reduction in the absence of cells does not lead to the formation of Pd(0) NPs (Bunge et al. 2010). This catalyst on "palladised cells" was used for reductive dehalogenation of chlorophenol (CP) and selected polychlorinated biphenyl (PCB) types. The same organism was used for dehalogenation of the other environmentally prevalent PCBs and polybrominated diphenyl ether (Harrad et al. 2007). The versatility of "bioPd" catalyst is also demonstrated in various reactions including dehalogenation of flame retardants such polybrominated diphenyl ether (PBDE) or tris(chloroisopropyl)phosphate (TCPP). Authors also compare effectiveness between biocatalyst, chemically reduced Pd(II) and commercial Pd(0) catalysts. Although chemically reduced Pd(II) and commercial Pd(0) were more effective debromination agents, "bioPd" dechlorinated TCPP was five times more effective than using commercial Pd(0) catalyst (Deplanche et al. 2009) (Table 2).

Table 2 Catalysis applications

NP	Organism used	Application	Reference
Pd	*Desulfovibrio vulgaris, D. desulfuricans*	Dehalogenation of CP and PCBs	Baxter-Plant et al. (2003)
Pd	*D. desulfuricans*	Dehalogenation of CP and PCBs	Baxter-Plant et al. (2004)
Pd	*D. desulfuricans*	Dehalogenation PCBs and polybrominated diphenyl ether	Harrad et al. (2007)
Pd	*D. desulfuricans*	Dehalogenation of flame retardant materials	Deplanche et al. (2009)
Pd	*D. desulfuricans, Rhodobacter sphaeroides*	Dehalogenation of (PCBs) and penta-CP	Redwood et al. (2008)
Pd	*D. desulfuricans, Bacillus sphaericus*	Hydrogenation of itaconic acid	Creamer et al. (2007)
Pd	*D. desulfuricans, B. sphaericus*	Hydrogenation, reduction and selective dehalogenation in non-aqueous solvents	Creamer et al. (2008)
Pd	*R. capsulatas, Arthrobacter oxidans*	Hydrogenation of 2-Butyne-1,4-diol	Wood et al. (2010)
Pd	*Cupriavidus necator, Pseudomonas putida*	Suzuki–Miyaura and Mizoroki–Heck reactions	Sobjerg et al. (2009)
Pd	*C. necator*	Catalysis of C—C bond formation	Gauthier et al. (2010)
Pd	*C. necator, P. putida, Paracoccus denitrificans*	Hydrogen production from hypophosphite	Bunge et al. (2010)
Pd	*Gardenia jasminoides* Ellis	Hydrogenation of p-nitrotoluene	Jia et al. (2009)
Pd	*Shewanella oneidensis*	Dehalogenation of chlorophenol and PCBs	De Windt et al. (2005)
Pd	*S. oneidensis*	Dehalogenation of perchlorate and PCBs	De Windt et al. (2006)
Pd	*S. oneidensis*	Dechlorination of lindane	Mertens et al. (2007)
Pd	*S. oneidensis*	Dechlorination of TCE, membrane reactor	Hennebel et al. (2009b)
Pd	*S. oneidensis*	Dechlorination of TCE, fixed bed reactor	Hennebel et al. (2009c)
Pd	*S. oneidensis*	Degradation process for diatrizoate, ICM	Hennebel et al. (2010)
Pd, Pt	*D. desulfuricans*	Continuous reduction Cr(VI) to Cr(III)	Mabbett et al. (2005)
Pd	*D. vulgaris, D. desulfuricans*	Reduction of Cr(VI) to Cr(III)	Humphries et al. (2006)

(continued)

Table 2 (continued)

NP	Organism used	Application	Reference
Pd	*Serratia sp.* (NCIMB)	Reduction of Cr(VI) to Cr(III)	Beauregard et al. (2010)
Pd	*E. coli* mutant strains	Reduction of Cr(VI) to Cr(III)	Deplanche et al. (2010)
Pd	*Clostridium pasteurianum*	Reduction of Cr(VI) to Cr(III)	Chidambaram et al. (2010)
Pt	waste yeast biomass	Fuel cell; energy production	Dimitriadis et al. (2007)
Pd, Pt	*D. desulfuricans*	Fuel cell; energy production	Yong et al. (2007)
Pd	*D. desulfuricans, E. coli, C. metallidurans*	Waste biorefining, fuel cells	Yong et al. (2010)
Pd	*E. coli* MC4100 (parent), mutant (IC007)	Fuel cell; energy production	Orozco et al. (2010)
Pd	*S. oneidensis*	Fuel cell; energy production	Ogi et al. (2011)
Au	*Sesbania drummondii*	Reduction of 4-nitrophenol	Sharma et al. (2007)
Au	*Cacumen platycladi*	Reduction of 4-nitrophenol	Huang et al. (2009)
Ag	*Sepia esculenta* cuttle-bone organic matrix	Reduction of 4-nitrophenol	Jia et al. (2008)

Using of *Desulfovibrio desulfuricans* in comparison with other bacterial strains has been also demonstrated: Redwood et al. (2008) reported comparison of catalytic efficiency of and *Rhodobacter sphaeroides* in dehalogenation of PCBs and penta-CP. Gram negative (G–) and Gram positive (G+) bacterial strains *D. desulfuricans* and *Bacillus sphaericus* took place as Pd(II) reducing agent for catalysis of itaconic (methylene succinic) acid (Creamer et al. 2007). Remarkably, the same research group published experiments in non-aqueous solvents (methanol). Specifically, experiments leading to hydrogenations of 4-azidoaniline hydrochloride and 3-nitrostyrene, and hydrogenolysis (reductive debromination) of 1-bromo-2-nitrobenzene were conducted (Creamer et al. 2008).

Another type of G– bacteria, *Shewanella oneidensis*, was also used for biofabrication of Pd(0) catalyst (with H_2, formate, lactate, pyruvate or ethanol as electron donors) for dehalogenation purpose (De Windt et al. 2005). The obtained bioPd(0) NPs had the ability to reductively dehalogenate (PCB) congeners in aqueous and sediment matrices from anonymous industrial plant. Moreover, the aforementioned paper offers a comparison with commercially available palladium powders. Further studies of *S. oneidensis* show differences between catalytic reactivity of Pd(0) crystals formed on viable or non-viable biomass. The relatively large and densely covering Pd(0) crystals (non-viable biomass) exhibited high catalytic reactivity towards hydrophobic molecules such as polychlorinated biphenyls. In contrast, the smaller and more dispersed nanocrystals on a viable bacterial carrier were catalytically active towards anionic pollutant perchlorate (De Windt et al. 2006).

