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The influence of chlorine compounds on the oxidation of nitrite and hydrogen sulfide ions by phototrophic sulfur bacteria

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In the process of anoxygenic photosynthesis phototrophic sulfur bacteria can use sulfides, thiosulfates, nitrites, bivalent iron, molecular hydrogen or organic compounds as exogenous electron donors and CO_2 as a carbon source. The influence of halides on transformed ecosystems, in particular, on their photosynthetic microbiota and its properties, remains insufficiently studied. The usage of nitrite and hydrogen sulfide ions as an electron donor of anoxygenic photosynthesis by cells of phototrophic purple and green sulfur bacteria *Thiocapsa* sp. Ya-2003, *Lamprocystis* sp. Ya-2003 and *Chlorobium limicola* IMV K-8, isolated from the Yavorivske Lake, under the influence of one of the most common toxicants, chlorine compounds, has been studied. Bacteria were cultivated under anaerobic conditions and constant lighting for 10 days in van Niel medium with $NaNO_2$ or $Na_2S\times9H_2O$ (4.2 mM). To study the influence of NaCl and $C_6H_4ClNO_3$ on biomass accumulation, nitrites or sulfides oxidation, nitrates or sulfates production, synthesis of intracellular carbohydrates, bacteria were sown in the media with chlorine compounds at concentrations that are equal to the maximum permissible concentration (MPC) of chloride ions – 9.859 mM, and 0.5–4.0 (in NaCl composition) or 0.03–4.0 (in $C_6H_4ClNO_3$ composition) times differed from the MPC. Biomass was determined by the turbidimetric method, the concentrations of nitrate, nitrite, hydrogen sulfide, sulfate ions in the cultural liquid – by the spectrophotometric method. The intracellular glucose and glycogen content was determined enzymatically in cell-free extracts of *C. limicola* IMV K-8, using the analytical kit "Diagluc-2". It was found that NaCl at concentrations 3.0–4.0 times higher than the MPC significantly inhibits the biomass accumulation (2.2–2.8 times), NO_2^- oxidation (by 26.3–35.7%), and NO_3^- formation (1.6–1.9 times) by all investigated strains of bacteria during growth in the medium with $NaNO_2$. Under the influence of NaCl at concentration 4.0 times exceeding the MPC the glycogen content in *C. limicola* IMV K-8 cells grown in the medium with $NaNO_2$ increased 2.1 times compared to the control. NaCl at concentrations 2.0–4.0 times higher than the MPC significantly inhibits the biomass accumulation (2.4–2.6 times), HS^- oxidation (by 42.9–47.5%), and SO_4^{2-} formation (2.9–3.1 times) by bacteria during growth in the medium with $Na_2S\times9H_2O$. Under the influence of NaCl at concentration 4.0 times higher than the MPC the glycogen content in *C. limicola* IMV K-8 cells grown in the medium with $Na_2S\times9H_2O$ increased 2.2 times compared to the control. $C_6H_4ClNO_3$ at concentration 4.0 times higher than the MPC of chloride ions slightly inhibited the biomass accumulation (1.3–1.5 times), HS^- oxidation (by 15.1–22.2%), and SO_4^{2-} formation (1.5–1.6 times) by bacteria in the medium with $Na_2S\times9H_2O$. Under the influence of $C_6H_4ClNO_3$ at concentration 4.0 times higher than the MPC the glycogen content in *C. limicola* IMV K-8 cells grown in the medium with $Na_2S\times9H_2O$ increased 2.0 times compared to the control. Chloronitrophenol revealed a less toxic effect on changing the physiological properties of bacteria than sodium chloride at the same concentrations. Glycogen content in *C. limicola* IMV K-8 cells grown in the medium with $NaNO_2$ and NaCl at concentration 4.0 times exceeding the MPC was the highest and amounted to 81.7 mg/g dry cell weight. Since the ability of all tested strains of phototrophic bacteria to oxidize nitrites or hydrogen sulfide remained sufficiently high even after adding chlorine compounds into the medium at concentrations 2.0–4.0 times exceeding the MPC of chloride ions, they are promising for use in technologies for cleaning environments with complex contamination by chlorine, sulfur, and nitrogen compounds.

Keywords: phototrophic bacteria; chlorine compounds; chloride ions; nitrite ions; hydrogen sulfide; glycogen.

Introduction

Phototrophic purple and green sulfur bacteria represent a group of Gram-negative anaerobic lithotrophic microorganisms inhabiting illuminated anaerobic zones of fresh, marine, or waste water with high H_2S content (Hallenbeck, 2017; Moroz et al., 2021; Imhoff, 2021). These bacteria are various in morphology and perform oxidative transformation of organic and inorganic compounds in aquatic ecosystems (Xie et al., 2023; Tsuji et al., 2024). They utilize CO_2 as a carbon source and sulfides, thiosulfates, nitrites, bivalent iron, molecular hydrogen, or organic compounds as exogenous electron donors of anoxygenic photosynthesis (Schott et al., 2010; Hemp et al., 2016; Dahl, 2017). Phototrophic microorganisms require exogenous electron donors to replenish the electron "vacancy" of the primary electron donor in the reaction center (cyclic electron transport) and for NAD^+ reduction (non-cyclic transport). Electron transfer along the electron transport chain to the oxidized bacteriochlorophyll molecule (the primary donor) occurs via an electrochemical gradient from carriers with lower oxidation-reduction potential (ORP) values to those with higher values. The transfer of electrons from exogenous donors to NAD^+, whose ORP is significantly higher than the ORP of the NAD^+ to $NADH_2$ reduction reaction, occurs via the mechanism of reverse transport against an electrochemical gradient with energy consumption (Kozlova et al., 2008). Electrons from oxidized inorganic compounds enter the respiratory chain carrier (level of quinones and cytochromes) whose ORP exceeds that of the donor oxidation reaction (Kozlova et al., 2008). In purple and green sulfur bacteria CO_2 converted into carbon compounds in the reductive pentose phosphate (Calvin-Benson) or tricarboxylic acid (Arnon-Buchanan) cycles, respectively. Representatives of *Chlorobium* genus can synthesize the intracellular reserve substances such as glucose and its polymerization product, glycogen (Gorishniy et al., 2008). Phototrophic green sulfur bacteria are promising for obtaining inexpensive organic carbon, making the study of the regulatory mechanisms of glycogen biosynthesis in the cells of these bacteria of significant theoretical and practical importance (Gorishniy et al., 2009; Tong et al., 2025).

Hydrogen sulfide disrupts respiration in prokaryotes and eukaryotes due to depolarization of membranes and inhibition of cytochrome oxidase activity. H_2S damages enzymes by binding to the heme centers of metalloproteins or through post-translational modification

of proteins by adding a thiol (-SH) group to reactive cysteine residues. It has been established that H_2S has pronounced genotoxic and carcinogenic action, causes numerous respiratory, cardiovascular, and eye diseases and leads to poisoning and death of animals (Halushka & Gudz, 2009; Corvino & Caliendo, 2024). Various species of the Chromatiaceae and Chlorobiaceae families oxidize hydrogen sulfide first to molecular sulfur and later to thiosulfates and sulfates (Dahl, 2017; Tanabe et al., 2024). The stages of thiosulfates oxidation to sulfates in sulfur bacteria are catalyzed by the periplasmic multienzyme Sox system (Dahl, 2017; Li et al., 2024). Sulfur globules can be detected in the cells of purple sulfur bacteria and outside the cells of green sulfur bacteria. Consequently, the formation of sulfur deposits is related to vital activity in particular of phototrophic sulfur bacteria (Garrity et al., 2001; Garrity et al., 2005; Rosenberg et al., 2014).

