

## Light-scattering properties of microorganisms *Desulfuromonas acetoxidans* by influence of silver

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### Abstract

The article deals with the concentration changes and relative content of bacterial cells of *Desulfuromonas acetoxidans* in the intervals of sizes 0.2–2.0 μm under the influence of nano silver particles. Correlation between these changes of light-scattering properties of bacterial cells and growth abilities of bacteria *Desulfuromonas acetoxidans* under influence of silver nanoparticles and ions has been shown. The purpose of the work was to research the intensity of processes the change of indexes of the antioxidant system the cells of *Desulfuromonas acetoxidans* at influence of silver nanoparticles and silver nitrate. The influence of various concentrations of silver nanoparticles and silver nitrate on enzymatic activity of catalase and reduced glutathione synthesis by *Desulfuromonas acetoxidans* cells under their cultivation with fumarate addition and with absence of sulphur has been determined. Specific catalase activity increased with enhancing of concentration and duration of bacterial cultivation under the addition of this salt. The highest specific catalase activity was determined on the second day of bacterial growth under the influence of all concentration range of investigated metal salt. The reduced glutathione content under silver nitrate and silver nanoparticles exposure varied depending on the cultivation time and metal concentration. The maximum reduced glutathione content has been observed. The result of catalase activity changes and glutathione content changes of sulfur-reducing *D. acetoxidans* bacteria cell-free extracts and has been investigated under the influence of different concentrations of Ag nanoparticles during four days of cultivation has been investigated.

**Key words:** *Desulfuromonas acetoxidans*, light, scattering.

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### 1. Introduction

*Desulfuromonas acetoxidans* are colorless strictly anaerobic sulfur bacteria that support reductive stage of sulfur cycle in the nature. In Bergey's Manual of Systematic Bacteriology they are described as "straight or slightly curved rods and elongated ovoid rods, 0.4–0.9 – 1.0–4.0 μm in length. Special resting forms such as spores are not known to occur. Gram negative. Motile, generally by means of a single flagellum located at a lateral or subpolar position; cells exhibit a characteristic propeller-like movement. Some strains have polar flagella. Strictly anaerobic. Possess mainly a respiratory type of metabolism with elemental sulfur serving as the terminal electron acceptor, and being reduced to H<sub>2</sub>S (dissimilatory sulfur reduction). l-Malate or fumarate may be fermented, giving succinate as the main product in the presence or absence of acetate. Betaine may be ferment-

ed to acetate, CO<sub>2</sub>, and trimethylamine. Optimal growth temperature, 30 °C" (Brenner et al., 2005). For researches bacterial cells *Desulfuromonas acetoxidans* IMB B-7384 strain was isolated from Yavoriv aquatic sulfur field (Lviv region, Ukraine). It was purified and identified at the Department of Microbiology of Ivan Franko National University of Lviv, Ukraine. It is registered in the Ukrainian Collection of Microorganisms of Institute of Microbiology and Virology NAS of Ukraine. The length is 0.4–0.8 μm and the width is about 1–4 μm.

*Desulfuromonas acetoxidans* are specific bacteria that belong to  $\delta$ -Proteobacteria group and possess an ability to reduce S<sup>0</sup> to H<sub>2</sub>S and Fe<sup>3+</sup> to Fe<sup>2+</sup> in the processes of dissimilation sulfur- and Fe(III)-reduction. It is set that to the bacterium *Desulfuromonas acetoxidans* is able to get energy at anaerobic terms at transformation of acetate to dioxide carbone in the loop of tricarbonic acid cycle by interactiv of

oxidation of acetate and proceeding in sulphur communication. The oxidation of acetate and other organic substances, in particular ethanol, propyl alcohol, butyl alcohol and others like that, with participation of bacteria of *Desulfuromonas acetoxidans* is considerably more intensive at presence of Fe (III) and Mn (IV) in an environment, that are electron acceptors and recommence at these terms (Rodén & Lovley, 1993). *Desulfuromonas acetoxidans* bacteria are considered to be used as substrate for microbial-anode fuel cells because of high electron recovery to the electric current as a result of electron transfer during the processes of acetate oxidation and  $S^0$ ,  $Fe^{3+}$  or  $Mn^{4+}$  reduction with  $Fe^{2+}$  or  $Mn^{2+}$  producing. Systematic researches of generation of electric current by the cells of bacteria of *Desulfuromonas acetoxidans* at different terms in double-chamber cells are driven to (Bilyy et al., 2014).

