DOI: 10.17725/rensit.2022.14.393

Methods for Studying Parameters Biogenic Metal Nanoparticles, Formed in situ

Dmitry A. Skladnev, Vladimir V. Sorokin
Vinogradsky Institute of Microbiology of RAS, http://www.inmi.ru/
Moscow 117312, Russian Federation
E-mail: skladda@gmail.com, vlvlsorokin@gmail.com

Sergey P. Karlov
Moscow Polytechnic University, http://mospolytech.ru/
Moscow 107023, Russian Federation
E-mail: karlovsp@yandex.ru

Vladimir I. Anisimkin
Kotelnikov Institute of Radioengineering and Electronics RAS, http://www.cplire.ru/
Moscow 125009, Russian Federation
E-mail: anis@cplire.ru

Received 15 November 2022, peer-reviewed 22 November 2022, accepted 29 November 2022

Abstract: **We propose an innovative method for assessing the integral level of metabolic activity of biological objects, based on the natural ability of living cells for generation metal nanoparticles from cations sources (artificially introduced into the samples), while in sterile samples or in the presence of inactive cells, the formation of nanoparticles does not occur. The method is called DBNG (detection of biogenic nanoparticles generation/growth). This article presents several examples of the use of the main analytical approaches applicable to obtain information about the parameters of biogenic nanocrystalline structures that formed in situ(directly in the tested samples), as well as options for the practical use of the proposed nanobiotechnological innovative approach DBNG to assess (estimate) the basic integral properties of living cells.**

Keywords: **metabolic activity of cells, generation de novo nanoparticles, detection of biogenic metal nanoparticles, fluorescence spectrometry, acoustoelectronic technologies**

UDC 57.016.6 +57.033

Acknowledgments: The work was done within the framework of the government task of the Ministry of Science and Higher Education of the Russian Federation, FRC "Fundamental Foundations of Biotechnology" of the RAS (№122040800164-6) and Kotel'nikov IRE RAS (№FFWZ-2022-0002). The work was partially funded by the Russian Foundation for Basic Research (grant # 20-57-7804).

For citation: Dmitry A. Skladnev, Sergey P. Karlov, Vladimir I. Anisimkin, Vladimir V. Sorokin. Methods for Studying Parameters Biogenic Metal Nanoparticles, Formed in situ. *RENSIT: Radioelectronics. Nanosystems. Information Technologies*, 2022, 14(4):393-414e. DOI: 10.17725/rensit.2022.14.393.

Contents

1. INTRODUCTION

Many areas of microbiology, ecology and medicine require measurements to assess the level of metabolism of living cells or the dynamics of its changes present in the investigated samples. There are various research tasks that require an answer to the question, are microorganisms present in the samples or are these samples sterile? Examples of such tasks include the study of samples taken in extreme natural zones of the Earth (Antarctic Lake Vostok), or when it is necessary to accurately determine whether the causative agent of the disease has been eliminated, or the task of detecting and identifying pathogenic bacteria and viruses, including in samples from other planets (Mars soil research project), and others similar to those. An exact and exhaustive answer to the question of whether the tested sample is sterile or not is very difficult, since in some cases the concentration of biological objects can be low. More often, a fairly accurate assessment of the types and abundance of biological objects, an assessment of the level of their metabolic activity is required.

At present, various methods for obtaining such information are widely used, from classical microbiological and biochemical methods to molecular genetics and metagenomics methods. However, some of them require considerable time to obtain a

reliable result, others are characterized by the high cost of the necessary reagents. Previously, an innovative method DBNG (detection of biogenic nanoparticles generation/growth) was proposed to quickly detect the presence of living cells in samples, as well as to assess the integral level of metabolic activity of biological objects. This approach is based on the natural ability of living cells to form metal nanoparticles from sources of cations artificially introduced into the samples, while in the presence of inactive cells or in sterile samples, the generation of nanoparticles does not occur [1].

Nowadays, commercial productions of nanoparticles for practical applications in medicine and biological research are produced *de novo* (from the very beginning) by chemical reduction of cations in reaction with molecules or molecular groups that have reducing properties (electron donors). To date, methods have been developed quite well that allow one to effectively and with a high yield obtain monodisperse preparations of metal nanoparticles with specified sizes, properties, and certain stabilizing coatings, which are necessary for use. Such progress would not have been possible without the development of the measurement and analytical capabilities of scientific instrumentation, without expanding the possibilities of statistical processing of the results obtained.

1.1. Modern concepts on the de novo generation of biogenic metal nanoparticles

Biological objects in natural habitats are constantly encountered with certain cations. Some of them are necessary for the normal functioning of the enzyme systems of cell metabolism. At the same time, excess concentrations of most cations are toxic. Metabolically active cells have a natural ability to reduction of cations, which allows being transferred to a solid non-toxic state by generation metal nanoparticles [2]. Thus, cation detoxification is one of the main protective reactions for the survival of microbial cells from the very first years of their habitat in the environment on Earth.

It is important to emphasize that the *de novo* generated nanoparticles create a new solid nanocrystalline phase in the reaction mixture. The appearance in cell suspensions (i.e. among amorphous structures in the liquid phase) of a new crystalline phase can be detected with high accuracy by many physical methods. It is this phase transition that we propose to use for the detection of biological objects. Using many biological model objects, we have shown that metabolically active cells are able to very quickly generate *de novo* metal nanoparticles from salt solutions as sources of cations artificially introduced into samples, while in the presence of inactive cells or in sterile samples, the formation of nanoparticles does not occur.

The fundamental natural ability of living cells to protect their metabolic activity from the toxic effects of cations (Me^{n+}) is realized by reducing cations to the zero-valent state (Me^0). The absence of a charge on the reduced atoms triggers the process of their agglutination, which leads to the formation of insoluble, much less toxic nanoclusters (Me^0NCs). Primary clusters with a small number of atoms can partially retain their charge, since they can include atoms of both types (Me^{n+} and Me^0) [3,4]. In this case, if electron donors are constantly present in the reaction mixture, the proportion of cations in nanoclusters proportionally decreases.

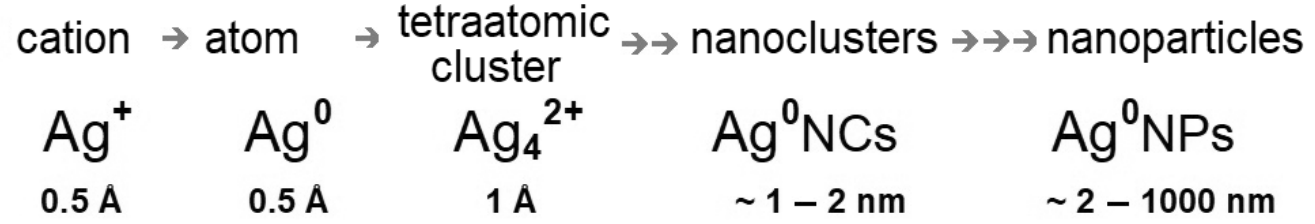

Fig. 1. *Successive stages of cation reduction and de novo formation of nanocrystalline structures.*

Continued agglutination of less and less charged nanoclusters leads to the generation of larger and larger crystalline structures called metal nanoparticles (Me^0NPs) (**Fig. 1**).

When developing the proposed approach, we took into account the observed significant differences in the parameters and properties of nanoparticles generated in cultures of different microorganisms, in cultures of different ages or the physiological state of their cells, differing in the chemical composition of surface cell biopolymers, as well as in cases of different compositions of growth media and reaction conditions [5,6,7]. It should be noted that during the generation of nanoparticles, active cells can secrete various organic compounds – stabilizers that interfere with the process of self-assembly of larger nanocrystalline structures [1,8,9] (see also Fig. 13).

In the last twenty years, metal nanoparticles have attracted increased attention due to their unique optical properties due to the phenomenon of surface plasmon resonance. It should also be noted that preparations of metal nanoparticles have a highly developed surface, high capacitance of the electric double layer, and high catalytic activity [10]. Due to these specific properties of nanosized crystals, the dynamics of their generation can be reliably recorded using many high-precision analytical methods. Along with spectroscopy in the UV and visible range (UV–vis absorption), which is common for biological studies, this can be *X*-ray fluorescence (RFS), as well as electronic (transmission electron microscopy TEM) and scanning electron microscopy (field emission scanning electron microscopy FESEM). To study metal nanoparticles, it is possible to carry out surface enhanced Raman scattering spectroscopy (surface enhanced Raman scattering SERS), registration of the distribution of linear sizes of nanoparticles (particle size distribution PSD), dynamic light scattering (DLS), *X*-ray angular scattering (*X*-ray powder diffraction XRD), thin layer chromatography (TLC) and others [3,11,12]. It should be emphasized that it is the crystal structure of nanoparticles that makes it possible to use most of the above methods for recording the parameters of generated *de novo* biogenic nanoparticles directly against the background of the presence of microorganism cells or other biological objects that ensured the *in situ* reduction (directly in the samples under study) of artificially introduced cations. It has been accurately shown that all optical and other properties of synthesized nanocrystals are completely and strictly determined by their linear dimensions, shape, and atomic composition [13,14]. Since the experimenter has the opportunity to control the physicochemical parameters and conditions when de novobiogenic nanocrystals, it becomes possible to isolate the influence of the biological object present in the reaction mixture, which is the main reducing agent

of cations. Thus, since the use of traditional high-precision analytical methods for studying the parameters of nanocrystals can be effectively used to monitor the generation of biogenic nanoparticles. All of them can be used to solve many important problems in microbiology, biotechnology, and medicine.

