THE EFFECT OF METAL NANOPARTICLES ON THE CULTIVATION AND CONTENT OF BIOLOGICALLY ACTIVE SUBSTANCES IN CHLORELLA VULGARIS

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Abstract

Nanoparticles are microscopic objects ranging in size from 1 to 100 nm. The distinctive properties of nanoparticles are their size, morphology and bioactivity. Metallic nanoparticles make up to 90% of all used nanomaterials. This paper presents a new approach to obtaining silver nanoparticles by chemical reduction of silver cations in the presence of biopolymer reductants and stabilizers sodium alginate and sodium carboxymethylcellulose. The obtained silver nanoparticles were used to cultivate the biomass of Chlorella vulgaris. The results of the experimental studies showed the environmental safety and bioactivity of the obtained nanoparticles for the growth of microalgae. The most pronounced stimulating effect was found for silver nanoparticles obtained in the presence of the biopolymer sodium carboxymethylcellulose.

Keywords: *Chlorella vulgaris*, silver nanoparticles, biopolymer, sodium alginate, sodium carboxymethylcellulose

1. INTRODUCTION

The addition of metal nanoparticles (NPs) to the culture of the microalgae *Chlorella vulgaris* is of practical interest, since they can directly or indirectly interact with the microalgae, leading to an increase in the production of biomass and biologically active substances [1].

Physicochemical and biological characteristics of NPs, i.e. shape, size and bioactivity affect the metabolism of microalgae, but at the same time, to increase the value of microalgae biomass, it is necessary to synthesize optimal NPs. One of the most common and promising NPs for agriculture and biotechnology are silver NPs. AgNPs act as nanofertilizers and stimulate seed germination and plant growth, increase the efficiency of the photosynthesis process. AgNPs also have antibacterial, antiviral and antifungal properties and provide non-toxic control of pests and pathogens [2-5].

Physical, chemical and biological methods are used to synthesize NPs, each with its own advantages and disadvantages. Among them, chemical methods are the most economical and easily scalable. Chemical synthesis is based on the reaction of reducing silver cations from the $AgNO_3$ matrix with various reducing agents according to the following scheme:

$$AgNO_3 \leftrightarrow Ag^+ + NO_3^- \qquad Ag^+ + e^- \rightarrow Ag^0$$

As a result, the chemical synthesis of NPs must be carried out using non-toxic, biocompatible reagents, such as natural polymers.

Sodium alginate (SA) is a biopolymer isolated from brown seaweed, a water-soluble polysaccharide with gelling properties, widely used in medicine, biotechnology, and the food industry (Fig. 1) [6].

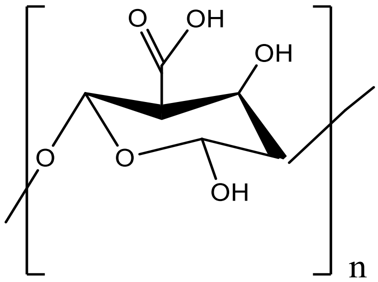

Fig.1. Structural formula of the SA biopolymer

Sodium carboxymethyl cellulose (Na-CMC) is a water-soluble biopolymer obtained from wood cellulose. It is used as a stabilizer and thickener in the food and medical industries (Fig. 2) [7].

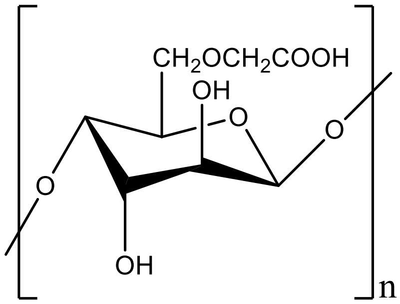

Fig. 2. Structural formula of the biopolymer Na-CMC

The aim of this research was to evaluate the effect of AgNPs obtained using Na-CMC and SA biopolymers on the cultivation of *Chlorella vulgaris* biomass.

2. MATERIALS AND METHODS

2.1. Materials

The objects of the study were colloidal solutions of AgNPs and a suspension of the microalgae Chlorella vulgaris.

