INFLUENCE OF ZNS NANOPARTICLES ON PRODUCTIVITY OF RED ALGAE *PORPHYRIDIUM CRUENTUM*

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Наночастицы ZnS были синтезированы с использованием коллоидных методов, Для получения водного коллоидного раствора, поверхность частиц была модифицирована тиоглицеролом. Наличие тиоглицерола на поверхности наночастиц было подтверждено с помощью инфракрасной спектроскопии. Водный раствор наночастиц ZnS был протестирован на красных водорослях Porphyridium cruentum, которые были использованы в качестве биологического объекта.

Introduction

At present in connection with the development and implementation of nanotechnologies, the problems associated with possible influence of the man-made nanoparticles on vital functions of living organisms are of special concern. Algae are diverse and available objects, the use of which in screening studies makes it possible to assess both the specificity of exposure to nanoparticles and dose-related effects, i.e. sensitivity can be regarded as indicator of the environmental toxicity of nanomaterials [1].

The purpose of this study was to investigate the impact of nanoparticles of zinc sulfide (ZnS) on vital functions of aquatic organisms – red microalga Porphyridium cruentum, which is known as a biotechnological object with practical application in medicine and food technology. Porphyridium cruentum is being used in the production of arachidonic and eicosapentaenoic fatty acids, phycobiliproteins and sulphated polysaccharides [2]. Zinc sulfide is an important II-IV semiconductor material and has a wide range of applications such as optical sensors, solid state solar window layers, photoconductors, phosphors and catalysts.

Materials and methods

Chemicals. Tri-*n*-octylphosphine (TOP), Aldrich, 90%, sulfur shot (Aldrich, 99.999%), squalane (Aldrich, 99%), zinc acetate dihydrate (Aldrich, 99.99%), and oleic acid (cis-9-octadecenoic acid, Aldrich, 90%),1-thioglycerol (3-mercaptopropane-1,2-diol, Aldrich, 90%). Anhydrous methanol, hexane, chloroform, acetone were used without further purification. Trioctylphosphine sulfide (0.75 M, TOP-S) was prepared by the complete dissolving of the necessary amount of sulfide in 50 ml of TOP at 60–70°C under moderate stirring. The TOP-S solution described above was prepared and stored in a nitrogen glove box.

The microalgae cultivating and obtaining of extracts from biomass. The strains of *Porphyridium cruentum* were cultivated under laboratory conditions. Water extracts were prepared from native biomass by freezing, and ethanol extracts were derived with 70% ethanol in ratio 1/10 (w/v).

Synthesis of nanoparticles. Zinc sulfide nanoparticles were prepared by the colloidal method [3]. A standard synthesis of ZnS nanoparticles was performed in a round-bottom three-neck flask equipped with a magnetic stirrer, a thermocouple, and a temperature control unit. Zinc oleate was prepared by heating a mixture of 2 mmol zinc acetate, 4 mmol of oleic acid, and 20 mmol of squalane. Oleic acid (OA) was employed both for group II precursor formation and nanoparticle stabilization during the synthesis intended for nucleation and reaction rate control. This solution was heated under vacuum at 80–100°C for 5–6 h in order to form zinc oleate and to remove the already formed acetic acid. The subsequent synthesis of ZnS nanoparticles was carried out by rapid injection of trioctylphosphine sulfide (TOP-S) solution maintained at room-temperature into a vigorously stirred mixture containing cadmium oleate heated from 140°C to 200°C under N_2 atmosphere. The reaction mixture was maintained at a fixed temperature for 10 min and then promptly cooled to room temperature using an ice-water bath. The solution quickly turned dark red during the synthesis due to the formation of ZnS colloidal solution. The TOP-S/lead oleate molar ratios varied from (1.0–3) to 1. In order to use hydrophobic nanoparticles for biological applications, they first must be transferred into aqueous solution.