The chemical reduction of cations itself implies the participation of various sources/ donors of free electrons in the reaction. Both inorganic substances and molecular groups of organic biogenic compounds with reducing properties (for example, –OH) can serve as electron donors during the generation of metal clusters and nanoparticles [7]. The level of presence of molecular electron donors in the reaction mixture significantly affects the ratio of reduced atoms and unreduced (residual) cations ingenerated *de novo* nanocrystalline structures. With a constantly high presence of reducing agents in the reaction mixture, the proportion of cations and, accordingly, the charge level of the surface of nanocrystals constantly decreases, which contributes to the acceleration of self-assembly of nanoclusters and the rapid enlargement of nanoparticles. It is important to note that it is metabolically active cells that have a natural ability to constantly secrete low molecular weight compounds into the external environment, many of which have reducing properties. It is this property of living metabolically active cells that allows them to act as electron donors for a long time, i.e., serve as stable cation reducers and, accordingly, ensure the generation of metal nanoparticles [15]. Less active biological objects (resting dormant forms of microorganisms, spores, viral particles) can reduce cations only at the expense of various electron donor groups localized on the cell surface or included in surface biopolymers. Virus particles can only act as extremely weak cations reducing agents due to the presence of reducing amino acid groups in the proteins of their capsids [6,16,17].

Another specific natural factor that negatively affects the possibility of enlargement of biogenic metal nanoparticles due to a decrease in the interaction forces of nanoclusters has been well studied. Many organic compounds capable of being adsorbed on the surface of growing nanocrystals have this effect. Based on modern concepts, biogenic molecules react with residual positively charged Me^{n+} atoms of nanoclusters and form a surface layer that prevents further self-assembly and enlargement. Such compounds are called size stabilizers at the early stages of nanoparticles generation [6].

1.2. Main research principles and provisions applied in the development protocol of the DBNG method

At all stages of the biosynthesis of metal nanoparticles *de novo*, the ongoing processes of cation reduction and the formation of a solid crystalline phase in the reaction mixture significantly and quite definitely depend on the reaction conditions and on the combination of the components present. That is why we proposed to use the measured dynamic parameters of nanocrystalline products obtained as a result of the reduction of cations to assess the general physiological state of cells [1,7,15]. In general, the experimentally measured actual parameters of biogenic nanostructures generated *in situ* are an indicator of the properties of those cells that ensure the restoration of introduced cation sources.

First of all, the protocol of the DBNG method involves the introduction of salt

as a source of cations (in the form of low concentrated sterile solution) directly into the aqueous suspension of the biological object under study (i.e., *in situ*). To confirm the fact that the reducing agents are of biogenic origin, the protocol provides for the preparation of a control aliquot of the tested sample, freed from cells using a mechanical method (filtration, centrifugation). The obligatory use of control (sterile, cell-free) variants of the studied samples is necessary, since the cells of biological samples are usually found in growth media, that is, in multicomponent solutions of organic compounds and the necessary growth factors, many of which are electron donors. At the same time, it should be emphasized that the reducing ability of such compounds, even in the composition of full-fledged growth media, cannot ensure the full generation of metal nanoparticles, but only leads to the appearance of their precursors – nanoclusters (**Fig. 2**).

As emphasized above, the physicochemical conditions in the reaction mixture during the in situgeneration of both nanoclusters and larger nanoparticles significantly affect the parameters of the final crystalline self-assembly products [16,17]. An increase in temperature, as well as stirring (vibration) of test tubes with reaction mixtures during the reduction of cations, accelerates the process of self-assembly of nanoclusters, which ultimately leads to an increase in the size of the generated nanoparticles.

It is important to note that the use of different salts as a source of cations can give different results. In particular, it was noted that the use of Tollens' reagent $Ag(NH_3)_2NO_3$ as silver cations source makes it possible to form larger nanoparticles in the presence of microbial cells than when using the $AgNO_3$ salt under identical conditions.

The DBNG protocol involves the introduction of very small amounts of cation sources (~mM) into the test samples, which are sufficient only to form a noticeable amount of nanoparticles with minimal toxic effect on the metabolism of tested cells under study. It is important to note that the use of low concentrations of cation sources retains the possibility of isolating pure cultures of the detected microorganisms from the studied aliquots.

Metabolically active cells are able to generate silver nanoparticles in a few minutes. For standard experiments according to the DBNG protocol, the duration of all reactions for the reduction of introduced cations by microorganism cells was limited to 20 minutes [1]. This time does not exceed the length of normal metabolic responses of cells to the addition of excess concentrations of various salts to the medium [6,18], and also does not

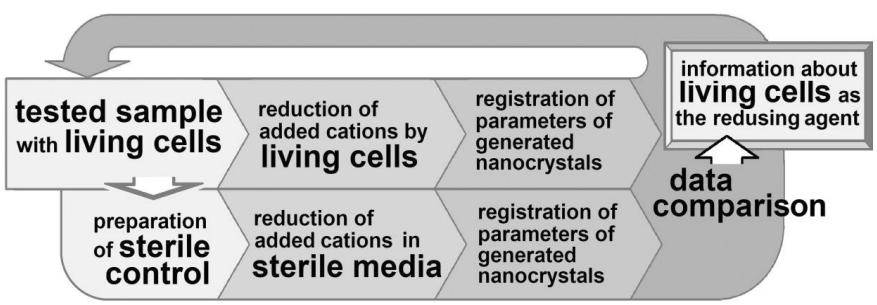

Fig. 2. *The concept of the DBNG methodological approach*.

exceed the generation time of most microorganisms. Thus, a short time of carrying out the reduction reaction makes it possible to fix the physiological state of the studied cultures (samples) precisely at the moment of introducing the source of cations. To detect viral particles (as weak reducing agents), the reaction time with introduced cations was increased to 50 minutes.

Since the generation of metal nanoparticles is carried out *in situ* directly in the studied cell suspensions, it was proposed for the first time to record the spectral characteristics of nanoparticles in the *vis*-range using an Ulbricht integrating sphere [1]. This method of measuring spectral characteristics in a reflected light flux makes it possible to register even nanoparticles that form directly on the surfaces of biological objects, which is impossible with a standard transmitted light flux.

It has been shown that the DBNG protocol (using TEM) makes it possible to register metabolically active cells of microorganisms by their ability to generate biogenic nanoparticles with a sensitivity of ~10 ml^{-1}. The use of other analytical methods for the detection of nanocrystalline structures can increase the sensitivity of the method.

For generation *in situ* of metal nanoparticles by cells or tissue fragments, it was proposed to place the studied biomaterial itself directly into a solution of a cation source. In such cases, standard inactive preparations should be used as controls.

According to the DBNG protocol, the generation of biogenic metal nanoparticles is carried out in microliter volumes of the reaction mixture. This allowed us to develop a concept for applying this innovative method in a microfluidic version (Lab-on-Chip).

2. MATERIALS, METHODS, EQUIPMENT

Pure cultures of microorganisms were stored and prepared for research using standard growth media LB [1] or M9 [20] according to standard microbiological methods. Natural water samples were taken with standard instruments in an aseptic way, transported and stored at a low temperature.

Preparations of tumor tissue cells from neoplasms of the adnexal apparatus of the eye, as well as healthy tissue from the same eye as a control, were surgically removed. Paired tissue samples were placed under aseptic conditions in equal volumes of a sterile solution of a cation source.

The sources of cations were sterile aqueous solutions of ammonia silver $Ag(NH_3)_2NO_3$ obtained according to the protocol of the Tollens reaction [1, 19]. To obtain Na_2PdCl_4, $PdCl_2$ and an excess of NaCl in distilled water were used [20].

The generation of metal nanoparticles was carried out in situ directly in the studied water samples or in bacterial suspensions at the late logarithmic phase of culture growth. The reaction mixture contained aliquots of the studied cell suspensions (about 10^6 mL^{-1}) with a volume of 45 μL and 5 μL of a sterile solution of the cation source in a final salt concentration of 0.1 mM. The cation reduction reaction time was 20 min. Aliquots of the studied suspensions freed from microorganisms after complete sedimentation of cells by centrifugation at 12000 g for 15 min were used as control [1].

Hydrogels were prepared using low EEO agarose (0.4 wt %) and silver nitrate (5 μg/mL) [21].

Microorganisms were visualized using an Amplival microscope (DDR) with a phase contrast device.

Registration of the generation of biogenic nanoparticles *in situ* directly in cell suspensions was performed using an OceanOptics USB-2000 spectrometer (USA) with an Ulbricht integrating sphere.

