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SYNTHESIS OF COPPER NANOPARTICLES IN PRESENCE OF MICROORGANISMS

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Abstract: Application of nanoparticles in different industries attracts special attention nowadays. Use of microorganisms is one of the actively studied directions for nanoparticles production. The strain of Pseudomonas stutzeri possessing capacity to bioformation of copper nanoparticles was selected as a result of conducted screening among collection cultures. Earlier this strain revealed activity in terms of biosynthesis of silver nanoparticles too. The possibility to regulate size and form of produced nanoparticles by regulating concentration of ions in the solution was established. The strain is considered as a potential source for development of the technology for production of copper nanoparticles.

Keywords: nanoparticles, copper nanoparticles, microorganisms, Pseudomonas stutzeri

INTRODUCTION

Nowadays interest paid to metal nanoparticles (Nps) and their production grows considerably due to their innovative application in different industrial fields [1]. Extensive practical application of Nps is stipulated by a number of their unique features. Nps opened different approaches for development of new materials and evaluation of their properties by the modulation of the particles' size, morphology and distribution [2, 3]. Nps of metals are extensively studied due to their unique characteristics such as antimicrobial, antitumor and catalytic activities, and magnetic and optical properties as well.

The last decade is characterized by extensive study on biosynthesis of Nps of different metals with application of microbial agents such as actynomycetes, fungi, yeasts, viruses and sea-weeds [4, 5]. Respectively, results of these studies tremendously affected development of the green nanotechnology [6, 7]. Microorganisms may be used as potential agents for environmentally safe and reasonably inexpensive synthesis of different Nps of metals, such as silver, gold, palladium and copper, and metal oxides such as zinc oxide, titanium oxide and so on. It is well known that many types of microorganisms may serve as biocatalysts in the process of formation of Nps of metals and their compounds and secure biosynthesis of microfine and stable Nps at large scales. In particular, bacteria possess outstanding ability to reduce ions of heavy metals and are one of the best candidates for Nps synthesis. Some types of bacteria developed ability to use specific protective mechanisms to suppress stresses such as toxicity of ions of heavy metals. Moreover, they are capable to grow at extremely high concentrations of ions of metals, e.g. strains *Pseudomonas stutzeri* and *Pseudomonas aeruginosa* [8, 9].

Bacteria from genus *Pseudomonas* are among the most extensively applied microorganisms in nanotechnology. The application of the innovative approach for synthesis of copper Nps by the strain of bacteria *Pseudomonas stutzeri*, earlier isolated from soil, resulted in receipt of 50-150 nm cubic copper Nps [10]. Varshney et al reported the possibility of use of nonpathogenic strain of *Pseudomonas stutzeri* for the fast method of biological synthesis for

production of 8-15 nm spherical copper Nps [11]. It was revealed that strain *Pseudomonas stutzeri* AG259 produces silver Nps [12], which are accumulated in the periplasmic space of bacterial cells. Widely distributed bacterial species *Lactobacillus* was reported to synthesize both Nps of gold and silver in standard conditions [13]. Prakash et al described extracellular synthesis of silver Nps by bacteria *Bacillus cereus*. Synthesized Nps were spherical and 10-30 nm in size [14].

It is known that, as a rule, production of metal Nps by bacteria takes place during the stationary phase of their growth. This is because, as compared to other phases of growth, the highest metabolic stress takes place during the stationary phase of growth, during which metabolites, capable to reduce metals, are accumulated, and presence of ions of metals in the growth medium results in formation of Nps. Moreover, the initial concentration of copper ions heavily affects the production of Nps by living cells [15, 16]. It was established that metabolites received from *Pseudomonas stutzeri* promote to formation of copper Nps, moreover, they stabilize them.

Thus, it may be assumed that microorganisms are capable to decrease toxicity of metals by formation of Nps via their integration with proteins, polysaccharides and other metabolites. Genes responsible for resistance to metals, cytochromes of c-type, peptides, cellular enzymes (such as nitrate reductase) and reducing co-factors play considerable role in synthesis of both silver and copper Nps in bacteria. Organic substances produced by bacteria act as natural stabilizing agents for metal Nps, thus preventing their aggregation and securing stability during extended period of time [17].

Singh et al reported the biological synthesis CuO Nps by culture of bacteria *Escherichia coli* [18]. Obtained Nps were represented by different sizes and shapes. Studies conducted by several authors with use of endophytic actynomycetes *Streptomyces sp*. revealed that extract of biomass of these strains stimulates synthesis of CuO NPs[19]. Results obtained in this study clearly revealed that biosynthesized CuO NPs express effective bioactivity and thus may become the ground for development of the universal biotechnological applications [19]. Usha et al proved that CuO Nps synthesized by *Streptomyces sp*. may be used for development of fabrics with antimicrobial properties for hospital application aiming prevention or minimization infections with pathogenic bacteria [20].

