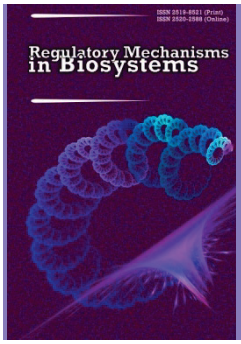

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Dissimilatory reduction of sulfate, nitrate and nitrite ions by bacteria *Desulfovibrio* sp. under the influence of potassium dichromate

O. M. Moroz, S. O. Hnatush, G. V. Yavorska, G. I. Zvir

Ivan Franko National University of Lviv, Lviv, Ukraine

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Ivan Franko National University of Lviv, Hrushevsky st., 4, Lviv, 79005, Ukraine.
Tel.: +38-067-811-86-44.
E-mail: oksana.moroz@lnu.edu.ua

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In the process of anaerobic respiration, sulfate reducing bacteria, besides sulfates, can use other electron acceptors: nitrates, nitrites, oxidized forms of heavy metals, in particular, hexavalent chromium, which are harmful for organisms. Selection of pollutant-resistant stains of this kind of bacteria isolated from technogenically altered ecotopes, capable of reductive transformation of various nature pollutants, is an especially relevant task for the creation of new effective remediation biotechnologies. The purpose of this work was to investigate the regularities of usage of sulfate, nitrate or nitrite ions by bacteria of the *Desulfovibrio* genus, isolated from Yavorivske Lake, at conditions of simultaneous presence in the medium of another electron acceptor – Cr(VI), to establish a succession of electron acceptors' reduction by investigated sulfidogenic bacteria and to evaluate the efficiency of their possible application in technologies of complex purification of the environment from metal, sulfur and nitrogen compounds. Bacteria were grown under anaerobic conditions for 10 days in Kravtsov-Sorokin medium without Mohr's salt. To study the efficiency of sulfate, nitrate, or nitrite ions' reduction at simultaneous presence in the medium of Cr(VI), bacteria were sown in media with $Na_2SO_4{\times}10H_2O$, $NaNO_3$, $NaNO_2$ or $K_2Cr_2O_7$ to final SO_4^{2-}, NO_3^-, NO_2^- or Cr(VI) concentration in the medium of 3.47 (concentration of SO_4^{2-} in medium of standard composition) or 1.74, 3.47, 5.21, 6.94, 10.41 mM. Biomass was determined turbidimetrically, and the concentrations of sulfate, nitrate, nitrite, ammonium ions, hydrogen sulfide, Cr(VI), Cr(III) in cultural liquid were determined by spectrophotometric method. It has been established that Cr(VI) inhibits the biomass accumulation, sulfate ions' reduction and hydrogen sulfide production by *Desulfovibrio* sp. after simultaneous introduction into the medium of 3.47 mM SO_4^{2-} and 1.74–10.41 mM Cr(VI). In the medium with the same initial content (3.47 mM) of SO_4^{2-} and Cr(VI), bacteria reduced 2.1–2.3 times more Cr(VI) than sulfate ions with Cr(III) production at concentrations up to 2.2 times higher than hydrogen sulfide. It has been shown that $K_2Cr_2O_7$ inhibits the biomass accumulation, the nitrate ions reduction and the ammonium ions production by bacteria after simultaneous addition into the medium of 3.47 mM NO_3^- and 1.74–10.41 mM Cr(VI) or 1.74–10.41 mM NO_3^- and 3.47 mM Cr(VI). In the medium with the same initial content (3.47 mM) of NO_3^- and Cr(VI) bacteria reduced 1.1–1.3 times more nitrate ions than Cr(VI) with the production of ammonium ions at concentrations up to 1.3 times higher than that of Cr(III). It has been established that $K_2Cr_2O_7$ inhibits the biomass accumulation, the nitrite ions' reduction and the ammonium ions' production by bacteria after simultaneous addition into the medium of 3.47 mM NO_2^- and 1.74–10.41 mM Cr(VI) or 1.74–10.41 mM NO_2^- and 3.47 mM Cr(VI). In the medium with the same initial content (3.47 mM) NO_2^- and Cr(VI) the reduction of Cr(VI) by bacteria practically did not differ from the reduction of nitrite ions (was only slightly lower – up to 1.1 times), almost the same concentrations of trivalent chromium and ammonium ions in the cultural liquid were detected. The processes of nitrate and nitride reduction, carried out by bacteria of *Desulfovibrio* genus, were revealed to be less sensitive to the negative influence of sodium dichromate, as compared with the process of sulfate ions' reduction, which in the medium with 3.47 mM SO_4^{2-} and 1.74–10.41 mM Cr(VI) decreased by 3.2–4.6 times as compared with this process in the medium with only $Na_2SO_4{\times}10H_2O$. The investigated strains of bacteria are adapted to high concentrations of toxic pollutants (up to 10.41 mM) and therefore are promising for application in technologies of complex environment purification from hexavalent chromium, sulfur and nitrogen compounds.

Keywords: anaerobic respiration; hexavalent chromium; sulfate; nitrate; nitrite ions.

Introduction

Facultative or obligate anaerobic bacteria of *Desulfovibrio*, *Desulfotomaculum*, *Desulfobacterium*, *Desulfobacter*, *Geobacter*, *Shewanella*, *Wolinella*, *Desulfurella*, *Desulfuromusa*, *Desulfuromonas*, *Pseudomonas* etc. genera in the process of anaerobic respiration oxidize a number of simple organic compounds or molecular hydrogen using electron acceptors with high (Cr(VI), Mn(IV), NO_3^-, Fe(III), NO_2^-, L-malate, fumarate, tri- or tetrachlorethylene) or low (SO_4^{2-}, elemental or polysulfide sulfur, HCO_3^-) oxidation-reduction potential (Richter et al., 2012; Abdulina et al., 2018; Teng et al., 2019). Dissimilatory reduction of sulfate ions to H_2S in sulfate reducing bacteria occurs in their cytoplasm with formation of adenosine-5'-phosphosulfate (APS) as an intermediate product. The stages of sulfate reduction are catalyzed by ATP sulfurylase, APS reductase, a number of sulfite reductases (Lengeler et al., 2005). Nitrates, nitrites and other oxidized nitrogen compounds can be reduced by microorganisms, which synthesised nitrate and nitrite reductases (Mitchell et al., 1986; Lengeler et al., 2005). Facultative anaerobic bacteria carry out dissimilatory reduction of nitrates with the formation of NO_2^-, NO, N_2O and N_2 or nitrite ions with the participation of NAD(P)H or reduced menaquinone can be directly reduced to NH_4^+ (Lengeler et al., 2005; Xia et al., 2018). Nitrate reductase NarGHI is an enzyme complex that includes multi-heme b-type cytochrome, proteins with Fe-S clusters and Mo-containing cofactor (Morozkina & Zvyagilskaya, 2007; Kozlova et al., 2008). Nitrate reduction takes place with the formation of nitrite and its further reduction by complex of periplasmic dissimilatory nitrite reductases to NH_4^+ in *Desulfovibrio desulfuricans*, *Desulfotomaculum* sp., *Desulfobacter* sp., *Desulfuromonas* sp. and *Wolinella succinogenes* is described (Bokranz et al., 1983; Keith & Herbert, 1983; Peretyatko & Gudz, 2011; Moroz, 2013; Xia et al., 2018; Chayka & Peretyatko, 2018).

Bacteria which reduce sulfates, nitrates, nitrites and oxidized forms of heavy metals occupy close ecological niches, providing various links in the cycle of chemical elements in nature (Barton et al., 2015; Yan et al., 2018). The structure and properties of the components of electron transport chain and enzymes, involved in the process of dissimilatory reduction of oxidized metal forms, have been intensively studied in recent years in connection with the ability of metal reducing bacteria in the process of anaerobic respiration to release electrons into the medium (Gescher & Kappler, 2012; Richter et al., 2012; Breuer et al., 2015), due to which exoelectrogenic anaerobic bacteria are considered as the high effective anode biocatalysts in microbial fuel cells (Fitzgerald et al., 2013; Prokhorova et al., 2017; Simonte et al., 2017; Hnatush & Maslovska, 2018). Soluble and insoluble metal compounds are reduced outside the cells of metal reducing bacteria by a system of membrane-bound metal reductases (multi-heme c-type cytochromes) (Gescher & Kappler, 2012; Richter et al., 2012; Breuer et al., 2015), therefore electrons are released into the medium. Metal ions or oxoanions (depending on the concentration, physical and chemical conditions of medium) can be reduced by microorganisms not only on the cell surface but also between the inner and outer membranes, in the periplasm, internal compartments and cytoplasm (Kozlova et al., 2008; Richter et al., 2012). In cells, they interact with intracellular reductants (such as amino acids, nucleotides, sugars, organic acids, glutathione, flavoenzymes, vitamins), generate chemical active intermediates, free radicals and can cause oxidative stress (Viti et al., 2012; Hnatush & Maslovska, 2018).

Sulfidogenic bacteria of the *Desulfovibrio* genus attract the attention of researchers as potential agents for the purification of waters contaminated with hydrogen sulfide and heavy metals. These bacteria as a result of dissimilatory sulfate reduction produce the hydrogen sulfide that interacts with divalent metal ions with formation of insoluble sulfides, which are thus removed from the natural cycle (Gudz et al., 2011; Kiran et al., 2017; Moroz et al., 2018). Sedimentation of sulfides can occur outside the cells of microorganisms, in the cells or on the cell surface at pH 3–9 (Kuznetsov et al., 2015). The efficiency of microbiological precipitation of metal ions in the form of MeS by hydrogen sulfide, produced by bacteria, depends on the concentration of hydrogen sulfide, which they produce in the process of dissimilatory sulfate reduction (Gudz et al., 2011; Kiran et al., 2017; Moroz et al., 2018). On the other hand, the intensity of anaerobic respiration of microorganisms in contaminated ecotopes is determined by the level of their adaptation to unfavourable conditions of environment (Viti et al., 2014; Mustapha & Halimoon, 2015; Kiran et al., 2017). These bacteria oxidize organic substrates using metals with variable valence as electron acceptors, reduce and transform them into nontoxic or less toxic forms for living organisms (Kuznetsov et al., 2015; Moroz et al., 2017a).