Measurement of the intensity of small-angle *X*-ray scattering of preparations was carried out on an automatic diffractometer "AMUR-K" with a single-coordinate position-sensitive detector OD3M (in a capillary with a volume of 20 μL) at a fixed radiation wavelength of $\lambda_{0.1542}$ nm with a signal accumulation time of 40 minutes according to a certified method [1]. The scattering measurement results were processed to calculate the volumetric size distribution function of nanoparticles $D_V(R)$ using the MIXTURE program.

Particle size was measured by PCS on a Delsa Nano Submicron Particle Size and Zeta Potential Particle Analyzer (USA).

Images of nanoparticles were obtained using thetransmission electron microscope JEM-1400 (JEOL, Japan) at the UNIQEM Collection Core Facility, Research Center of Biotechnology of the Russian Academy of Science equipped with the microanalyzer (Oxford Instruments, Great Britain) at an accelerating voltage of 80 keV.

The determination of the linear dimensions of metal nanoparticles was carried out using the computer program "Compass 3*D-V*14" and a specially developed calculation algorithm for electronic photographs, at least 300 nanoparticles for each sample.

3. RESULTS

3.1. Basic protocol of the method DBNG

The basic protocol of the method DBNG was tested on a model organism of gram-negative mycobacteria *Mycobacterium smegmatis* cultivated in a rich LB growth medium. It was obvious that a significant amount of various organic growth factors in the composition of the LB medium can serve as electron donors, i.e., they can participate in the reduction reaction of Ag^+ cations introduced into the cell suspension, and thereby initiate the formation of reduced Ag^0 silver nanoparticles. A series of initial experiments to study the dynamics of the formation of biogenic silver nanoparticles was carried out using the method of measuring small-angle *X*-ray scattering (SAXS). The SAXS method is based on the effects of the elastic interaction of *X*-rays with bound electrons in a sample. This method of structural diagnostics provides a resolution in the range from 1 to 10^3 nm, which is most accurately suited for in-depth analysis of the structure and properties of biogenic nanocrystalline materials generated *de novo* in biological solutions. Indeed, according to the results of the determination using SAXS, the introduction of a sterile $Ag(NH_3)_2NO_3$ solution into a sterile LB growth medium resulted in a rapid (in 1–2 minutes) mass formation of silver nanoclusters with sizes up to 2 nm. Over a longer time, larger nanocrystalline structures formed in the reaction mixture (**Fig. 3***A*). Similar measurements in cell suspensions of *M. smegmatis* revealed two types of nanoparticles with contrasting differences in size: small nanoparticles up to 3 nm in diameter and large nanoparticles with a wide size distribution of 20-40 nm (Fig. 3*C*).

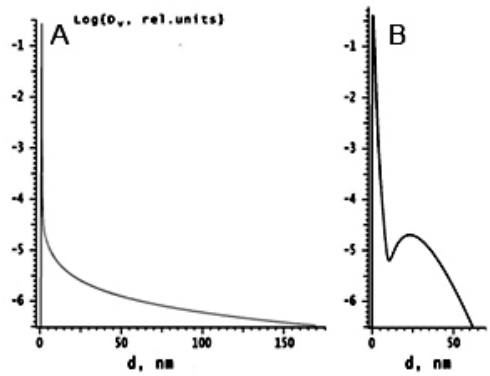

Fig. 3. *Formation of reduced silver nanoclusters in the LB growth medium (A) and biogenic Ag^0NPs nanoparticles in the presence of Mycobacterium smegmatis cells (B).*

Thus, in sterile samples, there is a reduction according to the "chemical" type – with the depletion of reducing agents and slowing down the agglutination of nanoclusters. In a suspension of active cells, a high level of secreted electron donors activates (according to the "green" type) the reduction of cations sorbed on nanoclusters and, accordingly, activates the generation of ever larger nanostructures. In fact, the detection of living cells by the formation of nanocrystals *in situ* is reduced to discrimination of the "chemical" or "green" type of this process.

3.2. Evaluation of the size distribution of biogenic silver and palladium nanoparticles from dynamic light scattering

Light scattering methods are indispensable for determining the hydrodynamic radii of nanocrystals and biopolymer molecules in biological fluids, their molecular weights, as well as the intensity distributions of scattered light over their sizes. We used this method in experiments with an isogenic pair of obligate methylotroph *Methylopilusquaylei* to compare the ability of their cells to generate nanoparticles of two metals differing in the valence of silver Ag^+ and palladium Pd^{2+} cations. It can be noted that comparisons of the properties of the initial wild-type microbial culture and the mutant derived from it are widely used in genetic engineering and biotechnological studies, but have practically not been used to assess the possibilities of synthesizing biogenic metal nanoparticles.

The linear dimensions and distributions of biogenic nanoparticles in these experiments were recorded using a Delsa Nano Analyzer that implements the principles of dynamic light scattering (DLS). The growth characteristics of the original wild type strain *M. quaylei* WT and its streptomycin-resistant derivative *M. quaylei* Sm^R did not differ under standard cultivation conditions on media with methanol as the sole carbon and energy source [20]. It was shown that the cells of the *M. quaylei* Sm^R mutant are resistant to the presence of up to 4 mg/mL of streptomycin, are characterized by reduced secretion of exopolysaccharides, and have a reduced surface hydrophobicity. In the presence of cells of both methylotrophic cultures *M. quaylei* WT and *M. quaylei* Sm^R under standard conditions of the DBNG protocol, large reduced silver nanoparticles of average size 45 nm and 70 nm, respectively, were generated (**Fig. 4**). The survival rate of the streptomycin-resistant mutant *M. quaylei* Sm^R in these reaction mixtures was 8 times lower than that of the initial culture. Under similar conditions, after the introduction of a source of palladium cations, the formation of Pd^0NPs nanoparticles occurred only in the presence of cells of a *M. quaylei* Sm^R culture, which correlated with a much higher degree of resistance of such cells (12 times higher) to palladium salt than in wild-type cells. Since the generation of numerous optically dense nonmetallic particles (not containing palladium nanoparticles) was observed in the reaction mixture in which *M. quaylei* WT cells were present, it can be concluded that

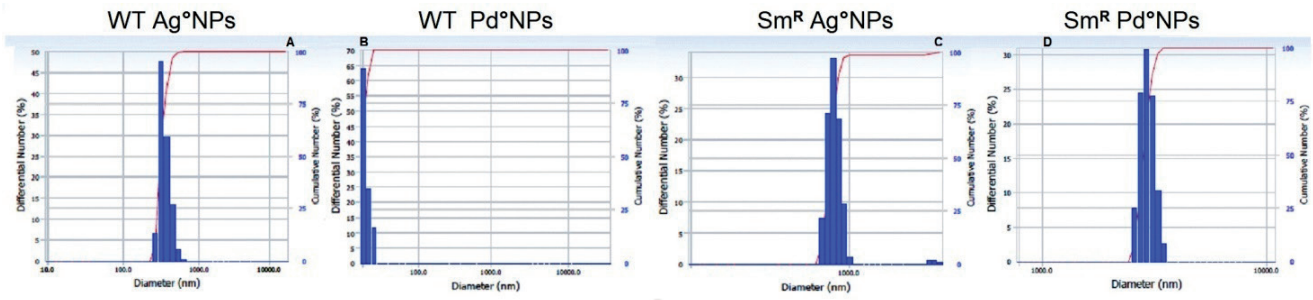

Fig. 4. *Sizes of biogenic silver and palladium nanoparticles formed in the presence of cells of methylotrophic cultures of M. quaylei wild type (WT) and isogenic streptomycin-resistant derivative (Sm^R).*

the reduction of Pd^{2+} cations was inhibited at the stage of nanoclusters present in the form of a high polymer complex with secreted methylotrophic exopolysaccharides. Thus, the preservation of the natural level of exopolysaccharide biosynthesis in the wild-type *M. quaylei* WT culture does not compensate for the high sensitivity of cells to Pd^{2+} cations, which generally does not allow them to generate Pd^0NPs nanoparticles. It is obvious that both cultures are almost equally capable of reducing silver cations and can serve as producers of Ag^0NPs nanoparticles when cultivated on simple synthetic media with methanol.

3.3. Spectrometry of preparations of biogenic silver nanoparticles

Absorption spectrophotometry is one of the most common physical and chemical methods for studying solutions and solids, based on recording absorption spectra in the λ range from 200 to 1000 nm. The recorded dependence of the intensity of absorption of incident light on the wavelength reflects the structure and composition of various compounds, the qualitative and quantitative composition of biological solutions, and shows the presence of trace elements in the objects under study. The atoms of each chemical element have strictly defined resonant frequencies, as a result of which it is at these frequencies that they emit or absorb light. This property is especially important (for us) when analyzing the composition and size of (biogenic) metal nanoparticles. Obtaining absorption spectra is easy to perform, does not require complex preparation of samples for analysis, and is possible in the presence of a small amount of the analyte. Currently, a large number of different models of spectrophotometers are being produced, including very compact ones, which allows them to be used even in the field studies.