In this work the results of researches of bacterial growth and the distribution of bacteria cells in size intervals 0.2–2.0  $\mu\text{m}$  under the influence of cooperation of bacteria cells of *Desulfuromonas acetoxidans* with ions and nanoparticles of silver are determined. The results of researches of changes of enzyme antioxidant system by determination of specific activity of catalase and not enzyme antioxidant system by determination of content of intracellular reduced glutathione in the cells *Desulfuromonas acetoxidans* under the action of silver nitrate and silver nanoparticles are presenting.

## 2. Materials and methods

**Microbial cells, medium and cultivation.** Microbial cells *Desulfuromonas acetoxidans* IMV B-7384, which was applied in these investigations, belongs to the Ukrainian Collection of Microorganisms of D. K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine. Bacteria have been cultivated under the anaerobic conditions in the modified Postgate C medium (Pfennig & Biebl, 1976; Biebl & Pfennig, 1977) in which sterile sulfur, biotin, fumaric acid were added before cultivation. Fumaric acid served as carbon source. Optimal pH for growth was 6.8–7.5 and optimal temperature was 30 °C.

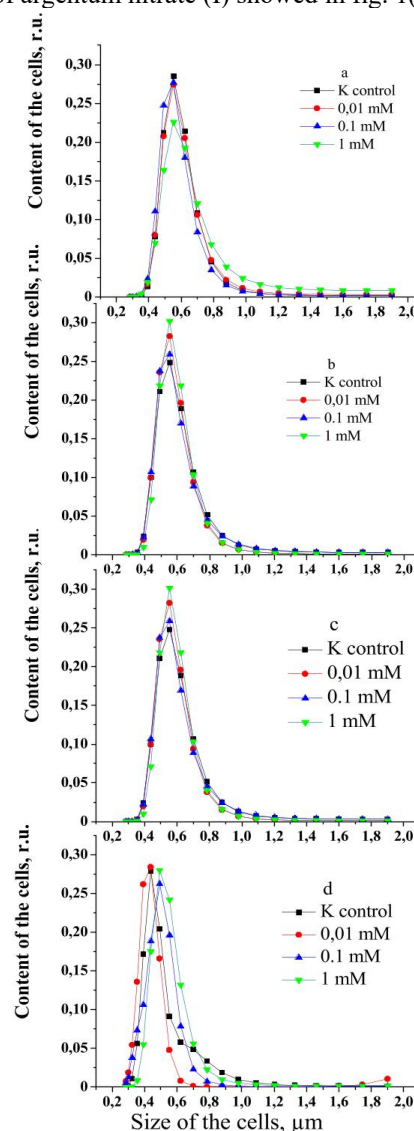
**Cell size distribution and relative content measurement.** Bacterial growth commonly can be investigated by the registration of bacterial suspension turbidity at  $\lambda = 410$  nm. The distribution of particles in size is by the light scattering methods is proposed in (Bilyy et al., 2008; 2011). It includes the sounding of flow suspended bacterial cells by monochromatic coherent light, the registration of cooperative signals of sounding radiation and the explored microbiological objects by detecting of amplitudes and duration of scattered light impulses. Investigation of light scattering properties of bacterial *Desulfuromonas acetoxidans* cells under the influence of silver nitrate ( $Ag^+$  ions) and silver nanoparticles were carried out during four days and concentrations of  $AgNO_3$  and Ag nanoparticles into the growth medium variation in intervals  $10^{-3} - 10^{-6}$  M/L. The control samples didn't contain any investigated metal salts. After the appropriate time of growth 1 ml of bacteria suspension was diluted in 100 ml of deionized water and measures were carried out by using the apparatus PRM-6M, which was constructed at the Laboratory of Optical-Electronic Device of Ivan Franko National University of Lviv. For the estimation of concentration influence of metal of Ag in case of nanoparticles of silver initial solution of titrated with multiplicity 2, and in

case of salt of  $AgNO_3$  – multiplicity 10. The errors of cell size distribution measurement of constructed equipment are 5 %.