Oxidized nitrogen compounds (nitrates, nitrites, nitrogen oxides) are the most hazardous pollutants of the environment. The primary toxic effect of nitrites on eukaryotes is the conversion of hemoglobin to methemoglobin. Additionally, nitrites in eukaryotic and prokaryotic cells cause alterations in extracellular and intracellular levels of Cl^- and K^+, leading to a strong electrolyte imbalance and the formation of mutagenic and carcinogenic N-nitrosamines (Camargo & Alonso, 2006; Kuypers et al., 2018). Phototrophic sulfur bacteria utilize nitrogen compounds in the processes of assimilation or dissimilation (Murali et al., 2022; Murali et al., 2024). They can fix the molecular nitrogen (Proctor, 1997). In the absence of ammonium some species assimilate nitrates or nitrites (Olmo-Mira et al., 2006). Aerobic nitrite oxidation (aerobic nitrification) is carried out by chemolithotrophic bacteria of the second phase of nitrification with nitrite oxidoreductase as the key membrane-bound enzyme (Kozlova et al., 2008). Anaerobic nitrite oxidation (anaerobic nitrification) is carried out only by phototrophic bacteria (Griffin et al., 2007). In this process, NO_2^- serves as the electron donor of anoxygenic photosynthesis, and the electrons are used for autotrophic CO_2 fixation. Phototrophic nitrite oxidation by purple sulfur bacteria of the *Thiocapsa* and *Lamprocystis* genera (Schott et al., 2010; Moroz et al., 2021) and purple nonsulfur bacteria *Rhodopseudomonas yavorovii* (Tarabas et al., 2019a) has been described. In *Thiocapsa* KS1 oxidation of nitrites to nitrates catalyze Mo-bis-MGD-bound nitrite oxidoreductase (Tanaka et al., 1983; Hemp et al., 2016). The ORP of nitrite ions oxidation reaction ($NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^- = +350$ mV) is significantly higher than the ORP of hydrogen sulfide oxidation reactions ($H_2S \rightarrow S^0 + 2H^+ + 2e^- = -250$ mV; $S^0 + 3H_2O \rightarrow SO_3^{2-} + 6H^+ + 4e^- = +5$ mV; $SO_3^{2-} + H_2O \rightarrow SO_4^{2-} + 2H^+ + 2e^- = -280$ mV) (Lengeler et al., 1999; Kozlova et al., 2008).

Pollution of water bodies results from the discharge of harmful inorganic (metals, acids, alkalis, mineral salts) and organic contaminants (dye, humic substances, phenolic compounds, petroleum, surfactants, pesticides, pharmaceuticals, detergents, etc.) through wastewater (Biletsky, 2004; Kochmar & Karabyn, 2023; Kochmar et al., 2024). Microorganisms, due to their physiological and genetic characteristics, respond more rapidly than other organisms to changes in environment quality and the effects of stress factors. The efficiency of biological methods of environment purification depends on both the metabolic activity of selected strains of bacteria and their resistance to pollutants (Martínez-Jardines et al., 2025). The influence of most halides (compounds containing univalent anions of the halogens) on the properties of photosynthetic microbiota remains poorly studied. In nature, halides are found in minerals, rocks, volcanic gases, and halides are used in the food industry, in the production of fertilizers, fluxes in metallurgy, acids, solvents, in the cultivation of artificial crystals, in photography, and in the pharmaceutical production (Biletsky, 2004; Kurta, 2009; Palencia et al., 2021; Yang & Zhu, 2024). It is known that chlorides have a complex effect on various structures of microorganisms: the cytoplasmic membrane, cytoplasmic proteins, genome, and respiratory chain enzymes by blocking SH-groups. Another function of Cl^- is in Na^+ homeostasis: the efficiency of sodium ions export can increase with simultaneous chloride ions export via sodium channels (Roessler et al., 2003; Fang et al., 2018).

The aim of this study was to investigate the influence of sodium chloride and 2-chloro-4-nitrophenol (organochlorine compound) on the oxidation of nitrite and hydrogen sulfide ions by phototrophic purple and green sulfur bacteria *Thiocapsa* sp. Ya-2003, *Lamprocystis* sp. Ya-2003, and *Chlorobium limicola* IMV K-8, isolated from Yavorivske Lake, as well as on the accumulation of intracellular carbohydrates by green sulfur bacteria.

Materials and methods

Strains of phototrophic bacteria *Thiocapsa* sp. Ya-2003, *Lamprocystis* sp. Ya-2003, and *Chlorobium limicola* IMV K-8, isolated from Yavorivske Lake, were identified at the Microbiology Department of Ivan Franko National University of Lviv (Kit & Gudz, 2007; Gorishniy et al., 2008). Strain *C. limicola* IMV K-8 was registered in the depository of D. K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine in 2010.

Bacteria were grown in the van Niel medium (Gudz et al., 2014) of such composition (g/L): NH_4Cl (0.4); $MgSO_4 \times 7H_2O$ (0.33); KH_2PO_4 (0.4); $CaCl_2 \times 2H_2O$ (0.05); CH_3COONa (2.55); $C_3H_3O_3Na$ (1.0); $NaNO_2$ (0.29) or $Na_2S \times 9H_2O$ (1.0) (4.2 mM – concentration of electron donor in the medium of standard composition); $NaHCO_3$ (6.0); inositol (1.0); vitamin B_{12} (0.000005); microelements solution – 2 mL. Solutions of CH_3COONa, $C_3H_3O_3Na$, inositol, $NaHCO_3$, $NaNO_2$ or $Na_2S \times 9H_2O$, vitamin B_{12} and microelements of such composition (g/L): $FeSO_4 \times 7H_2O$ (2.0), diluted in 25% HCl; $CoCl_2 \times 6H_2O$ (0.19); $MnCl_2 \times 4H_2O$ (0.1); $ZnCl_2$ (0.07); $Na_2MoO_4 \times 2H_2O$ (0.036); $NiCl_2 \times 6H_2O$ (0.024); H_3BO_3 (0.006); $CuCl_2 \times 2H_2O$ (0.002), were sterilized separately and placed into the medium before seeding of the cells. The pH of the medium was adjusted to the optimum by phosphoric acid solution (10%). The pH value of the medium for purple bacteria was slightly alkaline (pH ~ 7.5–8.0) and the pH value for green bacteria was neutral (pH ~ 7.0). The initial cell concentration in the medium for seeding was 0.2 mg/mL. Bacteria were cultivated under anaerobic conditions and constant lighting for 10 days at temperature +25…+28 °C. To create anaerobic conditions, 25 mL tubes were filled with medium to the top and closed with rubber stoppers. During bacteria growth the whole day lighting was provided by incandescent lamps (60–75 W). The illumination intensity was determined using a lux-meter U-116. Purple sulfur bacteria were illuminated by light with a wavelength of more than 800 nm with the use of red interference filter, the illumination intensity was 150–200 lux. Green sulfur bacteria were illuminated by rays with a wavelength of 700–800 nm, the illumination intensity was 40 lux.

To study the influence of chlorine compounds: NaCl and $C_6H_4ClNO_3$, on biomass accumulation, nitrites or sulfides oxidation, nitrates or sulfates production, synthesis of intracellular carbohydrates, bacteria were sown in test tubes, grown until the middle of the exponential growth phase under anaerobic conditions and optimal illumination in the media with 4.2 mM $NaNO_2$ or $Na_2S \times 9H_2O$ and chlorine compounds at concentrations that are equal to the maximum permissible concentration (MPC) of chloride ions – 350 mg/L or 9.859 mM (Ministry of Health of Ukraine, 2010: Hygienic requirements for drinking water intended for human consumption. Order of the Ministry of Health of Ukraine on approval of State sanitary norms and rules No. 400 dated 12.05.2010), and 0.5, 2.0, 3.0, 4.0 (in NaCl composition), 0.03, 0.06, 0.11, 0.17, 0.22, 0.5, 2.0, 3.0, 4.0 or 0.05, 0.1, 0.2, 0.5, 2.0, 3.0, 4.0 (in $C_6H_4ClNO_3$ composition) times differed from the MPC. Since van Niel medium (excluding microelements) contains chloride ions which are necessary for optimal growth of bacteria at concentration of 5.402 mM, the test compounds (NaCl and $C_6H_4ClNO_3$) were added to the medium additionally. The control was a medium without pollutants. After 10 days biomass, NO_2^-, NO_3^-, HS^-, SO_4^{2-} content in the cultural liquid, glucose and glycogen content in cell-free extracts were determined. The concentration of sulfate ions: 0.524 mM, available in van Niel medium (excluding microelements), was subtracted from that measured in the cultural liquid after bacteria growth in the medium with $Na_2S \times 9H_2O$.