3.3.1. Study of microbial cultures of active slut

Many technological processes include, at the final stages, the treatment of water waste with the use of activated sludge for the biosorption of toxic metal ions in the form of nanoparticles. Thus, in the effluents of electroplating, textile, uranium and silver processing industries, Ni^{2+}, Zn^{2+}, Cu^{2+}, and U(VI) cations can be present in significant amounts [22]. The DBNG approach was applied in the selection of microbial cultures capable of efficiently extracting metals from solutions for a long time without loss of vital activity in artificially created activated sludge. Since cations Ag^+ are much more toxic for most microorganisms than cations of other elements, it was silver salts that were used in assessing the levels of resistance of sludge preparations and pure cultures isolated from it to other metals. Several cultures capable

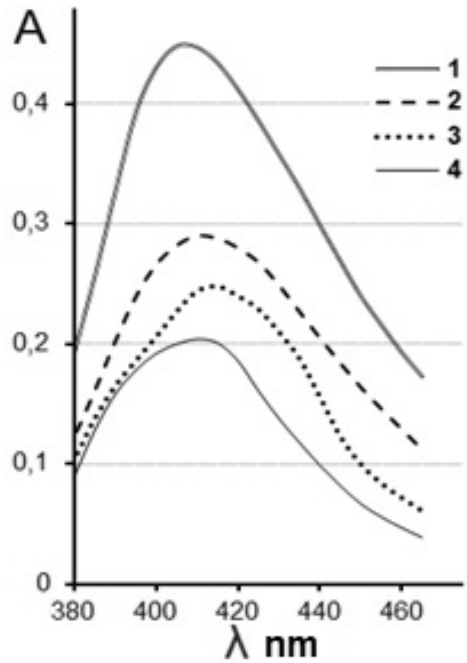

Fig. 5. *Levels of production of biogenic Ag^0NPs by cells of pure cultures of Penicillium glabrum (1), Fusarium nivale (2), Trichosporon cutaneum (3), Agrobacterium tumifaciense (4).*

of rapidly forming Ag^0NPs were selected. The most promising crops for biosorption of toxic cations were identified as *Fusarium nivale* and *Penicilliumglabrum* (**Fig. 5**). Artificial activated sludge, created on their basis, had the form of multilayer granules with a given composition and structure: the cells of cultures less resistant to metals were shielded by a layer of cells, the most resistant to high concentrations of toxic metals. It was shown that at a content of 20 mg/L Ag^+ in the initial sludge, the level of cell survival did not exceed 7%; for the most stable pure cultures of *F.nivale* and *P.glabrum*, the survival rate was about 12-24%. The artificial activated sludge arranged in this way remained viable and developed even in the presence of up to 70 mg/L Ag^+ in the medium. Such sludge was capable of accumulating silver up to 50% of the dry mass of cells, which corresponds to a sorption capacity of up to 380 mg/g and showed the degree of silver extraction up to 95% in just 3-5 minutes (at a concentration of cations Ag^+ ~100 mg/L).

3.3.2. Growth of yeast culture in the top layer of hydrogel

In recent years, 3*D* bioprinting technologies have been increasingly used in practice for the formation of artificial biomaterials for restoration of damaged human organs. Usually, living cell structures are included in a shaping gel, which ensures their optimal physiological state for normal cell growth. In our experiments, the model eukaryotic microorganism *Yarrowia lipolytica* Y-3603 was grown for up to 7–8 days in cuvettes filled with two-layer hydrogels with an agarose concentration of 0.4 wt% in a complete LB medium [21]. The bottom sterile layer of the gel contained 5 μg/mL of silver nitrate as a source of cations Ag^+ (**Fig. 6**). It is important to emphasize that for the implementation of the DBNG approach, we used low electroendoosmotic agarose (EEO type), since it was shown that only this type of agarose is not able to reduce introduced cations without the participation of cells (Fig. 6*B*).

The growth of the *Y. lipolytica* culture in the upper layer of the hydrogel was controlled by increasing the optical density of the samples using a fiber optic spectrometric system. Similarly, the dynamics of the generation of biogenic Ag^0NPs (by reducing the optical

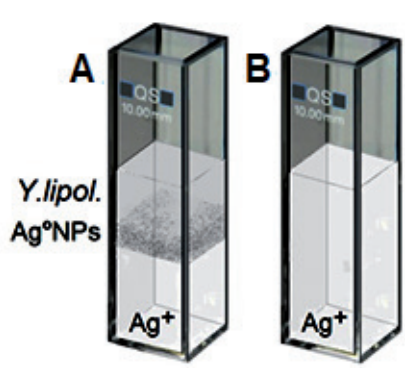

Fig. 6. *Formation of silver nanoparticles in the presence of Y. lipolytica yeast culture growing in the EEO agarose hydrogel.*

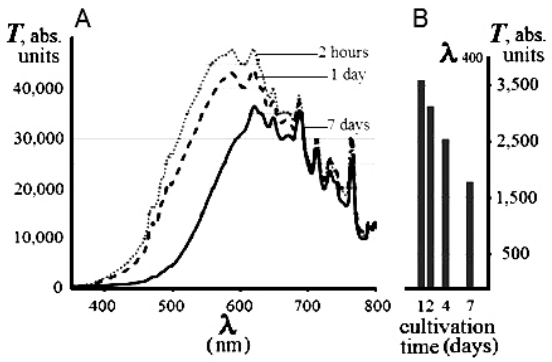

Fig. 7. *Growth dynamics of Y. lipolytica yeast and in situ formation of biogenic Ag^0NPs in EEO agarose hydrogel layer.*

permeability of the hydrogel) was recorded at a specific wavelength of silver nanoparticles λ_{400} nm. As can be seen from the presented data (**Fig. 7**), the transparency of the samples containing yeast steadily decreased over several days as the culture grew in the thickness of the hydrogel (Fig. 7*A*).

Since the decrease in transparency measured at λ_{400} was also observed throughout this time, it can be concluded that the level of reductive activity of the *Y. lipolytica* culture remains at a sufficiently high level for long-term generation of nanoparticles *in situ* (Fig. 7*B*). In general, the application of the DBNG approach in these experiments showed that the methodology for assessing the possibility of generating biogenic nanoparticles *in situ* allows one to control the physiological state of living *Y. lipolytica* yeast cells during submerged cultivation in an agarose hydrogel medium [21].

3.3.3. Comparison of malignant and benign tumors

Malignant neoplasms are among the most common tumors of all types of tissues. The clinical course of malignant tumors is characterized by high polymorphism, due to which their differential diagnosis can present significant difficulties. Preoperative diagnostics in order to determine the volume of surgical intervention makes it possible to determine the structure and localization of the pathological process, the exact size of neoplasms, and the relationship with surrounding tissues. But the issue of urgent intraoperative morphological diagnosis of benign and malignant tumors is still relevant. As is known, the defining feature of the tumor process is abnormal uncontrolled proliferation, to maintain which tumor cells undergo oncogene-oriented metabolic reprogramming [23,24]. A dramatic increase in the metabolic activity of cancer cells leads to a noticeable increase in their ability to generate biogenic nanoparticles during the interaction of cell metabolites with added salts [25,26]. We used the innovative DBNG approach to study adnexal tumors identified clinically in patients. Healthy tissue from the same eye was used as controls for each patient (**Fig. 8**).

Photometric determination at wavelengths close to those specific for silver nanoparticles (λ_{400}) showed that for all studied pairs of

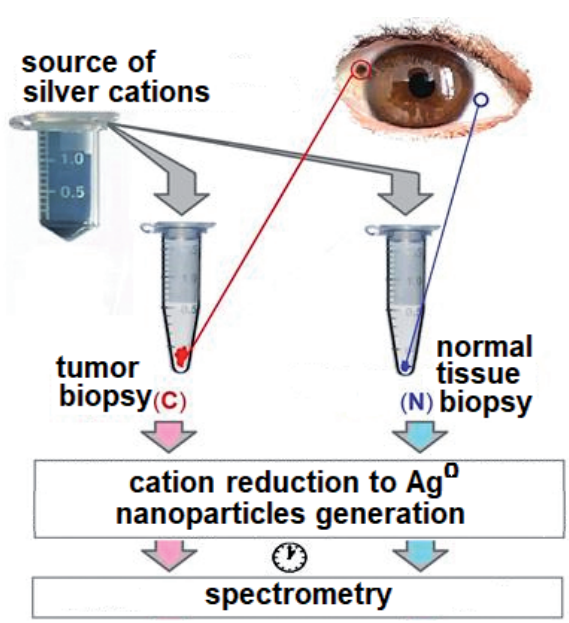

Fig. 8. *Scheme of sample preparation for studying the restorative activity of eye conjunctival cells.*

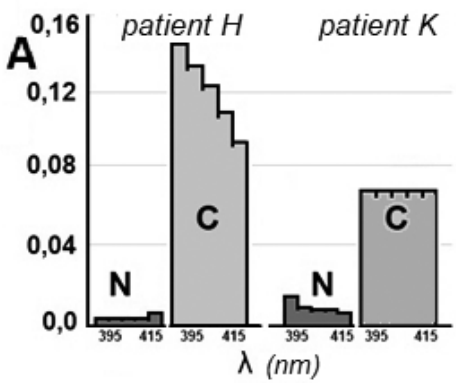

Fig. 9. *Comparison of the vis spectra of silver nanoparticles formed from tumor (C) and healthy (N) conjunctival tissues.*

samples (Cancer *vis* Normal tissue), the optical density of reaction mixtures (that is, the volume of silver nanoparticles formed in 3-5 minutes) in all samples with in malignant and benign types of tumors of the adnexal apparatus of the eye is higher than in control samples of similar healthy tissues (**Fig. 9**).