Chromium is the most abundant heavy metal in the lithosphere (69 μg/g). This metal is introduced into the environment from natural sources such as volcanic eruptions, forest fires, and weathering, but the largest contribution to the deposition of chromium in the biosphere is the result of anthropogenic activities (electroplating, steel, and automobile manufacturing, wood treatment, leather tanning, pigments in dyes, paints, inks, plastics, and military defense applications) (Viti et al., 2014). The Cr(VI) is soluble, highly toxic to living organisms, mutagenic, and carcinogenic to humans. Cr(III) is relatively insoluble under natural conditions and therefore less toxic than Cr(VI) (Sobol & Schiestl, 2012; Viti et al., 2014). Nonetheless Cr(III) affects DNA replication, causes mutagenesis, and alters the structure and activity of enzymes, reacting with their carboxyl and thiol groups. Sulfate uptake in bacteria is carried out by sulfate permeases that belong to the SulT (CysPTWA), SulP, CysP/(PiT), and CysZ families. The chromate oxyanions are structurally related to sulfate ions and its uptake occurs mainly through sulfate permeases (Aguilar-Barajas et al., 2011; Hoffmann et al., 2017). Cr(VI), penetrating the cell membrane of *Shewanella oneidensis* MR-1, *Pseudomonas putida* F1, *Cupriavidus metallidurans* CH34, *Arthrobacter* sp. FB24 by the ABC sulfate transporter system (Aguilar-Barajas et al., 2011), interacts with intracellular reductants and generates chemically active intermediates Cr(V) or Cr(IV), free radicals and Cr(III) as the end product (Viti et al., 2014). Reduction of Cr(VI) to Cr(V) is combined with the formation of H_2O_2. The interaction of Cr(V) with H_2O_2 leads to the formation of hydroxyl radicals. It is believed that the genotoxicity and toxicity of Cr(VI) is due to damage to DNA and proteins, respectively, oxygen radicals, formed as a result of its reduction (Sobol & Schiestl, 2012).

Cr(VI) at low concentrations may slightly stimulate the growth of microorganisms, but at high content in the medium it modifies the expression of genes whose products are involved in transport and metabolism of carbohydrates, amino acid conversion, production and use of energy in the form of ATP etc. Under the influence of 1 and 5 mM Cr(VI) in *S. oneidensis* MR-1 and *Arthrobacter* sp. FB24, respectively, decreases the number of enzymes involved in the synthesis of phosphoenolpyruvate and pyruvate, components of pyruvate dehydrogenase complex, aldehyde dehydrogenase, which leads to decrease in biomass accumulation (Viti et al., 2014).

To persist in Cr(VI)-contaminated environments, microorganisms must have efficient systems to neutralize the negative effects of this form of chromium. The systems involve detoxification or repair strategies such as Cr(VI) efflux pumps, Cr(VI) reduction to Cr(III), and activation of enzymes involved in the detoxification of active oxygen forms, repair of DNA damage. Determinants of chromate and dichromate resistance localized in chromosomal and/or plasmid DNA (genes encoding proteins involved in their transport across the membrane) have been described (Caballero-Flores et al., 2011). Other known mechanisms of resistance to Cr(VI) are specific removal of chromate ions from the cytoplasm by pumping systems or extracellular reduction of Cr(VI) to Cr(III) (Belchik et al., 2011; Richter et al., 2012; Viti et al., 2014). Under anaerobic conditions *Deinococcus radiodurans* R1, *S. oneidensis* MR-1 and others use Cr(VI) as an electron acceptor (Belchik et al., 2011). Cytochromes MtrA, MtrB, MtrC and OmcA *S. oneidensis* MR-1 are involved in dissimilatory reduction of not only Cr(VI) but also Fe(III), U(VI), Tc(VII), and moreover it was found that MtrC and OmcA are terminal reductases of extracellular Cr(VI) reduction (Belchik et al., 2011; Jing et al., 2020). Membrane-bound metal reductases in gram-negative bacteria are associated with the outer side of the membrane to reduction metal ions outside the cell (Lengeler et al., 2005; Richter et al., 2012). Three-, tetra- and deca-heme c-type cytochromes in *Shevanella frigidimarina*, *Desulfovibrio vulgaris*, *Desulfuromonas acetoxidans* are localized between the inner and outer membranes and in the periplasm, through which electrons from the cytoplasm from the reactions of organic compounds oxidation are transferred to the outside the cells, where the metal ions are actually reduced (Gescher & Kappler, 2012).

Sewage of many industrial enterprises, flooded sulfur deposits, waste heaps of coal mines, landfills for municipal and industrial waste contain, in addition to sulfur, carbon and nitrogen compounds, heavy metals and radionuclides, the content of which exceeds the maximum permissible concentrations (Kuzmishyna-Diakiv & Hnatush 2015; Tarabas et al., 2017). The danger of heavy metals is due to their bioaccumulation and concentration during the trophic chains movement. At high content in natural media heavy metals cause a toxic effect on living organisms, which reduces biodiversity and productivity of ecosystems (Kuznetsov et al., 2015). In natural conditions, where there are mainly several possible electron acceptors of anaerobic respiration, bacteria first of all reduce acceptors with higher standard oxidation-reduction potential. Although the succession of electron acceptors' reduction by microorganisms is determined by electrochemical laws, the issue it is not sufficiently clear. In various microorganisms, the succession of reduction of elements with variable valence is determined genetically and controlled by complex regulatory mechanisms (Lengeler et al., 2005; Kozlova et al., 2008; Rosenberg et al., 2014).

Unlike *Desulfovibrio vulgaris*, *D. desulfuricans* is able to use nitrate as an alternative to sulfate as the terminal electron acceptor to support growth. Nitrate reduction is catalysed by a periplasmic nitrate reductase encoded in the napCMADGH genes (Marietou et al., 2009). Expression of the nitrate reductase operon in the strain *D. desulfuricans* 27774 is induced during growth in the presence of nitrate, but repressed by sulfate, even in the presence of nitrate (Marietou et al., 2009). Nitric oxide is an obligate intermediate during denitrification, but bacteria that reduce nitrate to ammonia also generate small quantities of NO, which in turn activates a protective nitrosative stress response. Hcp in sulfate reducing bacteria is a high affinity nitric oxide reductase that protects cytoplasmic proteins from nitrosative damage by NO generated as a side product of nitrite reduction to ammonia (Cadby et al., 2017). Although nitrate and nitrite reduction are

tightly regulated in response to substrate availability, the global responses to nitrate or NO were largely regulated independently. In *D. desulfuricans* 27774 multiple NADH dehydrogenases, transcription factors of different function and genes for iron uptake were differentially expressed in response to electron acceptor availability or nitrosative stress (Cadby et al., 2017).

In technogenic reservoirs there are often several possible electron acceptors of anaerobic respiration. The succession of their reduction by microorganisms is not well understood, it may be different in bacteria strains of the same genus (Rosenberg et al., 2014). Selection of pollutant-resistant strains of sulfate reducing bacteria isolated from technogenically altered ecotopes, capable of reductive transformation of various nature pollutants, is an especially relevant task for the creation of biotechnologies for purification (Kozlova et al., 2008; Kuznetsov et al., 2015; Basniwal et al., 2017; Li et al., 2018; Teng et al., 2019). Previously we have shown that bacteria of *Desulfovibrio* genus in addition to oxidized forms of sulfur or nitrogen can reduce oxidized forms of heavy metals, in particular, Cr(VI), transforming them into compounds less toxic to living organisms (Moroz et al., 2016, 2017a, 2017b). The purpose of this work was to investigate the regularities of sulfate, nitrate or nitrite ions usage by these bacteria at conditions of simultaneous presence in the medium of another electron acceptor – Cr(VI), to establish a succession of electron acceptors' reduction by strains of sulfidogenic bacteria of *Desulfovibrio* genus isolated by us from Yavorivske Lake and to evaluate the efficiency of their possible application in technologies of complex purification of the environment from metal, sulfur and nitrogen compounds.

Materials and methods

Sulfate reducing bacteria *Desulfovibrio desulfuricans* IMV K-6, *Desulfovibrio* sp. Yav-6, *Desulfovibrio* sp. Yav-8, isolated by us earlier from Yavorivske Lake, were identified at Microbiology Department of Ivan Franko National University of Lviv (Peretyatko et al., 2006; Moroz, 2010). Strain *D. desulfuricans* IMV K-6 has been stored in the depository of D. K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine since 2009.

Bacteria were grown for 10 days in Kravtsov-Sorokin medium (Gudz et al., 2014) without SO_4^{2-} and without Mohr's salt of the folowing composition (g/L): $NaH_2PO_4 \times 12H_2O$ (0.84), K_2HPO_4 (0.50), NH_4Cl (0.16), $MgCl_2 \times 6H_2O$ (0.10), sodium lactate ($NaC_3H_5O_3$) (2.00), at a temperature of 30 ºC in test tubes (25 mL), completely topped up by the medium. Before bacteria seeding, 0.05 mL of $Na_2S \times 9H_2O$ (1%) sterile solution was added to the medium. A sterile 10 N NaOH solution was used to provide pH of the medium to 7.2. Bacteria were sown in the medium to initial concentration of cells of 0.1 mg/mL. Solutions of sodium fumarate ($C_4H_3NaO_4$), $Na_2SO_4 \times 10H_2O$, $NaNO_3$, $NaNO_2$, $K_2Cr_2O_7$ were sterilized separately and placed into the medium before seeding of the cells at different concentrations. Into media with $C_4H_3NaO_4$, $K_2Cr_2O_7$ or $NaNO_3$ or $NaNO_2$ 0.017 mM cysteine ($C_3H_7NO_2S$) was introduced to provide the assimilation needs of bacteria in sulfur (Lengeler et al., 2005). To media with $NaNO_3$, $NaNO_2$ and $K_2Cr_2O_7$ or without it the NH_4Cl was not added.

To determine the efficiency of sulfate, nitrate, or nitrite ions reduction at simultaneous presence in the medium of potassium dichromate (the medium with two electron acceptors: SO_4^{2-}, NO_3^- or NO_2^- and Cr(VI)), cells were previously cultivated in the medium with sodium fumarate (3.47 mM) as an electron acceptor and sodium lactate (17.86 mM) as an electron donor to the middle of the exponential growth phase. Bacteria were sown in a media with sodium lactate (17.86 mM), to which 1 M solutions of $Na_2SO_4 \times 10H_2O$, $NaNO_3$ or $NaNO_2$ were added to their final concentration in the medium of 3.47 mM (concentration of SO_4^{2-} in medium of standard composition) and different volumes of the sterile 1 M solution of $K_2Cr_2O_7$ to final Cr(VI) concentrations in the medium of 1.74, 3.47, 5.21, 6.94, 10.41 mM, which is in 0.5, 1.0, 1.5, 2.0 and 3.0 times different from the standard electron acceptor content in Kravtsov-Sorokin medium. Bacteria were also sown in a medium with sodium lactate at the same concentration, to which different volumes of the sterile 1 M solutions of $NaNO_3$ or $NaNO_2$ were added to their final concentrations in the medium of 1.74, 3.47, 5.21, 6.94, 10.41 mM and the sterile 1 M solution of $K_2Cr_2O_7$ to final Cr(VI) concentration in the medium of 3.47 mM. The cells were also sown in a media with sodium lactate, to which were added sterile 1 M solutions of $Na_2SO_4 \times 10H_2O$, $NaNO_3$, $NaNO_2$ or $K_2Cr_2O_7$ to final SO_4^{2-}, NO_3^-, NO_2^- or Cr(VI) concentration in the medium of 3.47 mM, to test the bacteria growth in media with sulfate, nitrate, nitrite ions or Cr(VI) as the sole electron acceptor (control). Into the media without bacteria the solutions of $Na_2SO_4 \times 10H_2O$, $NaNO_3$, $NaNO_2$ or $K_2Cr_2O_7$ were added to final SO_4^{2-}, NO_3^-, NO_2^- or Cr(VI) concentration in the medium of 3.47 mM to verify their spontaneous reduction. Different volumes of the 1 M solution of $K_2Cr_2O_7$ in the medium to obtain required Cr(VI) concentrations were calculated taking into account the molecular weight of the compound and atomic mass of chromium (two atoms), since it is known that in aqueous solutions dichromate ions are hydrolysed to form of $HCrO_4^-$: $Cr_2O_7^{2-} + H_2O \rightleftarrows 2HCrO_4^-$ (Mandich, 1997). Therefore, to obtain the same content SO_4^{2-}, NO_3^-, NO_2^- or Cr(VI) the molar concentration of $K_2Cr_2O_7$ in the medium was twice lower than other compounds ($Na_2SO_4 \times 10H_2O$, $NaNO_3$, $NaNO_2$) that contain one atom of sulfur or nitrogen. Biomass, the concentrations of sulfate, nitrate or nitrite ions, Cr(VI), Cr(III), hydrogen sulfide or ammonium ions in cultural liquid were determined on 10 day or 1, 2, 4, 6, 8, 10 days of growth. By the difference between the initial and residual content of electron acceptors in the medium was calculated the efficiency (%) of their reduction by bacteria, based on the ratio of molar concentrations of reduced by bacteria sulfate, nitrate, nitrite ions or Cr(VI) in the process of anaerobic respiration and their concentrations at the beginning of cultivation, which were taken as 100%.