Synthesis of thiol-coated nanoparticles. Ligand exchange and water solubilization. ZnS nanoparticles were water solubilized by replacing the initial hydrophobic surfactants (OA) with 1-thioglycerol(TG) [4]. A 10 mg amount of TG and 5.0 mg of QDs were dissolved in 30 mL of solvent. Two solvent systems were compared: methanol and a 1:1 mixture of acetone and methanol. The solution was adjusted to pH10 with tetramethylammonium hydroxide pentahydrate (TMAHP). The flask was purged with N2, and the tempera-

ture was raised to 70 °C. Samples were removed from the reaction at specific times during the exchange. Hydrophilic ZnS nanoparticles were water solubilized and purified from hydrophobic nanoparticles.

Nanocrystalline sample characterization. The XRD-ray analysis of the resultant material was performed using a DRON-3.0 linear diffractometer (Fe K_α-radiation, the θ-2θ technique).

Spectrum-100 Fourier transform spectrometer (Perkin Elner). Each spectrum was obtained at room temperature by averaging over 64 interferograms with a resolution of 1 cm^{-1}. The samples for the IR measurements were prepared as tablets.

Culture condition and experimental design. The unicellular red alga *Porphyridium Cruentum* CNM – AR – 01 was cultivated in a nutritive mineral medium that was optimized at temperature of 27˚C, constant and continuous illumination and periodic agitation. The cultivation time was 10 days. The biomass was separated from the cultural liquid by centrifugation and standardized for the biomass content up to 10 mg/1 ml [5].

The experiments were carried out by inoculating algae from a stock culture (on 10^{th} day of growth) to a fresh medium at an initial cell density of 0,4 abs to 560 nm. The aliquots of 50 ml of this culture were spiked with suitable amounts of aqueous solution of ZnS nanoparticles in order to obtain a final range of ZnS between 0,01 – 10 mg/L.

The productivity of the microalgae was determined spectrophotometrically at a wavelength of 560 nm. The maximum extinction of the microalgae suspension is 0,3. The second calculus coefficient for g/L is 1.64.

Results and discussion

A typical powder diffraction pattern of the produced ZnS show three broad peaks positioned at 2θ = 28.57°, 33.19°, 47.63° and 56.49° with correspondence to the diffraction planes (111), (200), (220) and (311) respectively. The peaks were perfectly indexed to the cubic zinc blende. The broadening of the diffraction pattern for ZnS implies a reduction in particle size. An average size of 30–35 nm for the ZnS nanoparticles was estimated from the X-ray powder diffraction data using the Scherrer equation.

As determined by FTIR-Spectroscopy, stabilization of ZnS nanoparticles by oleic acid occurs in the process of nanoparticles high-temperature colloidal synthesis.

A feature of typical spectrum of nanoparticles obtained by high-temperature synthesis using oleic acid as a stabilizer is a broad absorption band at 2500– 3500 cm^{-1}, which is related to the OH bond of the carboxyl group of oleic acid. Two sharp peaks at 2924 cm^{-1} and 2854 cm^{-1} are assigned as (CH2 -) 2924 cm^{-1} vs (CH2-) -2854 cm^{-1}. A strong peak at 1710 cm^{-1} belongs to C=O bonds, while the band at 1285 cm^{-1} corresponds to the C-O bonds. Flat features related to the OH groups appear at 1462 cm^{-1} and 1937 sm^{-1}. A shift towards lower frequencies of absorption bands in the region of 2925 to 2921 cm^{-1} and 2854 to 2852 cm^{-1} is observed in the spectrum of ZnS nanoparticles. The absorption band at 1710 cm^{-1} which is related to stretching vibrations of carbonyl groups splits in two bands, and new bands appear at 1556 cm^{-1} and 1410 cm^{-1} which are characteristic of (COO-) and vas (COO-) vibrations. This suggests that the carboxyl group of oleic acid is not simply adsorbed on the surface of ZnS nanoparticles, but zinc is symmetrically coordinated by two oxygen atoms.