S. oneidensis bacterial strain was also used for removal of the pesticide lindane (γ-hexachlorocyclohexane or γ-HCH) by catalytic reduction of γ-HCH to benzene (as more efficient than with commercial powdered Pd(0) – Mertens et al. 2007). The same study introduces a membrane reactor technology suitable for dechlorination of γ-HCH polluted wastewater at a low-flux synthetic dialysis membrane. Similar implementation of membrane reactor was introduced for degradation process of diatrizoate – iodinated contrast media (ICM). Although currently applied techniques such as advanced oxidation processes exhibit only limited removal efficiencies of ICM, work by Hennebel et al. (2010) showed that membrane contactors with encapsulated biogenic NPs can be effective for contaminated water treatment. Topic of reactor technology for "bioPd" catalysts is further pursued in works dealing with dechlorination of trichloroethylene (TCE) in a pilot-scale membrane reactor (Hennebel et al. 2009b) and dechlorination of TCE by encapsulated palladium NPs in a fixed bed reactor (Hennebel et al. 2009c). Polyurethane cubes empowered with "bio-Pd" were implemented in a fixed bed reactor for the treatment of water containing TCE. This study shows that the influent recycle configuration resulted in a cumulative removal of 98% TCE after 22 h (with ethane as main reaction product). The same reactor in a flow through configuration achieved removal rates up to 1,059 mg TCE g $(Pd)^{-1} \cdot day^{-1}$.

Feasibility of another organisms for reduction of Pd(II) to Pd(0) for organic catalysis was also demonstrated by other studies. Bacterial strains *Rhodobacter capsulatas* and *Arthrobacter oxidans* were employed in "bioPd" formation for partial hydrogenation of 2-butyne-1,4-diol to 2-butene-1,4-diol (Wood et al. 2010). This "bioPd" was proven to be a highly selective catalyst for partial hydrogenation reactions. Bunge et al. (2010) tested possibilities of three bacterial strains (*Cupriavidus necator, Pseudomonas putida, Paracoccus denitrificans*) on bioPd(0) catalysis of hydrogen production from hypophosphite and further discuss the hypothetical mechanism of bacterial reduction of Pd(II) to Pd(0). Remarkably, Pd(0) catalysts fabricated by the organisms mentioned above were used also for catalysis of Suzuki–Miyaura and Mizoroki–Heck reactions (briefly C—C bond formation) by Sobjerg et al. (2009) and Gauthier et al. (2010). The enormous importance of these reactions for organic synthesis may be confirmed by the long-anticipated Nobel prize in Chemistry 2010 for their discoverers – Richard F. Heck, Ei-ichi Negishi and Akira Suzuki (more about these reactions and NPs in review article by Narayanan (2010)). Moreover, aforementioned studies also contribute to the hot issue of metal waste management and waste recovery.

Interestingly, Jia et al. (2009) published bioreduction method – reduction of palladium chloride by water crude extract – with plant *Gardenia jasminoides* Ellis'. Abilities of this "bioPd(0)" nanocatalyst were tested and documented on hydrogenation reaction of p-nitrotoluene. The catalysts showed a conversion of 100% under conditions of 5 MPa, 150°C for 2 h. The selectivity of the product – p-methylcyclohexylamine – achieved 26.3%. The "bioPd(0)" catalyst was recycled five times without any agglomeration and with highly maintained activity.

It is also well known that aforementioned bacterial species such as *Shewanella alga*, *Pseudomonas putida* or *Desulfovibrio vulgaris* (Mabbett et al. 2002) may be

used to biologically treat contaminated wastewaters by reduction of Cr(VI) – known as carcinogen and mutagen – to Cr(III) – relatively non-toxic and non-carcinogenic form. Nevertheless, some studies showed that "bio-Pd(0)" is more efficient at Cr(VI) reduction than live cells of *D. desulfuricans* or chemically reduced Pd(II), using hydrogen as the electron donor (Mabbett and Macaskie 2002). Pd(0) mediates hemolytic bond cleavage of H_2, with the production of radical H^*, which can then donate its electron to Cr(VI). Continuous-flow studies using *D. vulgaris* Bio-Pd(0) with agar as the immobilization matrix were also investigated (Humphries et al. 2006), showing the effect of Bio-Pd(0) loading, inlet Cr(VI) concentration, and flow rate on the efficiency of Cr(VI) reduction. Mabbett et al. (2005) presents possibility of mixed-metal-bioPd(O) catalysts employing *D. desulfuricans*, Pd(II) and Pt(IV) or industrial waste leachates (contains e.g. Rh, Cu, Fe, Al, Pt). Two flow-through reactor systems were also compared by aforementioned work. Similar experiments were performed by Beauregard et al. (2010) using *Serratia sp.* and formate as the electron donor. Remarkably, Cr species concentrations within the reactor were controlled by spatial mapping using magnetic resonance imaging technique ($Cr(VI)_{(aq)}$ is non-paramagnetic while $Cr(III)_{(aq)}$ is paramagnetic).

Moreover, Chidambaram et al. (2010) published experiments where the electron donor is substituted by fermentation process (fermentatively produce hydrogen in presence of glucose) in bacteria *Clostridium pasteurianum*, which also serves for reduction Pd(II) ions to form PdNPs "bio-Pd(0)" that primarily precipitated on the cell wall and in the cytoplasm. Finally, the most scientifically explored organism in the world, *Escherichia coli* (or its mutants), contribute to the "bioPd(0)" catalyst knowledge. Experiments with three types of hydrogenases encoded by bacterial DNA were performed by Deplanche et al. (2010), based on optimal catalytic activity in Cr(VI)/Cr(III) system.

3.2.2 "BioPd(0)" and "BioPt(0)" as a Fuel Cells Electro-Catalysts

Similar approach to utilization of microorganisms with ability to enzymatically reduce and absorb palladium, platinum and other precious metals was also used for manufacturing of a bio-fuel cell for power production. Since fuel cells have been identified as a future technology to power motor vehicles, generators and portable electronic device, authors recommend overall review papers (Andújar and Segura 2009; Winter and Brodd 2005) for more information and historical context in fuel cells topic.

In work by Yong et al. (2007) Pt(0) and Pd(0) bio-accumulated by *D. desulfuricans* was applied onto carbon paper and tested as anodes in a polymer electrolyte membrane (PEM) fuel cell for power production and compared to commercial fuel cell grade Pt catalyst. A similar strategy is also suggested using yeast-based biomass, immobilized in polyvinyl alcohol cryogels, for the manufacture of fuel cell Pt(0). This is then used to generate electrical energy from renewable sources such as glucose and ethanol (Dimitriadis et al. 2007). Finally, the dried biomass-supported palladium (*Shewanella oneidensis*) was tested as an anode catalyst in a PEM fuel

cell for power production. It was shown to have a maximum power generation comparable to the commercial catalyst (Ogi et al. 2011).

Fusion of waste biorefining and cheap nanocatalyst for fuel cells and power generation employing *D. desulfuricans, E. coli* and *C. metallidurans*, carbon paper and proton exchange membrane fuel cell was recently published (Yong et al. 2010). Using an *E. coli* MC4100 strain, a mixed metallic catalyst was manufactured from an industrial processing waste. This mixed-metal biocatalyst gave approximately 50% of the power output compared to commercial or "bioPd" *D. desulfuricans* catalyst. Electrical energy production efficiency of biocatalyst fabricated by aforementioned *E. coli* (parent) strain and its derived mutant strain IC007 (as well as a comparison with *D. desulfuricans*) is further discussed by Orozco et al. (2010).