Biomass was determined turbidimetrically using the photoelectrocolorimeter KFK-3 by the optical density of the cell suspension by measuring it at a wavelength of 340 nm in a cuvette with an optical way of 3 mm and calculated using the formula: C, g/L = ($E_{340} \times n$) / K, where E_{340} – extinction (λ = 340 nm); n – dilution factor; 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 to 0.19 (Gudz et al., 2014). In a cultural liquid, separated from the cells by centrifugation (4025 g, 15 min), were determined the concentrations of sulfate ions by turbidimetric method for the formation of barium sulfate after precipitation of sulfates by barium chloride, 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) (Granger, 1996), Cr(VI) colorimetrically by interaction with 1,5-diphenylcarbazide in acid solution, Cr(III) by reaction with chromazurol S, hydrogen sulfide by spectrophotometric method for the formation of methylene blue and ammonium ions by colorimetric method for the formation of indophenol (Gudz et al., 2014).

Experiments were repeated three times with three parallel formulations for each variant of experimental and control conditions. The obtained data were processed by standard methods of variation statistics. The data of research results were expressed as mean value (x) and standard deviation (± SD) of three measurements. The reliability of the difference between experimental and control variants was evaluated using ANOVA. Differences were considered statistically significant at $P < 0.05$.

Results

The efficiency of biological methods for purifying the environment from pollutants depends not only on the metabolic activity of the selected strains of bacteria, but primarily on their resistance to metal compounds. Therefore, we studied the ability of *Desulfovibrio* sp. bacteria to reduce in the process of anaerobic respiration sulfate, nitrate or nitrite ions with the simultaneous presence in the medium of $K_2Cr_2O_7$ at different concentrations. The bacteria were sown in media, to which 3.47 mM $Na_2SO_4 \times 10H_2O$ and $K_2Cr_2O_7$ at different concentrations were added.

The bacteria were also sown in media with $Na_2SO_4 \times 10H_2O$ or $K_2Cr_2O_7$ to final SO_4^{2-} or Cr(VI) concentration in the medium of 3.47 mM, to test the bacteria growth in the media with sulfate ions or hexavalent chromium as the sole electron acceptor (Table 1). After 10 days of growth the biomass of bacteria in the medium with $Na_2SO_4 \times 10H_2O$ was 2.1–2.2 times higher than in the medium with $K_2Cr_2O_7$. After the simultaneous addition of $Na_2SO_4 \times 10H_2O$ and $K_2Cr_2O_7$ to the cultiva-

tion medium with growing of Cr(VI) concentrations a gradual decrease in the biomass accumulation by bacteria was observed, compared to growth in the media with only $Na_2SO_4 \times 10H_2O$. In the medium with 3.47 mM SO_4^{2-} and 10.41 mM Cr(VI) the growth of bacteria was decreased 1.9–2.6 times, compared with growth in the medium with $Na_2SO_4 \times 10H_2O$. In the media with $Na_2SO_4 \times 10H_2O$ and $K_2Cr_2O_7$ with increasing of Cr(VI) concentrations also a gradual (3.2–4.6 fold) decreasing in the efficiency of sulfate ions reduction by bacteria was detected as compared with their reduction in the medium with only $Na_2SO_4 \times 10H_2O$ (94.0–96.0%, Fig. 1a). In this medium the cells produced 0.67–1.01 mM hydrogen sulfide (control – 2.73–2.94 mM, Table 1). The efficiency of Cr(VI) reduction by bacteria in a medium with $Na_2SO_4 \times 10H_2O$ and $K_2Cr_2O_7$ was found to be 1.2–2.2 times lower as compared with its reduction in the medium with only $K_2Cr_2O_7$ (71.8–74.4%, Fig. 1b). In the medium with $Na_2SO_4 \times 10H_2O$ and $K_2Cr_2O_7$ bacteria produced 1.02–3.64 mM of Cr(III) (control – 2.41–2.56 mM, Table 1).

In the medium with $Na_2SO_4 \times 10H_2O$ and $K_2Cr_2O_7$ without bacteria the efficiency of sulfate ions and Cr(VI) reduction was found to be insignificant and did not exceed 3.2% and 4.6%, respectively (Fig. 1). Thus, it has been established that Cr(VI) inhibits the biomass accumulation, sulfate ions reduction and hydrogen sulfide production by bacteria *Desulfovibrio* sp. after simultaneous introduction into the medium of 3.47 mM SO_4^{2-} and Cr(VI) (1.74–10.41 mM). In the medium with the same initial content (3.47 mM) of SO_4^{2-} and Cr(VI) bacteria reduced 2.1–2.3 times more Cr(VI) than sulfate ions with Cr(III) production at concentrations up to 2.2 times higher than hydrogen sulfide.

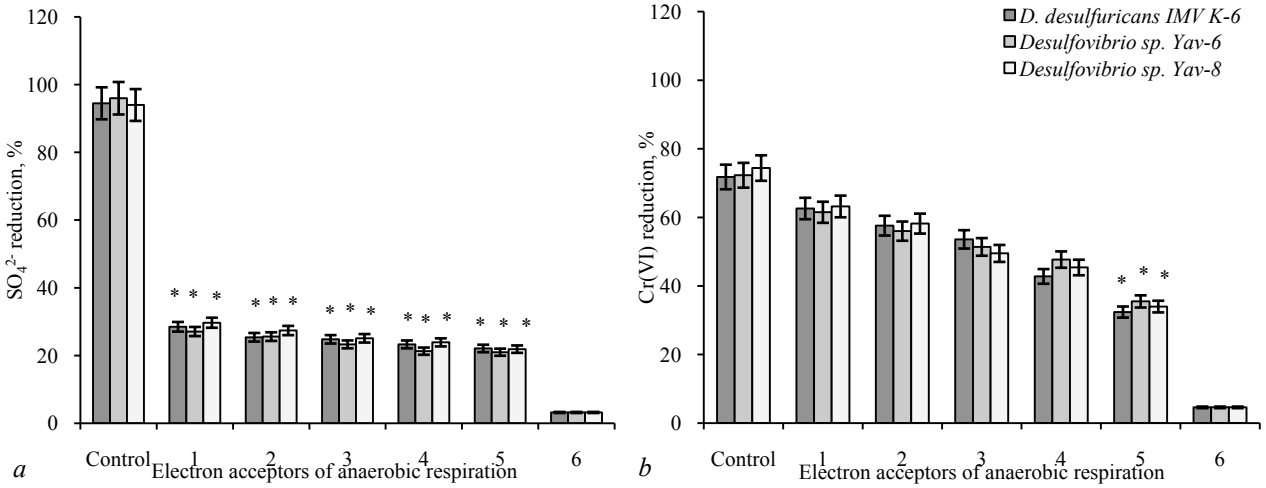

Fig. 1. Efficiency of 3.47 mM SO_4^{2-} (*a*) and 1.74–10.41 mM Cr(VI) (*b*) reduction by *Desulfovibrio* sp. after 10 days of growth in media with $Na_2SO_4 \times 10H_2O$ or $K_2Cr_2O_7$ (x ± SD, n = 3): designation on the horizontal axis: control – 3.47 mM sulfate ions (*a*), 3.47 mM Cr(VI) (*b*); *1* – 3.47 mM sulfate ions and 1.74 mM Cr(VI); *2* – 3.47 mM sulfate ions and 3.47 mM Cr(VI); *3* – 3.47 mM sulfate ions and 5.21 mM Cr(VI); *4* – 3.47 mM sulfate ions and 6.94 mM Cr(VI); *5* – 3.47 mM sulfate ions and 10.41 mM Cr(VI); *6* – 3.47 mM sulfate ions (without bacteria) (*a*), 3.47 mM Cr(VI) (without bacteria) (*b*); * – the data were statistically significant as compared with the control (P < 0.05)

Table 1

Reduction of 3.47 mM SO_4^{2-} and 1.74–10.41 mM Cr(VI) by *Desulfovibrio* sp. after 10 days of growth in media with $Na_2SO_4 \times 10H_2O$ or $K_2Cr_2O_7$ (x ± SD, n = 3)

