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BIOSYNTHESIS OF SILVER NANOPARTICLES USING THERMOPHILIC BACILLUS SP. $Б_1$

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Abstract. The presented work is devoted to the synthesis of silver nanoparticles by a thermophilic Bacillus Sp.$Б_1$ that was isolated from a hot spring Babazanan in Salyan region of Azerbaijan. The formation of silver nanoparticles was determined primarily visually by darkening the color of the reaction medium and by the absorption wavelength of 410-420 nm in a UV-spectrophotometer. Also, the formation of silver nanoparticles was determined by XRD analysis using a Rigaku Mini Flex 600 X-ray diffractometer. It was found that the synthesized silver nanoparticles are represented by Ag and AgCl. The size of the nanoparticles was determined using the Scherrer equation. The size of silver nanoparticles was 22.9 nm and 64.6, 75.7 nm for Ag and AgCl, respectively.

Keywords: *green synthesis, silver nanoparticles, thermophilic bacteria, XRD-analysis, UV-spectrophotometer.*

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1. Introduction

Green synthesis of metal nanoparticles using various biological objects is widespread today. Despite the fact that there are other methods for obtaining metal nanoparticles such as physical and chemical methods the biological method is cost effective, ecofriendly and profitable method (Sinha, 2009). Chemical and physical methods have various limitations in terms of synthesis procedures which may involve use of drastic experimental conditions coupled what may involve use of toxic byproducts, thus damaging the environment (Ganbarov, 2017).

Many microorganisms can synthesise nanoparticles like silver, gold, magnesium and etc. Among metal nanoparticles silver nanoparticles are most obtained using various microorganisms. The resistance caused by the bacterial cell for silver ions in the environment is responsible for its nanoparticles synthesis. Thermophilic microorganisms are also widely used in the formation of silver nanoparticles. Since thermophilic microorganisms live at high temperatures, their metabolism proceeds at a high rate and the reaction product is obtained in a short period of time. This makes thermophilic microorganisms an indispencable object for the synthesis of silver nanoparticles (Deljou, 2016).

Silver nanoparticles have attracted and demandable research of interest in the field of nanotechnology due to its distinct properties such as good conductivity, chemical stability, catalytic and antimicrobial activity (Li *et al.*, 2006, Chen *et al.*, 2005, Setua *et al.*, 2007, Wei, 2015). Given that many pathogenic microorganisms have developed resistance to antibiotics it is necessary to produce alternative antibacterial agents that can replace antibiotics. The antibacterial and antioxidant effects of silver and its salts had been already known to science. Silver nanoparticles are currently used as an antibacterial agent in the treatment of infections that cause wounds and chronic ulcers (Chastre, 2008). Silver nanoparticle have capacity inactivates bacterial enzyme by releasing ionic silver which inactivates the thiol groups (Sharma, 2009). The silver ions inhibits bacterial DNA replication, damage cell cytoplasm, depleting levels of adenosine triphosphate (ATP) and finally death of cell (Feng *et al*., 2000).

Based on this information, we synthesized silver nanoparticles using thermophilic Bacillus Sp. Strain $Б_1$ in our experiment.

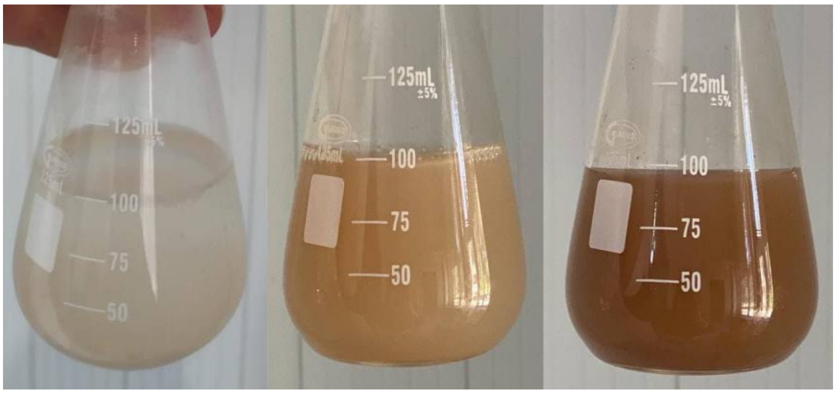
2. Materials and methods

The Bacillus strain used in the present study was isolated from a hot spring “Babazanan” (pH=7.0, t=50^0) in Salyan region of Azerbaijan. This strain was grown in liquid medium with the following composition: beef extract-1.0 g/l, yeast extract-2.0 g/l, peptone-5.0 g/l, sodium chloride-5.0 g/l. The strain Bacillus sp. $Б_1$ was incubated in thermostat at 56^0 C for 72 hours. After 72 hours of incubation, the biomass was separated from the culture fluid by centrifugation at 3000 rpm for 15 minutes. The settled biomass was centrifuged thrice by adding distilled water at 3000 rpm for 5 minutes for complete purification from the nutrient medium. The 10 g washed biomass was suspended in 50 ml distilled water and 50 ml (1mM) $AgNO_3$ solution was added. Then obtained mixture was incubated in thermostat at 55^0 C for 8 days.

The formation of silver nanoparticles was observed primarily by the darkening of the color of the reaction medium. Also, the presence of silver nanoparticles was checked by measuring the absorption spectrum in a UV-spectrophotometer (UV-Vis specord 250 plus) and using XRD analysis (Rigaku Mini Flex 600 X-ray diffractometer). The size of the silver nanoparticles was calculated using the Scherrer equation.