Another electrocatalysis application with modified electrodes is also mentioned in Sect. 3.5.1.

3.2.3 Catalysis of 4-Nitrophenol Reduction Reactions

Presence of toxic pollutants such as nitro-aromatic compounds in soil and water is a result of incomplete combustion of fossil fuels and their usage as chemical feedstock for synthesis of explosives, pesticides, herbicides, dyes, pharmaceuticals, etc. The headlong and reckless utilization of these pollutants in the past has resulted in wide-ranging environmental pollution. The usage of biosynthesized NPs capable to catalyze degradation of, among other chemicals, nitro-aromatics (and then together with microbial remediation), would be a great contribution to this particularly topical issue (take a look at the following reviews for more information – Kulkarni and Chaudhari 2007; Liotta et al. 2009; Lewis et al. 2004; Guimarães et al. 2010).

As the first report of a NP-bearing biomatrix directly reducing a toxic pollutant 4-nitrophenol (*p*-nitrophenol; 4-NP), Sharma et al. (2007) published experiments of growth of *Sesbania* seedlings in chloroaurate Au(III) solution. This procedure resulted in the accumulation of gold with the formation of stable AuNPs in plant tissues. The catalytic effectiveness of the biomass with Au(0)NPs was documented by the reduction of aqueous 4-nitrophenol (4-NP).

Remarkably, extensive research was performed using 21 traditional Chinese medicinal plant and herb species (Huang et al. 2009). After classification into four categories including leaves, flowers, fruits, and grasses, effectiveness of the protocol in producing AuNPs was demonstrated usually after 30 min of incubation with aqueous $HAuCl_4$. Potential application of the these biogenic AuNPs as catalysts was exhibited in *Cacumen Platycladi* (which exhibited biosynthesis of very small and monodisperse NPs). Catalytic reduction of 4-NP showed excellent catalytic performance compared to the aforementioned study (Sharma et al. 2007).

For instance of AgNPs, Jia et al. (2008) reported the use of a cuttlebone-derived organic matrix (from *Sepia esculenta*) as scaffold and reducer for the formation of AgNPs. The resulting composite was applied to catalyze the reduction of 4-NP. Possibilities of separation from the liquid-phase reaction and reusability in more cycles have been also reported.

3.3 Medical Applications

The application of metallic NPs in the medical and biopharmaceutical fields are both numerous and promising. AgNPs can be utilized as infection protection (wound coatings, bone cements and implants) or prophylactic environment (paints, disinfectants) due to their antibacterial effects (see specialized section [Sect. 3.4] for detail information). Other qualities of silver include regenerative properties (skin regeneration) and wound-healing ability (dressing for burns and ulcers) (Chaloupka et al. 2010). Another review article by Nair and Laurencin (2007) is dedicated to therapeutic applications of AgNPs, particularly to their antibacterial, antiviral, antifungal properties (remarkably, with regard to properties of bulk silver). Additionally, wound healing ability and the potential usage for surface enhanced Raman scattering (SERS) and metal enhanced fluorescence (both powerful tools for detection and identification) is discussed (Table 3).

The authors also recommend two recent review articles dealing with nanogold pharmaceutical applications. The first article by Alanazi et al. (2010) describes properties such as surface plasmon absorption, surface plasmon light scattering, and the biosensing, diagnostic and therapeutic applications of AuNPs. The second review by Patra et al. (2010) concerns the specific application and fabrication of AuNPs for targeted therapy in pancreatic cancer.

Also magnetic NPs appear as very promising for targeted drug delivery or hyperthermia applications. For further reading, see the prominent review by Pankhurst et al. (2003) and its continuance (Pankhurst et al. 2009) or other useful studies by the following: (Cherukuri et al. 2010; Adarsh et al. 2010; Veiseh et al. 2010; Chen and Schluesener 2008). On the other hand, we strongly disagree with the statement and references dedicated to medical applications of biosynthesized metallic NPs in paper by Rodríguez-Carmona and Villaverde (2010). Cited literature is misleading and, excluding one study, has nothing to do with proposed applications.

Despite wide possibilities and theoretical application, only few records can be found in recent literature. As previously mentioned, the obvious drawback of

Table 3 Medical applications

NP	Organism used	Application	Reference
Au	*Cymbopogon citratus* – lemon grass	NIR tunable absorption; coating technology and hyperthermia of cancer cells	Shankar et al. (2005)
Au	*Psidium guajava* leaf	Antidiabetic study, inhibition of PTP1B	Basha et al. (2010)
Ag	Tea leaf extract	Biocompatible	Moulton et al. (2010)
Fe_3O_4	*Magnetospirillum gryphiswaldense*	Drug carrier	Sun et al. (2008)
Ag	*Aspergillus niger*	Wound healing activity	Sundaramoorthi et al. (2009)
Au	Egg shell	Blood serum glucose sensor	Zheng et al. (2011)

biological methods is the need to purify the sample and extract the NPs. This is due to potential pathogens or poisons that might contaminate material and is particularly important for medical applications. Despite these problems, we can still find some useful studies and potential applications.

Biocompatibility is a very important property for all material possibly dealing with medical usage in living organisms. Moulton et al. (2010) reported biosynthesis experiments with tea leaf extract as a reducing and capping agent for AgNP fabrication. Evaluation of mitochondrial function to assess cell viability and membrane integrity in human keratinocytes showed that the AgNPs were nontoxic. This may be attributed to the tea antioxidants on the NP surface. Although this method of synthesis appears to be promising based on the initial *in vitro* studies, they need to be followed by future *in vivo* tests to accurately evaluate the biocompatibility.

Wound healing activity of AgNPs, synthesized extracellularly using *Aspergillus niger*, was evaluated on rat model for case of excision wound and thermal wound (Sundaramoorthi et al. 2009). Researchers illustrated the efficient antimicrobial property of AgNPs and also confirmed the ability of nanosilver to modulate the cytokines involved in wound healing.

Shape of NPs can critically influence their properties, especially the optical. Gold nanotriangles with tunable size were biofabricated by a simple method involving the reduction of aqueous gold ions with an extract of the lemongrass plant (Shankar et al. 2005). Interestingly, absorption in the near-infrared region of the electromagnetic spectrum is "expected to be of application" in hyperthermia of cancer cells and in infra red (IR) – absorbing optical coatings.

As an elegant application of bacterial magnetosomes (BM), Sun et al. (2008) presented experiments leading to employment of BM from *Magnetospirillum gryphiswaldense* as a chemotherapy drug carrier (doxorubicin was loaded isolated and cleaned BMs using a bifunctional crosslinker). As drug efficiency and toxicity may be significantly altered by structural modification, evaluation of the drug effect (drug coupled with BM) was also performed. The antitumor effects of doxorubicin loaded BMs were evaluated by HL60 and EMT-6 carcinoma cells. They were cytotoxic to the cancer cells, inhibited cancer cell proliferation and suppressed the mRNA levels of the significant oncogene *c-myc*. The drug releasing process from BMs was also monitored. The assets of this approach (compare to artificial magnetic particles) may be the ability to carry larger amounts of drug, ease for preparation and dispersion, high stability and more uniformity. Being surrounded with membrane that consists of lipids and proteins, purified and sterilized magnetosomes were not toxic to mouse fibroblasts in vitro (Li et al. 2007). This indicates the advantage of biocompatibility.