Strain	Electron acceptors of anaerobic respiration	Residual content in cultural liquid, mM		Cr(III), mM	S^{2-}, mM	Biomass, g/L
		SO_4^{2-}	Cr(VI)			
D. desulfuricans IMV K-6	3.47 mM SO_4^{2-}	0.19 ± 0.01	0	0	2.85 ± 0.02	2.50 ± 0.03
	3.47 mM SO_4^{2-} (wb)	3.36 ± 0,03	0	0	0.08 ± 0.01	0
	3.47 mM SO_4^{2-} and 1.74 mM Cr(VI)	2.48 ± 0.01	0.65 ± 0.07	1.02 ± 0.02	0.91 ± 0.04	2.46 ± 0.08
	3.47 mM SO_4^{2-} and 3.47 mM Cr(VI)	2.59 ± 0,04	1.47 ± 0.02	1.93 ± 0.09	0.86 ± 0.09	2.22 ± 0.03
	3.47 mM SO_4^{2-} and 5.21 mM Cr(VI)	2.61 ± 0.02	2.42 ± 0.06	2.66 ± 0.07	0.83 ± 0.07	1.83 ± 0.04
	3.47 mM SO_4^{2-} and 6.94 mM Cr(VI)	2.66 ± 0.07	3.97 ± 0.04	2.92 ± 0.04	0.74 ± 0.08	1.47 ± 0.07
	3.47 mM SO_4^{2-} and 10.41 mM Cr(VI)	2.70 ± 0.09	7.04 ± 0.02	3.32 ± 0.02	0.69 ± 0.07	1.02 ± 0.07
	3.47 mM Cr(VI)	0	0.98 ± 0.06	2.41 ± 0.03	0	1.22 ± 0.05
	3.47 mM Cr(VI) (wb)	0	3.31 ± 0.05	0.14 ± 0.01	0	0
Desulfovibrio sp. Yav-6	3.47 mM SO_4^{2-}	0.14 ± 0.02	0	0	2.94 ± 0.05	2.61 ± 0.02
	3.47 mM SO_4^{2-} (wb)	3.36 ± 0.03	0	0	0.08 ± 0.01	0
	3.47 mM SO_4^{2-} and 1.74 mM Cr(VI)	2.53 ± 0.02	0.67 ± 0.04	1.04 ± 0.04	0.92 ± 0.09	2.52 ± 0.05
	3.47 mM SO_4^{2-} and 3.47 mM Cr(VI)	2.58 ± 0.05	1.53 ± 0.02	1.89 ± 0.07	0.87 ± 0.01	2.33 ± 0.04
	3.47 mM SO_4^{2-} and 5.21 mM Cr(VI)	2.66 ± 0.07	2.53 ± 0.07	2.63 ± 0.01	0.79 ± 0.02	1.98 ± 0.02
	3.47 mM SO_4^{2-} and 6.94 mM Cr(VI)	2.73 ± 0.01	3.63 ± 0.05	3.29 ± 0.09	0.71 ± 0.06	1.72 ± 0.09
	3.47 mM SO_4^{2-} and 10.41 mM Cr(VI)	2.74 ± 0.05	6.71 ± 0.01	3.64 ± 0.08	0.68 ± 0.03	1.40 ± 0.09
	3.47 mM Cr(VI)	0	0.96 ± 0.08	2.45 ± 0.01	0	1.23 ± 0.08
	3.47 mM Cr(VI) (wb)	0	3.31 ± 0.05	0.14 ± 0.01	0	0
Desulfovibrio sp. Yav-8	3.47 mM SO_4^{2-}	0.21 ± 0.02	0	0	2.73 ± 0.08	2.62 ± 0.06
	3.47 mM SO_4^{2-} (wb)	3.36 ± 0.03	0	0	0.08 ± 0.01	0
	3.47 mM SO_4^{2-} and 1.74 mM Cr(VI)	2.44 ± 0.08	0.64 ± 0.04	1.02 ± 0.04	1.01 ± 0.08	2.41 ± 0.02
	3.47 mM SO_4^{2-} and 3.47 mM Cr(VI)	2.52 ± 0.04	1.45 ± 0.02	1.97 ± 0.07	0.93 ± 0.09	2.24 ± 0.03
	3.47 mM SO_4^{2-} and 5.21 mM Cr(VI)	2.60 ± 0.01	2.63 ± 0.08	2.55 ± 0.01	0.82 ± 0.05	1.74 ± 0.02
	3.47 mM SO_4^{2-} and 6.94 mM Cr(VI)	2.64 ± 0.07	3.79 ± 0.04	2.99 ± 0.09	0.78 ± 0.02	1.35 ± 0.09
	3.47 mM SO_4^{2-} and 10.41 mM Cr(VI)	2.71 ± 0.05	6.87 ± 0.01	3.49 ± 0.08	0.67 ± 0.09	1.00 ± 0.04
	3.47 mM Cr(VI)	0	0.89 ± 0.04	2.56 ± 0.01	0	1.19 ± 0.08
	3.47 mM Cr(VI) (wb)	0	3.31 ± 0.05	0.14 ± 0.01	0	0

Note: (wb) – the medium without bacteria.

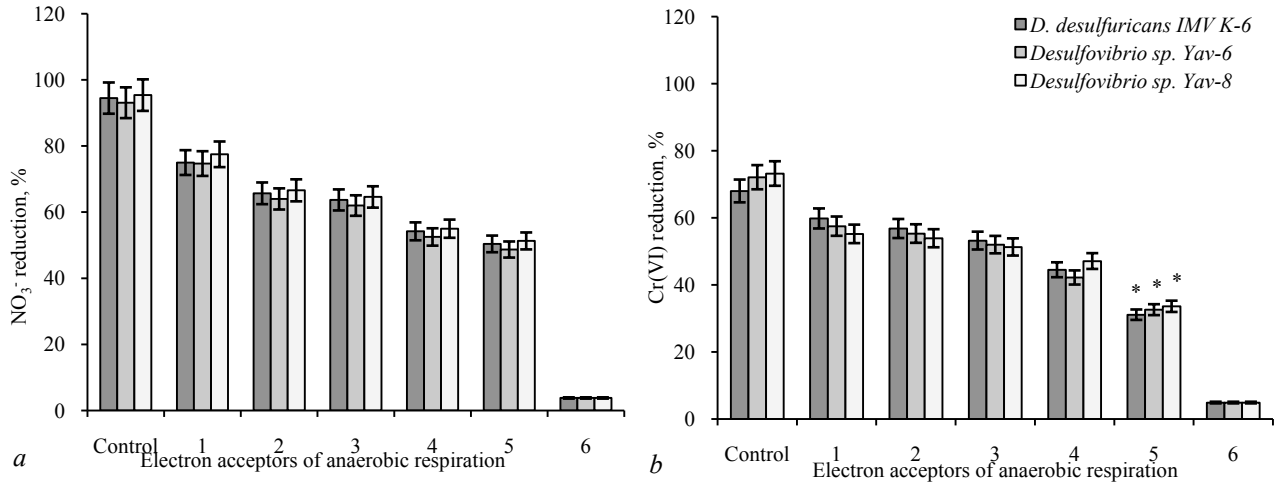

Fig. 2. Efficiency of 3.47 mM NO_3^- (*a*) and 1.74–10.41 mM Cr(VI) (*b*) reduction by *Desulfovibrio* sp. after 10 days of growth in media with $NaNO_3$ or $K_2Cr_2O_7$ (x ± SD, n = 3): designation on the horizontal axis: control – 3.47 mM nitrate ions (*a*), 3.47 mM Cr(VI) (*b*); *1* – 3.47 mM nitrate ions and 1.74 mM Cr(VI); *2* – 3.47 mM nitrate ions and 3.47 mM Cr(VI); *3* – 3.47 mM nitrate ions and 5.21 mM Cr(VI); *4* – 3.47 mM nitrate ions and 6.94 mM Cr(VI); *5* – 3.47 mM nitrate ions and 10.41 mM Cr(VI); *6* – 3.47 mM nitrate ions (without bacteria) (*a*), 3.47 mM Cr(VI) (without bacteria) (*b*); * – the data were statistically significant as compared with the control (P < 0.05)

Table 2

Reduction of 3.47 mM NO_3^- and 1.74–10.41 mM Cr(VI) by *Desulfovibrio* sp. after 10 days of growth in media with $NaNO_3$ or $K_2Cr_2O_7$ (x ± SD, n = 3)

Strain	Electron acceptors of anaerobic respiration	Residual content in cultural liquid, mM		Cr(III), mM	NH_4^+, mM	Biomass, g/L
		NO_3^-	Cr(VI)			
D. desulfuricans IMV K-6	3.47 mM NO_3^-	0.19 ± 0.03	0	0	2.62 ± 0.02	2.38 ± 0.03
	3.47 mM NO_3^-(wb)	3.34 ± 0.04	0	0	0.10 ± 0.01	0
	3.47 mM NO_3^- and 1.74 mM Cr(VI)	0.87 ± 0.02	0.70 ± 0.07	0.99 ± 0.03	2.45 ± 0.09	2.36 ± 0.05
	3.47 mM NO_3^- and 3.47 mM Cr(VI)	1.19 ± 0.06	1.50 ± 0.02	1.91 ± 0.07	2.25 ± 0.01	2.35 ± 0.04
	3.47 mM NO_3^- and 5.21 mM Cr(VI)	1.26 ± 0.07	2.44 ± 0.06	2.71 ± 0.01	2.16 ± 0.02	2.01 ± 0.02
	3.47 mM NO_3^- and 6.94 мМ Cr(VI)	1.59 ± 0.08	3.85 ± 0.04	3.04 ± 0.09	1.82 ± 0.06	1.74 ± 0.09
	3.47 mM NO_3^- and 10.41 mM Cr(VI)	1.72 ± 0.04	7.17 ± 0.02	3.19 ± 0.08	1.71 ± 0.03	1.42 ± 0.06
	3.47 mM Cr(VI)	0	1.11 ± 0.06	2.32 ± 0.05	0	1.25 ± 0.06
	3.47 mM Cr(VI) (wb)	0	3.30 ± 0.05	0.15 ± 0.03	0	0
Desulfovibrio sp. Yav-6	3.47 mM NO_3^-	0.24 ± 0.02	0	0	2.65 ± 0.03	2.53 ± 0.02
	3.47 mM NO_3^-(wb)	3.34 ± 0.04	0	0	0.10 ± 0.01	0
	3.47 mM NO_3^- and 1.74 mM Cr(VI)	0.88 ± 0.02	0.74 ± 0,07	0.94 ± 0.04	2.43 ± 0.09	2.37 ± 0.05
	3.47 mM NO_3^- and 3.47 mM Cr(VI)	1.25 ± 0.06	1.55 ± 0,02	1.87 ± 0.07	2.19 ± 0.01	2.33 ± 0.04
	3.47 mM NO_3^- and 5.21 mM Cr(VI)	1.32 ± 0.07	2.50 ± 0,06	2.69 ± 0.01	2.11 ± 0.02	1.98 ± 0.02
	3.47 mM NO_3^- and 6.94 мМ Cr(VI)	1.65 ± 0.08	4.01 ± 0,04	2.87 ± 0.09	1.79 ± 0.06	1.72 ± 0.09
	3.47 mM NO_3^- and 10.41 mM Cr(VI)	1.78 ± 0.04	7.02 ± 0,02	3.37 ± 0.08	1.64 ± 0.03	1.40 ± 0.06
	3.47 mM Cr(VI)	0	0.97 ± 0,06	2.45 ± 0.01	0	1.23 ± 0.08
	3.47 mM Cr(VI) (wb)	0	3.30 ± 0,05	0.15 ± 0.03	0	0
Desulfovibrio sp. Yav-8	3.47 mM NO_3^-	0.16 ± 0.02	0	0	2.69 ± 0.02	2.48±0.06
	3.47 mM NO_3^-(wb)	3.34 ± 0.04	0	0	0.10 ± 0.01	0
	3.47 mM NO_3^- and 1.74 mM Cr(VI)	0.78 ± 0.02	0.78 ± 0.09	0.96 ± 0.04	2.58 ± 0.04	2.42±0.04
	3.47 mM NO_3^- and 3.47 mM Cr(VI)	1.16 ± 0.06	1.60 ± 0.05	1.83 ± 0.07	2.27 ± 0.07	2.38±0.06
	3.47 mM NO_3^- and 5.21 mM Cr(VI)	1.23 ± 0.07	2.54 ± 0.01	2.63 ± 0.01	2.20 ± 0.01	1.77±0.02
	3.47 mM NO_3^- and 6.94 мМ Cr(VI)	1.56 ± 0.08	3.67 ± 0.09	3.24 ± 0.09	1.89 ± 0.09	1.64±0.03
	3.47 mM NO_3^- and 10.41 mM Cr(VI)	1.69 ± 0.04	6.91 ± 0.03	3.45 ± 0.08	1.73 ± 0.08	1.43±0.01
	3.47 mM Cr(VI)	0	0.93 ± 0.06	2.49 ± 0.01	0	1.21±0.02
	3.47 mM Cr(VI) (wb)	0	3.30 ± 0.05	0.15 ± 0.03	0	0

Notes: (wb) – the medium without bacteria; to media with NO_3^- and Cr(VI) or without it the NH_4Cl was not added.