Thus, organic molecules around the nanoparticles form a tightly-packed monolayer. Moreover, even after prolonged washing procedure of nanoparticles with various solvents, including those dissolving the oleic acid, is not possible to achieve full removal of oleic acid.

Water-soluble thiolated molecules are among the most commonly used ligands to render nanoparticles in water-soluble and biocompatible form. These ligands maintain a relatively small total size of nanoparticles, which makes them useful for many biological applications.

The transfer of nanoparticles in aqueous solution is necessary for biological applications. This is achieved by exchange reactions. By comparing the IR spectra of the free thioglicerol with a model of nanoparticles associated with thiol groups was observed broadening of the spectrum and the emergence of absorption bands characteristic for the OH and SH-groups. The inclusion of sulfur thiol groups in the crystal lattice of ZnS occurs in the process of ligand exchange [6, 7].

To check the effects of ZnS nanoparticles on Porphyridium they were supplemented to the cultivation environment of the microalgae in the first day of the vital cycle.

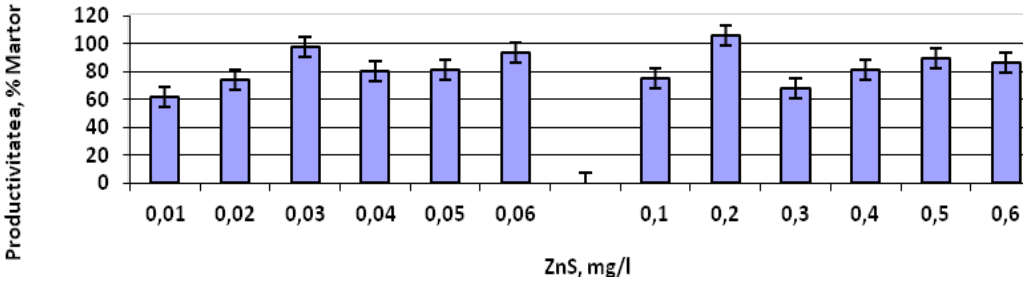


Fig. 1. *Productivity of Porphyridium cruentum (% of control sample) in the presence of ZnS nanoparticles (0,01-0,6 mg/l)*

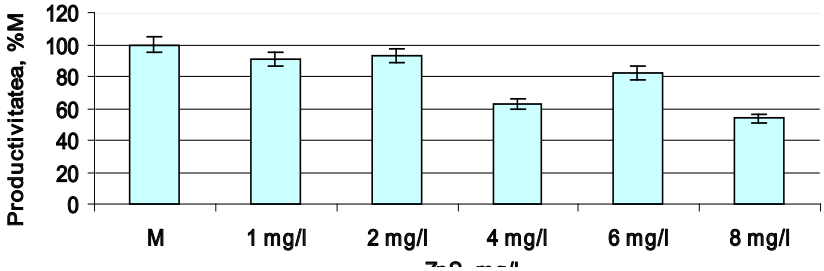


Fig. 2. *Productivity of Porphyridium cruentum (% of control sample) in the presence of ZnS nanoparticles (1–8 mg/l)*

The influence of ZnS nanoparticles on productivity of *Porphyridium cruentum* is manifested by their involvement in structural lipid oxidation of cell membranes.

In the range of concentration of nanoparticles between 0,01-0,6 mg/l was observed slight monotonic increase of productivity by 10-15% (Fig. 1).

At further increase of the concentration of nanoparticles in the range of 1–8 mg/l was observed a clear reduction in productivity (Fig. 2).

Conclusion

Nanoparticles with dimensions of 35 nm with hydrophobic shell consisting of oleic acid were obtained by colloidal synthesis. The change of the shell state from hydrophobic to hydrophilic was demonstrated with 1-thioglicerol. Because growth and metabolism of algae are fundamental to the functioning of ecosystems and the structure of aquatic food-chains, our study suggests nano-ZnS has potential to alter important community and ecosystem properties of aquatic habitats.

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