Biomass was measured turbidimetrically using the photoelectrocolorimeter KFK-3 by detecting the optical density of the cell suspension (optical way $l = 3$ mm) at wavelengths $\lambda = 450$ nm for green and 660 nm for purple sulfur bacteria and calculated using the formula:

C, g/L = (E×n)/K, where E − extinction; n − dilution factor, times; K – coefficient of recalculation, obtained from the calibration curve of the dependence of extinction from the mass of dry cells, determined by the weight method, and equal 0.131 for green and 0.17 for purple sulfur bacteria (Gudz et al., 2014). The cells were separated from the cultural liquid by centrifugation (4000 g, 15 min). The concentrations of nitrate ions after their reduction to nitrites in the presence of Zn:$MnSO_4$ (1:100) powder as a reducing agent and nitrite ions by spectrophotometric method which relies on a diazotization reaction with a Griess reagent (n-(1-naphthyl)ethylenediamine dihydrochloride, sulfanil and acetic acid) were determined in the cultural liquid (Granger, 1996; Gudz et al., 2014). The concentrations of hydrogen sulfide by spectrophotometric method (based on the formation of methylene blue as a result of the interaction of n-amino-dimethylaniline (N, N-dimethyl-n-phenylenediamine dihydrochloride) and hydrogen sulfide) and sulfate ions by turbidimetric method (after precipitation of sulfates with barium chloride in the form of $BaSO_4$) also were determined in the cultural liquid (Gudz et al., 2014). The intracellular glucose and glycogen contents were determined enzymatically in cell-free extracts of *C. limicola* IMV K-8, using the analytical kit "Diagluc-2" (Gonchar, 1998). *C. limicola* IMV K-8 cells were precipitated by centrifugation (4000 g, 30 min) and resuspended in 3 mL of extraction buffer (50 mM potassium phosphate buffer, pH 7.5; 10^{-5} M EDTA (ethylenediamine tetraacetate); 10^{-5} M PMSF (phenylmethylsulfonyl fluoride)). Tubes with cells were frozen in a freezer camera at –10 °C and used to preparation of cell-free extracts. Cells were disrupted with an ultrasound disintegrator UZDN-2T (22 kHz, 5 min) in tubes immersed in ice. Cell fragments were separated by centrifugation (9000 g, 45 min) at 4 °C. The obtained cell-free extracts were immediately used to determine the content of glucose and glycogen. Glycogen concentration was calculated from the difference in glucose levels before and after acid hydrolysis. Glycogen hydrolysis was performed by boiling cell-free extracts in the presence of 1 N H_2SO_4 for 3 h with subsequent neutralization of $Ba(OH)_2$ (Gorishniy et al., 2008).

The experiments were repeated three times with three parallel formulations for each variant of experimental and control conditions. Data obtained were expressed as mean (x) and standard deviation (±SD) of three measurements. The obtained data were processed by generally accepted methods of variation statistics using the Statistica 8.0 software package. The reliability of the difference between experimental and control variants was evaluated using ANOVA. Differences between the samples were considered statistically significant at $P < 0.05$, $P < 0.01$, $P < 0.001$ (Petrovska et al., 2022).

Results

The investigation of the properties of phototrophic microorganisms under the influence of stress factors and the evaluation of the expediency of their use for detoxification of aquatic environments from toxicants is a highly relevant issue in protecting anthropogenically altered ecosystems. The impact of halides, particularly chlorides, on the ability of purple and green sulfur bacteria to carry out the oxidative transformation of nitrites or sulfides in the process of anoxygenic photosynthesis remains insufficiently studied. Therefore, we studied the ability of *Thiocapsa* sp. Ya-2003, *Lamprocystis* sp. Ya-2003 and *Chlorobium limicola* IMV K-8 to oxidize nitrite and hydrogen sulfide ions with the simultaneous presence in the medium of chlorine compounds at different concentrations.

To examine the influence of sodium chloride on biomass accumulation, nitrites oxidation, nitrates formation, and intracellular carbohydrate synthesis, phototrophic bacteria were cultivated for 10 days in the medium containing $NaNO_2$ (4.2 mM) and NaCl at concentrations that are equal or 0.5–4.0 times differed from the MPC (Fig. 1–3). Medium without NaCl was used as the control. Sodium chloride at all tested concentrations inhibited the growth, nitrites' oxidation, and nitrates' accumulation by phototrophic sulfur bacteria (Fig. 1). In the medium with NaCl at concentration 4.0 times higher than the MPC the biomass accumulated by bacteria was 2.22–2.81 times lower compared to the control (Fig. 1a). Nitrites' oxidation by all strains in the medium with $NaNO_2$ and the investigated contaminant slowed down, therefore, the residual content of nitrite ions increased with increasing NaCl concentrations in the cultivation medium, while the content of nitrate ions accumulated by bacteria decreased. The residual content of nitrite ions in the medium with chloride ions at concentration 4.0 times higher than the MPC exceeded the NO_2^- content in the control variants 2.12–2.66 times after 10 days of bacteria cultivation (Fig. 1b). While in the medium without pollutant bacteria oxidized 75.7–78.5% of nitrite ions present in the medium, then in the medium with chloride ions at concentration 4.0 times higher than the MPC bacteria oxidized nitrite ions 26.3–35.7% less than in the control (Fig. 2). The oxidation of a lower quantity of nitrites by cells of all strains of bacteria in the medium with NaCl resulted in the formation of a lower quantity of nitrates compared to the control. In the medium with chloride ions at a concentration 4.0 times higher than the MPC, the nitrate ions' content was 1.60–1.87 times lower than in the control (Fig. 1c).

Sodium chloride stimulated the synthesis of intracellular carbohydrates by bacteria *C. limicola* IMV K-8 during growth in the medium containing $NaNO_2$ (4.2 mM, Fig. 3). In phototrophic green bacteria cells grown in the medium with contaminant at all tested concentrations, an increase in intracellular glucose content was observed. While the glucose content in cells grown in the control medium was 8.03 ± 0.23 mg/g dry cell weight, then in cells grown in the medium with NaCl at concentration 4.0 times higher than the MPC, its content increased by 98.1%. The glycogen content in cells grown in the contaminant-free medium was 39.02 ± 0.61 mg/g dry cell weight. Sodium chloride at concentration 4.0 times higher than the MPC stimulated glycogen synthesis in the cells by 109.3%. The glycogen content in cells grown in the medium with $NaNO_2$ and NaCl at concentration 4.0 times exceeding the MPC was 81.68 ± 0.92 mg/g dry cell weight.

Thus, it was determined that NaCl at concentrations 3.0–4.0 times higher than the MPC slowed the growth, nitrites' oxidation, and inhibited nitrates' formation by phototrophic purple and green sulfur bacteria in the medium containing $NaNO_2$. Chloride ions stimulated glucose and glycogen synthesis by phototrophic green sulfur bacteria cells while they used nitrite ions as electron donors of anoxygenic photosynthesis. Under the influence of sodium chloride at concentration 4.0 times exceeding the MPC, the glycogen content in *C. limicola* IMV K-8 cells grown in the medium with $NaNO_2$ increased 2.09 times compared to the control. Stimulation of intracellular carbohydrate synthesis in green sulfur bacteria by this inorganic toxicant indicates a slowdown of processes of constructive and energy metabolism, since glycogen, being a secondary metabolite, serves as a reserve compound.

To study the influence of sodium chloride on biomass accumulation, hydrogen sulfide ions' oxidation, sulfates' formation, and intracellular carbohydrate synthesis, the strains of phototrophic bacteria were cultivated for 10 days in the medium containing Na2S×9H2O (4.2 mM) and NaCl at concentrations that are equal or differed 0.5–4.0 times from the MPC (Fig. 4–6). The control medium did not contain NaCl. Sodium chloride at all tested concentrations had a negative influence on biomass accumulation, hydrogen sulfide ions' oxidation, and sulfates' formation by phototrophic sulfur bacteria (Fig. 4). The lowest biomass values, 2.37–2.63 times lower than in the control, were observed in the medium with NaCl at concentration 4.0 times higher than the MPC (Fig. 4a). With increasing contaminant concentrations in the medium of bacteria growth, the residual hydrogen sulfide ions content increased, while the content of sulfate ions accumulated as a result of HS^- oxidation by bacteria decreased.