To study the influence of sodium dichromate at Cr(VI) concentration in the medium of 1.74–10.41 mM on the usage of 3.47 mM nitrate ions by bacteria, they were cultivated in the media without NH_4Cl, to which 3.47 mM $NaNO_3$ and $K_2Cr_2O_7$ at different concentrations were added. The bacteria were also sown in the media with $NaNO_3$ or $K_2Cr_2O_7$ to final NO_3^- or Cr(VI) concentration in the medium of 3.47 mM, to test the bacteria growth in the media with nitrate ions or hexavalent chromium as the sole electron acceptor (Table 2). After 10 days of growth the biomass of bacteria in the medium with $NaNO_3$ was revealed to be 1.9–2.1 times higher than in the medium with $K_2Cr_2O_7$. After simultaneous addition into the medium of $NaNO_3$ and $K_2Cr_2O_7$ with increasing concentrations of Cr(VI) a gradual inhibition of bacteria growth was observed, compared with growth in the medium with only $NaNO_3$. In the medium with NO_3^- and 10.41 mM Cr(VI) the growth of bacteria decreased 1.7–1.8 times, compared with the growth in the medium with $NaNO_3$ as the sole electron acceptor. In the media with $NaNO_3$ and $K_2Cr_2O_7$ with increase of Cr(VI) concentrations there was also a gradual (1.2–1.9 times) decrease of the efficiency of nitrate ions reduction by cells, compared with their reduction in the medium with only $NaNO_3$ (93.1–95.4%) (Fig. 2a). In the media with $NaNO_3$ and $K_2Cr_2O_7$ bacteria 1.64–2.58 mM of ammonium ions were produced (control – 2.62–2.69 mM, Table 2). The efficiency of Cr(VI) reduction by cells with increase in its concentrations in the media with $NaNO_3$ and $K_2Cr_2O_7$ was revealed to be from 1.1 to 2.2 times lower than its reduction in the medium only with $K_2Cr_2O_7$ (68.0–73.2%, Fig. 2b). In the media with $NaNO_3$ and $K_2Cr_2O_7$ bacteria were produced 0.94–3.45 mM of Cr(III) (control – 2.32–2.49 mM, Table 2). In a medium with $NaNO_3$ or $K_2Cr_2O_7$ without bacteria the efficiency of NO_3^- and Cr(VI) reduction did not exceed 3.8% and 4.9%, respectively (Fig. 2). Thus, it has been shown that $K_2Cr_2O_7$ inhibits the biomass accumulation, the nitrate ions' reduction and the ammonium ions' production by bacteria

of *Desulfovibrio* sp. after simultaneous addition into the medium of 3.47 mM NO_3^- and Cr(VI) (1.74–10.41 mM). In the medium with the same initial content (3.47 mM) of NO_3^- and Cr(VI) bacteria reduced 1.2 times more nitrate ions than Cr(VI) with the production of ammonium ions at concentrations in the same number of times higher than that of Cr(III).

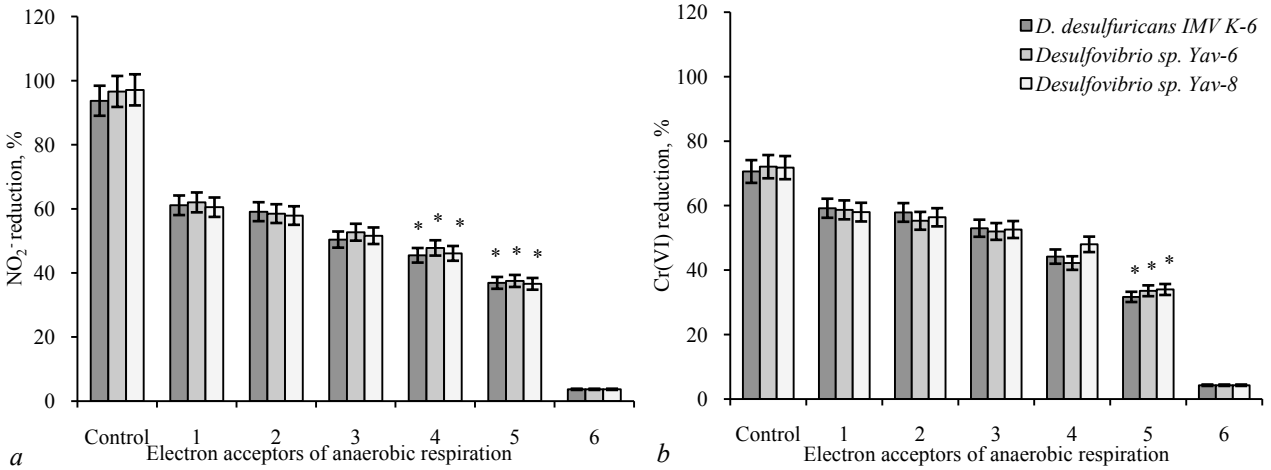

Fig. 3. Efficiency of 3.47 mM NO_2^- (*a*) and 1.74–10.41 mM Cr(VI) (*b*) reduction by *Desulfovibrio* sp. after 10 days of growth in media with $NaNO_2$ or $K_2Cr_2O_7$ (x ± SD, n = 3): designation on the horizontal axis: control – 3.47 mM nitrite ions (*a*), 3.47 mM Cr(VI) (*b*); *1* – 3.47 mM nitrite ions and 1.74 mM Cr(VI); *2* – 3.47 mM nitrite ions and 3.47 mM Cr(VI); *3* – 3.47 mM nitrite ions and 5.21 mM Cr(VI); *4* – 3.47 mM nitrite ions and 6.94 mM Cr(VI); *5* – 3.47 mM nitrite ions and 10.41 mM Cr(VI); *6* – 3.47 mM nitrite ions (without bacteria) (*a*), 3.47 mM Cr(VI) (without bacteria) (*b*); * – the data were statistically significant as compared with the control ($P < 0.05$)

Table 3

Reduction of 3.47 mM NO_2^- and 1.74–10.41 mM Cr(VI) by *Desulfovibrio* sp. after 10 days of growth in media with $NaNO_2$ or $K_2Cr_2O_7$ (x ± SD, n = 3)

Strain	Electron acceptors of anaerobic respiration	Residual content in cultural liquid, mM		Cr(III), mM	NH_4^+, mM	Biomass, g/L
		NO_2^-	Cr(VI)			
D. desulfuricans IMV K-6	3.47 mM NO_2^-	0.22 ± 0.03	0	0	2.58 ± 0.02	2.44 ± 0.03
	3.47 mM NO_2^- (wb)	3.34 ± 0.04	0	0	0.09 ± 0.01	0
	3.47 mM NO_2^- and 1.74 mM Cr(VI)	1.35 ± 0.02	0.71 ± 0.07	1.01 ± 0.03	2.05 ± 0.09	2.41 ± 0.05
	3.47 mM NO_2^- and 3.47 mM Cr(VI)	1.42 ± 0.06	1.46 ± 0.02	1.95 ± 0.07	1.98 ± 0.01	2.31 ± 0.04
	3.47 mM NO_2^- and 5.21 mM Cr(VI)	1.72 ± 0.07	2.45 ± 0.06	2.72 ± 0.01	1.72 ± 0.02	1.94 ± 0.02
	3.47 mM NO_2^- and 6.94 мМ Cr(VI)	1.89 ± 0.08	3.87 ± 0.04	3.04 ± 0.09	1.53 ± 0.06	1.70 ± 0.09
	3.47 mM NO_2^- and 10.41 mM Cr(VI)	2.19 ± 0.04	7.11 ± 0.02	3.28 ± 0.08	1.25 ± 0.03	1.40 ± 0.06
	3.47 mM Cr(VI)	0	1.02 ± 0.06	2.34 ± 0.05	0	1.09 ± 0.06
	3.47 mM Cr(VI) (wb)	0	3.32 ± 0.05	0.14 ± 0.03	0	0
Desulfovibrio sp. Yav-6	3.47 mM NO_2^-	0.12 ± 0.02	0	0	2.65 ± 0.03	2.45 ± 0.02
	3.47 mM NO_2^- (wb)	3.34 ± 0.04	0	0	0.09 ± 0.01	0
	3.47 mM NO_2^- and 1.74 mM Cr(VI)	1.32 ± 0.02	0.72 ± 0.07	0.97 ± 0.04	2.08 ± 0.09	2.42 ± 0.05
	3.47 mM NO_2^- and 3.47 mM Cr(VI)	1.44 ± 0.06	1.55 ± 0.02	1.87 ± 0.07	1.94 ± 0.01	2.33 ± 0.04
	3.47 mM NO_2^- and 5.21 mM Cr(VI)	1.64 ± 0.07	2.50 ± 0.06	2.69 ± 0.01	1.75 ± 0.02	1.98 ± 0.02
	3.47 mM NO_2^- and 6.94 мМ Cr(VI)	1.81 ± 0.08	4.01 ± 0.04	2.87 ± 0.09	1.62 ± 0.06	1.72 ± 0.09
	3.47 mM NO_2^- and 10.41 mM Cr(VI)	2.17 ± 0.04	6.91 ± 0.02	3.47 ± 0.08	1.22 ± 0.03	1.42 ± 0.05
	3.47 mM Cr(VI)	0	0.97 ± 0.06	2.45 ± 0.01	0	1.23 ± 0.08
	3.47 mM Cr(VI) (wb)	0	3.32 ± 0.05	0.14 ± 0.03	0	0
Desulfovibrio sp. Yav-8	3.47 mM NO_2^-	0.10 ± 0.02	0	0	2.72 ± 0.02	2.41 ± 0.06
	3.47 mM NO_2^- (wb)	3.34 ± 0.04	0	0	0.09 ± 0.01	0
	3.47 mM NO_2^- and 1.74 mM Cr(VI)	1.37 ± 0.02	0.73 ± 0.09	0.95 ± 0.04	2.03 ± 0.04	2.40 ± 0.04
	3.47 mM NO_2^- and 3.47 mM Cr(VI)	1.46 ± 0.06	1.51 ± 0.05	1.88 ± 0.07	1.96 ± 0.07	2.38 ± 0.06
	3.47 mM NO_2^- and 5.21 mM Cr(VI)	1.68 ± 0.07	2.47 ± 0.01	2.66 ± 0.01	1.72 ± 0.01	1.77 ± 0.02
	3.47 mM NO_2^- and 6.94 мМ Cr(VI)	1.87 ± 0.08	3.61 ± 0.09	3.29 ± 0.09	1.57 ± 0.09	1.64 ± 0.03
	3.47 mM NO_2^- and 10.41 mM Cr(VI)	2.20 ± 0.04	6.87 ± 0.03	3.51 ± 0.08	1.25 ± 0.08	1.43 ± 0.01
	3.47 mM Cr(VI)	0	0.98 ± 0.06	2.36 ± 0.01	0	1.11 ± 0.02
	3.47 mM Cr(VI) (wb)	0	3.32 ± 0.05	0.14 ± 0.03	0	0

Notes: (wb) – the medium without bacteria; to media with NO_2^- and Cr(VI) or without it the NH_4Cl was not added.

