Synthesis of Metallic Nanoparticles Mediated by Microbes

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Abstract
An overview of metallic nanoparticles' synthesis methods, using microorganisms, is presented in this paper. Among the most common nanoparticles which have been reported to be obtained through biosynthesis in the presence of different microbes, silver, gold, cadmium sulphide, zinc oxide and titanium oxide are listed. The main characteristics of these nanoparticles are generally analysed by ultraviolet-visible spectrometry, X-ray diffraction, scanning electron microscopy, and transmission electron microscopy. The morphology and structure of the nanoparticles can be tailored due to the type of microorganisms (bacteria, fungi, yeasts, algae or actinomycetes), and furthermore due to the strain of each microorganism. Moreover, the synthesis process parameters, such as temperature, incubation time, solution pH, and the precursor molar concentration, can contribute to the change in nanoparticles' properties. The biosynthesized nanoparticles exhibit antimicrobial activity, and can be used for water treatment, cosmetics, catalysis and biosensor applications. This green synthesis method has gained interest lately due to the low-cost and environmental-friendly features.

Keywords
nanoparticles synthesis, microorganisms, environmental-friendly

1. Introduction

In the recent years the synthesis of nanoparticles has gained interest due to the potential application in a number of areas, such as medicine, catalysts industry and biosensors [1, 2]. The most important and commonly studied type of metallic nanoparticles have been found to be those of noble metals, like gold and silver, but also copper and cadmium sulphide, zinc oxide and titanium dioxide. It has been demonstrated that a high ratio volume to area offers better properties which improve their application. The nanoparticles enumerated previously have shown that they possess antibacterial activity [3, 7].

TiO_2 has been used in the production of quantum dot sensitized solar cells by preparing a uniform titanium dioxide film that facilitates electron transfer process [8].

In the paper of Kundu et al., the biosynthesis of ZnO nanoparticles was described, to be further used onto cotton fabrics. The properties of ZnO nanoparticles have been found to be higher in the photocatalytic and antimicrobial activities than other metallic nanoparticles. The MTT assays (colorimetric assay for measuring cell metabolic activity) revealed a concentration-dependent cytotoxicity of anthraquinone against HT-29 cancerous cells [9]. Moreover, the biosynthesized ZnO particles, using *Candida albicans* as mediator, were used as catalysts for the fast and efficient synthesis of steroidal pyrazolines [1]. Other authors have reported the formation of conjugate of ZnO nanoparticles with shinorine which may be useful in the manufacture of non-toxic sunscreen agent [5].

Silver nanoparticles are well known for their antimicrobial activity. To date, *Escherichia coli, Salmonella, Streptococcus pyogenes* and *Staphylococcus aureus* are the most common bacteria against which silver nanoparticles have shown significant antimicrobial activity. Silver nanoparticles could contribute in the treatment of certain types of cancer, due to their cytotoxicity properties [2, 10]. In Table 1 one can observe different applications of green synthesis of metallic nanoparticles using microorganisms. The types of microorganisms used for biosynthesis range from bacteria, to fungi, to cyanobacterium.

Table 1. Biosynthesized metallic nanoparticles

Type of nanoparticles	Name of Microorganism	Synthesis Location	Size (nm)	References
Ag	Yeast strain MKY3	Extracellular	2-5	[11]
Ag	Fungus *Aspergillus flavus*	Extracellular	17 ±5.9	[12]
Au	Bacteria *Pseudomonas aeruginosa*	Extracellular	15–30	[3]
Au	Fungus *Guignardia mangifera*	Extracellular	15 - 35	[13]
Au	Bacteria *Streptomyces fulvissimus*	Extracellular	10 - 50	[14]
ZnO	Actinobacteria *Rhodococcus pyridinivorans* NT2.	Extracellular	100-120	[9]
ZnO	Bacteria *Lactobacillus plantarum VITES07*	N.A.	7–19	[15]
ZnO	Fungus *Candida albicans*	N.A.	20	[1]
ZnO	Cyanobacterium *Anabaena strain L31*	Extracellular	80	[2]
TiO_2	Bacteria *Lactobacillus* sp.	N.A.	24.63±0.32	[6]
TiO_2	Yeast *Sachharomyces cerevisae*	N.A.	12.57±0.22	[6]
TiO_2	Bacteria *Bacillus mycoides*	Extracellular	40–60	[8]
CdS	Fungus *Coriolus versicolor*	Extracellular	100–200	[7]
CdS	Bacterium *Klebsiella aerogenes*	Intracellular	20–200	[16]
CdS	Bacteria *Rhodobacter sphaeroides*	Extracellular	2.3 ± 0.15 6.8 ± 0.22 36.8 ± 0.25	[17]