The residual hydrogen sulfide ions content in the medium with chloride ions at concentration 4.0 times higher than the MPC on the 10-th day 2.19–2.60 times exceeded the HS^- content in the control variants (Fig. 4b). While in the medium without pollutant bacteria oxidized 63.8–70.2% of HS^-, present in the medium, then in the medium with chloride ions at concentration 4.0 times higher than the MPC, bacteria oxidized hydrogen sulfide by 42.9–47.5% less than in the control (Fig. 5). The oxidation of fewer hydrogen sulfide ions by cells of all strains of phototrophic bacteria in the medium with inorganic pollutant resulted in the formation of a lower quantity of sul-

fates detected in the cultural liquid. In the medium with chloride ions at concentration 4.0 times higher than the MPC, the sulfate ions' content was significantly lower than in the control variant, 2.85–3.08 times (Fig. 4c).

The inorganic contaminant stimulated intracellular carbohydrate synthesis by *C. limicola* IMV K-8 during growth in the medium containing $Na_2S{\times}9H_2O$ (4.2 mM, Fig. 6). In cells of phototrophic green bacteria grown in the medium with the tested toxicant at all concentrations, an increase in intracellular glucose content was observed.

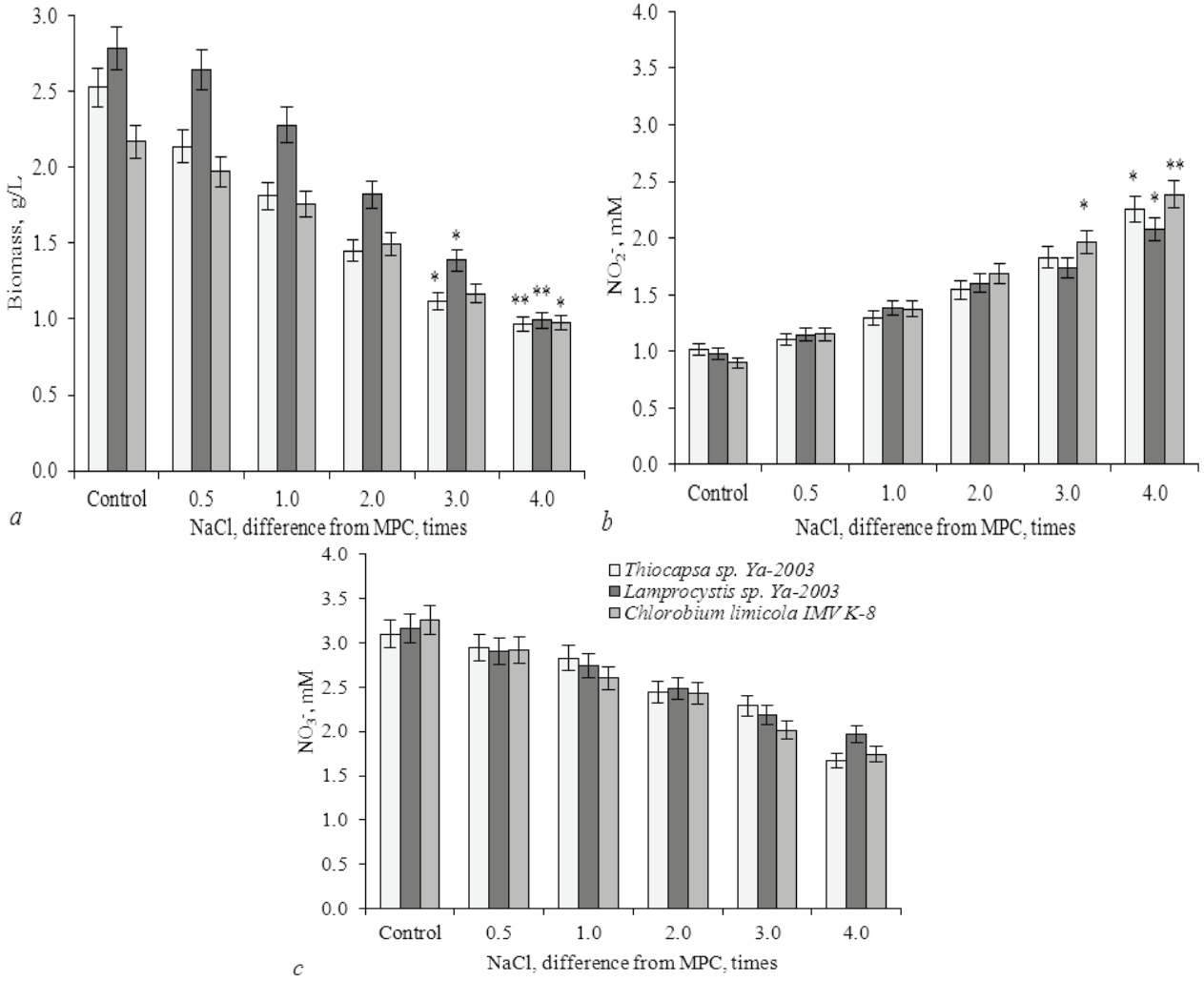

Fig. 1. Biomass (*a*), the content of NO_2^- (*b*) and NO_3^- (*c*) in the cultural liquid after 10 days of *Thiocapsa* sp. Ya-2003, *Lamprocystis* sp. Ya-2003 and *Chlorobium limicola* IMV K-8 cultivation in the medium with 4.2 mM $NaNO_2$ and NaCl at different concentrations (x ± SD, n = 3); Control – the medium without NaCl; *, ** − the data were statistically significant compared to the control (P < 0.05; P < 0.01)

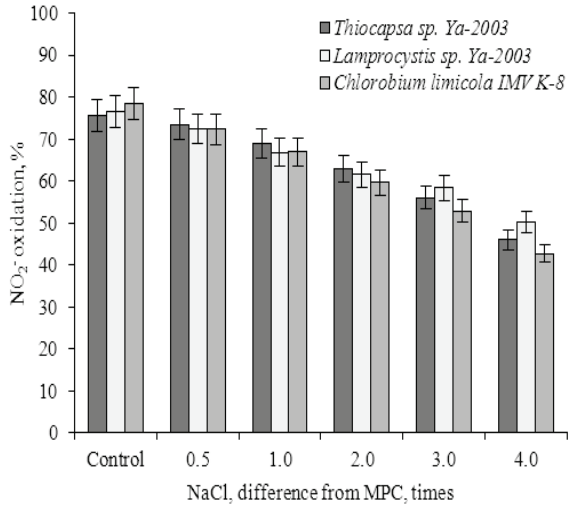

Fig. 2. Efficiency of NO_2^- oxidation by bacteria *Thiocapsa* sp. Ya-2003, *Lamprocystis* sp. Ya-2003 and *Chlorobium limicola* IMV K-8 after 10 days of cultivation in the medium with 4.2 mM $NaNO_2$ and NaCl at different concentrations (x ± SD, n = 3); Control – the medium without NaCl

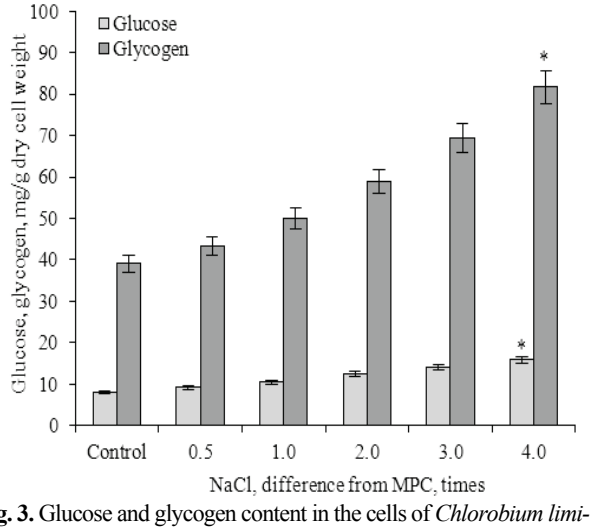

Fig. 3. Glucose and glycogen content in the cells of *Chlorobium limicola* IMV K-8 on the 10-th day of cultivation in the medium with 4.2 mM $NaNO_2$ and NaCl at different concentrations (x ± SD, n = 3); Control – the medium without NaCl; * − the data were statistically significant compared to the control (P < 0.05)

While the glucose content in cells grown in the medium without contaminant was 7.76 ± 0.77 mg/g dry cell weight, then its content increased by 126.0% in cells grown in the medium with NaCl at concentration 4.0 times higher than the MPC. The glycogen content in cells grown in the contaminant-free medium was 36.66 ± 0.12 mg/g dry cell weight. Chloride ions at concentration 4.0 times higher than the MPC introduced into the medium of bacteria cultivation stimulated glycogen synthesis in cells by 120.8%. The glycogen content in cells grown in the medium with $Na_2S \times 9H_2O$ and NaCl at concentration 4.0 times exceeding the MPC was 80.93 ± 0.95 mg/g dry cell weight.