**Measurement of catalase and intracellular reduced glutathione content.** Antioxidant defense system activity has been measured in the cell-free extract after the second, third and fourth day of bacterial growth. Cells were washed by 0.9 % NaCl solution and disintegrated on the ultrasonic homogenizer at 22 kHz at 0 °C during five minutes. Cell debris were sedimented by centrifugation at 5640–8800g at 4 °C during 30 minutes. Catalase activity was measured spectrophotometrically at  $\lambda = 410$  nm by the degree of breakdown of hydrogen peroxide in the cell-free extract (Goth, 1991). Reduced glutathione content has been measured by the degree of dithionitrobenzoic acid reduction in cell-free extract (absorbance maximum  $\lambda = 412$  nm) (Owen, 2010).

## 3. Results and discussion

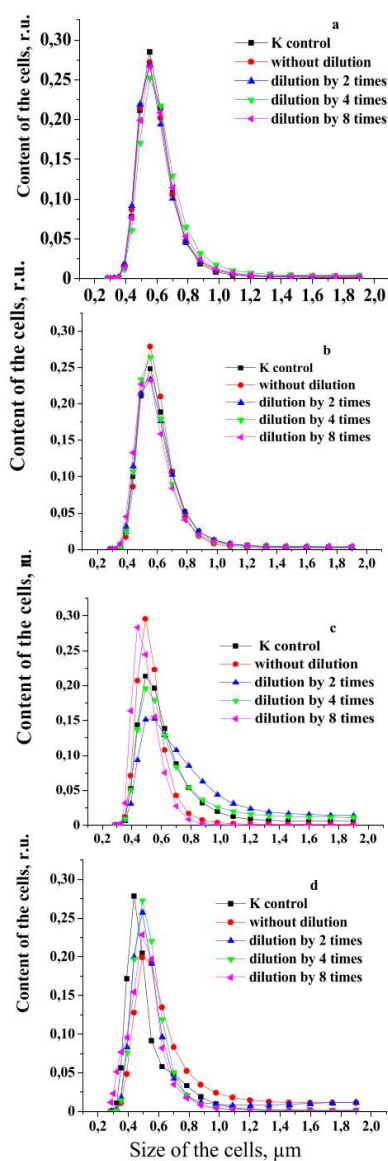
**Results of light scattering properties of bacterial *desulfuromonas acetoxidans* cells.** The cells' size distribution curve under the influence of all investigated concentrations of argenterum nitrate (I) showed in fig. 1(a-d).



**Fig. 1(a-d).** Concentration dependences of the cell size distribution of bacteria cells *Desulfuromonas acetoxidans* under the influence of Ag nanoparticles: Time of cultivation (a)–1 days, (b)–2 days, (c)–3 days, (d)–4 days

It was observed that the curve of cells' size distribution and maximum of cells' size distribution during the first, second and the third day didn't change under the influence of 0.01–10 mM of AgNO<sub>3</sub> and it equaled 0.55 mkm. On the fourth day of growth the maximum of cells' size distribution for curve with concentration 0.01 mM of AgNO<sub>3</sub> changed from 0.55 to 0.43 mkm. The maximum of cells' size distribution for curve with concentration 0.1 mM and 1.0 mM it 0.5 mkm.

