Analysis of the biocidal and photocatalytic properties of cadmium sulfide nanoparticles obtained by microbial synthesis

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Abstract. The article presents the results of the analysis of the bactericidal and photocatalytic properties of cadmium sulfide nanoparticles (NPsCdS) obtained in the process of microbial synthesis using cells of the bacterium *Bacillus subtilis* 168. This methodologically simple and environmentally friendly method allows one to obtain nanocrystals, the surface of which is coated with protein molecules synthesized by a bacterial strain and stabilizing the nanomaterial in colloidal suspensions. The physicochemical characteristics of biogenic nanoparticles make them competitive with nanomaterials of abiogenic origin. The biocidal activity of protein-coated NPsCdS against test cultures of various types of microorganisms was established. The photocatalytic activity of NPsCdS with respect to decolorization of organic dyes methylene blue and brilliant green using UV radiation with a wavelength of 365 nm and a power of 160 watts was demonstrated.

Keywords: biogenic nanoparticles of cadmium sulfide, *Bacillus subtilis* 168, protein coating, bactericidal properties, photodecolorization of dyes, methylene blue, brilliant green.

In recent years, significant progress has been made in research aimed at the synthesis and search for the functional application of fluorescent semiconductor nanoparticles of cadmium sulfide (NPsCdS), the optical, electronic, physicochemical, biological and photocatalytic properties of which are of interest for solving a wide range of applied problems [1, 2].

A wide variety of physical, chemical and hybrid methods of production allows to achieve large-scale production of nanomaterials of various chemical composition with adjustable properties, high yield and reproducibility, which is often accompanied by "harsh" technological synthesis conditions using toxic reagents, which negatively affects the ecological state of the environment and health person. Complex, energy-intensive methods for producing nanoparticles pushed the scientific community to search and develop an alternative, "green" approach for the synthesis of NPsCdS nanocrystals, based on the interaction of salts as sources of sulfur anions and Cd^{2+} cations with cells of living organisms, bacteria, fungi, yeast, algae, plant extracts, and the biomolecules secreted by them [3]. Among the variety of biological objects, bacteria are distinguished as the most studied and effective "bioplatforms" for the synthesis of nanomaterials. The main advantage of biogenic nanoparticles, which distinguishes them from industrially synthesized nanocrystals, is the presence of a biolayer, which simultaneously acts as a natural stabilizer, promotes functionalization, biocompatibility of the surface and an increase in the coordination centers of absorption of dye molecules, which characterizes biogenic NPsCdS as independent nanosized biocidal preparations and photocatalysts [4, 5].

This work presents the results of a study of the biocidal and photocatalytic properties of biogenic NPsCdS obtained by microbial biosynthesis using the bacterial strain *Bacillus subtilis* 168 (hereinafter NPsCdS/*Bacillus*). The nanomaterial was obtained by introducing aqueous solutions of Na_2S and $CdCl_2$ salts into a culture liquid containing cells, proteins, and other metabolites of the bacterial strain *B. subtilis* 168 in an equimolar ratio of 2 mM: 2 mM. All stages of the developed by us bacterial synthesis of NPsCdS/*Bacillus* and their main characteristics - morphology and elemental composition, quantitative and qualitative analysis of the protein coating adsorbed on the surface of nanocrystals, the value of the hydrodynamic diameter (HD) and zeta potential, fluorescence intensity - are described in detail in the previously published us article [6].

As a result of our studies, it was found that NPsCdS/*Bacillus* are nanosized (5±1 nm) spherical crystals with the confirmed presence of the elements Cd and S. On the surface of NPsCdS/*Bacillus*, the only protein flagellin FliC with a molecular weight of 35 kDa was detected, identified as one of the constituent components of the bacterium *B. subtilis* 168 flagella. The presence of flagellin on the surface of nanoparticles determines the HD (200–550 nm) and zeta potential with a negative value from –20 to –27 mV. NPsCdS/*Bacillus* has been shown to fluoresce in the blue region of the spectrum (300–450 nm) at an excitation wavelength of 270 nm [6].