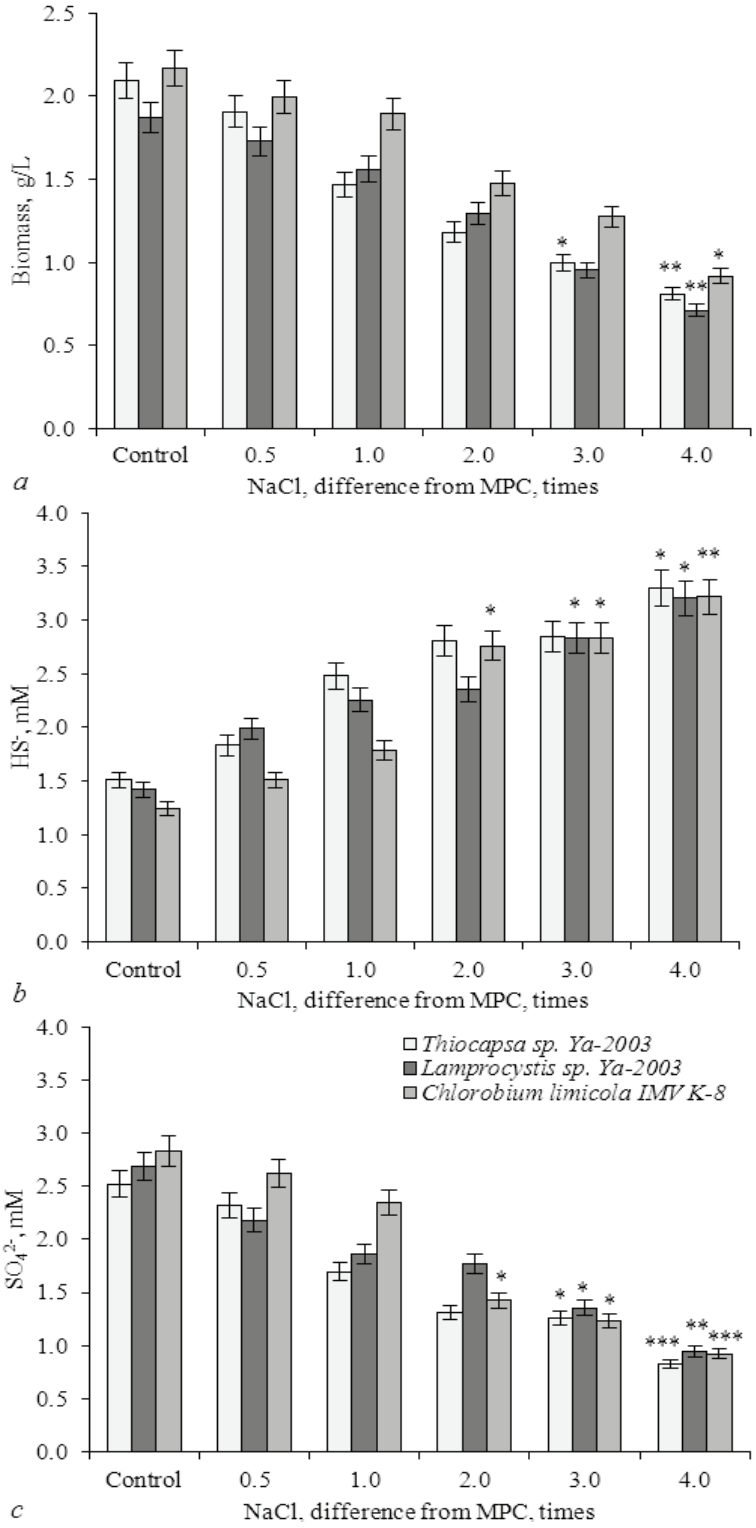

Fig. 4. Biomass (*a*), the content of HS^- (*b*) and SO_4^{2-} (*c*) in the cultural liquid after 10 days of *Thiocapsa* sp. Ya-2003, *Lamprocystis* sp. Ya-2003 and *Chlorobium limicola* IMV K-8 cultivation in the medium with 4.2 mM $Na_2S \times 9H_2O$ and NaCl at different concentrations (x ± SD, n = 3); Control – the medium without NaCl; *, **, *** – the data were statistically significant compared to the control ($P < 0.05$; $P < 0.01$; $P < 0.001$)

Thus, it was shown that NaCl at concentrations 2.0–4.0 times higher than the MPC inhibited growth, HS^- oxidation, and SO_4^{2-} formation by phototrophic purple and green sulfur bacteria in the medium containing $Na_2S \times 9H_2O$. Chloride ions stimulated glucose and glycogen synthesis by cells of phototrophic green bacteria while they used hydrogen sulfide ions as an electron donor of anoxygenic photosynthesis. Under the influence of sodium chloride at concentration 4.0 times higher than the MPC the glycogen content in *C. limicola* IMV K-8 cells grown in the medium with $Na_2S \times 9H_2O$ increased 2.21 times compared to the control.

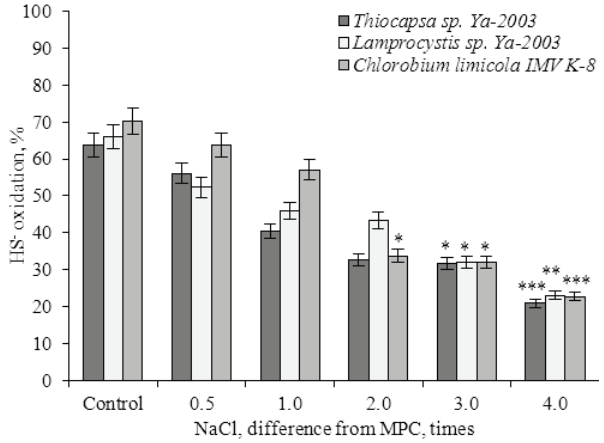

Fig. 5. Efficiency of HS^- oxidation by bacteria *Thiocapsa* sp. Ya-2003, *Lamprocystis* sp. Ya-2003 and *Chlorobium limicola* IMV K-8 after 10 days of cultivation in the medium with 4.2 mM $Na_2S{\times}9H_2O$ and NaCl at different concentrations (x ± SD, n = 3); Control – the medium without NaCl; *, **, *** – the data were statistically significant compared to the control ($P < 0.05$; $P < 0.01$; $P < 0.001$)

Despite the observed toxicity of chloride ions, the ability of all tested strains of phototrophic bacteria to oxidize nitrites or hydrogen sulfide remained sufficiently high even in the presence in the medium of NaCl at concentrations 2.0–4.0 times exceeding the MPC.