There are two kinds of biosynthesis, depending on the place where the process occurs, namely, intra- or extracellular. The intracellular synthesis occurs in the cell, while the extracellular synthesis takes place due to the enzymes secreted by the cells [18]. The most common phenomenon is the extracellular synthesis, as observed from the short selective reports in this field (Table 1). Generally, the shape and size of nanoparticles have a significant influence on their respective properties. The ones desired for nanoparticles are: spherical shape and less than 100 nm in diameter.

In this paper, some of the microorganisms used in nanoparticle biosynthesis are presented. The aim of the paper is to highlight the current state of the art regarding the biosynthesis of metallic nanoparticles using microorganisms. Moreover, to emphasize the importance of low-cost and low-toxicity factors involved in this process is emphasized. An important demand in the nanotechnology field is not represented only by the final material properties, but by the synthesis process aspects, which usually are desired to be eco-friendly.

2. Principles of Metallic Nanoparticles Synthesis, Mediated by Microorganisms

At least one class from all domains of microorganisms have shown the ability to synthesise metallic nanoparticles. The green synthesis of metallic nanoparticles can be divided into two important categories, namely, bioreduction and bioabsorption. In the first case, metal ions are chemically reduced into more stable forms biologically. Many organisms have the ability to utilise dissimilatory metal reduction, in which the reduction of a metal ion is coupled with the oxidation of an enzyme. This results in stable and inert metallic nanoparticles that can then be safely removed from a contaminated sample. The second category is bioabsorption. This involves the binding of metal ions from an aqueous or soil sample into the organism itself, such as on the cell wall, and does not require the input of energy [19]. P. Syngh et al., have described in their paper the principles of biosynthesis of nanoparticles and explanations on how one can achieve a good monodispersity, by changing the relevant critical parameters (Figure 1) [20].