Another medical application deals with diabetes, particularly with inhibion of enzyme protein tyrosine phosphatase (PTP), type PTP1B. Disturbance of the normal balance of PTP function has been implicated as the source of several human diseases, including diabetes, cancer, and inflammation (Tonks 2003). The rapid formation of AuNPs with guavanoic acid from the leaf extract of *Psidium guajava* was reported by Basha et al. (2010). These were used in an antidiabetic PTP 1B inhibitory assay and showed significant inhibitory effect with an IC_{50} of 1.14 μg/mL.

Zheng et al. (2011) reported the biofabrication of AuNPs (by means of egg shell membrane) and their application in the glucose biosensor for blood glucose determination (further information in Sect. 3.5.1 and Zheng et al. (2010a)). The biosensor has been also applied to measure the glucose content in human blood serum samples (showing agreement with a standard routine medical spectrophotometric test method).

3.4 Antimicrobial Applications

Although this chapter may have been included in discussion of medical usages (Sect. 3.3), authors have decided to create a new section to address the increasingly large amount of studies being published in this area (Table 4).

Table 4 Antimicrobial applications

NP	Organism used	Application	Reference
Ag	*P. aeruginosa*	Activity vs. G+ and G– bacteria	Suresh et al. (2010)
Ag	*Penicilium sp.*	Activity vs. G+ and G– bacteria	Maliszewska and Puzio (2009)
Ag	*Pleurotus sajor caju*	Activity vs. G+ and G– bacteria	Nithya and Ragunathan (2009)
Ag	*Bipolaris nodulosa*	Activity vs. G+ and G– bacteria	Saha et al. (2010)
Ag	*Streptomyces sp*	Activity vs. G+ and G– bacteria	Shirley et al. (2010)
Ag	*Aspergillus oryzae* var. *viridis*	Activity vs. *S. aureus*	Binupriya et al. (2009)
Au	*Trichoderma viride*	Activity vs. VRSA	Fayaz et al. (2011)
Ag	*T. viride*	Vegetable and fruit preservation	Fayaz et al. (2009)
Ag	*Aspergillus clavatus*	Activity vs. MRSA, MRSE	Saravanan and Nanda (2010)
Ag	*Garcinia mangostana* (Mangosteen) leaf	Activity against *E. coli, S. aureus*	Veerasamy et al. (2011)
Ag	*Candida albicans, E. coli, B. cereus, P. fluorescens*	Activity against *E. coli*	Ul'berg et al. (2010)
Ag	*Phoma glomerata*	Synergy with antibiotics	Birla et al. (2009)
Ag	*Opuntia ficus-indica*	Effect in combination with antibiotics	Gade et al. (2010)
Ag	*T. viridae*	Effect in combination with antibiotics	Fayaz et al. (2010a)
Ag	*Fusarium acuminatum*	Activity vs. G+ and G– bacteria	Ingle et al. (2008)

(continued)

Table 4 (continued)

NP	Organism used	Application	Reference
Ag	Varied microalgae species	Activity vs. G+ and G− bacteria	Merin et al. (2010)
Ag	*Acalypha indica* leaf	Activity vs *E. coli, Vibrio cholerae*	Krishnaraj et al. (2010)
Ag	*Fusarium oxysporum*	Cotton fabrics incorporated with AgNPs	Durán et al. (2007)
Ag	*Fusarium solani*	Cotton fabrics incorporated with AgNPs	El-Rafie et al. (2010)
Ag	*Azadirachta indica* (Neem) leaf	Bactericidal effect in cotton cloth against *E. coli*	Tripathi et al. (2009)
Ag	*Cinnamon zeylanicum* bark	Activity against *E. coli* BL-21	Sathishkumar et al. (2010a)
Ag	*Curcuma longa* tuber	Immobilization on cotton cloth	Sathishkumar et al. (2010a, b)
Ag	*Eucalyptus citriodora, Ficus bengalensis*	Antibacterial activity against *E. coli,* loaded on the cotton fibres	Ravindra et al. (2010)
Ce	*Leptothrix discophora, Pseudomonas putida*	Activity against bacteriophage UZ1	De Gusseme et al. (2010a)
Ag	*Lactobacillus fermentum*	Activity against bacteriophage UZ1	De Gusseme et al. (2010b)
Ag	*Amylomyces rouxii*	Antifungal and antibacterial activity	Musarrat et al. (2010)
Ag	*S. hygroscopicus*	Antifungal and antibacterial activity	Sadhasivam et al. (2010)
Ag	*A. niger*	Antifungal and antibacterial activity	Jaidev and Narasimha (2010)
Ag	*Sesuvium portulacastrum* callus and leaf	Antifungal and antibacterial activity	Nabikhan et al. (2010)
Ag	*Alternaria alternate*	Activity in combination with fluconazol	Gajbhiye et al. (2009)
Au	Genus *Musa* – banana peel	Antifungal and antibacterial activity	Bankar et al. (2010)
Au	*Rhizopus oryzae*	Antifungal and antibacterial activity	Das et al. (2009)
Ag	*Aspergillus clavatus*	Antifungal and antibacterial activity	Verma et al. (2010)
Ag	*Solanum torvum*	Antifungal and antibacterial activity	Govindaraju et al. (2010)

Due to the outbreak of infectious diseases caused by different pathogenic bacteria and fungi and the development of antibiotic or metal resistant strains, there is increasing need to find new antibacterial products. Although different types of

nanomaterials like titanium, copper, magnesium or alginate have promising antibacterial properties, Au and AgNP have showed best efficiency against bacteria, viruses and fungi (Rai et al. 2009). The broad-spectrum antimicrobial properties of metallic NPs (mostly silver and gold) encourage their use as disinfectants in purification processes (medicine, water and air), food production, cosmetics, clothing, and numerous household products (Marambio-Jones and Hoek 2010).

In this section, we will illustrate the antibacterial, antiviral and antifungal effects of biosynthesized NPs. This application promises to be very beneficial for both the industrial and medical fields. For further reading on AgNPs utilization and silver mechanism of action, see aforementioned review articles – Marambio-Jones and Hoek (2010), Rai et al. (2009), Nair and Laurencin (2007) or review papers by Sharma et al. (2009), Cho et al. (2005).

3.4.1 Antibacterial Activity

Bacteria have different membrane and cell wall structures. Therefore, we can classify them generally as Gram-negative (G−) or Gram-positive (G+). The key component of the membrane, peptidoglycan, is a decisive factor in the membrane organization. G− bacteria has only a thin peptidoglycan layer (~2–3 nm) between their two membranes, while G+ bacteria lack the outer membrane (is substituted by thick peptidoglycan layer).