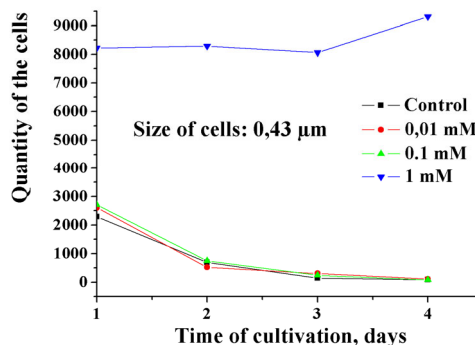
The cells' size distribution curve under the influence of all investigated concentrations of Ag nanoparticles showed in fig. 2 (a-d). Cells' size distribution curves on the fig. 2(a, b) of sulfur bacteria *Desulfuromonas acetoxidans* under the influence of all investigated Ag nanoparticles concentration range during second day of growth are presented on the fig. 2 (a, b). It was observed that the curve of cells' size distribution and maximum of cells' size distribution didn't change 3 and it equaled 0.55 mkm.



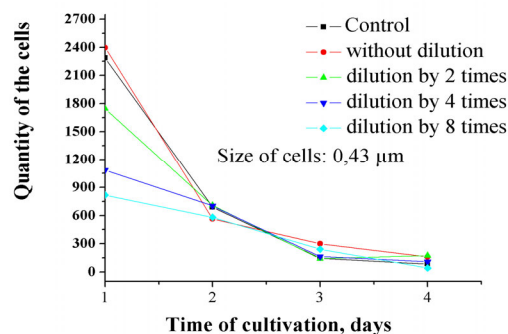
**Fig. 2(a-d).** Concentration dependences of the cell size distribution of bacteria cells *Desulfuromonas acetoxidans* under the influence of Ag nanoparticles: Time of cultivation (a)–1 days, (b)–2 days, (c)–3 days, (d)–4 days

The changes of cell's size distribution and their relative content in the chosen interval of sizes under the influence of all investigated Ag nanoparticles concentration it was observed on the third day of growth the cells *Desulfuromonas acetoxidans*. Under the influence of all minimum concentrations of Ag nanoparticles the maximum of cells' size distribution was 0.43 mkm. At the highest concentration of Ag nanoparticles the maximum of cells' size distribution was 0.50 μm. During fourth day of growth under the influence of all investigated concentrations of Ag nanoparticles the maximum of cells' size distribution was 0.50 mkm.

Estimation of influence of silver on the investigated bacterial cells built after dependences of absolute number of cells in the size interval of 0.43 mkm. Concentration dependences the quantity of the bacteria cells *Desulfuromonas acetoxidans* under the influence of AgNO<sub>3</sub> on time of growth are showed in fig. 3. Concentration dependences the quantity of the bacteria cells *Desulfuromonas acetoxidans* under the influence of Ag nanoparticles on time of growth are showed in fig. 4. The absolute number of cells *Desulfuromonas acetoxidans* for Ag nanoparticles on fourth day of growth during the influence of all investigated concentrations of Ag nanoparticles was 2000. The absolute number of cells *Desulfuromonas acetoxidans* under the influence of AgNO<sub>3</sub> with concentrations in intervals 0.01–0.01 mM on fourth day of growth was 2000. However, for solution from the concentration of AgNO<sub>3</sub> of 1 mM/L there was an opposite picture. An amount of particles on the first day of growth was in 2.5 times more than for solutions with the less concentration AgNO<sub>3</sub>, in times of growth grew and on a fourth day there were 9400 particles to the order in solution.



**Fig. 3.** Concentration dependences the quantity of the bacteria cells *Desulfuromonas acetoxidans* under the influence of AgNO<sub>3</sub> on time of growth

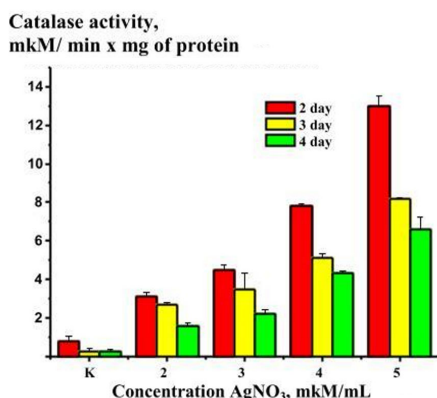


**Fig. 4.** Concentration dependences the quantity of the bacteria cells *Desulfuromonas acetoxidans* under the influence of Ag nanoparticles on time of growth