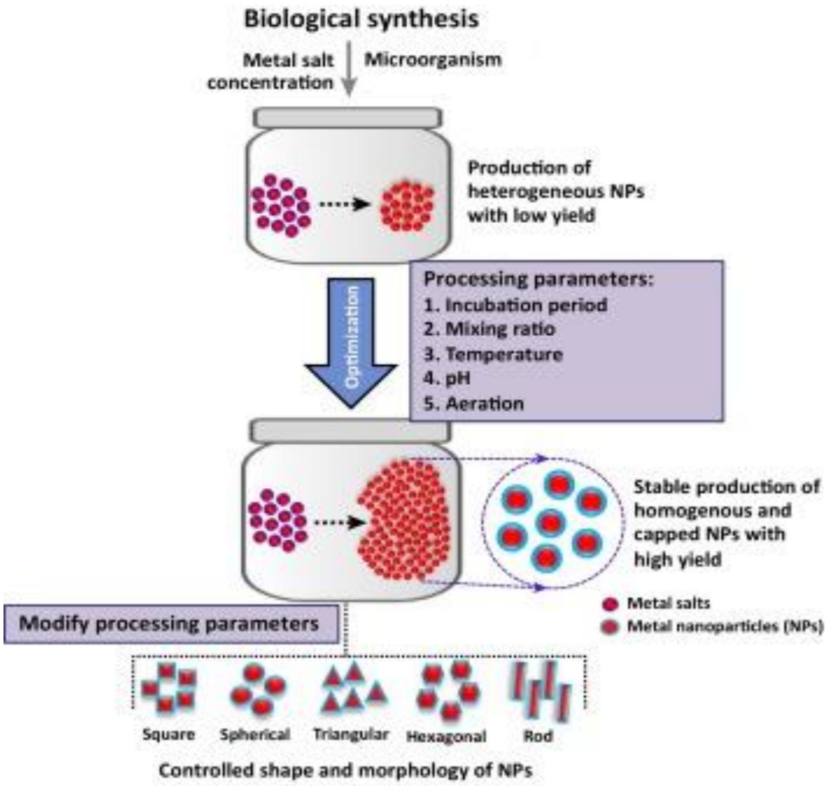

Fig. 1. Biological synthesis of metallic nanoparticles using microorganisms [20]

Even by carefully controlling several synthesis parameters, such as salt concentration, mixing ratio of biological extract and metal salt, pH value, temperature, incubation time, and aeration, there is still need of optimization in terms of producing homogenous nanoparticles of a similar size and shape. Biological synthesis can also provide an additional capping layer on synthesized nanoparticles with the attachment of several biologically active groups, which can enhance the efficacy of biological nanoparticles.

3.1. Silver nanoparticles

Silver nanoparticles have a large area of application, from non-linear optics, spectrally selective coatings, to solar energy absorption. There are different methods through which silver nanoparticles can be obtained, both by the top-down and bottom-up approaches. The production of silver nanoparticles using cyanobacteria *Spirulina platensis* and actinobacteria *Streptomyces* spp. 211A as mediators, it has been reported [21]. The synthesis method is simple and inexpensive. In this case the yellowish-brown color of the colloid solution has indicated the formation of silver nanoparticles. The second step to confirm the transformation from $AgNO_3$ to Ag is UV-vis analysis, which has shown the highest peak position of absorbance in the vicinity of 420 nm. Similarly, the surface plasmon peak located at around 420 nm was observed for the silver nanoparticles synthesized using yeast, MKY3 strain [11]. In Figure 2 the UV-visible spectra for silver nanoparticles mediated by *Myxococcus virescens* is presented.

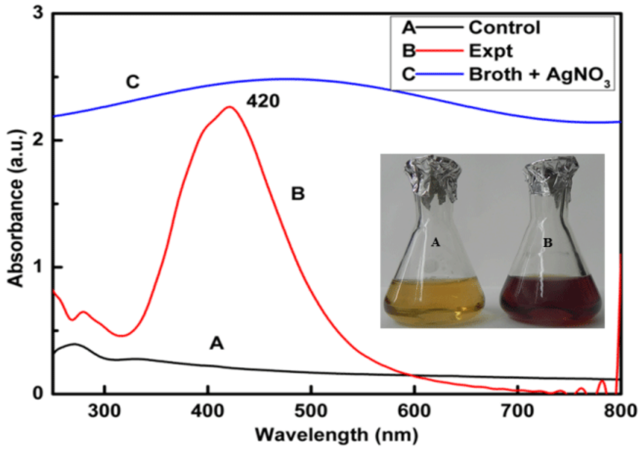

Fig. 2. UV-visible spectra of silver nanoparticles, with the highest peak at 420 nm [22]

It is very important to relate one's findings to a control solution, as shown in the graph from Fig. 2, containing the precursor (in this case, $AgNO_3$) in combination with liquid media, processed in the same conditions, in order to emphasize the role of microorganisms, as reducing agent and stabilizing agents. The absence of the absorption peak for the control solution signifies that the reaction from $AgNO_3$ to Ag does not occur.

3.2. Gold nanoparticles

The biosynthesis of gold nanoparticles using bacteria *Pseudomonas aeruginosa* was reported by Husseiny, M.I., et al. The recorded results, such as, the fluorescence and UV-visible spectra of the bacteria-synthesized nanoparticles, presented similar values as Au NPs (gold nanoparticles) prepared by chemical means.