Morones et al. (2005) published study regarding possible interactions between AgNPs and G− bacteria. Small NPs disturb the function of the membrane (such as permeability or respiration) by attaching to it's surface and, subsequently, penetrate the cell and cause further damage by interacting with the DNA.

Spectrum of organisms used for biosynthesis of NPs with antibacterial effect varies from bacteria, fungi and alga to leaf, root, bark and tuber extracts of higher plants and trees. As one of the first records, Ingle et al. (2008) reported a mycosynthesis of silver antibacterial NPs with biological activity against different human pathogens including multidrug resistant and highly pathogenic bacteria such as *Staphylococcus aureus, Salmonella typhi, Staphylococcus epidermidis*, and *Escherichia coli*. Similarly, fungal strain *Aspergillus clavatus* was used for extracellular biosynthesis of stable AgNPs with antibacterial activity against methicillin (antibiotics) resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* (Saravanan and Nanda 2010). Antibacterial activity against *Staphylococcus aureus* KCCM 12256 was also observed in case of AgNPs biosynthesized by filamentous mold *Aspergillus oryzae* (Binupriya et al. 2009). Bioreductive synthesis of nanosized Ag particles was performed using live and dead cell filtrates with NP size varying from 5 to 50 nm. Another phytopathogenic fungal specie *Bipolaris nodulosa* can serve as reducing agent for silver nitrate reduction with resulting Ag NPs active against *Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli* and *Micrococcus luteus* pathogens (Saha et al. 2010). Ag NPs biosynthesized by gilled mushroom specie Pleurotus sajor-caju (Nithya

and Ragunathan 2009) can serve against *Pseudomonas aeruginosa, Escherichia coli* (G−) and *Staphylococcus aureus* (G+). Identical bacterial species were used in a similar study (Maliszewska and Puzio 2009) employing the famous genus of ascomycetous fungi, *Penicillium sp*, with major importance in the natural environment as well as food and drug production.

Fungal plant pathogen *Phoma glomerata* was employed in synthesis of Ag NP and, together with antibiotics, proved effective against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* (Birla et al. 2009). Synthesized NPs showed comprehensive bactericidal activity against the aforementioned G− and G+ bacterial species and enhanced the antimicrobial activity of used antibiotics (ampicillin, gentamycin, streptomycin and vancomycin). Interestingly by using gold, a mold species *Trichoderma viride* (widely used as bio-fungicide) was used to biosynthesize vancomycin bound NPs and exhibited activity against vancomycin resistant *Staphylococcus aureus*, vancomycin sensitive *S. aureus* and *E. coli* (Fayaz et al. 2011). Additionally, all experiments were performed as comparison between vancomycin bound AuNPs and vancomycin as such.

Streptomyces sp. bacterially derived AgNPs were reported (Shirley et al. 2010) as biologically active against seven species of both G+ and G− bacteria (*Staphylococcus aureus, S. epidermidis, E. coli, S. typhi, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris)*. Also, already known metal reducing G− bacteria *Shewanella oneidensis* was used for silver nanocrystallites biofabrication (Suresh et al. 2010). Bacterial toxicity assessments showed that prepared biogenic Ag NPs have a greater bactericidal activity on *E. coli*, *S. oneidensis*, and *B. subtilis* strains than chemically synthesized colloidal-AgNPs. Ul'berg et al. (2010) reported usage of four bacterial species leading to bio-AgNPs active against *E. coli*.

Photosynthetic organisms are in antimicrobial biofabrication represented by algae, plants and trees. Four species of marine microalgae (normal and microwave irritated) were used in comparison and assessment of antimicrobial properties of resulting AgNPs against human pathogens *Escherichia coli, Klebsiella sp, Proteus vulgaricus, Pseudomonas aeruginosa* (Merin et al. 2010). Also higher plants can take a place in NP synthesis. Leafs extract of Garcinia mangostana (Mangosteen) were employed in AgNPs biofabrication and the antibacterial assays were done on human pathogenic *E. coli* and *Staphylococcus aureus* by standard disc diffusion method with considerable results (Veerasamy et al. 2011). Krishnaraj et al. (2010) investigated biosynthesis of AgNPs and its activity on water borne bacterial pathogens (*E. coli* and *Vibrio cholerae*). During the antibacterial experiments, alteration in membrane permeability and respiration of the AgNP treated bacterial cells were recorded.

Gade et al. (2010) reported *Opuntia ficus-indica* mediated synthesis of colloidal AgNPs and their antimicrobial assessment in combination with commercially available antibiotics (the maximum activity was demonstrated by ampicillin followed by streptomycin and vancomycin). Similarly, the extracellular biosynthesis of AgNPs from silver nitrate solution by fungus *Trichoderma viride* is reported (Fayaz et al. 2010a). Increasing of their antimicrobial activities with various antibiotics against gram-positive and gram-negative bacteria was described. Although antibacterial

activities of ampicillin, kanamycin, erythromycin, and chloramphenicol were increased in the presence of AgNPs against test strains, ampicillin showed the highest enhancing effect.

Also food-storage possibilities of biofabricated NPs were examined using the same organism (Fayaz et al. 2009). Antibacterial activities of AgNP-incorporated sodium alginate films were tested against *E. coli* ATCC 8739 and *S. aureus* ATCC 6538 strains – disk method exhibited antibacterial activity against both G+ and G– bacteria. Antimicrobial coating was applied to carrot and pear surface and conservation impact was examined compare to untreated samples.

3.4.2 Antifungal and Combined Activity

Although there are reports of biosynthesized NPs with antifungal activity, they usually exhibit it only in combination with antibacterial activity. It is in this context, then, that they will be mentioned in the following text.

Mycelia-free water extracts from *Amylomyces rouxii* facilitated the production of stable, monodispersed and spherical AgNPs (size range of 5–27 nm). Biosynthesized AgNPs exhibited antimicrobial activity against bacterial (*Shigella dysenteriae type I, Staphylococcus aureus, Citrobacter sp., E. coli, Pseudomonas aeruginosa, Bacillus subtilis*) as well as fungal (*Candida albicans, Fusarium oxysporum*) species. Biological reduction of aqueous silver ions by extracellular components of *Streptomyces hygroscopicus* (Sadhasivam et al. 2010) resulted in AgNPs which significantly inhibited the growth of medically important pathogenic gram-positive bacteria (*Bacillus subtilis, Enterococcus faecalis*), gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*) and yeast (*Candida albicans*). The colloidal AgNPs biosynthesized with filtrate from *Aspergillus niger* inhibited the growth of the fungus itself (seeded in the nutrient agar plate). Potential antifungal activity was due to inactivation of sulfhydryl groups in the fungal cell wall and disruption of membrane bound enzymes and lipids which causes the cell lysis (Jaidev and Narasimha 2010). Antibacterial activity against both G+ (*Staphylococcus sp., Bacillus sp.*) and G– (*E. coli*) bacterial species was observed. Similar results were obtained employing *Aspergillus clavatus* (against *Candida albicans, Pseudomonas fluorescens* and *Escherichia coli*) by Verma et al. (2010). Also, Govindaraju et al. (2010) published study utilizing *Solanum torvum* as mediator for biosynthesis of AgNPs eliciting antibacterial activity against pathogenic bacteria *Pseudomonas aeruginosa*, *Staphylococcus aureus*, pathogenic fungi *Aspergillus flavus* and *Aspergillus niger*.