To test the influence of chloronitrophenol on biomass accumulation, hydrogen sulfide ions' oxidation, sulfate ions' formation, and intracellular carbohydrate synthesis, phototrophic bacteria were cultivated for 10 days in the medium containing $Na_2S{\times}9H_2O$ (4.2 mM) and $C_6H_4ClNO_3$ at concentrations that are equal or 0.03–4.00 times differed from the MPC (Fig. 7–9). The control medium did not contain $C_6H_4ClNO_3$. Chloronitrophenol at all tested concentrations had little inhibitory effect on biomass accumulation, hydrogen sulfide ions' oxidation, and sulfates' formation by phototrophic sulfur bacteria (Fig. 7). The lowest biomass values, 1.29–1.45 times lower than in the control, were observed in the medium containing $C_6H_4ClNO_3$ at concentration 4.0 times exceeding the MPC of chloride ions (Fig. 7a). With increasing concentrations of chloronitrophenol in the medium, a slight increase in the residual content of HS^- was observed, indicating that the contaminant inhibits the ability of bacteria to oxidize hydrogen sulfide ions. Therefore, the content in the cultural liquid of sulfate ions, formed as a result of HS^- oxidation by bacteria, decreases. The residual hydrogen sulfide ions' content in the medium with $C_6H_4ClNO_3$ at concentration 4.0 times higher than the MPC on the 10-th day 1.44–1.78 times exceeded the HS^- content in the control variants (Fig. 7b). While in the medium without pollutant bacteria oxidized 65.8–71.7% of the available in the medium HS^-, then in the medium with chloronitrophenol at concentration 4.0 times higher than the MPC bacteria oxidized hydrogen sulfide ions by 15.1–22.2% less than in the control (Fig. 8). Oxidation of a smaller quantity of hydrogen sulfide ions by cells of all strains of bacteria in the medium with this organic contaminant resulted in the formation of a smaller quantity of sulfate ions detected in the cultural liquid. In the medium with chloronitrophenol at concentration 4.0 times higher than the MPC the sulfate ions' content was 1.51–1.60 times lower than in the control variants (Fig. 7c).

Chloronitrophenol stimulated intracellular carbohydrate synthesis by *C. limicola* IMV K-8 during growth in the medium containing $Na_2S{\times}9H_2O$ (4.2 mM, Fig. 9). In phototrophic green bacteria cells grown in the medium with the tested organochlorine compound at all concentrations, an increase in intracellular glucose content was observed. While the glucose content in cells grown in the medium without contaminant was 7.95 ± 0.43 mg/g dry cell weight, then in cells grown in the medium with $C_6H_4ClNO_3$ at concentration 4.0 times higher than the MPC its content increased by 58.8%. The glycogen content in cells grown in the medium without contaminant was 37.46 ± 0.33 mg/g dry cell weight. Chloronitrophenol, added into the medium of bacteria cultivation at concentration 4.0 times higher than the MPC of chloride ions, stimulated glycogen synthesis in cells by 99.2%. The glycogen content in cells grown in the medium containing $Na_2S{\times}9H_2O$ and $C_6H_4ClNO_3$ at concentration 4.0 times exceeding the MPC was 74.65 ± 0.52 mg/g dry cell weight.

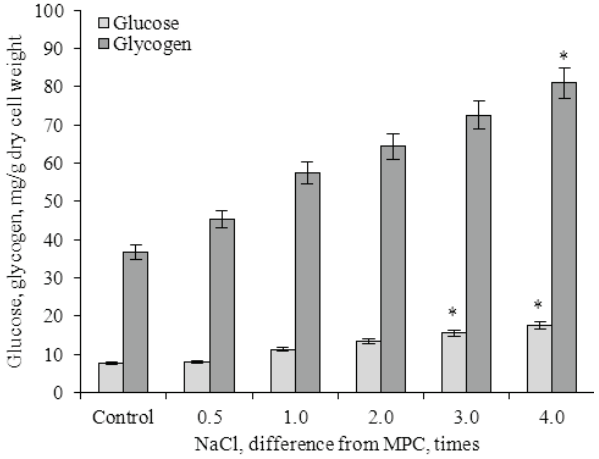

Fig. 6. Glucose and glycogen content in the cells of *Chlorobium limicola* IMV K-8 on the 10-th day of cultivation in the medium with 4.2 mM $Na_2S{\times}9H_2O$ and NaCl at different concentrations (x ± SD, n = 3); Control – the medium without NaCl; * – the data were statistically significant compared to the control ($P < 0.05$)

Thus, it was shown that $C_6H_4ClNO_3$ at concentration 4.0 times higher than the MPC of chloride ions slightly inhibited growth, HS^- oxidation, and SO_4^{2-} formation by phototrophic purple and green sulfur bacteria in the medium containing $Na_2S{\times}9H_2O$. Chloronitrophenol stimulated glucose and glycogen synthesis by cells of phototrophic green bacteria, which utilized hydrogen sulfide ions as electron donor of anoxygenic photosynthesis. Under the influence of chloronitrophenol at concentration 4.0 times higher than the MPC the glycogen content in *C. limicola* IMV K-8 cells grown in the medium containing $Na_2S{\times}9H_2O$ increased 1.99 times compared to the control.

Discussion

Phototrophic sulfur bacteria perform anoxygenic photosynthesis, using molecular hydrogen, Fe^{2+}, reduced sulfur or nitrogen compounds as electron donors (Hemp et al., 2016; George et al., 2020; Adessi et al., 2021; Imhoff, 2021; Moroz et al., 2023). The products of nitrite and hydrogen sulfide ions' oxidation by these bacteria are nitrates and sulfates, respectively, which accumulate in the medium (Dahl, 2017; Hallenbeck, 2017; Moroz et al., 2021). Phototrophic bacteria grow photoautotrophically or photoheterotrophically, utilizing organic acids, alcohols, carbohydrates, and polysaccharides (Dahl, 2017; Adessi et al., 2021). It has been established that phototrophic nonsulfur bacteria *Rhodopseudomonas yavorovii* IMV B-7620, isolated from Yavorivske Lake, purifies water of the settler No 3 filtrate of the Lviv Solid Household Waste Landfill, enriched with organic compounds, from Cl^- by 99.8% in the process of their cultivation (Tarabas et al., 2019b). Phototrophic bacteria *Rhodopseudomonas palustris* are effective in the bioremediation of wastewater contaminated with Cr(VI) and in the degradation of aromatic compounds, in particular 2,4,6-trichlorophenol (McGrath & Harfoot, 1997). *Rh. palustris*, together with *Rhodospirillum rubrum* and *Rhodospirillum photometricum*, grow phototrophically in the presence of CO_2 due to reductive dehalogenation of halogen-containing carboxylic acids. These bacteria also produce H_2, which can be used as biofuel, and generate an electric current (McGrath & Harfoot, 1997; Sepúlveda-Muñoz et al., 2023; Kamaraj & Rusyn, 2024; Morrison & Bose, 2024; Teke et al., 2024). Chlorinated nitrobenzene (or nitrophenol) products, in particular, such as the organochlorine nitro compound 2-chloro-4-nitrophenol with antibacterial properties, are widely used in the pharmaceutical and chemical industries as precursors for organic compound synthesis. In general, organochlorine compounds are used for the synthesis of plastics and synthetic resins, for the production of household

chemicals, in the electronics and metalworking industries, agriculture, the pharmaceutical industry, as well as a component of solvents for cleaning internal combustion engines from deposits, for lubricating the upper part of cylinders, engines and valves, as a component of flushing oils (Biletsky, 2004; Kurta, 2009; Palencia et al., 2021; Yang & Zhu, 2024). The influence of chloride ions on the physiological properties of phototrophic sulfur bacteria, in particular their ability to adapt to high concentrations of chlorine compounds, remain insufficiently studied.