Thus, the proposed methodological approach of DBNG makes it possible to quickly solve the problem of determining the degree of malignancy of tumors based on the spectrometric rapid assessment of their metabolic activity in order to optimize the tactics of surgical intervention.

3.3.4. Registration of nanoparticles using the Ulbricht integration sphere

The observation of generation *in situ* biogenic silver nanoparticles by their specific absorption spectrum at a wavelength of about λ_{400} nm can be hindered by the high density of the studied cell suspension. Performing spectrometric measurements using an Ulbricht integrating sphere as a detector eliminates this problem (**Fig. 10**). In addition, monitoring the dynamics of the generation of nanoparticles from the very beginning of the cation reduction reaction makes it possible not to carry out measurements in control (freed from cells) sample aliquots. Indeed, a fresh culture of

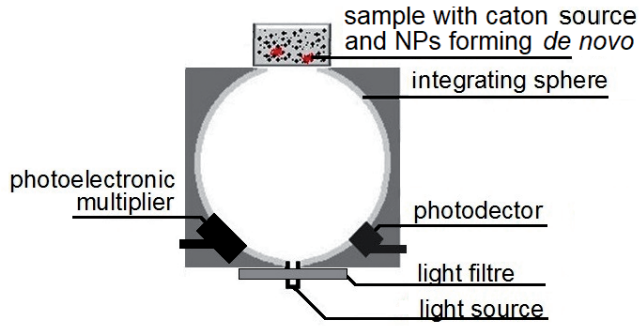

Fig. 10. *Scheme of an integrating sphere for registration of biogenic metal nanoparticles.*

M. smegmatis grown in complete LB growth medium does not show the characteristic Ag^0NPs peak (**Fig. 11**).

After adding of silver cations source (4 μg/mL $Ag(NH_3)_2NO_3$), a series of measurements of the spectra of the suspension of the reaction mixture was carried out. On Fig. 11 shows that as early as 10 minutes after the addition of cations Ag^+, a specific peak for Ag^0NPs at λ_{400} nm can be recorded, followed by a gradual increase in its amplitude. Such a bathochromic shift of the peak maximum indicates a constant increase in the size of silver nanoparticles generated *in situ* in the studied reaction mixture.

When carrying out similar experiments with higher concentrations of Tollen's reagent, as well as when using $AgNO_3$ salt as a source of cations, there was a delay in

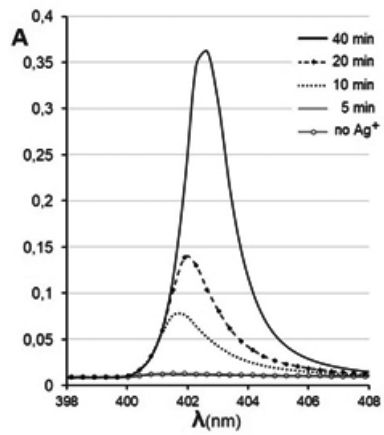

Fig. 11. *Dynamics of formation of Ag^0NPs nanoparticles in the presence of M. smegmatis cells.*

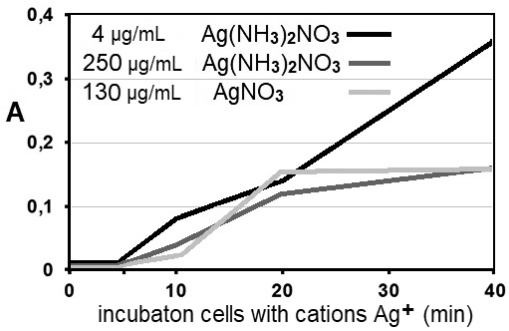

Fig. 12. *Dependence of the dynamics of in situ formation of biogenic nanoparticles in the presence of M. smegmatis cells on the source of silver cations.*

the appearance of the peak at an early stage of nanoparticle formation and a cessation of their enlargement already by the 20th minute of the reaction (**Fig. 12**). Such a change in the dynamics of the generation of biogenic nanoparticles indicates a significant suppression of the reductive activity of mycobacteria cells. Such effects should be taken into account when developing special protocols for applying the method DBNG to various biological objects.

3.3.5. Fluorescence spectrometry of biogenic metal nanoparticles

As noted above, for the detection of metal nanoparticles, their specific optical properties due to the phenomenon of surface plasmon resonance, highly developed surface, high capacitance of the electrical double layer, and the ability of nanoparticles to amplify the signal in Ramanscattering, light and fluorescence spectrometry are widely used. One of the unique features of nanoclusters of many metals ranging in size from 2-5 nm is their pronounced fluorescent properties under certain parameters of exciting illumination. If the sizes of nanoclusters of reduced metal atoms are commensurate with the Fermi wavelength for electrons, the supramolecular properties of nanocrystals manifest themselves due to discrete electronic states caused by strong quantum confinement of free electrons. The fluorescent properties are retained during the self-assembly of nanoclusters during generation *de novo* of nanoparticles. As the size of metal nanocrystalline structures increases (during the self-assembly of nanoclusters), the ratio of surface and deep atoms changes, which leads to a shift in fluorescence radiation and even its complete quenching when nanocrystalline structures reach a certain size [27,28].

We propose to use this natural feature of the fluorescence of metal nanoclusters/nanoparticles, i.e., the dependence on the size of nanocrystalline structures, in the method for determining the metabolic activity of cells (when assessing the reductive cellular activity necessary for *de novo* formation of nanoparticles from cation solutions). In this case, the generation of biogenic nanoparticles occurs precisely *de novo*, that is, it goes through the stages from the "primary" (equimolar) reduction of cations to zero-valent atoms, then clustering with the gradual formation of di- and tetraatomic nanoclusters (Fig. 1), then self-assembly and gradual enlargement of nanocrystalline structures (**Fig. 13**). This means that at the moment when a certain amount of nanoclusters capable of exhibiting their fluorescence is formed in the reaction mixture,

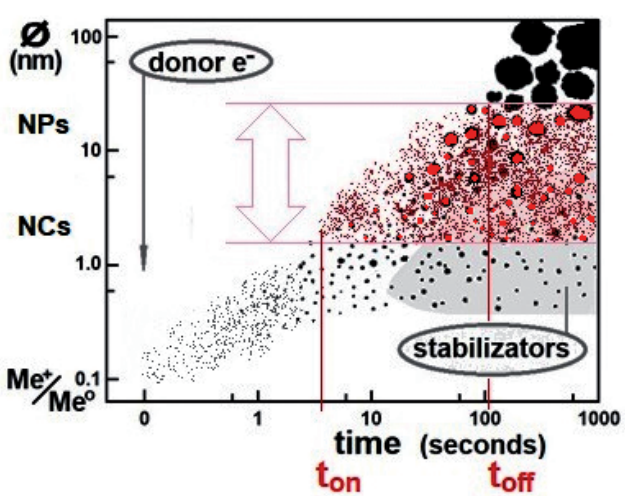

Fig. 13. *Schematic diagram of the dependence of the fluorescence of metal nanoparticles on their size.*

it can be detected by standard methods. The fluorescence of the nanocrystalline structures will continue to persist, all the time in the process of self-assembly of nanoclusters and enlargement of nanoparticles, until all (most) of the formed nanoparticles exceed the fluorescence quenching size.

Thus, by periodically measuring the presence (or intensity) of fluorescence in a sample, it is possible to determine two points that characterize the reducing activity of cells present in the sample under study: *i.* is the time required for the formation (measured) amount of fluorescent nanoclusters of reduced metal atoms (Fig. 13, t_{one}); *ii.* is the time required for all formed nanoparticles to exceed the size corresponding to fluorescence quenching (Fig. 13, t_{off}).

Crystal structures, including atoms of various elements, also differ in their fluorescent properties and in the parameters of exciting illumination. It is known that the fluorescent properties of nanoclusters can be enhanced by including reduced atoms of different metals in them [29]. We propose to use this natural feature of the fluorescence of nanocrystalline structures in the framework of the method DBNG to determine the integral metabolic activity of cells. To enhance the fluorescence of biogenic nanoclusters generated *de novo*, use a sterile solution as a source of cations for introduction into the reaction mixture, for example, containing a silver salt with the addition of, for example, lanthanide salts.