The bactericidal activity of protein-coated NPsCdS/*Bacillus* was assessed by the diffusion method in agar medium against test cultures obtained at the National Bioresource Center of the All-Russian collection of industrial microorganisms RC "Kurchatov Institute" - State Research Institute of Genetics: *Bacillus licheniformis* (B-7360), *Rhodococcus rhodochrous* (AC-1093), *Escherichia coli* K-12 (B-3345), *Pseudomonas putida* (B-4492) and yeast *Saccharomyces cerevisiae* (Y-3251). Bacterial strains belonged to gram + and gram taxonomic groups. The wells were filled with 50 μl of nanoparticles (3 mg/ml) and incubated at 30–37°C for 24 h, then the diameter of the zones of no growth of test cultures was estimated.

It was shown that the most susceptible microorganisms to the action of NPsCdS are the yeast *S. cerevisiae* (inhibition zone 33 mm). For gram + bacteria *B. licheniformis* and *R. rhodochrous*, diameters of growth inhibition zones of 28 mm and 16 mm were observed, respectively. Among gram-bacterial cultures, NPsCdS had an inhibitory effect only on *P. putida* (16 mm). Thus, the different sensitivity of microorganisms to the action of NPsCdS/*Bacillus* was established, which can be explained by the structure of the cell walls of microorganisms of various groups and species and, as a consequence, the degree of penetration of Cd^{2+} ions into the cells [7]. At the same time, NPsCdS/*Bacillus* showed the greatest efficiency against test strains belonging to different groups - eukaryotic yeast *S. cerevisiae* and prokaryotic gram (+) bacteria *B. licheniformis.* These results indicate the possibility of using NPsCdS of bacterial origin as biocidal drugs for various medical applications.

The photocatalytic properties of NPsCdS/*Bacillus* were studied using the example of decolorization of organic dyes brilliant green (BG) and methylene blue (MB). The concentration of dyes was 25 ppm, the concentration of nanoparticles was 0.5 mg/ml, UV radiation with a wavelength of 365 nm and a power of 160 W for 3 hours. The experiments have shown that there is a difference in the degree of efficiency of photodecolorization of the claimed dyes, depending on their belonging to a certain chemical family. Thus, BG as a representative of the triphenylmethane family was discolored in the presence of NPsCdS by 85%, which is ~ 2.4 times higher than that of the thiazine dye MB, which is more resistant to fading. Despite the insignificant photodecolorization index obtained for MB, it is worth noting that, in the absence of nanoparticles, UV irradiation only leads to 8% discoloration of the dye. These results indicate the need to include NPsCdS in the photocatalysis process in order to achieve greater destruction of dyes of various chemical nature.

The possibility of using NPsCdS/*Bacillus* as a photonanocatalyst in three cycles of BG and MB photodecolorization under the same experimental conditions has been established. The efficiency of photodecolorization of dyes decreased in the third cycle to 40%, but this was enough to continue the process of decolorization of dyes. The reasons for the decrease in the efficiency of decolorization of dyes with repeated use of biogenic nanoparticles may be incomplete removal of dye molecules from the surface of the protein layer of nanoparticles, or the loss of a small part of nanoparticles during their isolation, purification, and resuspension. Thus, we have demonstrated the fundamental possibility of using biogenic NPsCdS/*Bacillus* as photocatalysts for decoloration of dyes of various chemical groups, as well as the possibility of effective threefold photocatalytic decolorization of dyes using UV irradiation of 160 W, which contributes to the further operation of biogenic nanoparticles under real decolorization conditions.

Conclusions. Biogenic NPsCdS/*Bacillus* obtained by microbial synthesis and containing an adsorbed protein layer on the surface, along with physicochemical analogs, can be used as independent biocidal preparations of natural origin, especially against the growth of yeast and bacterial gram (+) cultures, as well as for photocatalytic discoloration of synthetic organic dyes of various chemical families. The possibility of multiple participation of NPsCdS/*Bacillus* in the photocatalysis of dyes under UV irradiation shows the promise of using biogenic nanoparticles for purifying polluted wastewater and maintaining a favorable environmental situation.

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