To investigate the influence of sodium dichromate at Cr(VI) concentration in the medium of 1.74–10.41 mM on the 3.47 mM nitrite ions reduction by sulfate reducing bacteria, they were grown in a medium without NH_4Cl to which 3.47 mM $NaNO_2$ and $K_2Cr_2O_7$ at different concentrations were added. The bacteria were also sown in a media with $NaNO_2$ or $K_2Cr_2O_7$ to final NO_2^- or Cr(VI) concentration in the medium of 3.47 mM, to test the bacteria growth in the media with nitrite ions or hexavalent chromium as the sole electron acceptor (Table 3). Biomass of bacteria in the medium with $NaNO_2$ was revealed up to 2.2 times higher than in the medium with $K_2Cr_2O_7$. After simultaneous addition into the medium of $NaNO_2$ and $K_2Cr_2O_7$ with increasing concentrations of Cr(VI) there was a decrease in the bacteria growth, compared with growth in a medium with $NaNO_2$. In the medium with NO_2^- and 10.41 mM Cr(VI) the growth of bacteria decreased 1.7 times, compared with growth in medium with only $NaNO_2$. In the media with $NaNO_2$ and $K_2Cr_2O_7$ with increase of Cr(VI) concentrations there was a gradual (1.5–2.7 times) decrease in the efficiency of nitrite ions reduction by bacteria, as compared with their reduction in the medium with $NaNO_2$ (93.7–97.1%, Fig. 3a). In the media, containing $NaNO_2$ and $K_2Cr_2O_7$, the cells produced 1.22–2.08 mM of ammonium ions (control – 2.58–2.72 mM, Table 3). The efficiency of the Cr(VI) reduction by bacteria with increasing its concentration in media with $NaNO_2$ and $K_2Cr_2O_7$ was re-

vealed to be from 1.2 to 2.2 times lower than its reduction in the medium with $K_2Cr_2O_7$ (70.6–72.1%) (Fig. 3b). In the media with $NaNO_2$ and $K_2Cr_2O_7$ cells produced 0.95–3.51 mM of the Cr(III) (control – 2.34–2.45 mM, Table 3). In the media with $NaNO_2$ and $K_2Cr_2O_7$ without bacteria the reduction of NO_2^- and Cr(VI) did not exceed 3.7% and 4.3%, respectively (Fig. 3). Thus, it has been established that $K_2Cr_2O_7$ inhibits the biomass accumulation, the nitrite ions reduction and the ammonium ions production by bacteria of *Desulfovibrio* sp. after simultaneous addition into the medium of 3.47 mM NO_2^- and Cr(VI) (1.74–10.41 mM). In the medium with the same initial content (3.47 mM) NO_2^- and Cr(VI) the reduction of Cr(VI) by bacteria practically did not differ from the reduction of nitrite ions (was only slightly lower), almost the same concentrations of trivalent chromium and ammonium ions were detected in the cultural liquid.

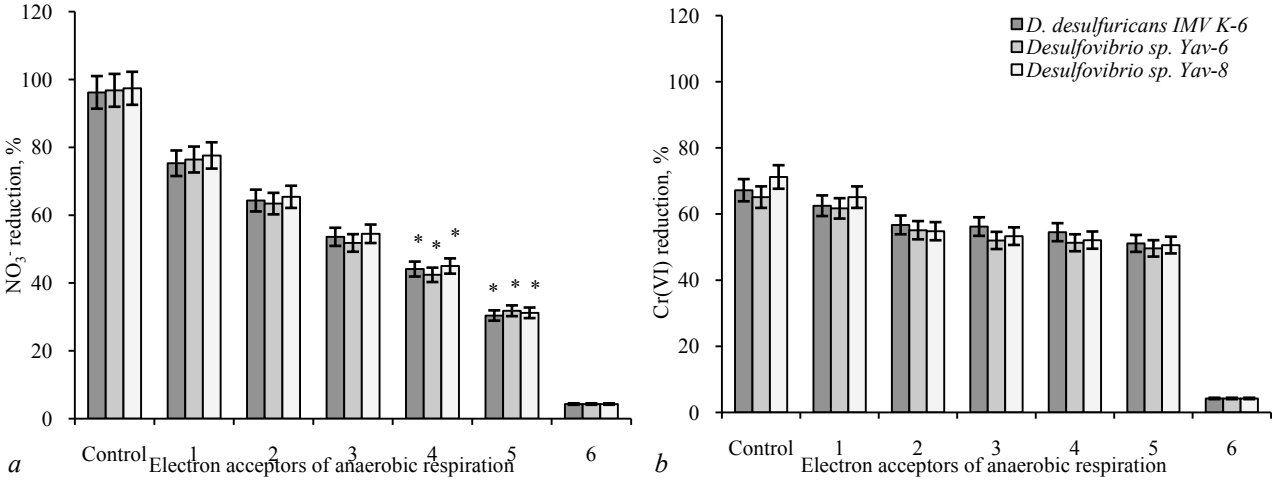

Fig. 4. Efficiency of 1.74–10.41 mM NO_3^- (*a*) and 3.47 mM Cr(VI) (*b*) reduction by *Desulfovibrio* sp. after 10 days of growth in media with $NaNO_3$ or $K_2Cr_2O_7$ (x ± SD, n = 3): designation on the horizontal axis: control – 3.47 mM nitrate ions (*a*), 3.47 mM Cr(VI) (*b*); *1* – 1.74 mM nitrate ions and 3.47 mM Cr(VI); *2* – 3.47 mM nitrate ions and 3.47 mM Cr(VI); *3* – 5.21 mM nitrate ions and 3.47 mM Cr(VI); *4* – 6.94 mM nitrate ions and 3.47 mM Cr(VI); *5* – 10.41 mM nitrate ions and 3.47 mM Cr(VI); *6* – 3.47 mM nitrate ions (without bacteria) (*a*), 3.47 mM Cr(VI) (without bacteria) (*b*); * – the data were statistically significant as compared with the control ($P < 0.05$)

Table 4
Reduction of 1.74–10.41 mM NO_3^- and 3.47 mM Cr(VI) by *Desulfovibrio* sp. after 10 days of growth in media with $NaNO_3$ or $K_2Cr_2O_7$ (x ± SD, n = 3)

Strain	Electron acceptors of anaerobic respiration	Residual content in cultural liquid, mM		Cr(III), mM	NH_4^+, mM	Biomass, g/L
		NO_3^-	Cr(VI)			
D. desulfuricans IMV K-6	3.47 mM NO_3^-	0.13 ± 0.03	0	0	2.67 ± 0.02	2.54 ± 0.03
	3.47 mM NO_3^- (wb)	3.32 ± 0.04	0	0	0.12 ± 0.01	0
	1.74 mM NO_3^- and 3.47 mM Cr(VI)	0.43 ± 0.02	1.30 ± 0.07	2.04 ± 0.03	1.25 ± 0.09	2.54 ± 0.05
	3.47 mM NO_3^- and 3.47 mM Cr(VI)	1.24 ± 0.06	1.50 ± 0.01	1.95 ± 0.07	2.21 ± 0.01	2.35 ± 0.04
	5.21 mM NO_3^- and 3.47 mM Cr(VI)	2.42 ± 0.07	1.52 ± 0.06	1.91 ± 0.01	2.72 ± 0.02	2.01 ± 0.02
	6.94 mM NO_3^- and 3.47 мМ Cr(VI)	3.88 ± 0.08	1.58 ± 0.04	1.79 ± 0.09	3.02 ± 0.06	1.74 ± 0.09
	10.41 mM NO_3^- and 3.47 mM Cr(VI)	7.25 ± 0.04	1.70 ± 0.02	1.75 ± 0.08	3.08 ± 0.03	1.42 ± 0.06
	3.47 mM Cr(VI)	0	1.11 ± 0.06	2.26 ± 0.05	0	1.25 ± 0.06
	3.47 mM Cr(VI) (wb)	0	3.32 ± 0.05	0.11 ± 0.03	0	0
Desulfovibrio sp. Yav-6	3.47 mM NO_3^-	0.11 ± 0.02	0	0	2.66 ± 0.03	2.61 ± 0.02
	3.47 mM NO_3^- (wb)	3.32 ± 0.04	0	0	0.12 ± 0.01	0
	1.74 mM NO_3^- and 3.47 mM Cr(VI)	0.41 ± 0.02	1.33 ± 0.07	2.06 ± 0.04	1.23 ± 0.09	2.52 ± 0.05
	3.47 mM NO_3^- and 3.47 mM Cr(VI)	1.27 ± 0.06	1.56 ± 0.04	1.90 ± 0.07	2.17 ± 0.01	2.33 ± 0.04
	5.21 mM NO_3^- and 3.47 mM Cr(VI)	2.51 ± 0.07	1.67 ± 0.06	1.78 ± 0.01	2.65 ± 0.02	1.98 ± 0.02
	6.94 mM NO_3^- and 3.47 мМ Cr(VI)	4.00 ± 0.08	1.69 ± 0.04	1.74 ± 0.09	2.92 ± 0.06	1.72 ± 0.09
	10.41 mM NO_3^- and 3.47 mM Cr(VI)	7.10 ± 0.04	1.75 ± 0.02	1.70 ± 0.08	3.25 ± 0.03	1.40 ± 0.06
	3.47 mM Cr(VI)	0	1.21 ± 0.06	2.19 ± 0.01	0	1.23 ± 0.08
	3.47 mM Cr(VI) (wb)	0	3.32 ± 0.05	0.11 ± 0.03	0	0
Desulfovibrio sp. Yav-8	3.47 mM NO_3^-	0.09 ± 0.02	0	0	2.70 ± 0.02	2.62 ± 0.06
	3.47 mM NO_3^- (wb)	3.32 ± 0.04	0	0	0.12 ± 0.01	0
	1.74 mM NO_3^- and 3.47 mM Cr(VI)	0.39 ± 0.02	1.21 ± 0.09	2.15 ± 0.04	1.33 ± 0.04	2.54 ± 0.04
	3.47 mM NO_3^- and 3.47 mM Cr(VI)	1.20 ± 0.06	1.57 ± 0.05	1.75 ± 0.07	2.23 ± 0.07	2.38 ± 0.06
	5.21 mM NO_3^- and 3.47 mM Cr(VI)	2.37 ± 0.07	1.62 ± 0.01	1.79 ± 0.01	2.78 ± 0.01	1.77 ± 0.02
	6.94 mM NO_3^- and 3.47 мМ Cr(VI)	3.82 ± 0.08	1.66 ± 0.09	1.71 ± 0.09	3.08 ± 0.09	1.64 ± 0.03
	10.41 mM NO_3^- and 3.47 mM Cr(VI)	7.16 ± 0.04	1.71 ± 0.03	1.73 ± 0.08	3.21 ± 0.08	1.43 ± 0.01
	3.47 mM Cr(VI)	0	1.00 ± 0.06	2.35 ± 0.01	0	1.21 ± 0.02
	3.47 mM Cr(VI) (wb)	0	3.32 ± 0.05	0.11 ± 0.03	0	0

Notes: (wb) – the medium without bacteria; to media with NO_3^- and Cr(VI) or without it the NH_4Cl was not added.