2.2. Methods

Preparation of AgNPs in the Na-CMC biopolymer matrix: 1 ml of 10^{-4} M $AgNO_3$ was added to 5 ml of 0.4% Na-CMC, stirred and heated to 55-65°C on a magnetic stirrer with heating IKA® RH basic 2 (Germany), then 1 M NaOH was added dropwise to an alkaline pH of 9-10. The color of the solution changed within 15 min from colorless to yellow (Fig. 3).

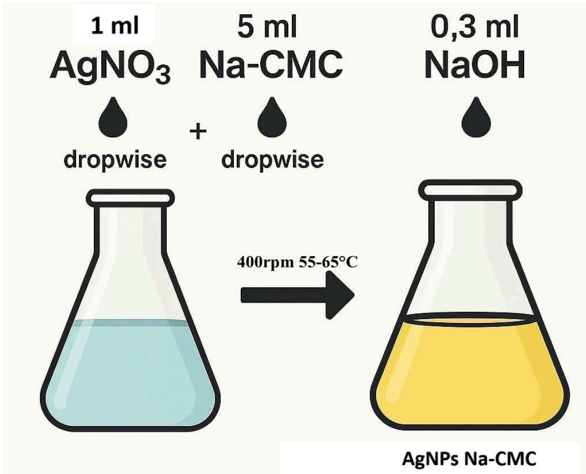

Fig. 3. Production of AgNPs using Na-CMC biopolymer

Preparation of AgNPs in the SA biopolymer matrix: 5 ml of an aqueous solution of 10^{-3} M $AgNO_3$ were added to 5 ml of SA (0.75%) in a vial to obtain a complex of silver ion with the substrate $[Ag(SA)]^+$. The reaction mixture was stirred and heated to 55-65°C on a magnetic stirrer with heating IKA®RH basic 2 (Germany). Then, an aqueous solution of 1 M NaOH (0.5 ml) was added to the prepared vial with 10 ml of the solution. The reaction continued for 15 minutes. The color of the colloidal solution changed from colorless to yellow. The change in the color of the solution indicates the formation of NPs (Fig. 4).

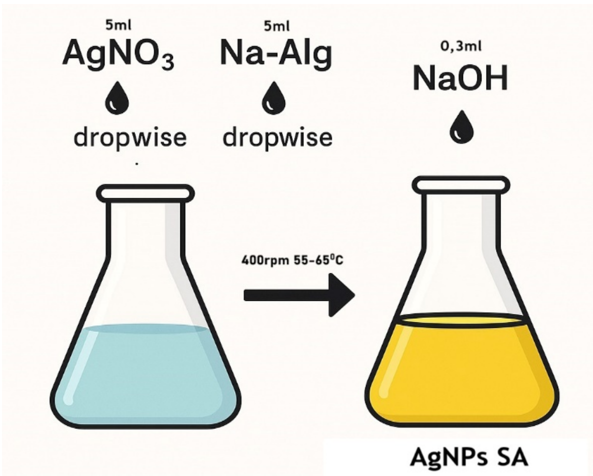

Fig. 4. Production of AgNPs using SA biopolymer

To confirm the successful synthesis of AgNPs, as well as to study their structural and colloid-chemical characteristics, UV-visible spectroscopy was carried out on a Specord 210 plus UV spectrophotometer (Analytik Jena, Germany) with measurement of the optical density of the solution in the range of 300-700 nm. The hydrodynamic radius of the particles was determined by three-dimensional light scattering spectroscopy on a goniometric device with a variable light scattering angle 3D LS Spectrometry (Switzerland).

Microalgae were cultivated using the commercial strain *Chlorella vulgaris* IFR No. C-111 on Bogdanov's nutrient medium in a grow box with controlled physicochemical conditions [8, 9]. Biomass growth was determined by optical and microscopic methods [10].