Currently numerous studies established the wide spectrum of microorganisms that may serve as biocatalysts in the process of formation of Nps of metal and their compounds and secure biosynthesis of microfine and stable Nps in large scale. Efficiency of such biosynthesis of Nps depends on many factors, that is why study of their influence on the process of Nps formation by microorganisms represents certain interest.

MATERIALS AND METHODS

The aim of study was receipt of copper Nps with use of microorganisms. The standard solutions of different concentration of $CuSO_4$ in distilled water were prepared (25-100 mg/l (Cu^{2+})). The aliquots of solution were added to the cultural broth of the microorganisms. Mixture of the cells and copper ions were incubated on the rotary shakers at 180 rpm and 28°C for 48-72 h. Formation of Nps were determined visually by staining solutions into characteristic colors.

Microorganisms were cultivated in standard conditions on the beef extract peptone broth diluted in 2 times.

Strains of microorganisms isolated from mixed populations of the microorganisms inhabiting soils polluted with different xenobiotics were used in this study. Selection of the objects was determined by their resistance to the different pollutants, including heavy metals, thus it is

assumed that ability to form Nps of metals is a protective function of the microorganisms [21].

Antimicrobial activity was determined by standard protocol [22].

UV-spectroscopy was conducted with use of spectrophotometer Specord 210 (Germany) within range 190-1000 nm. Accuracy of UV photometry with potassium dichromate was in accordance with Ph.Eur.±0.01.

Optical study was conducted with use of the optical microscope Leica 1000 (Germany) with magnification rate ×40 - ×1300.

Morphology of films of nanostructured systems was determined with use of atomic force microscope Agilent 5500 (USA) at the room temperature. Silicon cantilevers with hardness 9.5 N/m with frequency 145 kHz were used. Maximum field of scanning on AFM by X,Y was 15×15 μm^2, by Z – 1 μm.

RESULTS AND DISCUSSION

To determine ability of the microorganisms to synthesize copper Nps the studied strains were cultivated on elective media. Aliquots of solution of the copper sulphate were added into the cultural broth of the studied microorganism. Formation of the Nps was initially determined visually by the change of color of the solution, which is characteristic to the copper Nps (table 1).

Table 1. Screening of bacteria by Nps synthesis

№	Culture	Change in color	Intensity of staining		
			24 h	48 h	72 h
1.	*Pseudomonas putida*	green	+	+	-
2.	*Pseudomonas stutzeri*	brown	+	2+	3+
3.	*Pseudomonas fluorescens*	light green	+	+	+
4.	*Rhodococcus erytropolis*	yellow	+	2+	2+
5.	*Rhodococcus sp.*	yellow	+	2+	2+
6.	*Arthrobacter globiformis*	brown	+	+	+
7.	*Bacillus megatherium*	greenish	+	2+	2+
8.	*Bacillus subtilis*	light-yellow	-	+	+
9.	*Bacillus sp.*	light-yellow	-	+	+
10.	*Acinetobacter sp.*	greenish-blue	-	+	-

In one way or another all studied microorganisms expressed ability to synthesis of Nps (table 1). It should be noted that not all studied strains revealed standard staining. Some strains revealed change in solution color from blue to light blue, in some cases formation of the copper Nps was accompanied by light yellow staining. It is also should be noted that maximum activity in preliminary screening was expressed by strain *Pseudomonas stutzeri.* Moreover, Nps synthesized by this strain were stable for two and more weeks. That is why, further study focused on this strain.

Further, the reduction of copper ions at the presence of bacteria *Pseudomonas stutzeri* was studied at pH 7.0 and concentration of (Cu^{2+}) 25 and 50 mg/l.

Studied solutions were analyzed by UV-spectroscopy. Spectra of studied samples revealed presence of absorption bands characteristic to copper Nps, which are located within range 200-350 nm, depending on the size of the Nps (figure 1). Absence of absorption bands of copper ions (Cu^{2+}) within 700-900 nm range additionally confirmed restoration of copper ions.