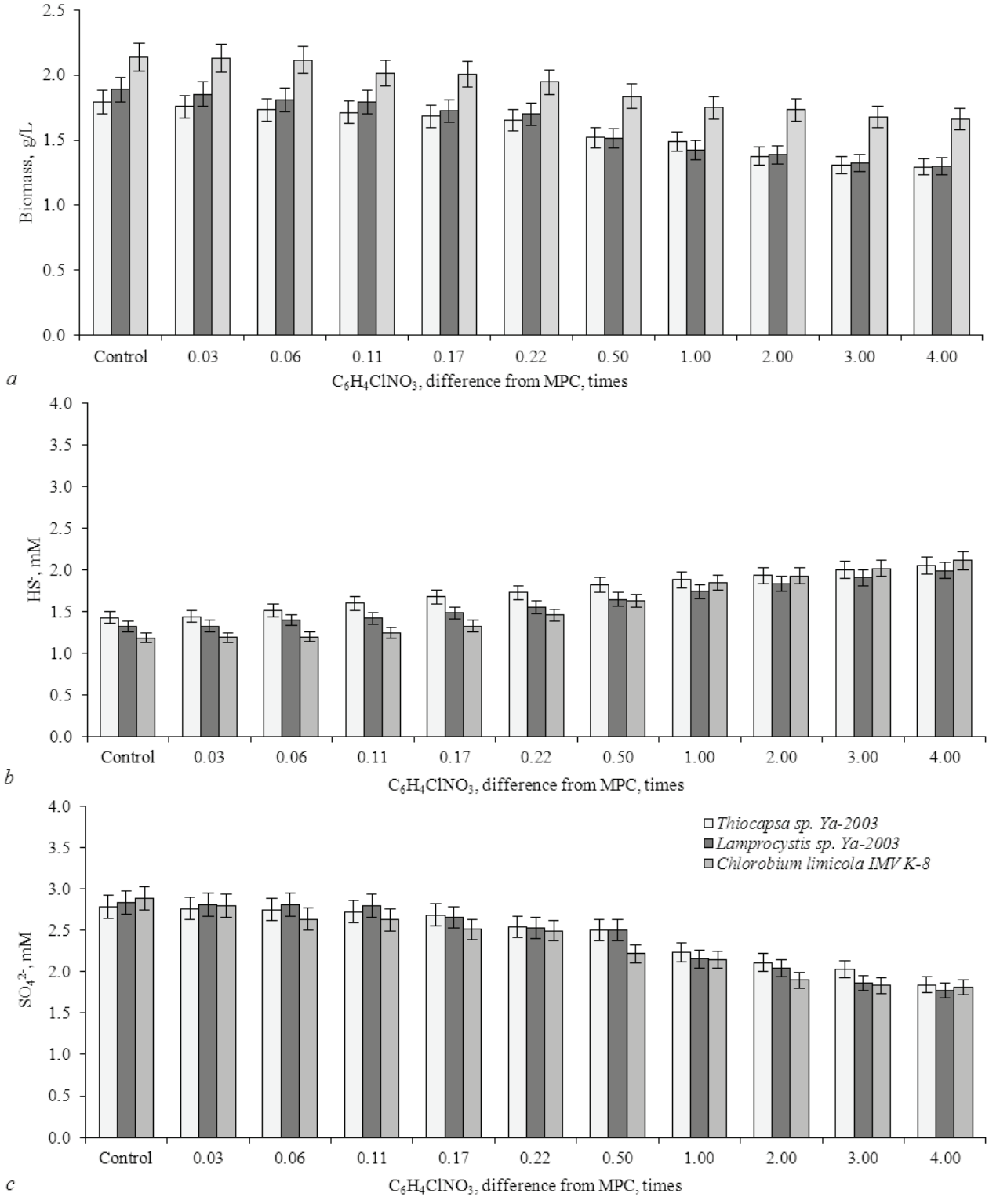

Fig. 7. Biomass (*a*), the content of HS^- (*b*) and SO_4^{2-} (*c*) in the cultural liquid after 10 days of *Thiocapsa* sp. Ya-2003, *Lamprocystis* sp. Ya-2003 and *Chlorobium limicola* IMV K-8 cultivation in the medium with 4.2 mM $Na_2S\times9H_2O$ and $C_6H_4ClNO_3$ at different concentrations (x ± SD, n = 3); Control – the medium without $C_6H_4ClNO_3$

Water in living organisms serves not only as a solvent but also as a substrate for enzymatic reactions. It influences molecular conformation and maintains cellular turgor. Chloride and sodium ions, sugars and other compounds dissolved in water compete for water molecules and bind them, i.e. convert them into a form inaccessible to microorganisms (Lengeler et al., 1999). Conditions of high salt or sugar content in the liquid medium are similar to conditions of water shortage, although the mechanisms of adaptation of microorganisms to them are different. Most marine microorganisms (seawater contains about 3% NaCl) require elevated concentrations of Na^+, so they were termed halophiles (Lengeler et al., 1999). Halophiles are categorized into three types based on the optimal salt concentration for growth:

slight (1–3%), moderate (3–15%), and extreme (15–30%) (Kumar et al., 2021). Typical marine bacteria grow poorly or not at all in media without NaCl, while most freshwater species are inhibited by seawater NaCl concentrations. Thus, the addition of 3% NaCl acts as a selective factor for marine purple nonsulfur bacteria. Salt concentrations above 10% are highly selective for moderately halophilic species, such as *Rhodothalassium salexigens*, *Rhodovibrio salinarum*, and *Rhodovibrio sodomensis*, which do not grow in media designed for freshwater or marine phototrophic bacteria (Imhoff, 2006; George et al., 2020).

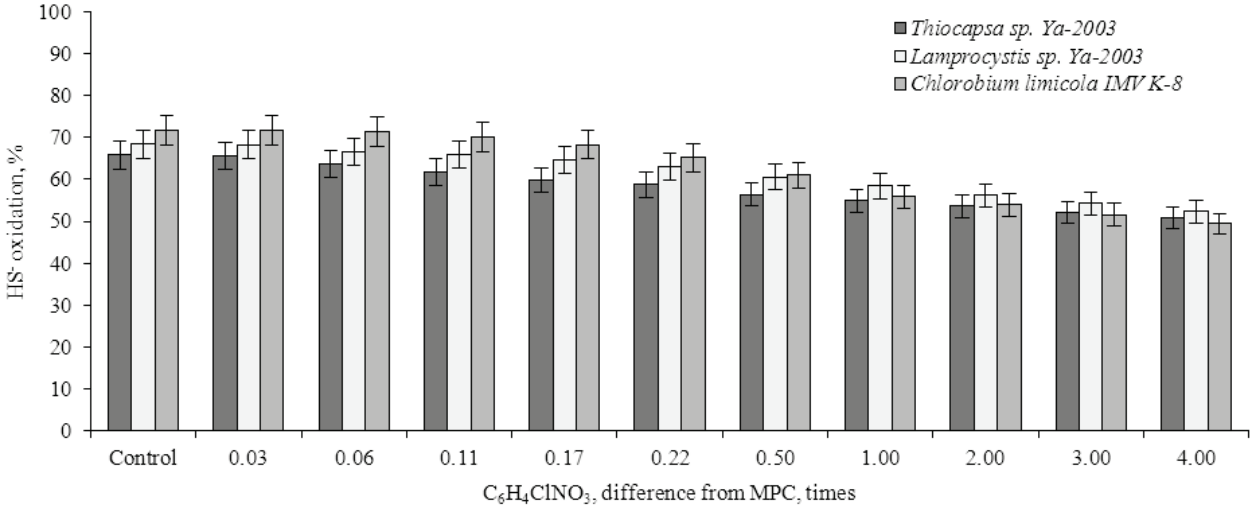

Fig. 8. Efficiency of HS^- oxidation by bacteria *Thiocapsa* sp. Ya-2003, *Lamprocystis* sp. Ya-2003 and *Chlorobium limicola* IMV K-8 after 10 days of cultivation in the medium with 4.2 mM $Na_2S{\times}9H_2O$ and $C_6H_4ClNO_3$ at different concentrations (x ± SD, n = 3); Control – the medium without $C_6H_4ClNO_3$