Determination of the mass fraction of total nitrogen and crude protein

The mass fraction of total nitrogen (W_N, %) and the content of crude protein (Wpr, %) in the chlorella biomass were determined by the Kjeldahl method [11] using the Keltran complex (Russia) and calculated using formulas 1 and 2:

$$W_N = \frac{(V_1 \cdot C_{N1} - V_2 \cdot C_{N2}) \cdot 14 \cdot 100}{m \cdot 1000} \qquad (1)$$

where V_1 is the volume of H_2SO_4 in the receiver, ml; CN_1 is the normal concentration of H_2SO_4, mmol/ml; V_2 is the volume of sodium hydroxide solution used for titration, ml; C_{N2} is the normal concentration of NaOH, mmol/ml; 14 is the atomic molar mass of nitrogen, m is the sample weight of dry chlorella biomass, g; 1000 is the conversion factor of mg of the atomic mass of nitrogen to g.

$$W_{pr} = 6.25 \cdot N \cdot 0{,}1 \qquad (2)$$

where 6.25 is the coefficient for converting the mass fraction of nitrogen to the mass fraction of crude protein, N is the mass of nitrogen in the analyzed sample (g/kg), recalculated from W_N (%); 0.1 is the coefficient converting units of mass fraction (g/kg) to percentages.

3. RESULTS AND DISCUSSION

Silver NPs were obtained by a chemical method using polyanionic biopolymers Na-CMC and SA as reducing agents and stabilizers. The mechanism for obtaining AgNPs using SA and Na-CMC is as follows: the polyanion plays the role of a reducing agent and stabilizer. The oxygen atoms of the hydroxyl and carboxyl groups are electron donors for the Ag^+ chelate. When heated, the Ag^0 chelate is formed. In this case, the hydroxyl group is oxidized to a carbonyl group, which can prevent the aggregation of AgNPs-SA and AgNPs-Na-CMC [12].

The formation of AgNPs was confirmed visually by yellow coloration of the solutions (Fig. 5), as well as by UV-visible spectroscopy and 3D LS (Fig. 6, 7).