These results are may be related to nature of the nvestigated objects. It is known that Bacterial dissimilation sulfur reduction is a process in which elemental sulfur is reduced to hydrogen sulfide under the specific bacterial polysulfid-ereductase (Psr) activity. This process is conducted by sulfurbacteria of different genus, such as *Sulfurospirillum*, *Desulfurella*, *Desulfuromonas* etc. *Desulfuromonas acetoxidans* are uncoloured gram-negative obligatory anaerobic sulfurreducing bacteria that inhabit sulfur containing aquatic environments. It's possibly can be explained by the interaction between  $\text{AgNO}_3$  and sulfur, which was added to the growth medium and produced  $\text{H}_2\text{S}$  by *Desulfuromonas acetoxidans* bacteria. Interaction between hydrogen sulfide as a final product of bacterial dissimilation sulfurreduction and  $\text{Ag}^+$  forming the nanoparticles of silver sulphide.

**Results the measurement of catalase and intracellular reduced glutathione content of bacterial *Desulfuromonas acetoxidans* cells**

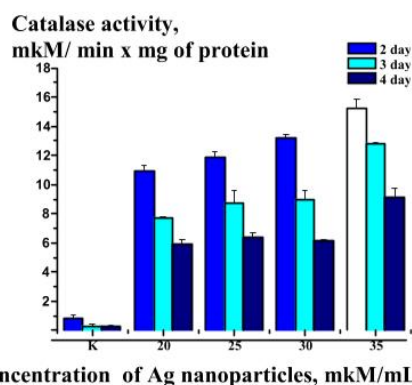
*Results the measurement of catalase content of cells D. Acetoxidans IMV B-7384.* The result of catalase activity of sulfur-reducing *Desulfuromonas acetoxidans* bacteria cell-free extracts has been investigated under the influence of different concentrations of silver (I) nitrate during four days of cultivation are showing on fig. 5. At influence of  $\text{AgNO}_3$  with the increase of concentration of salt of metal in an environment from 20 to 50  $\mu\text{M}$  activity of catalase grew in 3–8 times during the second time of cultivation, comparatively with control. At the increase of time cultivation took place gradual decline of activity of catalase in 0.5–1.5 times and 1.7–2 times on the third and fourth twenty-four hours accordingly at influence of all investigated concentrations of  $\text{AgNO}_3$ , comparatively with the second time of cultivation.



**Fig. 5.** Catalase activity of sulfur-reducing *Desulfuromonas acetoxidans* IMB B-7384 under the influence of different concentrations of  $\text{AgNO}_3$  during four days of cultivation

The result of catalase activity chaingees of sulfur-reducing *Desulfuromonas acetoxidans* bacteria cell-free extracts has been investigated under the influence of different concentrations of Ag nanoparticles during four days of cultivation are showing on fig. 6. The maximum of catalase activity of sulfur-reducing *Desulfuromonas acetoxidans* bacteria of IMB B-7384 under the influence of different concentrations of Ag nanoparticles was observed on the second day of growth. Catalase activity was higher by 11; 20 and 15 times respectively on the second, third and fourth days of growth in comparison with control samples. Maximal activity of enzyme was fixed at presence of in an environment 35  $\text{mkM/mL}$  of Ag nanoparticles and it equaled

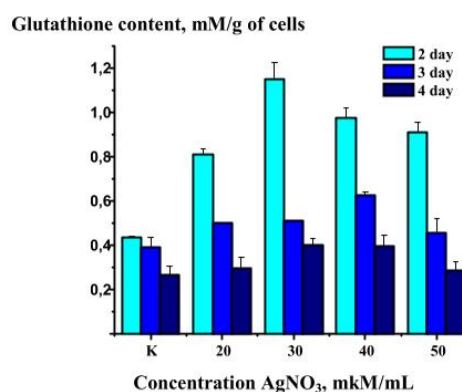
$15.2 \pm 0.86 \text{ mkM/min} \times \text{mg}$  of protein. The increase of concentration of Ag nanoparticles in the environment of Ag nanoparticles in the environment of incubation caused the gradual increase of specific activity of enzyme.