3.4. Use of transmission electron microscopy in the analysis of reaction mixtures in which metal nanoparticles are generated de novo

The use of transmission electron microscopy in the analysis of reaction mixtures in which metal nanoparticles are generated *de novo* makes it possible not only to observe optically dense crystalline structures of various shapes and sizes, but also to record the distribution of such nanocrystals in the samples under study. It is the results obtained using TEM that can be considered exhaustive for the characterization of cells of taxonomically different bacterial species, spores of bacilli, preparations of bacterial components of activated sludge granules, preparations of viral particles. It is TEM photographs that show that the generation of nanocrystals in the presence of cells of different cultures differs significantly, while the degree of association of the generated nanocrystals with biological objects present in the samples reflects the integral properties of the cell surface and their physiological state. It is important to emphasize that TEM studies of preparations prepared according to the protocol of the DBNG method provide high sensitivity to the presence of biological objects in the studied samples. When adding Tollens' reagent solution to a series of samples of *Bacillus* cell suspension, successively diluted with a sterile LB growth medium, it was found that even with a strong dilution of the cell suspension (up to $3\text{-}10^2 \cdot 10$ cells/mL) using TEM, one can easily detect the generated silver nanoparticles (in their complete absence in the control sterile samples of a complete growth medium).

3.4.1. Comparison of biogenic silver nanoparticles generated by metabolically active and dormant bacterial cells

The use of TEM made it possible to compare the features of the generation of metal nanoparticles in the presence of metabolically active and resting microorganism cells. For example, for mycobacteria *M. smegmatis*, the peculiarity

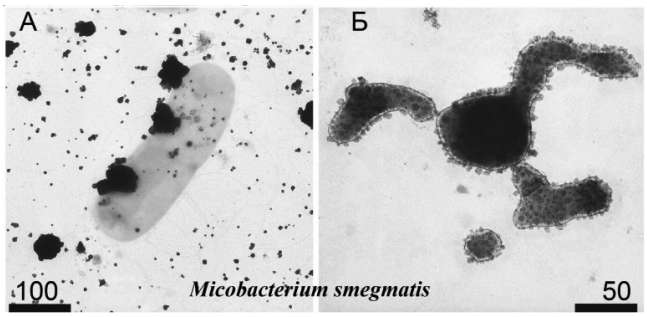

Fig. 14. *Biogenic silver nanoparticles formed by metabolically active (A) and resting (B) M. smegmatis cells.*

of cells, when cultivated in a long stationary phase, goes into an inactive, dormant state [30]. It was shown that growing cultures of *M. smegmatis* generate silver nanoparticles with a clearly uniform distribution in the sample volume (**Fig. 14***A*), while preparations of resting cells of these mycobacteria generated silver nanoparticles associated with cell wall surfaces and no larger than 10 nm in size (Fig. 14*B*). Such localization of the obtained nanoparticles indicates that the main contribution to their formation is made by the reductive chemical groups of surface biopolymers. The almost complete absence of nanoparticles in the intercellular space is in good agreement with the suppression of the secretion of low molecular weight reducing agents, which is typical for the transition of metabolically active *M. smegmatis* cells to a dormant state.

3.4.2. Generation of silver nanoparticles by psychroactive bacteria Cryobacterium arcticum under suppression of cell division

The study of the ability to generate silver nanoparticles when modeling the "turn off" of biosystems responsible for the synthesis of nucleic acids and cell division was carried out using a structural fluorine-containing analogue of uracil 5-fluorouracil (5-FUra). Cell suspensions of a pure culture of psychoactive bacteria *Cryobacterium arcticum* were incubated for 24 hours at their optimal temperature (+8°C) in a liquid medium with 5-FUra. To assess the reducing activity, suspensions fully grown in the presence of 0.32 and 1.0 μg/mL 5-FUra were used (a higher concentration of 3.2 μg/mL significantly suppressed bacterial viability). The use of TEM made it possible to establish that with a clear inhibition of cell division (the number of separate, unpaired cells did not exceed 10%), almost all silver nanoparticles were clearly associated with cells (**Fig. 15***A*), similar to silver nanoparticles generated by resting mycobacteria cells (see Fig. 14). It is important to note that the biogenic Ag^0NPs in these preparations had an anisotropic needle shape (Fig. 15*B*). The generation of such nanoparticles may indicate a special physiological state of *C. arcticum* bacteria, which had a normal metabolism prior to the introduction of the 5-FUra analogue, but are under stress due to intracellular DNA/protein imbalance. In preparations with an even lower content of 5-FUra (0.32 μg/mL), a significant proportion of cells of the standard uninhibited appearance (Fig. 15*C*), similar to cells not treated with the analogue, were retained (Fig. 15*D*).

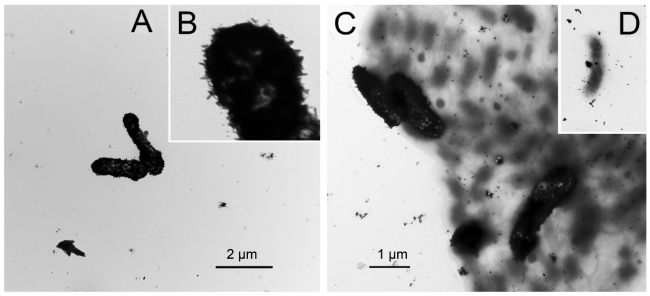

Fig. 15. *Formation of silver nanoparticles by Cryobacterium arcticum bacteria incubated with the addition of 1.0 μg/mL 5-FUra (A), anisotropic (needle) form of Ag^0NPs nanoparticles (B), Cryobacterium arcticum bacteria incubated with the addition of 0.32 μg/mL 5-FUra (C), the formation of silver nanoparticles by metabolically active cells (D).*

Generally, it can be concluded that the ability of the cells of psychoactive bacteria *C. arcticum* to generate certain forms of silver nanoparticles may reflect a change in the composition of biogenic reducing agents (forcations Ag^+) secreted into the growth medium. In addition, the heterogeneity of the cell population becomes apparent, which is detected by pronounced differences in the linear dimensions and zones of localization of generated silver nanoparticles.

3.4.3. Nanocells are able to reduce cations and generate nanoparticles

The use of TE microscopy made it possible for the first time to demonstrate the ability of nanocells of filtering bacteria to act as silver cation reducers. The interest in these living biological objects with dimensions of 0.2–0.5 μm and a volume of about 0.004–0.04 $μm^3$ is associated with their ability to pass through sterilizing filters with a pore size of 0.22 μm [32,33]. Nanocells are perceived as microbial&biochemical water pollutants, as sources of DNA, proteins, components of cell membranes and, therefore, are undesirable, for example, in drinks consumed by humans.

We have shown that the presence of metabolically active nanocells in water samples can be detected by their ability to generate silver nanoparticles with an average size of about 8 nm (**Fig. 16**). When the cell membrane is destroyed, the nanocells quickly lose their reductive ability, the lysis products become stabilizers of silver nanoclusters, which leads to a slowdown in their self-assembly during the formation of Ag^0NPsparticles.

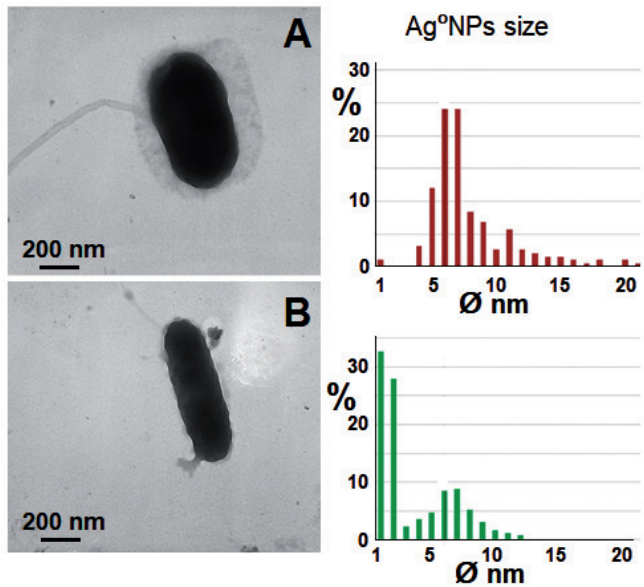

Fig. 16. *Biogenic silver nanoparticles formed by metabolically active and damaged nanocells.*

3.4.4. Viral particles enlarged with metal nanocrystals

The ability of viral capsid proteins to ensure the generation of metal nanoparticles makes it possible to use the DBNG approach to detect viruses and bacteriophages when they are in the extracellular space (even in the complete absence of metabolic reactions controlled by them). The generation of biogenic metal nanoparticles in the presence of viral particles occurs due to reducing amino acid groups exposed on the surface of capsids (**Fig. 17**). Due to this localization of electron donors and reduction products (nanocrystals), the word combination "encrusted viral particles" has become fixed in the literature. It should be noted that the differences in amino acid groups of capsids of different virus strains are reflected in the

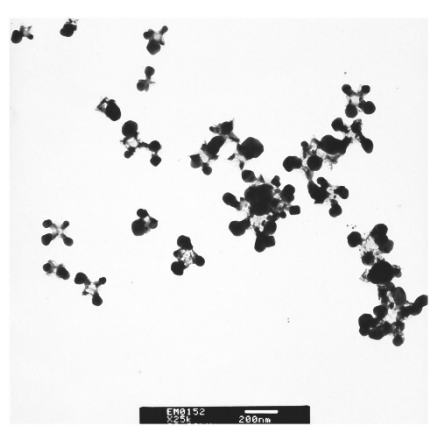

Fig. 17. *Silver nanoparticles encrusted with G7C coliphage particles.*

form of differences in the generated metal nanocrystals [17]. The application of the DBNG protocol showed the acceptability of this innovative method to detect (using TEM) in water samples viral particles of coliphage G7C [33] at a concentration of 10^2 ml^{-1}.