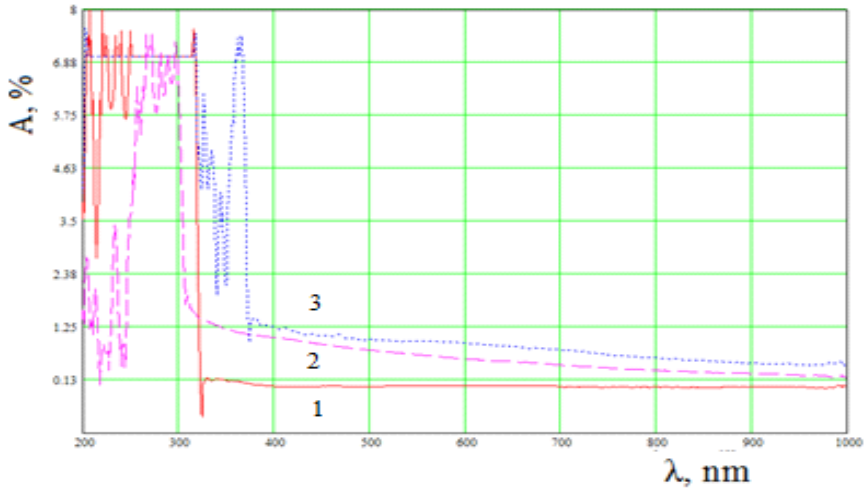
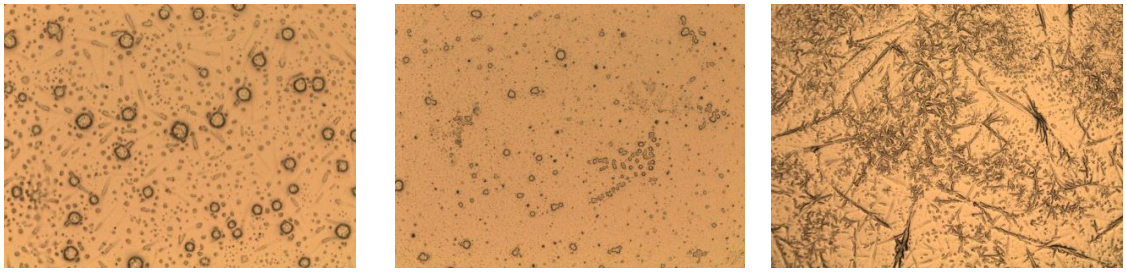


Figure 1. UV spectra of systems at the different content of copper Nps: 1) beef extract peptone broth; 2) beef extract peptone broth + bacteria + 25 mg/l (Cu^{2+}); 3) beef extract peptone broth + bacteria + 50 mg/l (Cu^{2+}).

Results revealed that with increased concentration of the copper ions (Cu^{+2}) the bathochromic absorption wavelength shift is observed in direct ratio with the size of Nps. Possibly, with increased size of copper Nps electrons located on the same level form zone where the width of this zone depends on size of Nps.

Morphology of the films obtained on basis of hydroxyethyl cellulose and copper Nps was studies with use of optical microscopy. Films produced on basis of bacteria in cultural broth and with copper Nps revealed presence of the particles in shape of spherulites expressing growth in radial direction; prolonged particles were observed as well (figure 2).



1 2 3

Figure 2. Optical pictures of systems containing copper Nps: 1 - beef extract peptone broth+ bacteria; 2 - beef extract peptone broth + bacteria + 25 mg/l (Cu^{2+}); 3 - - beef extract peptone broth + bacteria + 50 mg/l (Cu^{2+})

In the system the beef extract peptone broth + bacteria + 25 mg/l (Cu^{2+}) the size of Nps reduces and the spots begin to appear where particles agglomeration takes place. With increased concentration of copper ions (Cu^{2+}) in the system the needle like particles are produced and dendritic structures are formed. Formation of the dendritic structures is characteristic for ions and Nps of copper.

Size and distribution of the copper Nps within matrix were studied with application of the ASM (figure 3). Obtained results reveal that bacteria in the nutrient medium at the absence of copper ions (Cu^{2+}) form dendritic structure comprising spherical Nps with size ranging from

500 nm to 1.5 micron. Surface roughness reveals that there is a size variation and particles are unevenly distributed within the matrix (figure 3. a, b).

It should be noted that cubic copper Nps are formed in the studied samples containing 25 mg/l (Cu^{2+}) and 50 mg/l (Cu^{2+}) (figure 3; c-d). Nps with size 35-60 nm are formed at 25 mg/l (Cu^{2+}), while increasing concentration to 50 mg/l (Cu^{2+}) results in 6 times increased polydispersity of Nps, thus 75-300 nm Nps are formed. Surface roughness reveals that at 25 mg/l (Cu^{2+}) considerably small particles are formed, which have relatively thin degree of dispersion, whereas at 50 mg/l (Cu^{2+}) the surface of the films is not even, which is probably linked with high degree of dispersion by size and distribution of the nanoparticles within matrix.