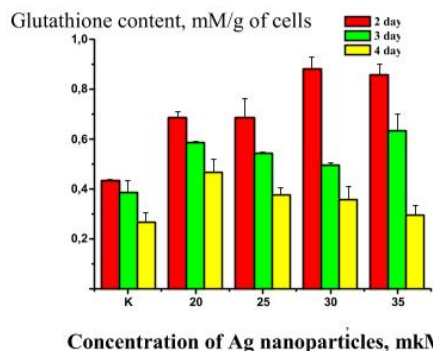
**Fig. 6.** Catalase activity of sulfur-reducing *Desulfuromonas acetoxidans* IMB B-7384 under the influence of different concentrations of Ag nanoparticles during four days of cultivation

*Results the measurement of intracellular reduced glutathione content of bacterial *Desulfuromonas acetoxidans* IMV B-7384.* The result of glutathione content changes of sulfur-reducing *Desulfuromonas acetoxidans* bacteria cell-free extracts has been figure 7. It is shown that during the four days of cultivation bacteria cells *Desulfuromonas acetoxidans* on the second day at influence of concentration of  $\text{AgNO}_3$  in the environment of incubation from 20 to 30  $\text{mkM/mL}$  the glutathione content grows, and from 40 to 50  $\text{mkM}$  – goes down. Maximal glutathione content was looked after on the second twenty-four hours by cultivations for the concentrations of 30  $\text{mkM/mL}$  silver nitrate. On the third and fourth twenty-four hours there was a decline of glutathione content on 37.5–50 % and on 63–72 % accordingly at all investigated concentrations.

The result of glutathione content changes of sulfur-reducing *Desulfuromonas acetoxidans* bacteria cell-free extracts has been investigated under the influence of different concentrations of Ag nanoparticles duing four days of cultivation are showing on fig. 8. An increase of concentration of Ag nanoparticles in the environment of incubation of *Desulfuromonas acetoxidans* of IMB B-7384 from 20 to 35  $\text{mkM/mL}$  predetermines the increase the glutathione content on the second day of cultivation.



**Fig. 7.** Glutathione content of sulfur-reducing *Desulfuromonas acetoxidans* IMB B-7384 under the influence of different concentrations of  $\text{AgNO}_3$  during four days of cultivation



**Fig. 8.** Glutathione content of sulfur-reducing *Desulfuromonas acetoxidans* IMB B-7384 under the influence of different concentrations of Ag nanoparticles during four days of cultivation

Maximal glutathione content was looked after on the second twenty-four hours by cultivations for the concentrations of 30 mkM/mL Ag nanoparticles and it equaled 1.1 mM/g of cells, that in 2 times largest, comparatively with control. Further cultivation caused the decline of glutathione content on 19–24 % and on 33–65 % on the third and fourth twenty-four hours accordingly comparatively with the second time of growing at all investigated concentrations.

#### 4. Conclusions

The influence of different concentrations of AgNO<sub>3</sub> and Ag nanoparticles on light scattering properties of sulfur-reducing *Desulfuromonas acetoxidans* bacteria on the base of their cells' size distribution and relative content with the maximum of cells' size distribution changes has been investigated. It was observed that under the influence of all concentration AgNO<sub>3</sub> and Ag nanoparticles the maximum of cells' size distribution changed from 0.55 to 0.5 mkm. The result of catalase activity changes and glutathione content changes of sulfur-reducing *D. acetoxidans* bacteria cell-free extracts and has been investigated under the influence of

different concentrations of Ag nanoparticles during four days of cultivation has been investigated.

#### Conflict of interest

The authors declare that there is no conflict of interest.

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