3.5. Analysis of the elemental composition of biogenic nanocrystals

All modern transmission electron microscopes are equipped with microanalyzers that allow elemental analysis of samples. This possibility is very important and useful in the study of biogenic metal nanoparticles. Since growth media for cultivating microorganisms always contain certain salts that can enter into substitution reactions with cation sources added (according to the protocol of the method DBNG), it is necessary to constantly monitor the elemental composition of biogenic nanocrystals. Confirmation of the fact that in our experiments optically dense nanoparticles generated de novo from introduced silver cations observed in the TEM field of view was obtained by *X*-ray microanalysis. The presence in the studied nanoparticles (regardless of their size) of reduced silver atoms, as well as other metals, was judged by the presence of the corresponding specific peaks in the spectra.

3.5.1. Study of natural metal nanoparticles of Lake Onega

Lake Onega is located at the junction of two large geological structures – the Baltic Shield in the northern part of the lake and the Russian Platform in the southern. Many areas of the lake are characterized by thick ferromanganese ore formations with accumulation of Cu, Fe, Zn, Mo, As, Ni, Cd, and Pb due to subaqueous discharge groundwater [34]. According to the available data, in such zones, extremely low rates of silt accumulation, and the microbial communities of the surface layer of bottom sediments are characterized by low taxonomic diversity. We assumed that the formation of ore deposits begins with the reduction of cations supplied with subaqueous discharge, that it should be accompanied by the generation of biogenic metal nanoparticles formed in the presence of cells of a community of autochthonous resistant microorganisms. Accordingly, it was of interest to show the participation of cells of autochthonous microorganisms from the bottom horizon of Lake Onega in the formation of such metal nanoparticles. In all samples of lake water from various parts of Lake Onega and Unitskaya Bay, the presence of natural polymetallic nanoparticles, similar in elemental composition to cations, which are most represented in the corresponding samples of lake water, was recorded. The concentration of autochthonous microbiota cells in different samples varied from 10^2 to 10^8 mL^{-1}.

Enrichment cultures of aboriginal microbiota were prepared from aseptically taken water samples from the bottom layer in a liquid medium depleted in organic matter (5% LB), which were then used to obtain biogenic nanoparticles (**Fig. 18**). Under standard conditions for the DBNG protocol, when using natural water samples as a source of cations, the formation of biogenic iron nanoparticles was recorded

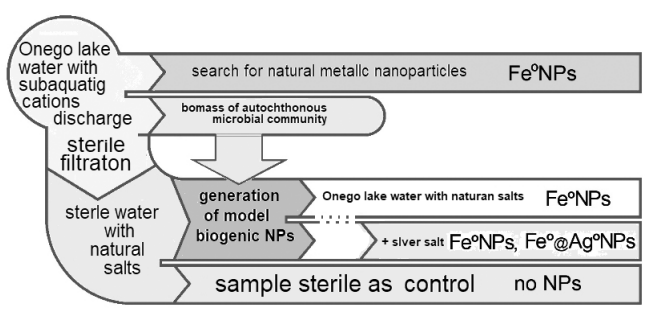

Fig. 18. *Scheme of the experiment showing the participation of the autochthonous microbial community of the bottom layer of Lake Onega in the formation of ore iron-bearing deposits.*

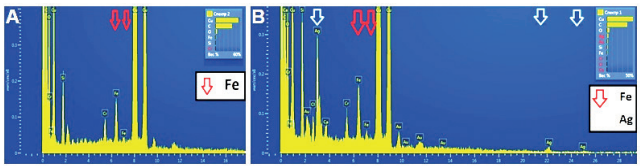

Fig. 19. *Spectra showing the elemental composition of natural (A) and model (B) nanoparticles formed in the presence of autochthonous microorganisms of Lake Onega*

(in the control sterile variants, without the addition of suspensions of microorganisms, nanoparticles were not formed). Elemental analysis of the obtained biogenic nanoparticles showed that they consist of reduced iron atoms.

In those variants of the experiment, where Tollens' reagent was added to the cell suspension of enrichment cultures of native microbiota together with lake water, silver nanoparticles were found, as well as nanoparticles containing both iron and silver atoms (**Fig. 19**). Simultaneous inclusion of zero-valent iron and silver atoms into biogenic nanoparticles unambiguously indicates the ability of cells of autochthonous microorganisms to actively reduce various cations, both those naturally present in the bottom waters of the Onega Basin and artificially added cations Ag^+ as a model element.

3.6. Acoustic methods for registration of nano- and microsized biological objects

Currently, to detect the presence of viral particles and microorganism cells in liquid solutions (and hydrogels), rather complex innovative solutions are increasingly being used, such as molecular ligands, functionalized magnetic structures, microfluidic devices with nanostructured surfaces to retain bacteria, volumetric acoustics, and advection [35]. One of the most common acoustoelectronic methods for studying various materials and structures is the use of acoustic delay lines (ADL). Acoustic sensors are able to detect various chemical and biological types of objects by measuring the corresponding changes in density, viscosity, conductivity, dielectric constant and temperature of liquids, while the sensors do not require large sample volumes. Optimization of acoustic modes for electrical and viscous measurements in liquid and hydrogel media made it possible for the first time to develop an acoustoelectronic technique and prototypes of acoustoelectronic sensors that do not contain sensitive coatings for detecting the presence of viral particles and microorganism cells in various liquids [36]. When the properties of the medium changed, for example, due to the presence of biological objects or their derivatives, the wave changed its speed, phase and amplitude. Changes in the acoustic characteristics were recorded at the output of the device as phase and amplitude "responses" of the acoustoelectronic sensor.

It was shown that the proposed technique and sensors are applicable to the detection in liquid growth media of such biological objects as viral particles, bacterial cells, as well as yeast cells and nanoparticles immobilized in hydrogels. It was found that the response threshold of the sensors used is 0.04% for a sterile diluted 10% LB growth medium, 0.07% for an aqueous suspension of bacterial cells, and 0.6% for a cell suspension in a 10% LB medium.

4. DISCUSSION

Assessment of the physiological state of certain biological objects is a constantly encountered problem in industrial microbial biotechnology, ecology, and medicine. However, the well-studied ability of living metabolically active cells to generate metal nanoparticles (when interacting with salt solutions by reducing cations) is not used to

solve these issues in practice. We propose to use this fundamental natural ability to study various aspects of the functioning of cells as such. In this work, we have shown that the dynamics of generation *de novo* of biogenic nanoparticles makes it possible to compare the integral properties (levels of metabolic activity) of pure cultures of microorganisms, their natural communities, and preparations of human cells.

We postulate a fundamental feature of the interaction of clusters of reduced atoms generated *de novo* in the presence of living cells: only in the presence of metabolically active cells, due to their reducing ability, can the relative fraction of residual cations in nanoclusters of metal atoms be reduced. Only in the presence of such cells, the process of enlargement of nanoclusters by agglutination and successive self-assembly leads to a fairly rapid generation of ever larger nanosized metal particles. Instrumental determination of the presence of biogenic nanoparticles generated *in situ* in reaction mixtures can provide a fairly accurate assessment of the level of metabolic activity and other properties of cells acting as cation reducers. The developed DBNG approach eliminates the need to use ready-made preparations of nanoparticles in research, reduces the duration and overall laboriousness of experiments.

5. CONCLUSION

The paper analyzes the possibilities of measurements that are most suitable for instrumental registration of the parameters of biogenic metallic nanocrystalline structures generated *in situ* according to the protocol of the innovative method DBNG. Examples of the application of the high-precision SAXS method and DLS nanosizer, several options for using spectrometry and transmission electron microscopy to assess the level of metabolic activity of cells by their ability to reduce cations and generate nanoparticles of silver and other metals are given. Generally, it has been shown that the application of traditional analytical methods for studying the parameters of generated biogenic nanoparticles can be effectively used to solve some of the most important problems of microbiology, biotechnology, and medicine.