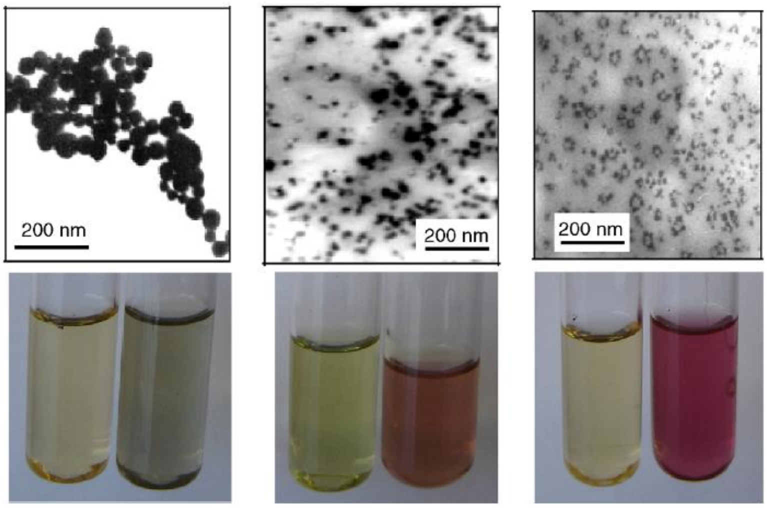

Fig. 3. The transmission electron microscopy and photo of Au NPs and colloids prepared using the supernatant of *P. aeruginosa* ATCC 90271, *P. aeruginosa* (2), and *P. aeruginosa* (1) respectively [3]

From Figure 3 one can conclude that there is an important difference between the gold nanoparticles synthesized by three distinct strains of the same bacteria [3].

The highest peaks obtained through UV-visible spectra for gold nanoparticles should be in the interval of 510-570 nm. V. Nachiyar et al. have obtained gold nanoparticles using endophytic fungi, showing an absorption peak at 560 nm [13]. Generally, metallic nanoparticles exhibit superior properties compared to the ones of bulk materials. Here the electronic, magnetic, catalytic and optical properties of gold nanoparticles can be mentioned. If the nanoparticles are obtained through green methods, the area of application could be extended in the biomedical field.

3.3. Zinc oxide nanoparticles

ZnO NPs have been investigated in the last years due to their unique properties, as optical, photocatalytic, electrical, electronic, dermatological and antibacterial properties. *Rhodococcus pyridinivorans* 6 NT2 strain has the ability to produce ZnO NPs. Those nanoparticles were loaded onto cotton fabrics which led to long term UV-protective, photocatalytic and antibacterial properties [9].

The biosynthesis process of ZnO nanoparticles using fungi, *Candida albicans*, is described in the paper of Shamsuzzaman and his co-workers, as eco-friendly reducing and capping agent. The synthesized nanoparticles were used as catalyst for the fast and efficient synthesis of steroidal pyrazolines. For those nanoparticles, X-ray diffraction was performed, in order to confirm the ZnO phase of the nanoparticles. In Figure 4 it can be observed that all peaks are very well matched with the wurtzite structure (hexagonal phase).

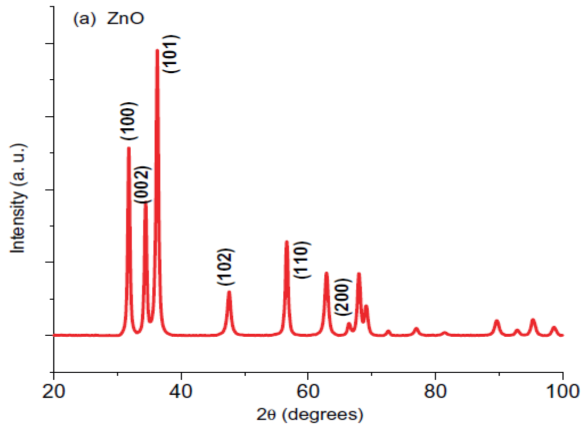

Fig. 4. XRD image of the ZnO nanoparticles biosynthesized using *Candida albicans* [1]

Usually, after UV-visible analysis of metallic nanoparticles, it is recommended to do the XRD analysis. The results can be used even for calculating the crystallite size of the nanoparticles, using the Debye Scherrer formula:

$$D = K\lambda \,/\, \beta\cos\Theta \,, \tag{1}$$

where K is constant,
λ is the wavelength of employed X-rays,
β is corrected full width at half maximum, and
Θ is Bragg's angle.