Interestingly, extracts from tissue culture-derived callus and leaf of the saltmarsh plant *Sesuvium portulacastrum* were used for AgNPs growth (Nabikhan et al. 2010). The antibacterial activity (*Pseudomonas aeruginosa, Staphylococcus aureus, Listeria monocytogenes, Micrococcus luteus, Klebsiella pneumoniae*) was more distinct compared to antifungal (*Alternaria alternata, Penicillium italicum, Fusarium equisetii*, *Candida albicans*) activity. Moreover, antimicrobial activity was enhanced when polyvinyl alcohol was added as a stabilizing agent (in comparison with samples prepared with distilled water).

Study of antifungal properties against large group of fungal species (*Phoma glomerata, Phoma herbarum, Fusarium semitectum, Trichoderma sp., Candida albicans*) in combination with triazole antifungal drug fluconazol was published by Gajbhiye et al. (2009). Biosynthesized AgNPs (by phyto-pathogenic fungus *Alternaria alternata*) enhanced antifungal activity of fluconazole against the test fungi (showing maximum inhibition against *C. albicans*, followed by *P. glomerata* and *Trichoderma sp*.). However, no significant enhancement was found against *P. herbarum* and *F. semitectum*.

Last but not least, formation of AuNPs with antifungal activity was also described. As a contribution to the water hygiene and treatment management, Das et al. (2009) describes simple on step procedure to obtain potable water free of pathogens and pesticides. AuNPs (10 nm average) were produced on the surface of fungus *Rhizopus oryzae* and showed antimicrobial activity against several G− and G+ pathogenic bacteria as well as the yeasts *Saccharomyces cerevisiae* and *Candida albicans*. Simulated contaminated water containing organophosphate pesticides (malathion, parathion, chloropyrifos, and dimethoate) along with E. coli was treated with gold bionanoconjugate and successful removal of contaminants was monitored by means standard disk method (*E. coli*) and gas chromatography analysis (pesticides). AuNPs with antibacterial (*Citrobacter kosari, Escherichia coli, Proteus valgaris, Pseudomonas aeruginosa, Enterobacter aerogenes and Klebsiella sp.*) and antifungal (*Candida albicans*) activity were also obtained employing banana peel extract (Bankar et al. 2010).

3.4.3 Antiviral Activity

Although humankind is waging an ongoing war against viruses in such wide ranging fields as medicine and agriculture, there has been only one recorded study about antiviral activity of biosynthesized NPs. Although not of the biosynthesized nature, the post-infected anti-HIV-1(BaL) activities of AgNPs (prepared chemically, 10 nm) toward Hut/CCR5 cells (cells derived from a human T cell line, which express the chemokine receptor CCR5) were evaluated by Sun et al. (2005). When compared to the control sample, AgNPs showed dose-dependent anti-retrovirus activities and showed high activity (at 50 mM – 98%) in inhibiting HIV-1 replication (for comparison, the AuNPs exhibited relatively low anti-HIV-1 activities 6–20%). This is an interesting example of the use of strictly chemically fabricated NPs. Remarkably, De Gusseme et al. (2010a) published study of virus removal by biogenic rare earth element cerium (produced by addition of aqueous Ce(III) to actively growing cultures of either freshwater manganese-oxidizing bacteria *Leptothrix discophora* or *Pseudomonas putida*). A model organisms for the antiviral assay was bacteriophage UZ1 (bacteriophage specific for common pathogenic bacterium *Enterobacter aerogenes*).

In study by the same research group (De Gusseme et al. 2010b), *Lactobacillus fermentum* served as a reducing agent and carrier matrix for AgNPs. The antiviral

qualities of biogenic AgNPs was confirmed in water containing aforementioned bacteriophage UZ1 (bacteriophage specific for common pathogenic bacterium *Enterobacter aerogenes*) and murine norovirus 1 (a model organism for human noroviruses). For continuous disinfection capability in water environment, the biogenic material was applied to electropositive filter (NanoCeram) and exhibited higher antiviral activity in comparison with the results obtained with the original filter.

3.4.4 Antibacterial Fabrics and Cloth

Another interesting utilization for biosynthesized NPs is immobilization on cotton cloth or cotton fibers. This approach demonstrates the possible use of such cloth in disinfection or sterilization.

As the first record, Durán et al. (2007) reported the extracellular production of AgNPs by fungus *Fusarium oxysporum* and antimicrobial effect of the NPs incorporated in cotton fabrics against *Staphylococcus aureus*. Moreover, effluent from impregnated fabrics (after several washing cycles) was treated with the suspension of *Chromobacterium violaceum*, metal binding bacteria, to reabsorb released NPs. Using a similar procedure and organism, fungi *Fusarium solani*, El-Rafie et al. (2010) prepared AgNPs and applied them to cotton fabrics with and without binder. The bleached cotton fabrics were padded through silver colloidal bath and squeezed with laboratory padder. Following the incorporation of a binder, after 20 washing cycles the material still exhibited effectiveness in antibacterial activity against *Staphylococcus aureus* and *E. coli*.

Azadirachta indica (Neem) is genus from mahogany family *Meliaceae*. Tripathi et al. (2009), studied the biosynthetic production of AgNPs by aqueous extract of Neem leaves and their immobilization on cotton cloth. Subsequently, utilizing standard disk method (including effect of consecutive washing in distilled water), their bactericidal effect against *E. coli* was observed. NP incorporation into cotton disks was performed by three approaches: (a) centrifuging the disks with liquid extract containing biosynthesized NPs; (b) *in-situ* coating process during synthesis, and (c) coating with dried and purified NPs. Antibacterial effect against E. coli BL-21 strain was also tested with AgNPs prepared by means of phyto-reductive extract and powder of *Cinnamon zeylanicum* (Sathishkumar et al. 2009) and *Curcuma longa* tuber (Sathishkumar et al. 2010b). The second work presents immobilization of AgNPs on cotton cloth. NPs were resuspended in water or polyvinylidene fluoride (PVDF) and sprayed over the pre-sterilized white cotton cloth in aseptic condition. PVDF immobilized cloth exhibited less antibacterial activity. However, consecutive washing drastically reduced antibacterial effectiveness of AgNPs immobilized in sterile water.

Topic of antibacterial NP containing cloth can be very promising for biofabrication approach and whole of nanotechnology. For further reading see following reviews: Dastjerdi and Montazer (2010), Perelshtein et al. (2008), Simoncic and Tomsic (2010).

3.5 Electrochemical and Sensing Applications

Metallic NPs are at the centre of intense research because an understanding of their surface chemistry might play a key role in effective utilization of technologies such as nanosensor, biosensor, electrocatalysis, nanodevice and nanoelectrochemistry (Guo and Wang 2007). From the view of electroanalytical chemistry, more attention has been paid to AuNPs because of their good biological compatibility, excellent conducting capability and high surface-to-volume ratio (Daniel and Astruc 2004). Usage of AuNPs in electrochemical interfaces has contributed to new vigor in electrochemistry. Development of new techniques and different electrode modified strategies may potentially enhance analytical selectivity and sensitivity of commonly used facilities (Xiong et al. 2007; Nguyen et al. 2011).