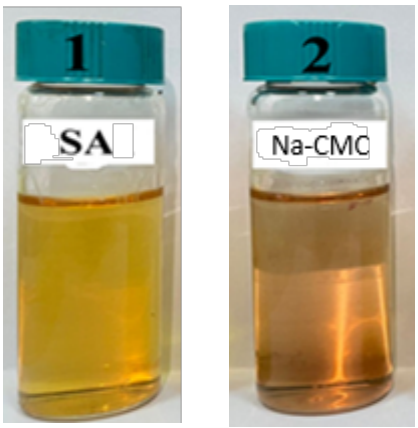

Fig. 5. Colloidal solutions of AgNPs

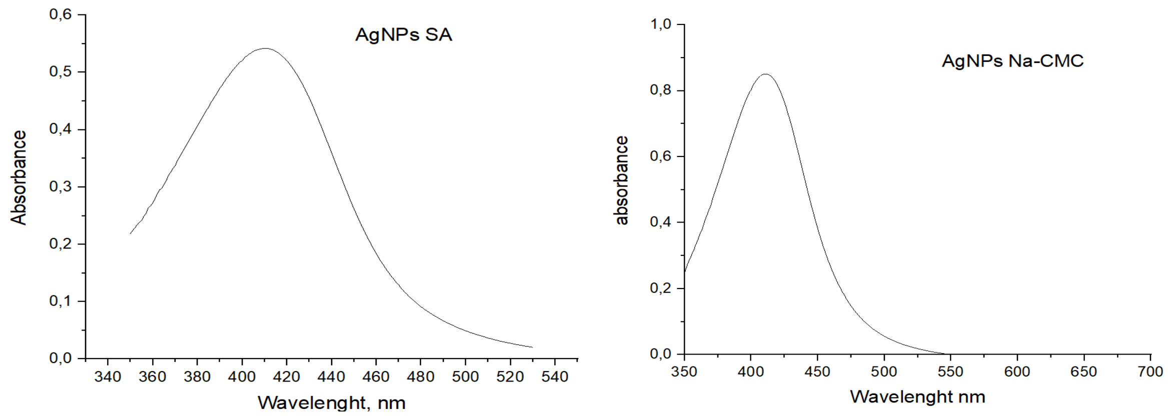

Fig. 6. UV study results of AgNPs

In the UV spectra of both the SA and Na-CMC systems, pronounced absorption bands were observed at a wavelength of about 420 nm (Fig. 6). This maximum corresponds to the surface plasmon resonance, which occurs as a result of the collective oscillation of free electrons on the surface of metal NPs under the influence of electromagnetic radiation. The appearance of such a peak indicates the formation of colloidal AgNPs, predominantly spherical in shape. The absence of additional peaks in the UV range confirms the purity of the obtained NPs and the absence of a significant amount of by-products, such as silver in ionic or oxide forms [13].

Comparative analysis of 3D LS data showed significant differences in the size characteristics of nanoparticles depending on the stabilizer used (Fig. 7).

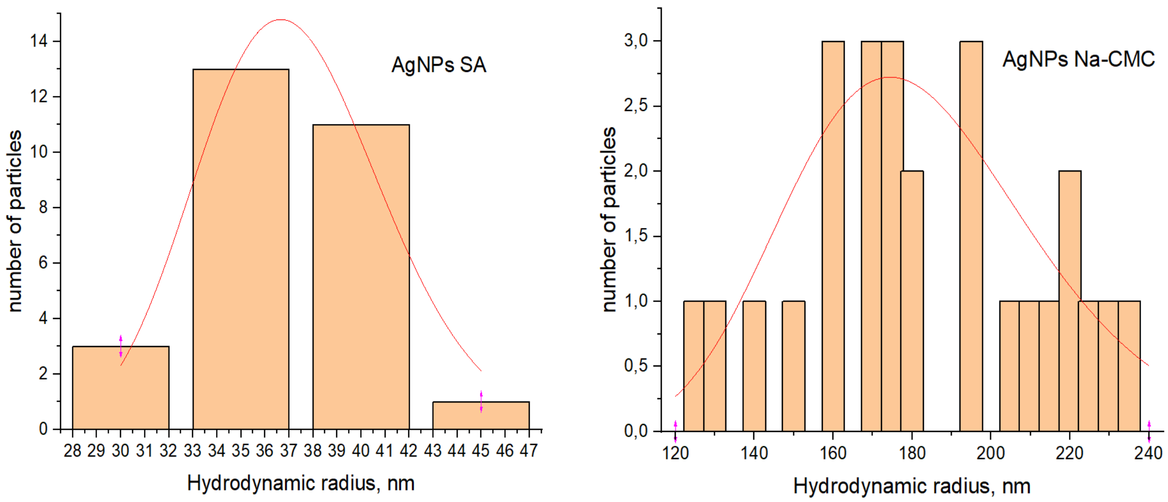

Fig. 7. Hydrodynamic radii of AgNPs obtained using SA and Na-CMC biopolymers

As can be seen from Fig. 7, in the case of using SA, AgNPs were characterized by a relatively narrow size distribution: the main range of radii was from 34 to 39 nm, with an average value of the hydrodynamic radius of about 36 nm. The narrow distribution indicates a high degree of stability and homogeneity of the colloidal system, which is probably due to the high coordination capacity of the functional groups (carboxyl and hydroxyl) of NaSA, which contribute to the effective encapsulation and stabilization of silver at the early stages of NP growth.

In contrast, AgNPs obtained using Na-CMC had significantly larger sizes: the hydrodynamic radius varied from 120 to 240 nm, with an average value of about 175 nm. The spectrum was characterized by a wide distribution region and pronounced polydispersity. The spatial structure of Na-CMC has an insignificant number of functional groups capable of complexation with silver ions. The obtained solutions of 3.10^{-8} M AgNPs with Na-CMC and 3.10^{-7} M with SA were separately introduced into Bogdanov's nutrient medium for cultivating *Chlorella vulgaris*. The effect of AgNPs on the growth of microalgae biomass is shown in Fig. 8,9.

