



THE IMPACT OF TEMPERATURE ON THE SYNTHESIS OF SILVER NANOPARTICLES BY *CANDIDA MACEDONIENSIS*

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Abstract: Nanoparticles are widely used in medical diagnosis and treatment, as carriers of drug preparations, in cosmetics, production packaging and transportation of foods and etc. Special attention is paid to the use of biological structures in the production of nanoparticles. The aim at the presented work was to investigate the influence of temperature on the formation of silver nanoparticles by *Candida macedoniensis* BSU-MI44. Wet biomass of yeast at AgNO₃ solution, was incubated at 25, 30, 35, 40°C. The samples have been analyzed on the UV spectrometer, the scanning electron microscope and the X-ray spectroscope. Spectrophotometric analyses showed 410 nm wavelength (peak), characteristic for silver nanoparticles in samples incubated at 25 and 30°C. By increasing temperature, the formation of silver nanoparticles has weakened and has finally stopped. The optimum temperature was between 25-30°C for the production of silver nanoparticles and the formed nanoparticles were spherical at both temperatures. The sizes of silver nanoparticles formed at 25°C and 30°C were 65.6 and 14.2-22.9 nm. The sizes of the first ones have been 2.8-4.7 times larger than the sizes of the others. Correspondingly X-ray spectroscopic analyses of the obtained samples showed the characteristic absorption peak for silver nanoparticles formed at temperatures 25 and 30°C.


Keywords: *Candida macedoniensis*, Silver nanoparticles, UV spectrum, SEM, X-ray spectrum


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1. Introduction

Currently, synthesis of nanoparticles is one of the fastest growing and leading fields of the nanotechnology. Unlike large-size materials, these particles have specific biological properties that differ in physical, chemical, magnetic, thermal, optical, and quantum sizes. Nanoparticles are widely used in medical diagnosis and treatment, as carriers of pharmaceutical preparations, in cosmetics, dyestuffs, food production and packaging, transportation of foods, oil production, agriculture and finally in environmental protection (Meenal Kowshik et al., 2002; Sastry et al., 2003; Narayanan and Sakthivel, 2010; Sadowski, 2010; Ganbarov et al., 2016a). The large-scale synthesis of metal nanoparticles using physical, chemical and biological methods is carried out, in many developed countries of the world (Ganbarov et al., 2015a; Ganbarov et al., 2015b; Muthupandian et al., 2013). Recently, special attention is paid to the use of the biological structures in the production of nanoparticles. The biological synthesis process of nanoparticles consists of three basic steps - to use medium-sized solvents for synthesis, select ecologically harmless agents, and select

non-toxic materials for stabilizing nanoparticles. For the production of metal nanoparticles by biological method are used fungi, bacteria and plants (Abo-State and Partila, 2015; Egorova and Revina, 2000; Sadowski, 2010). As a result of large-scale researches carried out by scientists, it was possible to synthesize metal nanoparticles such as silver, gold, zinc, selenium, titanium and platinum by using yeasts. Silver nanoparticles attract more attention due to the surface area, unique physical, chemical and biological properties (Xiangqian et al., 2011; Ganbarov et al., 2015a; Ganbarov et al., 2015b; Ganbarov et al., 2016a; Ganbarov et al., 2016b).

In our previous studies, the ability of *Candida macedoniensis* BSU-MI44 to form silver nanoparticles was studied. Depending on the amount of biomass, silver nanoparticles formation has been studied and the amount of optimal biomass was determined (Bhainsa and D'Souza, 2006; Bharde et al., 2006; Anal et al., 2008; Ganbarov and Musayev, 2012). The main purpose of the presented work was to study the influence of temperature on the silver nanoparticles production by



Candida macedoniensis BSU-MI44.

2. Materials and Methods

As an object of a research was used *Candida macedoniensis* BSU-MI44 taken from the culture collection of the Microbiology department. For the cultivation of *Candida macedoniensis* BSU-MI44 has been used the medium with the following composition: yeast extract - 10 g, sucrose - 20 g, peptone - 20 g, distilled water - 1 litre. The cultures were separately incubated at 25, 30, 35, 40°C, for 48 hours. The biomass was separated by filtration and washed 3 times in 100 ml distilled water. Then 10 g wet biomass was resuspended in 100 ml distilled water and 1 ml of 10⁻³ molar AgNO₃ solution was added and incubated at 25, 30, 35, 40°C until the color change.

The formation of silver nanoparticles was primarily visualized by the change of the color of the reaction mixture from light yellow to dark brown. Then the biomass was separated by filtration and the nanoparticles in the filtrate were analyzed on the

spectrophotometer at 400 to 450 nm wavelength.

Then preparation was made from cultured fluid and the shape, size of silver nanoparticles (nm) were determined on the scanning electron microscope (SEM JEOL.7600F, Japan). The nanoparticles, obtained by X-ray (EDAX) spectral analyses were determined to be silver.

3. Results and Discussion

The formation of silver nanoparticles by *Candida macedoniensis* BSU-MI44, depending on the temperatures (25, 30, 35, and 40°C) has been studied. It was shown that when the silver nanoparticles are accumulated, the colour of the reaction mixture darkens. This phenomenon is considered to be the initial indication of the existence of silver nanoparticles (Xiangqian et al., 2011; Ganbarov et al., 2015a; Ganbarov et al., 2015b; Ganbarov et al., 2016a; Ganbarov et al., 2016b). The appearance of silver nanoparticles due to the colour change was observed at 25 and 30°C (Figure 1).



