SYNTHESIS OF NANOPARTICLES OF Ag/Cu COMPOUNDS ON THE BASIS OF EXOPOLYSACCHARIDE MATRIX OF *AZOTOBACTER CHROOCOCCUM* XH2018 AND PHYSICAL AND CHEMICAL CHARACTERIZATION

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Abstract*. In current research it was biofabricated nanobiomaterial with 46.3% Ag-, 9.0% Ag_2O-, 3.5% AgO-, 20.6% Cu- and 20.6% Cu_2O-nanoparticles or 57.7% Ag, 39.0% Cu or 3.4% O on the basis of exopolysaccharide matrix of Azotobacter chroococcum XH2018. The crystal properties of the nanobiomaterial was characterized with the help of XRD (X-ray diffraction).*

Keywords*: microorganism, exopolysaccharide, (Ag/Cu) compounds, nanoparticle, spectroscopy, Azotobacter chroococcum.*

Introduction

Production of nanoparticles (NP) based on microorganism exopolysaccharide (EPS) matrices is currently one of the most convenient methods for obtaining nano biomaterials [1]. Based on bacterial EPS, a number of NPs, in particular Ag^-, $AgCl^-$, Ag/AgCl-NPs, have been synthesized and characterized.

Within the framework of this research, work was also carried out on the creation of nanobiomaterials containing nanoparticles of silver and copper compounds based on the EPS matrix of *Azotobacter chroococcum* XH2018 strain and their physico-chemical characterization.

Materials and methods

Bacterial strain. In this study, the exopolysaccharide of *Azotobacter chroococcum* XH2018 strain, studied in previous studies, was used. The strain was grown in conventional liquid Ashby medium (sucrose – 20 g/l; $MgSO_4 \cdot 7H_2O$ – 0.2 g/l; KH_2PO_4 – 0.2 g/l; $CaCO_3$ – 5 g/l; pH – 6.8-7.0) and in solid medium (above 15 g/l agar is added to the nutrient medium) was kept. In order for the strain to intensively produce EPS, it was grown in liquid Ashby medium for 3 days at a temperature of 28-30°C and in a deep method at a speed of 150-180 rpm [1].

Extraction of EPS from culture fluid of *A. chroococcum* strain XH2018. To isolate EPS samples from strain culture fluids, they were first centrifuged at 4°C at 10,000 rpm for 10 minutes, and the supernatant free of cell biomass was separated. EPS was precipitated by adding 1:2 volume of refrigerated absolute ethanol to the obtained supernatant. After separation, the resulting precipitate was again washed in absolute ethanol. After extracting the ethanol, the samples were dried in a vacuum desiccator for 24 hours [1].

Synthesis of Ag^- and Cu^-compound nanoparticles based on *A. chroococcum* XH2018 strain EPS matrix. Synthesis of nanoparticles of Ag^+ and Cu^{+2} compounds based on EPS matrix was carried out in two stages. First, NPs containing Ag compounds was formed on the basis of EPS matrix, then Cu/Cu_2O-NPs was impregnated into the same matrix.

Experimental conditions and standards of reagents were developed within the framework of this study. 0.2 g of Cu/Cu_2O-NPs and 0.34 g of $AgNO_3$ salt were mixed with 200 ml of *A. chroococcum* strain XH2018 EPS colloidal solution. The reaction mixture was kept at room temperature until the color changed. The mixture not absorbed into the EPS matrix was filtered off and the filtrate was mixed with absolute ethanol. Ethanol was poured until the precipitate was completely separated. The precipitate formed was separated and washed again with ethyl alcohol. The washed precipitate was dried at room conditions.

X-ray structural analysis (XRD) of nanobiomaterial. X-ray structural analysis of nanobiocomposites was performed on a Bruker D8 advance device. For this purpose, the dried sample was placed on the microscope window and the diffractogram was analyzed in Cu-K α radiation and nickel monochromator filter wave at 40 kV voltage and 30 mA current [1].

Results

Using the EPS macromolecule as both a reducing agent and a stabilizing agent, nanoparticles of silver and its compounds can be formed. In this process, the Ag^+ ion is returned to a neutral, i.e., atomic state. Atomic silver is placed in the EPS matrix in nanoscale [3, 4]. Based on this, in order to collect nanoparticles of silver and copper compounds, silver nanoparticles are first synthesized in the EPS matrix. This process is essentially a chemical process. At the same time, Cu/Cu_2O-NPs was added to the reaction mixture during the reduction of Ag^+ to Ag^0 in the EPS matrix.

It can be explained that this change consists of two parallel processes. The first is the reduction of silver ions and the deposition of atomic silver in the EPS matrix. The second is the direct absorption of Cu/Cu_2O-NPSs into the EPS matrix. As a result of this process, nanoparticles of Ag compounds and Cu/Cu_2O-NPS are simultaneously removed from the EPS matrix.

$AgNO_3$ 10 nM solution was used as silver source in our experiments. Based on the above process, a nanobiomaterial containing $Ag/Ag_2O/AgO/Cu/Cu_2O$-NPs was obtained. Notably, the silver cation in $AgNO_3$ formed Ag_2O- and AgO-NPs in the biological matrix in addition to Ag^0. It was determined that the crystal structure of the final nanobiomaterial consisted of 46.3% Ag^-, 9.0% Ag_2O^-, 3.5% AgO^-, 20.6% Cu^- and 20.6% Cu_2O-NPs (Table 1). According to the composition of the element, it was observed that it consists of 57.7% Ag, 39.0% Cu and 3.4% O (Table 2).