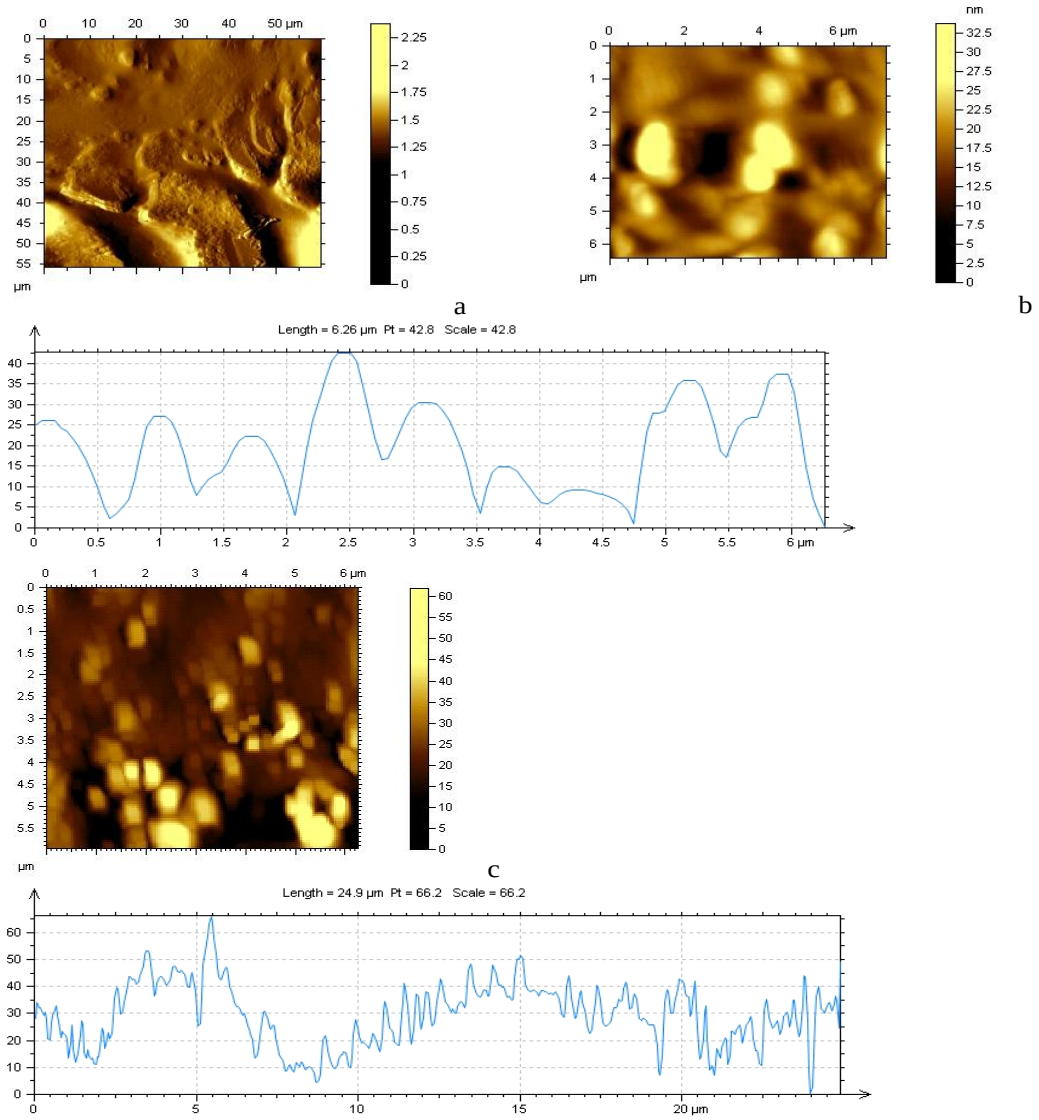


Figure 3. AFM pictures and surface roughness of the films containing copper Nps: a-b - beef extract peptone broth + bacteria; c - beef extract peptone broth + bacteria + 25 mg/l (Cu^{2+}); d - beef extract peptone broth + bacteria + 50 mg/l (Cu^{2+})

CONCLUSION

As result of conducted screening among studied bacterial cultures the strain of *Pseudomonas stutzeri* possessing ability to biological formation of the copper Nps was selected. This strain

in earlier study revealed activity in terms of biosynthesis of silver Nps as well [23]. The possibility to regulate size and shape of the produced Nps by variation of the concentration of metal ions was established. It was determined that increasing concentration of metal ions in the solution results not only in increased size, but leads to the change of the shape of Nps. Which in its turn may affect their biological activity. This feature provides vast opportunities for their application at the development of the new antimicrobial preparations.

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