As can be seen from Fig. 8 and 9, the optical density indicates the ongoing growth phases of the microalga: after a short-term adaptation stage, exponential cell growth is observed, and the cell death phase is absent. The optical density of the studied chlorella suspensions in the presence of AgNPs shows a coincidence of peaks at a wavelength of 683 nm at the same time of day and an increase in optical density over 3 days. This result is due to the absorption of light at a given wavelength by chlorophylls a and b and corresponds to their increase with the growth of the microalga biomass. At the same time, AgNPs with Na-CMC enhance the growth of chlorella cells and the concentration of chlorophylls *a* and *b* by 3 times.

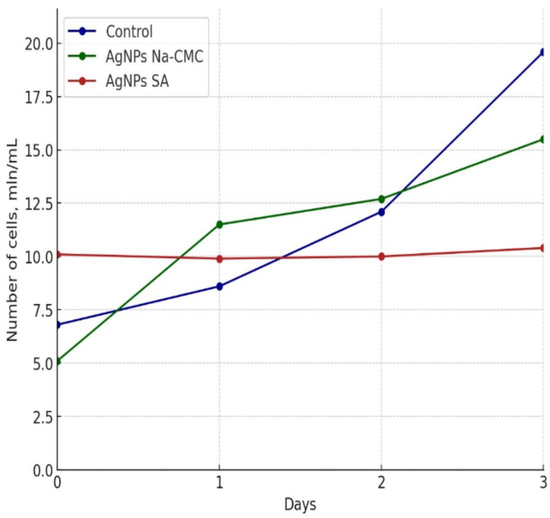

Fig. 8. Chlorella growth dynamics (number of cells, million/ml) for AgNPs Na-CMC and SA

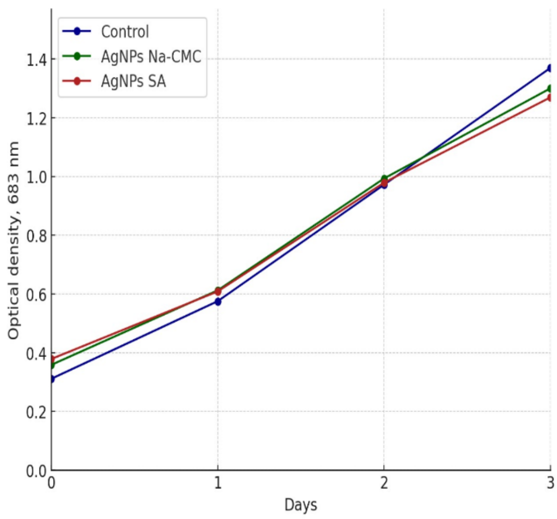

Fig. 9. Change in optical density (683 nm) for AgNPs Na-CMC and SA

The biostimulating properties of NPs are affected by their size and concentration (Fig. 7). Particles up to 50 nm are toxic, interact with the cell membrane and penetrate the cell, causing oxidative stress and the formation of reactive oxygen species [14, 15]. Particles larger than 50 nm do not exhibit a toxic effect on the vital activity of microalgae.

In general, modified NP nutrient media and Bogdanov's control medium have a stimulating effect on the growth of chlorella.

Analysis of total nitrogen and crude protein showed that the introduction of AgNPs into the nutrient medium does not have a toxic effect on the protein biomass of chlorella: the content of crude protein in the control and modified media was 42-47%, total nitrogen – 6-7%.

4. CONCLUSIONS

Thus, AgNPs were synthesized by the chemical reduction method using SA and Na-CMC biopolymers. The average size of monodisperse AgNPs with NaSA biopolymer is 36 nm, the average size of polydisperse AgNPs with Na-CMC biopolymer is 175 nm. The results indicate that SA is a more effective stabilizer of AgNPs compared to Na-CMC. The obtained AgNPs are not toxic for the growth of *Chlorella vulgaris* biomass. At the same time, AgNPs obtained using Na-CMC have the best biostimulating effect, which is explained by the lower concentration and larger sizes of NPs.

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