While not yet containing any direct applications, there are several studies which are potentially remarkable for their discussion of electrochemical properties. Biosynthesis of ferroelectric BaTiO3 NPs in assistance of *Lactobacillus sp*. was reported by Jha and Prasad (2010). After modification with PVDF, resulting nanocomposite exhibited enhancement in dielectric properties. AuNPs biosynthesized by alkalothermophilic actinomycetes *Thermomonospora curvata*, *Thermomonospora fusca*, and *Thermomonospora chromogena* and stabilized by cross-linker glutaraldehyde have potential usage as biosensor enhancer (Torres-Chavolla et al. 2010). Shilov et al. (2010) investigated electro-physical characteristics (cell ζ-potential, surface conductivity, electrophoretic mobility, dispersion of cell conductivity) of yeast cell with silver precipitate.

3.5.1 Sensors

Zheng et al. (2010b) reported biosynthesis of Au–Ag alloy NPs by yeast cells and their application to electrochemical vanillin sensing. Sensitive vanillin sensor based on glassy carbon electrode (GCE), modified by Au–Ag alloy NPs, was able to enhance the electrochemical response of vanillin. Electrochemical investigations confirmed a linear increase of the vanillin oxidation peak current at the sensor with its concentration in the range of 0.2–50 μM (detection limit 40 nm). Constructed sensor was successfully applied to the determination of vanillin in samples of vanilla bean and vanilla tea. This approach suggests possible replacement of commonly used methods in vanillin monitoring system (chromatography, capillary electrophoresis etc.).

Interestingly, Zheng et al. (2010a) also published green biosynthesis method for AuNPs based on usage of natural biomaterial, eggshell membrane ("fresh eggs were bought from a local supermarket in Hong Kong"). AuNPs on the eggshell surface were used to immobilize glucose oxidase (by cross-linking method with glutaraldehyde – Pingarrón et al. 2008) on the GCE for detection of glucose in solution. Enzyme activity of glucose oxidase was enhanced by the presence of highly

conductive AuNPs. Constructed biosensor showed linear response to glucose concentration ranged from 20 μM to 0.80 mM (with a detection limit of 17 μM) and has been successfully applied to detect the glucose level in glucose injections. Glucose sensor used for blood serum based on eggshell membrane and AuNPs (Zheng et al. 2011) is mentioned in medicinal applications in medical section (Sect. 3.3).

Even though it pertains to semi-metals, we will also mention a study dealing with synthesis of semiconductor selenium NPs employing cells of bacteria *Bacillus subtilis*. Two kinds (spherical, 1D-trigonal) of Se nanomaterial crystals with good adhesive ability and biocompatibility were employed as enhancing materials for hydrogen peroxide (H_2O_2) horseradish peroxidase biosensor, with the detection limit 8×10^{-8} M (for H_2O_2 concentration). Different types of Se crystals had no significant difference in sensor usage. Due to the obtained results, selenium nanomaterials GCE can be a promising instrument for applications dealing with the detection of H_2O_2 in food, pharmaceutical, clinical, industrial and environmental analyses (Wang et al. 2010).

3.5.2 Electrochemical Applications and Properties

Du et al. (2007) demonstrated in communication the bioreduction of aqueous Au(III) ions by the *E. coli* DH5α bacterial strain. AuNPs bound to the surface of the bacteria were used for application in direct electron transfer of protein hemoglobin (glass carbon electrode (GCE) coated by protein layer as well as the AuNPs biocomposite). Cyclic voltametry experiments were reported for different electrodes at scan rates of 0.1 V/s in pH 7.0 phosphate buffer solution. Although there are no obvious redox peaks at the blank electrodes (GCE, *E. coli*-GCE, hemoglobin-*E. coli*-GCE and AuNPs-*E. coli*-GCE), a pair of redox peaks (with formal potential of −0.325 V vs. Ag/AgCl reference electrode) was observed at AuNPs-hemoglobin-*E. coli*-GCE. These results proved the electron transfer between hemoglobin and GCE provided by means of the AuNPs modified electrode.

Similarly, extracellular synthesis of AuNPs using plant *Scutellaria barbata* as the reducing agent was observed (Wang et al. 2009). The obtained AuNPs were modified on the GCE and enhanced the electronic transmission rate between the electrode and the 4-NP.

Remarkably, biosynthesized CdSNPs were also used for construction of an ideal diode (Kowshik et al. 2002). Semiconducting NPs were biofabricated by *Schizosaccharomyces pombe* and were confirmed to have a Wurtzite ($Cd_{16}S_{20}$)-type structure. Diode was fabricated by means of tin-doped indium oxide coated glass substrate. This structure was spin coated by thin film of poly-phenylene vinylene (p-type material) and with washed *S. pombe* CdSNPs (n-type material) respectively. Silver contacts were also added. Obtained diode operated at low voltage and had forward current value, which makes the structure suitable for use as an ideal diode.

3.6 Optical, Bio-Imaging, Bio-Labeling Applications

The metallic nanocrystals are held at the center due to their photo-induced nonlinear optical properties. In particular, the unique optical properties associated with NPs and their composite materials include a high- or low-refractive index, high transparency, novel photoluminescence properties, photonic crystal, and plasmon resonance (Iskandar 2009). In nanoregime (hundreds to thousands of atoms) optical and electro-optical properties of materials can be tuned by varying the physical size of the crystal, leading to new phenomena, such as surface plasmon resonance in Au and AgNPs and the size dependent band gap of semiconductor (Talapin et al. 2009). Scientists are therefore able to tailor the electronic structure and properties without introducing any changes in the sample chemical composition. Methods for NP synthesis that allow control of NP characteristics, including size distribution, morphology, crystallinity, purity, and composition are of particular note (Iskandar 2009). Several methods for the synthesis of NPs and their composite materials have been reported previously. However, to be feasible for utilization in industrial scale, the process needs to be simple, low-cost, and able to operate continuously with a high production rate. Therefore, utilization of the biosynthesis approach may be beneficial (Table 5).