3.4. Titanium dioxide nanoparticles

Due to various technological utilization, the toxicity of titanium dioxide nanoparticles has turn into an important parameter. The biosynthesis of titanium dioxide nanoparticles using bacteria *Bacillus mycoides* was reported. Órdenes-Aenishanslins N.A et al. have proposed a possible mechanism (Figure 5) for TiO_2 biosynthesis using titanyl hydroxide as precursor and a still unknown organic molecule from *B. mycoides* [8]. An acidic group present into a nameless component of the extracellular matrix of *B. mycoides*, could have a key role in biotransformation, and would mediate the dehydration reaction. Due to organic coating, nanoparticles of TiO_2 byonsythesized by *B. mycoides* have exhibited low toxicity against *Escherichia coli*. Furthermore, the TiO_2 nanoparticles are good candidates for the manufacturing of solar cells [6, 8].

4. Conclusion

Metallic nanoparticles synthesized through biological methods present special properties. Due to the low toxicity involved in the process, the area of application can be greatly extended. The list of microorganisms which are able to be used for the biosynthesis of metallic nanoparticles is considerable, from bacteria, fungi to yeasts.

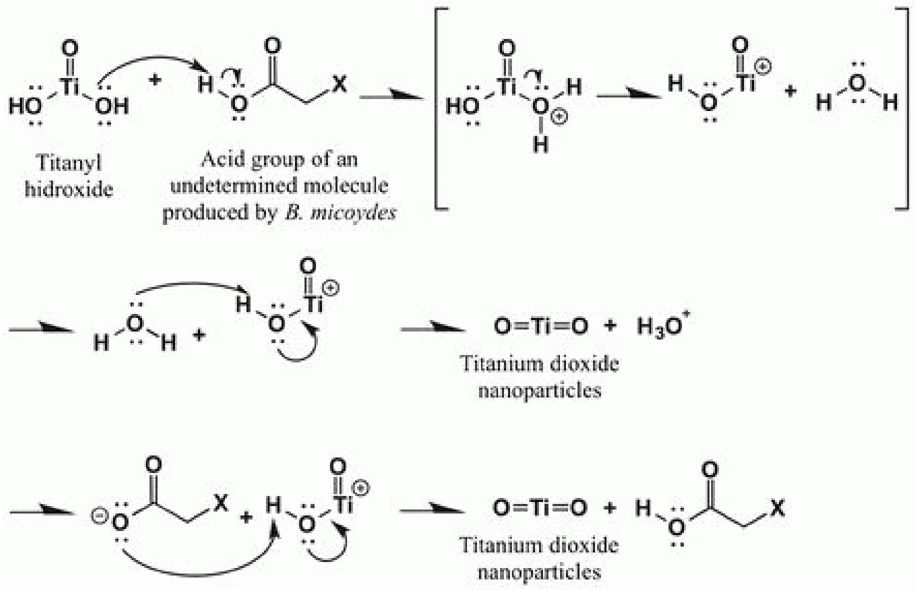

Fig. 5. Possible mechanisms for the biotransformation of titanyl hydroxide to titanium dioxide nanoparticles [8]

The bioreduction phenomenon is more common than bioabsorption. From the applications point of view, it would be important to harvest the metal nanoparticles formed within the biomass. Gold and silver nanoparticles exhibit striking colors (pink to blue for gold and light yellow to brown for silver) due to excitation of surface plasmon vibrations in the particles).

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