Figure 1. The color change of reaction mixture during the formation of silver nanoparticles by *Candida macedoniensis* BSU -MI44: a- experiment, b-control (1- 25°C, 2 - 30°C).

Spectrophotometric analyses of the samples showed that incubated samples at temperatures 25 and 30°C had absorption at 410 nm wavelength, which is characteristic for silver nanoparticles. Spectrophotometric analyses of samples taken from incubated variants showing no colour change (at temperatures 35 and 40°C) didn't have an absorption or the absorption was very weak (Figure 2).

All the samples were analyzed on the scanned electron microscope and nanoparticles were not observed in the variants incubated at 35 and 40°C. However, silver nanoparticles of different sizes were observed in variants incubated at 25 and 30°C.

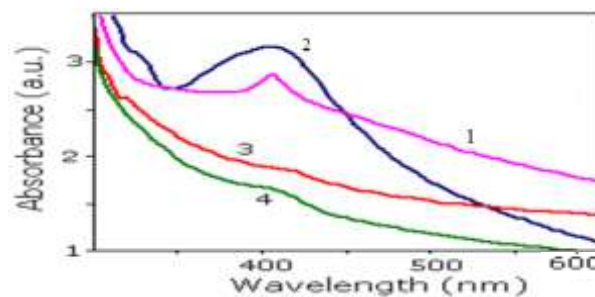


Figure 2. UV-visible absorption spectra of silver nanoparticles formed by *Candida macedoniensis* BSU-MI 44 depending on the temperatures: 1 - 25°C, 2 - 30°C, 3 - 35°C, 4 - 40°C.

The optimum temperature for the production of silver nanoparticles by the yeast cell strains was between 25 and 30°C. So, the nanoparticles formed at both temperatures had spherical forms (Xiangqian et al., 2011;

Ganbarov et al., 2015a; Ganbarov et al., 2015b; Ganbarov et al., 2016). However, the sizes of the silver nanoparticles formed at 25°C and 30°C were respectively, 22,6 nm and 22,9nm. It was determined that, the sizes of silver nanoparticles formed at 25°C was

2, 8 - 4, 7 times larger than those formed at 30°C. X-ray-phase spectroscopic analyses of the samples incubated at 25 (1) and 30°C (2) showed (Figure 3) characteristic adsorption peak of silver nanoparticles (Ag Lal).

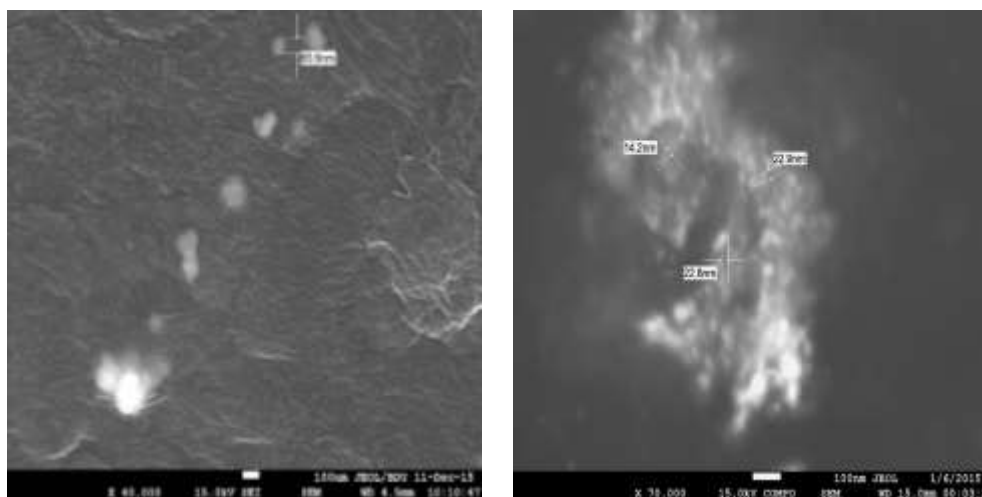


Figure 3. The shapes and sizes of silver nanoparticles, formed by *Candida macedoniensis* BSU-MI44 depending on the temperatures (1-25° and 2- 30°C) on the scanning electron microscope.

4. Conclusion

The impact of the temperature on the formation of silver nanoparticles by *Candida macedoniensis* BSU-MI44 was studied and it was determined that the optimum temperature for the synthesis of silver nanoparticles was between 25-30°C. The formation of silver nanoparticles at 35° and 40°C was not observed. The sizes of the formed nanoparticles depending on the temperatures were different. The sizes of silver nanoparticles formed at 30°C were 2.8-4.7 times smaller than the nanoparticles of 25°C.

Author Contributions

Percentages of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

| % | M.J. | K.G. | E.K. | S.H. | S.G. |
|-----|------|------|------|------|------|
| C | 20 | 20 | 20 | 20 | 20 |
| D | 20 | 20 | 20 | 20 | 20 |
| S | 20 | 20 | 20 | 20 | 20 |
| DCP | 20 | 20 | 20 | 20 | 20 |
| DAI | 20 | 20 | 20 | 20 | 20 |
| L | 20 | 20 | 20 | 20 | 20 |
| W | 20 | 20 | 20 | 20 | 20 |
| CR | 20 | 20 | 20 | 20 | 20 |
| SR | 20 | 20 | 20 | 20 | 20 |
| PM | 20 | 20 | 20 | 20 | 20 |
| FA | 20 | 20 | 20 | 20 | 20 |

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval/Informed Consent

Ethics committee approval was not required for this study because of there is no animal or human research.

In this research, no ethical statement was needed since artificial fungus cultures were used.

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