Non-pathogenic, fast-growing fungus *Trichoderma viride* (habited in dead organic materials) was used to biosynthesis small (2–4 nm), highly dispersed AgNPs (Fayaz et al. 2010b). Interestingly, photoluminescence measurements

Table 5 Electrochemical and sensing applications

NP	Organism used	Application	Reference
Ag	*Trichoderma viride*	Blue orange emission – photoluminiscence	Fayaz et al. (2010b)
Ag	*Parthenium hysterophorus*	Photoluminescence	Sarkar et al. (2010)
Ag	*Coriandrum sativum* leaf	Reverse sat. absorption, optical limiting	Sathyavathi et al. (2010)
CdTe	*Saccharomyces cerevisiae*	CdTe QDs for biolabeling and biosensing	Bao et al. (2010a)
CdTe	*Escherichia coli*	CdTe QDs for biolabeling and biosensing	Bao et al. (2010b)
Au-Ag	*Saccharomyces cerevisiae*	Vanillin sensor	Zheng et al. (2010a)
Au	Eggshell	Glucose sensor	Zheng et al. (2010b)
Se	*Bacillus subtilis*	H_2O_2 sensor	Wang et al. (2010)
CdS	*Schizosaccharomyces pombe*	Construction of ideal diode	Kowshik et al. (2002)
Au	*Escherichi coli*	Direct electrochemistry of hemoglobin	Du et al. (2007)
Au	*Scutellaria barbata*	Direct electrochemistry of 4-NP	Wang et al. (2009)

showed an emission in the range of 320–520 nm (fall in blue-orange region). This fact indicates such a method of AgNPs preparation as suitable for future bio-imaging and labeling application. Sarkar et al. (2010) published a similar study utilizing the flowering plant species *Parthenium hysterophorus* and AgNPs.

A simple, fast, and economical biological procedure using *Coriandrum sativum* leaf extract to synthesize AgNPs was reported by Sathyavathi et al. (2010). These NPs have an important application in nonlinear optics. Nonlinear refraction and absorption coefficients were measured using Z-scan technique with laser pulses. AgNPs were found to exhibit strong reverse saturable absorption, which has been identified as the main mechanism responsible for optical limiting. For more detailed information about optical limiting, see study by the same research group (Porel et al. 2007).

Extracellular synthesis cadmium telluride CdTe quantum dots (QDs) with tunable fluorescence emission employing *Saccharomyces cerevisiae* cells was published by Bao et al. (2010a). Fabricated CdTe QDs with uniform size (2–3.6 nm) were protein-capped, which makes them highly soluble in water. A similar approach (Bao et al. 2010b) was used for CdTe QDs by means of *E. coli*. Size-tunable optical properties were confirmed in both cases by ultraviolet–visible, photoluminescence, X-ray diffraction and transmission electron microscopy, with fluorescence emission from 488 to 551 nm. Moreover, QDs functionalized with folic acid and were used to image cultured cervical cancer cells *in vitro* (Bao et al. 2010b). The biosynthesized QDs may therefore have potential in broad bio-imaging and bio-labeling applications.

Shankar et al. (2005) published formation of gold nanotriangles with interesting absorption in the near-infrared region (see Sect. 3.3)

3.7 Further Applications and Properties

Throughout our study, we encountered various works that, while relevant, did not fit neatly in to any of aforementioned sections. Therefore, we chose several studies and publications with interesting applications or properties of biofabricated NPs for additional consideration (Table 6).

Table 6 Further applications and properties

NP	Organism used	Application	Reference
CuAlO2	*Humicola sp.*	Difficult-to-synthesize nanoparticles	Ahmad et al. (2007)
Au	*Emblica Officinalis*	Phase transfer, transmetallation	Ankamwar et al. (2005a)
Au	*Tamarindus indica* leaf	Nanotriangles, vapor sensing	Ankamwar et al. (2005b)
Au-Ag-Cu	*Brassica juncea* seed	Alloys	Haverkamp et al. (2007)
PbS, ZnS	Different *cocci* and *bacillus* cells	Hollow nanostructures; light harvesting and photocatalytic properties	Zhou et al. (2009)

Fabrication of otherwise difficult-to-synthesize NPs is undoubtedly an asset of bio methods. Ahmad et al. (2007) reported such a synthesis – multifunctional $CuAlO_2$ NPs (fungus based).

Phase transfer of biosynthesizes Au and AgNPs was published in a paper by Ankamwar et al. (2005a). NPs were fabricated using *Emblica officinalis* (amla, Indian Gooseberry) fruit extract. The experiments contained also their subsequent phase transfer to an organic solution (methanol) and the transmetallation reaction of hydrophobized AgNPs with hydrophobized chloroaurate ions (resulted in AuNPs).

Different shapes of resulting NPs also play a role in determining their properties. Tamarind leaf extract served as the reducing agent for the synthesis of gold nanotriangles ranged 20–40 nm in thickness (Ankamwar et al. 2005b). The effect of different organic solvent vapors like methanol, benzene and acetone on the conductivity of AuNPs triangles was investigated. Current-voltage characteristics were determined in presence of aforementioned organic solvent vapors and observation suggests possible application as chemical sensors. Biofabrication of anisotropic gold nanotriangles was also reported by Verma et al. (2010). On the other hand, Xie et al. (2007) performed experiments resulting in silver nanoplates employing green algal species *Chlorella vulgaris.*

Fabrication of alloys without sophisticated equipment or appropriately high temperature (Haverkamp et al. 2007) demonstrated the ability to synthesis the Au–Ag–Cu class of alloy by means of the *Brassica juncea* seed. Similarly, studies dealing with bimetallic Au-Ag alloy biosynthesis processes employing fungi were also published (Senapati et al. 2005; Sawle et al. 2008).

Morph-biotemplates, hollowed, former-bacterial cells, coated by chalcogenide NPs also represent an innovative approach to material science (Zhou et al. 2009). Moreover, these PbS and ZnS structures, prepared by the sonochemical method in presence of different *cocci* and *bacillus* (rod) templates, exhibited light harvesting and photocatalytic properties. Hollow structures possess superior photocatalytic activity to their solid counterparts during photocatalytic degradation of acid fuchsine and can be used as electromagnetic wave absorbers, ultraviolet shielding materials, photocatalysts or solar cells.

4 Summary, Conclusions, and Outlook

In this review, recent advances in the application of biosynthesized metallic NPs have been addressed. Applications have been categorized into a wide spectrum of sections such as catalysis, medicine, disinfection, sensors etc. When possible, comparison with the common chemical and physical synthesis approaches was investigated and discussed. In addition, the introduction of the relatively novel biosynthesized metallic NPs phenomenon into medicine, electrochemistry, optics and material science can reinforce their unique functions and properties, resulting in new methods and strategies for applied research and industrial utilization.

Although research in the field has been dramatically increasing since the beginning of the decade, biofabrication methods and approaches are still on the horizon of genuine applied research. This rapidly spreading, interdisciplinary field will require great research efforts from biochemists, physicists, biologists and materials scientists. A greater understanding of particular application mechanisms (how such technology will be utilized in the real world) will lead to mark crucial assets and drawbacks of bio-methods. In order to fully exploit biosynthesized metallic NPs, better stabilization techniques, more efficient material definition, better isolation processes and more effective immobilization possibilities should be designed and developed. To address these issues, the novel methods (organisms, substrates) should be incorporated and stabilization and capping mechanisms must be further investigated. Last but not least, possibilities of varied biosynthesized shapes (e.g. disk, plate, sponge, star, flake, urchin, prisms, wires and rods) are also quite promising.

Based on this study, we anticipate further development of biosynthesis methods and their utilization in many pertinent and advantageous real-world applications. In combination with other NP preparation methods, further investigation of processes and an expansion of potential applications will result in the deeper development of bionanotechnology.

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