3. Results and discussions

During the 8 days incubation period a darkening of the color of the reaction medium from white to brown was observed. The color changing was not observed in control flask, which incubated in the same condition. The darkening of reaction medium is one of the indications the presence of silver nanoparticles. The results are shown in the Fig.1.



A **B** **C**

Fig.1. Change in color of the reaction medium at 55^0 in a thermostat:
A - initial color before incubation; **B** - within 2 days; **C** - within 8 days

Biomass was separated by filtration and the resulting colloidal solution was analyzed and absorption wavelength of 410-420 nm was observed in UV-spectrophotometer (Fig.2). Thus this absorption was fit to the characteristic absorption of silver nanoparticles.

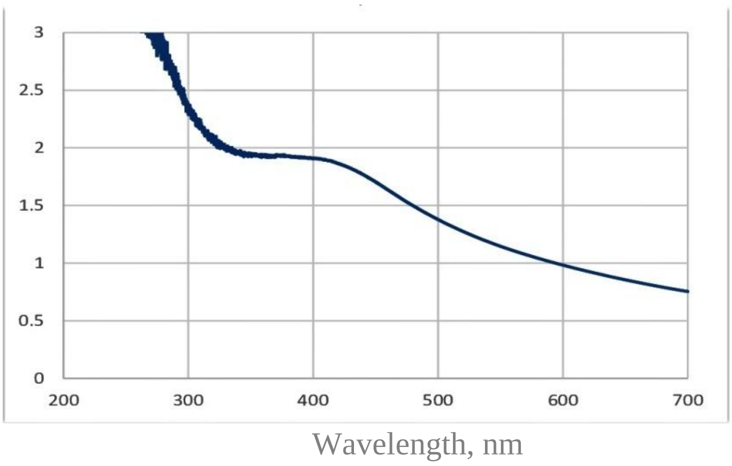


Fig. 2. UV-visible spectra of silver nanoparticles synthesized by Bacillus Sp. Б$_1$

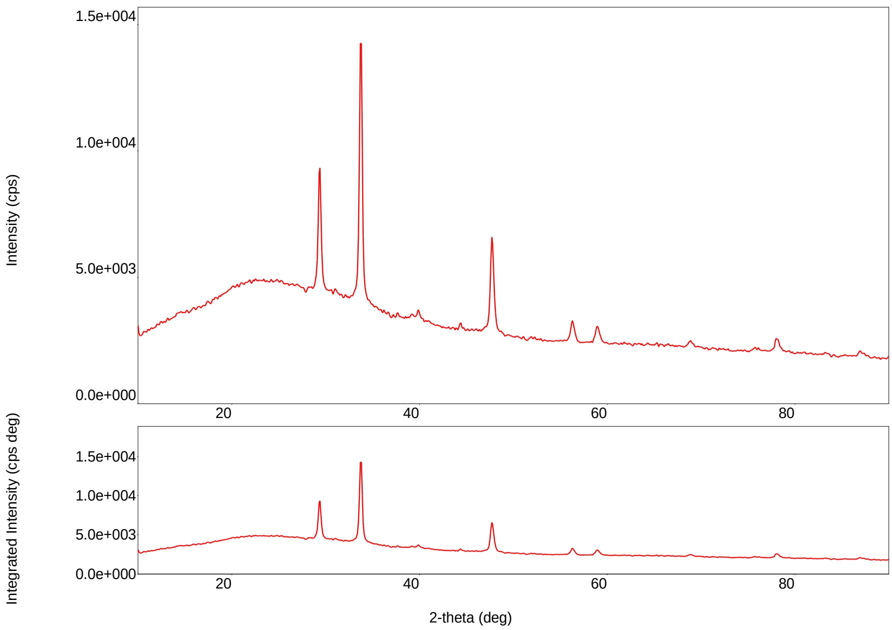


Fig. 3. X-ray diffraction (XRD) pattern of silver nanoparticles

Silver nanoparticles were characterized using X-ray diffractometer (Rigaku Mini Flex 600) in the scan range of 20-80 degree. As shown in Figure 3. The characteristic XRD peak at 77.9 were attributed to (311) reflection planes for cubic Ag (JCPDS No.65-2871). The peaks at 46.8, 67.4 were attributed to planes (220), (400) of cubic phase of AgCl crystal (JCPDS No.31-1238) (Table 1). Four unknown peaks, 29.3, 33.7, 39.9 and 58.8, were also observed, which may correspond to bacterial pellet. The particle size of Ag and AgCl is calculated by Scherrer equation. The calculated particle size is 22.9 nm and 64.6, 75.7 nm for Ag and AgCl, respectively.

Table 1. Peak list

2-theta (deg)	d (ang)	FWHM (deg)	Reflection
46.8	1.936(3)	0.15(3)	(220)
67.4	1.387975(8)	0.139(3)	(400)
77.9	1.22428(18)	0.37(3)	(311)

4. Conclusion

Green synthesis of silver nanoparticles using thermophilic bacteria is one of the most effective methods for producing silver nanoparticles. In this study, silver nanoparticles were synthesized using a thermophilic Bacillus Sp. $Б_1$. The formation of silver nanoparticles was observed by darkening the color of the reaction medium from white to brown, as well as by absorption wavelength of 410-420 nm in a UV-spectrophotometer. Using XRD analysis it was found that the synthesized silver nanoparticles are represented by Ag and AgCl with sizes of 22.9 nm and 64.6, 75,7 nm, respectively, according to the Scherrer equation.

Research in this direction continues.

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