Under the influence of chemicals, sulfate reducing bacteria isolated from technogenically transformed environments adapt to them, regulating the metabolic processes not only of sulfur compounds, but also of nitrogen, heavy metals, phosphorus, and carbon during chemotrophic growth. Pollution of the environment by nitrates and nitrites is growing every year. The peculiarities of the metabolism of microorganisms under the action of nitrogen compounds as stressors remain in many cases unclear. Therefore, we studied the ability of *Desulfovibrio* sp. bacteria to reduce in the process of anaerobic respiration nitrate or nitrite ions at different concentrations with the simultaneous presence in the medium of hexavalent chromium.

The influence of sodium dichromate at Cr(VI) concentration in the medium of 3.47 mM on the 1.74–10.41 mM nitrate ions usage by bacteria was studied. Bacteria were cultivated in the media without NH_4Cl, to which $NaNO_3$ at different concentrations and 3.47 mM Cr(VI) in form of

$K_2Cr_2O_7$ were added. The bacteria were also sown in the media with $NaNO_3$ or $K_2Cr_2O_7$ to final NO_3^- or Cr(VI) concentration in the medium of 3.47 mM, to test the bacteria growth in the media with nitrate ions or hexavalent chromium as the sole electron acceptor (Table 4). After 10 days of growth, the biomass of bacteria in the medium with $NaNO_3$ was revealed to be up to 2.2 times higher than in the medium with $K_2Cr_2O_7$. After simultaneous addition into the medium of $NaNO_3$ and $K_2Cr_2O_7$ with increasing concentrations of NO_3^- a gradual inhibition of bacteria growth was observed, compared with growth in the medium with only $NaNO_3$. In the medium with 10.41 mM NO_3^- and Cr(VI) the growth of bacteria decreased 1.8–1.9 times, compared with the growth in the medium with $NaNO_3$ as the sole electron acceptor. In the media with $NaNO_3$ and $K_2Cr_2O_7$ with increase in NO_3^- concentrations there was also a gradual (1.3–3.2 times) decrease in the efficiency of nitrate ions' reduction by cells, compared with their reduction in the medium with only $NaNO_3$ (96.2–97.4%, Fig. 4a). In the media with $NaNO_3$ and $K_2Cr_2O_7$ bacteria produced 1.23–3.25 mM of ammonium ions (control – 2.66–2.70 mM, Table 4). The efficiency of Cr(VI) reduction by cells with increase in NO_3^- concentrations in the media with $NaNO_3$ and $K_2Cr_2O_7$ was revealed to be 1.1–1.4 times lower than its reduction in the medium only with $K_2Cr_2O_7$ (65.1–71.2%, Fig. 4b). In the media with $NaNO_3$ and $K_2Cr_2O_7$ bacteria produced 1.70–2.15 mM of Cr(III) (control – 2.19–2.35 mM, Table 4). In a media with $NaNO_3$ or $K_2Cr_2O_7$ without bacteria the efficiency of NO_3^- and Cr(VI) reduction did not exceed 4.3% and 4.2%, respectively (Fig. 4). Thus, it has been shown that $K_2Cr_2O_7$ inhibits the biomass accumulation, the nitrate ions reduction and the ammonium ions production by bacteria of *Desulfovibrio* sp. after simultaneous addition into the medium of 1.74–10.41 mM NO_3^- and 3.47 mM Cr(VI). In the medium with the same initial content (3.47 mM) of NO_3^- and Cr(VI) bacteria reduced 1.1–1.2 times more nitrate ions than Cr(VI) with the production of ammonium ions at concentrations 1.1–1.3 times higher than that of Cr(III).

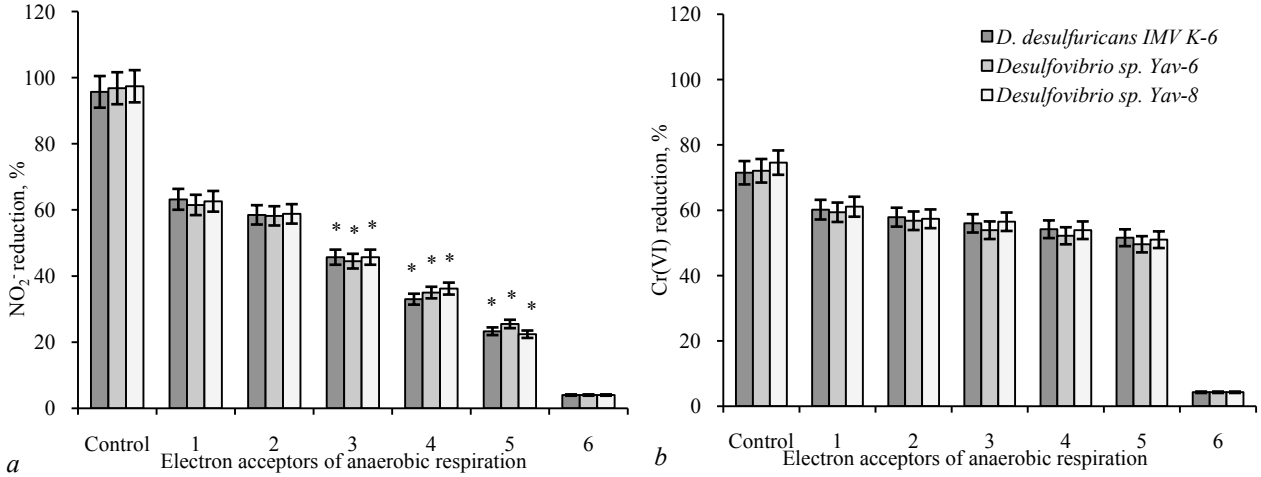

Fig. 5. Efficiency of 1.74–10.41 mM NO_2^- (*a*) and 3.47 mM Cr(VI) (*b*) reduction by *Desulfovibrio* sp. after 10 days of growth in media with $NaNO_2$ or $K_2Cr_2O_7$ (x ± SD, n = 3): designation on the horizontal axis: control – 3.47 mM nitrite ions (*a*), 3.47 mM Cr(VI) (*b*); *1* – 1.74 mM nitrite ions and 3.47 mM Cr(VI); *2* – 3.47 mM nitrite ions and 3.47 mM Cr(VI); *3* – 5.21 mM nitrite ions and 3.47 mM Cr(VI); *4* – 6.94 mM nitrite ions and 3.47 mM Cr(VI); *5* – 10.41 mM nitrite ions and 3.47 mM Cr(VI); *6* – 3.47 mM nitrite ions (without bacteria) (*a*), 3.47 mM Cr(VI) (without bacteria) (*b*); * – the data were statistically significant as compared with the control ($P < 0.05$)

Table 5
Reduction of 1.74–10.41 mM NO_2^- and 3.47 mM Cr(VI) by *Desulfovibrio* sp. after 10 days of growth in media with $NaNO_2$ or $K_2Cr_2O_7$ (x ± SD, n = 3)

Strain	Electron acceptors of anaerobic respiration	Residual content in cultural liquid, mM		Cr(III), mM	NH_4^+, mM	Biomass, g/L
		NO_2^-	Cr(VI)			
	3.47 mM NO_2^-	0.15 ± 0.01	0	0	2.71 ± 0.02	2.40 ± 0.03
	3.47 mM NO_2^-(wb)	3.33 ± 0.09	0	0	0.12 ± 0.01	0
	1.74 mM NO_2^- and 3.47 mM Cr(VI)	0.64 ± 0.02	1.38 ± 0.07	2.03 ± 0.03	1.03 ± 0.09	2.38 ± 0.05
D. desulfu	3.47 mM NO_2^- and 3.47 mM Cr(VI)	1.44 ± 0.06	1.46 ± 0.02	1.98 ± 0.07	1.96 ± 0.01	2.26 ± 0.04
ricans	5.21 mM NO_2^- and 3.47 mM Cr(VI)	2.83 ± 0.07	1.53 ± 0.06	1.89 ± 0.01	2.32 ± 0.02	1.85 ± 0.02
IMV K-6	6.94 mM NO_2^- and 3.47 мМ Cr(VI)	4.65 ± 0.08	1.59 ± 0.04	1.72 ± 0.09	2.23 ± 0.06	1.62 ± 0.09
	10.41 mM NO_2^- and 3.47 mM Cr(VI)	7.98 ± 0.04	1.68 ± 0.02	1.77 ± 0.08	2.38 ± 0.03	1.43 ± 0.06
	3.47 mM Cr(VI)	0	0.99 ± 0.06	2.38 ± 0.05	0	1.19 ± 0.06
	3.47 mM Cr(VI) (wb)	0	3.32 ± 0.05	0.12 ± 0.03	0	0
	3.47 mM NO_2^-	0.11 ± 0.02	0	0	2.73 ± 0.03	2.35 ± 0.02
	3.47 mM NO_2^-(wb)	3.33 ± 0.04	0	0	0.12 ± 0.01	0
	1.74 mM NO_2^- and 3.47 mM Cr(VI)	0.67 ± 0.02	1.41 ± 0.07	2.01 ± 0.04	1.01 ± 0.09	2.32 ± 0.05
Desulfo-	3.47 mM NO_2^- and 3.47 mM Cr(VI)	1.45 ± 0.06	1.50 ± 0.02	1.95 ± 0.07	1.93 ± 0.01	2.23 ± 0.04
vibrio sp.	5.21 mM NO_2^- and 3.47 mM Cr(VI)	2.89 ± 0.07	1.60 ± 0.06	1.82 ± 0.01	2.28 ± 0.02	1.86 ± 0.02
Yav-6	6.94 mM NO_2^- and 3.47 мМ Cr(VI)	4.51 ± 0.08	1.66 ± 0.04	1.77 ± 0.09	2.37 ± 0.06	1.67 ± 0.09
	10.41 mM NO_2^- and 3.47 mM Cr(VI)	7.76 ± 0.04	1.75 ± 0.02	1.70 ± 0.08	2.60 ± 0.03	1.35 ± 0.06
	3.47 mM Cr(VI)	0	0.97 ± 0.06	2.45 ± 0.01	0	1.26 ± 0.08
	3.47 mM Cr(VI) (wb)	0	3.32 ± 0.05	0.12 ± 0.03	0	0
	3.47 mM NO_2^-	0.09 ± 0.02	0	0	2.74 ± 0.02	2.38 ± 0.06
	3.47 mM NO_2^-(wb)	3.33 ± 0.02	0	0	0.12 ± 0.01	0
	1.74 mM NO_2^- and 3.47 mM Cr(VI)	0.65 ± 0.02	1.35 ± 0.09	2.09 ± 0.04	1.02 ± 0.04	2.34 ± 0.04
Desulfo-	3.47 mM NO_2^- and 3.47 mM Cr(VI)	1.43 ± 0.06	1.48 ± 0.05	1.95 ± 0.07	1.94 ± 0.07	2.28 ± 0.06
vibrio sp.	5.21 mM NO_2^- and 3.47 mM Cr(VI)	2.83 ± 0.07	1.51 ± 0.01	1.91 ± 0.01	2.34 ± 0.01	1.72 ± 0.02
Yav-8	6.94 mM NO_2^- and 3.47 мМ Cr(VI)	4.43 ± 0.08	1.60 ± 0.09	1.85 ± 0.09	2.48 ± 0.09	1.61 ± 0.03
	10.41 mM NO_2^- and 3.47 mM Cr(VI)	8.08 ± 0.04	1.70 ± 0.03	1.74 ± 0.08	2.29 ± 0.08	1.34 ± 0.01
	3.47 mM Cr(VI)	0	0.88 ± 0.06	2.47 ± 0.01	0	1.28 ± 0.02
	3.47 mM Cr(VI) (wb)	0	3.32 ± 0.05	0.12 ± 0.03	0	0

Notes: (wb) – the medium without bacteria; to media with NO_2^- and Cr(VI) or without it the NH_4Cl was not added.