REFERENCES

1. Sorokin VV, Skladnev DA, Volkov VV, Tereshchenko EY, Mulyukin AL, Gal'chenko VF. The pathways of silver nanoparticles formation by Mycobacterium smegmatis. *Dokl. Biol. Sci.*, 2013, 452:325-328.
2. Gadd G. Metals, minerals and microbes: geomicrobiology and bioremediation. *Microbiology*, 2010, 156:609-643.
3. Hilger A, Guppers N, Tenfelde H, Kreibig U. Surface and interface effects in the optical properties of silver nanoparticles. *Eur.Phys.J.*, 2000, 10:115-128.
4. Hussain S, Bashir O, Khan Z, Al-Thabaiti SA. Steroidal saponin based extracellular biosynthesis of AgNPs. *Journal of Molecular Liquids,* 2014, 199:489-494.
5. Kharissova OV, Dias HVR, Kharisov BI, Pérez BO, Pérez VMJ. The greener synthesis of nanoparticles. *Trends in Biotech.,* 2013, 31(4):240-248.
6. Zhou Y, Wang H, Lin W, Lin L, Gao Y, Yang F, Du M, Fang W, Huang J, Sun D, Li Q. Quantitative nucleation and growth kinetics of gold nanoparticles via model-assisted dynamic spectroscopic approach. *J. of Colloid and Interface Sci.*, 2013, 407:8-16.
7. Tan S, Chee S, Lin G, Mirsaidov U. Direct observation of interactions between nanoparticles and nanoparticle self-assembly in solution. *Acc. Chem. Res.*, 2017, 50(6):1303-1312.

8. Patil MP, Kim GD. Eco-friendly approach for nanoparticles synthesis and mechanism behind antibacterial activity of silver and anticancer activity of gold nanoparticles. *Appl. Microbiol. Biotechnol.*, 2017, 101:79-92, doi: 10.1007/s00253-016-8012-8.
9. Siddiqi K, Husen A, Rao R. A review on biosynthesis of silver nanoparticles and their biocidal properties. *J. Nanobiotechnol.*, 2018, 16:14-30.
10. Xie Y, Dong H, Zeng G, Tang L, Jiang Z, Zhang C, Deng J, Zhang L, & Zhang Y. The interactions between nanoscale zero-valent iron and microbes in the subsurface environment. *J. Hazard. Mater.*, 2017, 321:390-407.
11. Luo B, Smith JW, Ou Z, Chen Q. Quantifying the self-assembly behavior of anisotropic nanoparticles using liquid-phase transmission electron microscopy. *Acc. Chem. Res.*, 2017, 50(5):1125-1133, doi: 10.1021/acs.accounts.7b00048.
12. Wang J, Lin X, Shu T, Su L, Liang F, Zhang X. Self-assembly of metal nanoclusters for aggregation-induced emission. *Int. J. Mol. Sci.*, 2019, 20:1891-1904.
13. Siddiqi KS, Husen A, Rao RAK. A review on biosynthesis of silver nanoparticles and their biocidal properties. *J. Nanobiotechnol,* 2018, 16:14, doi: 10.1186/s12951-018-0334-5.
14. Alkilany AM, Thompson LB, Boulos SP, Sisco PN, Murphy CJ. Gold nanorods: their potential for photothermal therapeutics and drug delivery, tempered by the complexity of their biological interactions. *Adv. Drug Delivery Rev.*, 2012, 64:190-199.
15. Skladnev DA, Vasilyeva LV, Berestovskaya YuYu, Kotsyurbenko OR, Kalenov SV, Sorokin VV. Detection of microorganisms in low-temperature water environments by in situ generation of biogenic nanoparticles. *Front. Astron. Space Sci.,* 2020, 7:59, doi: 10.3389/fspas.2020.00059.
16. Priscyla D. Mechanistic aspects in the biogenic synthesis of extracellular metal nanoparticles by peptides, bacteria, fungi, and plants. *Appl. Microbiol. Biotechnol.*, 2011, 90:1609-1624.
17. Shukla S, Steinmetz NF. Virus-based nanomaterials as positron emission tomography and magnetic resonance contrast agents: from technology development to translational medicine. *Wiley Interdiscip Rev. Nanomed. Nanobiotechnol.*, 2015, 7(5):708-21, doi: 10.1002/wnan.1335.
18. Robinson CK, Webb K, Kaur A, Jaruga P, Dizdaroglu M, Baliga NS, Place A, Di Ruggiero J. A major role for nonenzymatic antioxidant processes in the radioresistance of Halobacterium salinarum. *J. Bacteriol.*, 2011, 193(7):1653-1662.
19. Anh-Tuan L, Huy P, Tam P. Green synthesis of finely-dispersed highly bactericidal silver nanoparticles via modified Tollens technique. *Cur. Appl. Physics,* 2010, 10:910-916.
20. Sorokin V, Pshenichnikova A, Kalenov S, Suyasov N, Skladnev DA. Comparison of the wild-type obligate methylotrophic bacterium Methylophilus quaylei and its isogenic streptomycin-resistant mutant via metal nanoparticle generation. *Trace Element Research,* 2020, 193:564-573, doi: 10.1007/s12011-019-01740-4.
21. Vyazmin A, Pokusaev B, Karlov S, Skladnev D, Shumova N, Volkova E. Features of biogenic nanoparticle formation in agarose gels and their effect on cell growth during bulk cultivation. *Chem. Engin. Transactions*, 2021, 84:73-78, doi: 10.3303/CET2184013.
22. Tyupa DV, Kalenov SV, Baurina MM, Yakubovich LM, Morozov AN, Zakalyukin RM, Sorokin VV, Skladnev DA. Efficient continuous biosynthesis of silver nanoparticles by activated sludge micromycetes with enhanced tolerance to metal ion toxicity. *Enzyme and Microbial Technology,* 2016, doi: 10.1016/j.enzmictec.2016.10.008.
23. Rinaldi G, Rossi M, Fendt S-M. Metabolic interactions in cancer: cellular metabolism at the interface between the microenvironment, the cancer cell phenotype and the epigenetic

landscape. *WIREs Syst. Biol. Med.*, 2017, e1397, doi: 10.1002/wsbm.1397.

24. Heiden MGV, DeBerardinis RJ. Understanding the intersections between metabolism and cancer biology. *Cell,* 2017, 168(4):657-669, doi: 10.1016/j.cell.2016.12.039.
25. Hassoun M, Schie IW, Tolstik T, Stanca SE, Krafft C, Popp J. Surface-enhanced Raman spectroscopy of cell lysates mixed with silver nanoparticles for tumor classification. *Beilstein J. Nanotechnol.,* 2017, 8:1183-1190, doi: 10.3762/bjnano.8.120.
26. Sánchez GJ, Maury P, Stefancikova L, Campion O, Laurent G, Chateau A, Bouraleh FH, Boschetti F, Denat F, Pinel S, Devy J, Porcel E, Lacombe S, Bazzi R, Roux S. Fluorescent radiosensitizing gold nanoparticles. *Int. J. Mol. Sci.*, 2019, 20:4618, doi: 10.3390/ijms20184618.
27. Zijlstra P, Orrit M. Single metal nanoparticles: optical detection, spectroscopy and applications. *Rep. Prog. Phys.*, 2011, 74, 106401 (55 pp), doi: 10.1088/0034-4885/74/10/106401.
28. Deng H, Yu H. Silver nanoparticle surface enabled self-assembly of organic dye molecules. *Materials,* 2019, 12:2592, doi: 10.3390/ma12162592.
29. Liaw J-W, Wu H-Y, Huang C-C, Kuo M-K. Metal-enhanced fluorescence of silver island associated with silver nanoparticle. *Nanoscale Res. Let.*, 2016, 11:26, doi: 10.1186/s11671-016-1247-6.
30. Kuznetsov BA, Davydova ME, Shleeva MO, Shleev SV, Kaprelyants AS, Yaropolov AI. Electrochemical investigation of the dynamics of Mycobacterium smegmatis cells' transformation to dormant, nonculturable form. *Bioelectrochemistry,* 2004, 64(2):125-131.
31. Wang Y, Hammes F, Düggelin M, Egli T. Influence of size, shape, and flexibility on bacterial passage through micropore membrane filters. *Environ. Sci. Technol.*, 2008, 42(17):6749-6754, doi: 10.1021/es800720n.
32. Belova SE, Fedotova AV, Dedysh SN. Prokaryotic ultramicroforms in a Sphagnum peat bog of upper Volga catchment. *Microbiology,* 2012, 81:614-620, doi: 10.1134/S0026261712050050.
33. Kulikov EE, Kropinski AM, Golomidova A, Lingohr E, Govorun VM, Serebryakova M, Prokhorov N, Letarova M, Manykin A, Strotskaya A, Letarov A. Isolation and characterization of a novel indigenous intestinal N4-related coliphage vB_EcoP_G7C. *Virology*, 2012, 426:93-99.
34. Slukovskii Z, Sidorova A, Kalinkina N. Estimation of heavy metal concentrations in organisms of the Baikalian amphipod G melinoides fasciatus Stebbing (Crustacea: Amphipoda) in Petrozavodsk Bay, Lake Onego. *J. of Elementology,* 2019, 24(1):267-279, doi: 10.5601/jelem.2018.23.2.1633.
35. Sande MG, Çaykara T, Silva CJ, Rodrigues LR. New solutions to capture and enrich bacteria from complex samples. *Med. Microbiol. Immunol.*, 2020, 209:335-341, doi: 10.1007/s00430-020-00659-1.
36. Anisimkin VI, Kuznetsova IE, Kolesov VV, Pytaikin II, Sorokin VV, Skladnev DA. Plate acoustic wave sensor for detection of small amounts of bacterial cells in micro-litre liquid samples. *Ultrasonics,* 2015, 62(9):156-159.