Table 1

Mass fraction of constituents of $Ag/Ag_2O/AgO/Cu/Cu_2O$-NPs based on A. chroococcum strain XH2018 EPS matrix

Component	Chemical formula	Mass fraction, %
Silver	Ag	46,3
Silver(I)-oxide	Ag_2O	9,0
Silver oxide	AgO	3,5
Copper	Cu	20,6
Copper (II)-oxide	Cu_2O	20,6

The X-ray structural composition of the resulting nanobiomaterial EPS@$Ag/Ag_2O/AgO/Cu/Cu_2O$-NPs showed the presence of peaks characteristic for each component (Fig. 1). It was found that Ag-, AgO-, Cu_2O-, Cu-NPs crystals in the nanobiomaterial are cubic, and AgO-NPs crystals are monoclinic. All other parameters of the obtained nanobiomaterial are detailed in Table 3.

Table 2

Elemental composition of Ag/Ag_2O/AgO/Cu/Cu_2O-NPs based on A. chroococcum XH2018 strain EPS matrix

Element	Mass fraction, %
Ag	57.7
Cu	39.0
O	3.4

Table 3.

Some characteristics of the crystal structure of Ag/Ag_2O/AgO/Cu/Cu_2O-NPs obtained on the basis of A. chroococcum XH2018 strain EPS matrix

№	*2θ [°]*	*d [Å]*	*I/I0 (peak height)*	*Peak surface*	*FWHM*
1	29,30	3,0457	41,13	78,44	0,2000
2	32,10	2,7861	34,56	115,33	0,3500
3	36,27	2,4745	179,01	597,46	0,3500
4	37,98	2,3675	1000,00	3337,53	0,3500
5	42,17	2,1410	62,68	388,49	0,6500
6	43,17	2,0937	234,19	781,63	0,3500
7	43,89	2,0641	329,54	942,73	0,3000
8	44,15	2,0497	238,90	1139,07	0,5000
9	46,08	1,9684	27,92	133,10	0,5000
10	50,30	1,8125	57,69	220,03	0,4000
11	61,28	1,5116	27,06	141,90	0,5500
12	64,28	1,4481	248,00	1300,68	0,5500

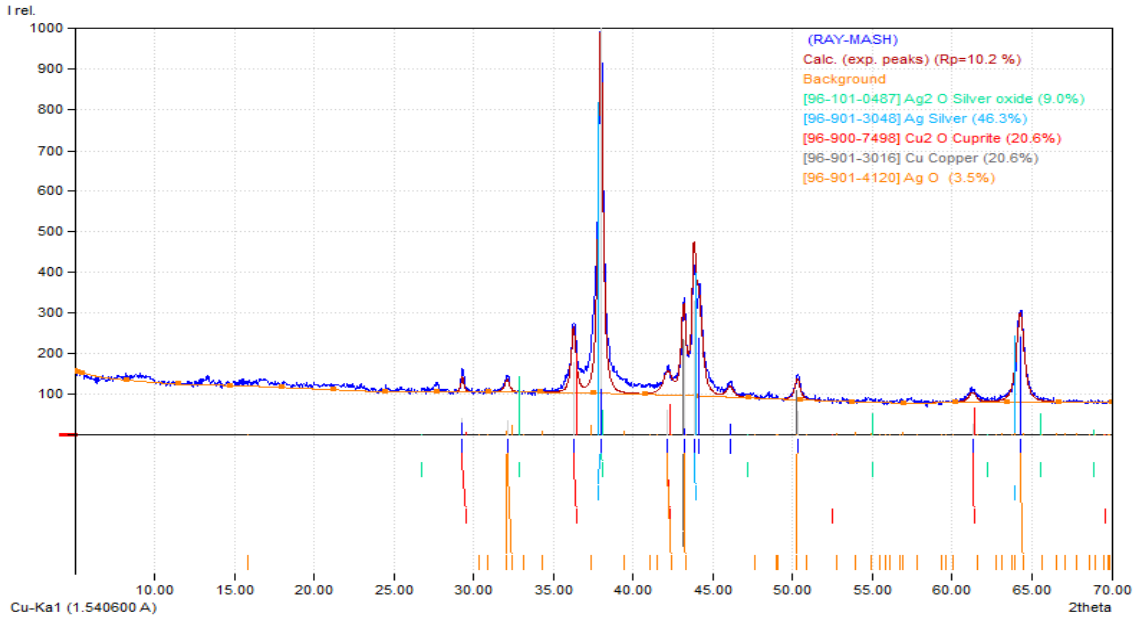

Fig. 1. X-ray structural spectrum of Ag/Ag_2O/AgO/Cu/Cu_2O-NPs based on A. chroococcum strain XH2018 EPS matrix

It can be concluded from the experiments that nanoparticles of silver and copper compounds, including Ag^-, Ag_2O^-, AgO^-, Cu^- and Cu_2O-NPs, were introduced into a single matrix.

This method makes it possible to combine the synergistic effects of different nanoparticles by summarizing their properties.

In general, our study also proved that bacterial EPS macromolecules serve as a means of assembling nanoparticles into a single matrix. In the next experiments, scientific studies on the introduction of nanoparticles into the matrix in a concentration-dependent manner will help to further fundamental research of this topic.

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