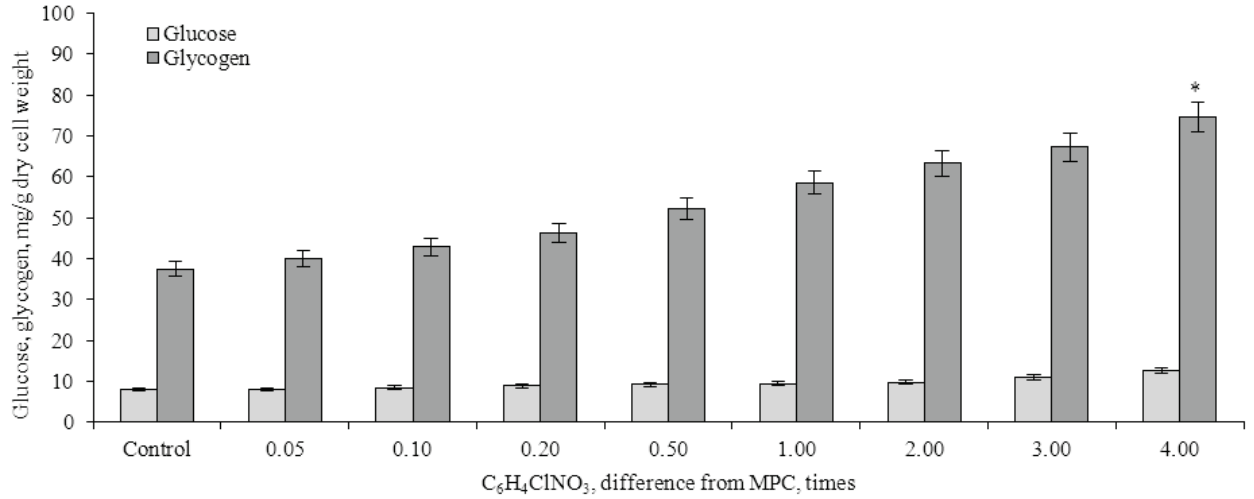

Fig. 9. Glucose and glycogen content in the cells of *Chlorobium limicola* IMV K-8 on the 10-th day of cultivation in the medium with 4.2 mM $Na_2S{\times}9H_2O$ and $C_6H_4ClNO_3$ at different concentrations (x ± SD, n = 3); Control – the medium without $C_6H_4ClNO_3$; * – the data were statistically significant compared to the control ($P < 0.05$)

Halophilic microorganisms adapt to high salt concentrations by osmoregulation with the participation of physiologically compatible organic osmolytes such as glycerol, ectoine, sugars (trehalose, arabitol) or amino acids and their derivatives (proline, betaine), which accumulate in cells as a result of biosynthesis and absorption from the environment. The structure and activity of enzymes of bacteria with this type of osmoregulation do not depend on the concentration of osmolytes in the cytoplasm. Halotolerance is also ensured by creating a high concentration of salts in the cell, i.e., by forming a gradient of Na^+ and K^+ concentrations due to the active removal of NaCl from the cell and the accumulation of KCl. The concentration of KCl inside the cell is maintained at the level of NaCl in the environment (~4 M) (Roessler et al., 2003; Kozlova et al., 2008; Bremer & Krämer, 2019). The conformation and activity of the enzymes of these microorganisms are supported by high concentration of salts, and a large number of acidic amino acids have been found in their structure. For example, the phototrophic purple halophilic bacteria *Halobacterium salinarium* use the gradient of electrochemical H^+ potential ($\Delta\mu_{H+}$) to synthesize ATP on ATPase and to remove Na^+ from the cell, which is essential for osmoregulation. The high concentration of K^+ in their cell is provided by uniport. Cl^- enters the cell due to symport with Na^+ and photochemical processes on halorhodopsin (Kozlova et al., 2008). In prokaryotes the *clcA* genes encode ClC transporters that ensure survival under acidic conditions. ClC chloride antiporter eliminates negatively charged intracellular chloride ions to prevent cytoplasmic membrane hyperpolarization (Kim et al., 2023).

Halophiles occur in all three domains of life: Bacteria, Archaea and Eukarya, they can be aerobic heterotrophs, aerobic or anaerobic phototrophs, reduce nitrates or sulfates, produce methane as a metabolic byproduct (Kumar et al., 2021; Saini et al., 2023). Due to the ability to grow in extreme ecotopes, they can be a source of various novel biomolecules with unique properties. For example, haloarchaea are adapted to survive under extreme saline conditions, accumulating osmolytes and salts to counteract the high osmotic pressure in their habitats. Haloarchaea synthesize halocins, proteinaceous antimicrobial substances that are stable at high temperature, elevated salt concentration, and alkaline pH. Halocins can be used in food preservation, medicine, and industries (Kumar et al., 2021; Martínez-Espinosa, 2024).

The concentrations of chlorine compounds, the influence of which on the properties of phototrophic microorganisms was studied, did not exceed the MPC of chloride ions by more than 4.0 times or 0.16%. Nevertheless, NaCl at the highest tested concentration, 4.0 times higher than the MPC, in the media with $NaNO_2$ or $Na_2S{\times}9H_2O$ inhibited the biomass accumulation by phototrophic sulfur bacteria 2.81 and 2.63 times and NO_2^- and HS^- oxidation by 35.7% and 47.5%, respectively. Furthermore, $C_6H_4ClNO_3$ at concentration 4.0 times higher than the MPC of chloride ions also inhibited the biomass accumulation by bacteria 1.45 times and HS^- oxidation by 22.2% in the medium with $Na_2S{\times}9H_2O$. Therefore, the strains *Thiocapsa* sp. Ya-2003, *Lamprocystis* sp. Ya-2003, and *Chlorobium limicola* IMV K-8, isolated from the fresh water of Yavorivske Lake, cannot be considered as halophiles. The slowing down of constructive and energy metabolism processes in the cells of green sulfur bacteria under the influence of both NaCl and $C_6H_4ClNO_3$ at concentrations 4.0 times higher than the MPC is evidenced by the stimulation by chlorine compounds of intracellular carbohydrates synthesis, in particular, glycogen, 2.09 times in the medium with $NaNO_2$, and 1.99–2.21 times in the medium with $Na_2S{\times}9H_2O$ compared to the control.

The results of our research indicate that chloride ions to varying extents change the physiological properties of phototrophic bacteria of the *Thiocapsa*, *Lamprocystis*, and *Chlorobium* genera, isolated from Yavorivske Lake, in particular, reduce the biomass accumulation, influence the nitrite and hydrogen sulfide ions oxidation. Chloronitrophenol in the cultivation medium of bacteria had a less toxic effect on changing their physiological properties than sodium chloride at the same concentrations, possibly due to the easier dissociation in solutions of the weaker ionic bond between the atoms of nonmetal chlorine and metal sodium in the NaCl molecule than of the stronger covalent polar bond between the atoms of chlorine and carbon in the $C_6H_4ClNO_3$ molecule (all bonds between the atoms of which are covalent). At concentrations exceeding the MPC by up to 4.0 times, chloride ions, which easily penetrate cells of bacteria, may cause oxidative stress, induce depolarization of the cytoplasmic membrane, and inactivate enzyme complexes involved in the process of anoxygenic photosynthesis by interacting, for example, with SH-groups of proteins or other cellular metabolites (Fang et al., 2018).

Despite the revealed toxicity of chlorine compounds, the ability of all tested strains of phototrophic bacteria to oxidize nitrites or hydrogen sulfide remained sufficiently high even after the introduction of chlorine compounds into the medium at concentrations that 2.0–4.0 times exceeded the MPC of chloride ions. Therefore, these strains can be applied in technologies for remediating environments with complex pollution by chlorine, sulfur and nitrogen compounds.

Conclusion

Thus, it has been established that NaCl at concentrations 2.0–4.0 times exceeding the MPC of chloride ions significantly inhibits biomass accumulation, NO_2^- and HS^- oxidation, and NO_3^- or SO_4^{2-} formation by phototrophic sulfur bacteria *Thiocapsa* sp. Ya-2003, *Lamprocystis* sp. Ya-2003, and *Chlorobium limicola* IMV K-8, isolated from Yavorivske Lake, during growth in van Niel medium containing $NaNO_2$ or $Na_2S{\times}9H_2O$. Under the influence of NaCl at concentration 4.0 times exceeding the MPC the glycogen content in the *C. limicola* IMV K-8 cells, grown in the media with $NaNO_2$ or $Na_2S{\times}9H_2O$, increased 2.09–2.21 times compared to the control. $C_6H_4ClNO_3$ at concentration 4.0 times higher than the MPC of chloride ions inhibited biomass accumulation, HS^- oxidation, and SO_4^{2-} formation by bacteria in the medium with $Na_2S{\times}9H_2O$. Under the influence of $C_6H_4ClNO_3$ at concentration 4.0 times exceeding the MPC, the glycogen content in the cells of *C. limicola* IMV K-8, grown in the medium with $Na_2S{\times}9H_2O$, increased practically twice compared to the control. Sodium chloride and chloronitrophenol at the same concentrations almost equally stimulate the synthesis of intracellular carbohydrates by green phototrophic sulfur bacteria during their use of both nitrite and hydrogen sulfide ions as electron donors of anoxygenic photosynthesis.

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