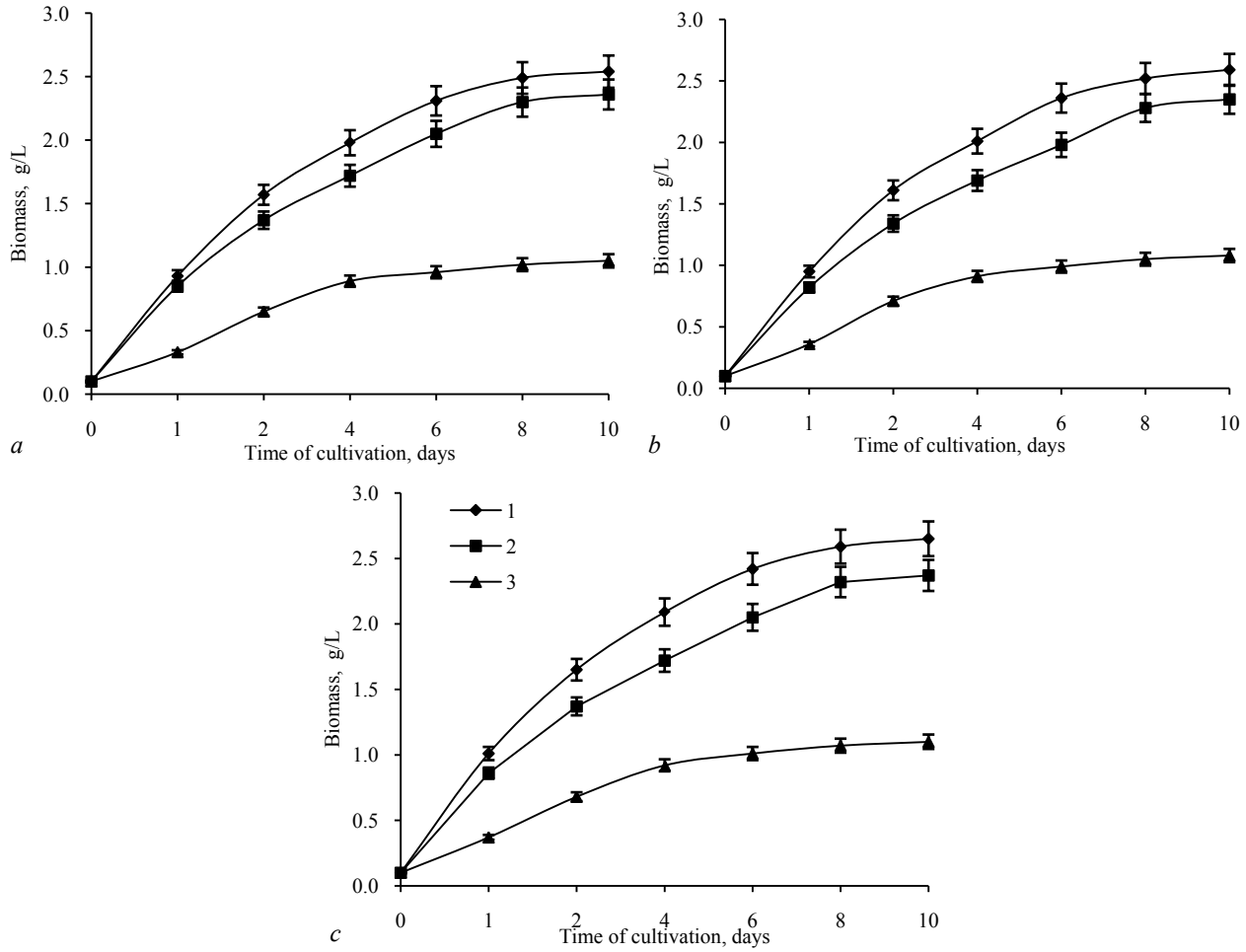

Fig. 6. Biomass accumulation by *Desulfovibrio desulfuricans* IMV K-6 (*a*), *Desulfovibrio* sp. Yav-6 (*b*) and *Desulfovibrio* sp. Yav-8 (*c*) during growth in media with $NaNO_3$ (*1*), $NaNO_3$ and $K_2Cr_2O_7$ (*2*), $K_2Cr_2O_7$ (*3*) (x ± SD, n = 3)

The influence of sodium dichromate at Cr(VI) concentration in the medium of 3.47 mM on the 1.74–10.41 mM nitrite ions usage by bacteria was studied. Bacteria were cultivated in the media without NH_4Cl, to which $NaNO_2$ at different concentrations and 3.47 mM Cr(VI) in form of $K_2Cr_2O_7$ were added. The bacteria were also sown in media with $NaNO_2$ or $K_2Cr_2O_7$ to final NO_2^- or Cr(VI) concentration in the medium of 3.47 mM, to test the bacteria growth in the media with nitrite ions or hexavalent chromium as the sole electron acceptor (Table 5). Biomass of bacteria in the medium with $NaNO_2$ was revealed to be 1.9–2.0 times higher than in the medium with $K_2Cr_2O_7$. After simultaneous addition into the medium of $NaNO_2$ and $K_2Cr_2O_7$ with increasing concentrations of NO_2^- there was a decrease in the bacteria growth, compared with growth in a medium with $NaNO_2$. In the medium with 10.41 mM NO_2^- and Cr(VI) the growth of bacteria was decreased 1.7–1.8 times, compared with growth in medium with only $NaNO_2$. In the media with $NaNO_2$ and $K_2Cr_2O_7$ with increase of NO_2^- concentrations there was a gradual (1.5–4.3 times) decrease in the efficiency of nitrite ions reduction by bacteria, as compared with their reduction in the medium with $NaNO_2$ (95.7–97.4%) (Fig. 5a). In the media, containing $NaNO_2$ and $K_2Cr_2O_7$, the cells produced 1.01–2.60 mM of ammonium ions (control – 2.71–2.74 mM, Table 5). The efficiency of the Cr(VI) reduction by bacteria with increase of NO_2^- concentrations in the media with $NaNO_2$ and $K_2Cr_2O_7$ was revealed to be from 1.2 to 1.5 times lower than its reduction in the medium with $K_2Cr_2O_7$ (71.5–74.6%, Fig. 5b). In the media with $NaNO_2$ and $K_2Cr_2O_7$ cells produced 1.77–2.01 mM of the Cr(III) (control – 2.38–2.47 mM, Table 5). In the media with $NaNO_2$ and $K_2Cr_2O_7$ without bacteria the reduction of NO_2^- and Cr(VI) did not exceed 4.0% and 4.3%, respectively (Fig. 5). Thus, it has been established that $K_2Cr_2O_7$ inhibits the biomass accumulation, the nitrite ions' reduction and the ammonium ions production by bacteria of *Desulfovibrio* sp. after simultaneous addition into the medium of 1.74–10.41 mM NO_2^- and 3.47 mM Cr(VI). In the medium with the same initial content (3.47 mM) NO_2^- and Cr(VI) the reduction of Cr(VI) by bacteria did not differ from the reduction of nitrite ions (was only slightly lower), almost the same concentrations of trivalent chromium and ammonium ions in the cultural liquid were detected.

Bacteria were cultivated during 10 days in the media without NH_4Cl, to which $NaNO_3$ and $K_2Cr_2O_7$ were added to equal NO_3^- and Cr(VI) concentrations in the medium of 3.47 mM. The bacteria were also sown in the media with $NaNO_3$ or $K_2Cr_2O_7$ to final NO_3^- or Cr(VI) concentration in the medium of 3.47 mM, to test the bacteria growth in the media with nitrate ions or hexavalent chromium as the sole electron acceptor (Fig. 6–8). On day 10 of growth the biomass of bacteria in the medium with $NaNO_3$ was revealed to be 2.4–2.5 times higher than in the medium with $K_2Cr_2O_7$. In the medium with $NaNO_3$ and $K_2Cr_2O_7$ the biomass of bacteria was 1.1 times lower than in the medium with only $NaNO_3$, but 2.2 times higher than in the medium with $K_2Cr_2O_7$ as the sole electron acceptor (Fig. 6). In the medium with $NaNO_3$ and $K_2Cr_2O_7$ there was a 1.5 times decrease in the efficiency of nitrate ions' reduction by cells, compared with their reduction in the medium with only $NaNO_3$ (95.7–96.8%) (Fig. 7a, 7b). In the medium with $NaNO_3$ and $K_2Cr_2O_7$ bacteria produced 2.18–2.25 mM of ammonium ions (control – 2.63–2.78 mM, Fig. 8a, 8b, 8c). The efficiency of Cr(VI) reduction by cells in the medium with $NaNO_3$ and $K_2Cr_2O_7$ was revealed to be from 1.2 to 1.4 times lower than its reduction in the medium only with $K_2Cr_2O_7$ (68.6–70.9%, Fig. 7c, 7d). In the medium with $NaNO_3$ and $K_2Cr_2O_7$ bacteria produced 1.73–1.94 mM of Cr(III) (control – 2.31–2.38 mM, Fig. 8d, 8e, 8f). In the medium with $NaNO_3$ and $K_2Cr_2O_7$ bacteria reduced 1.1–1.3 times more nitrate ions than Cr(VI) with the production of NH_4^+ at concentrations 1.2–1.3 times higher than that of Cr(III) (Fig. 8). In a medium with $NaNO_3$ or $K_2Cr_2O_7$ without bacteria the efficiency of NO_3^- and Cr(VI) reduction did not exceed 3.2–3.7% and 4.0–4.3%, respectively (Fig. 7). Thus, it has been shown that $K_2Cr_2O_7$ inhibits the biomass accumulation, the nitrate

ions' reduction and the ammonium ions production by bacteria of *Desulfovibrio* sp. after simultaneous addition into the medium of 3.47 mM NO_3^- and 3.47 mM Cr(VI).

Bacteria were grown during 10 days in the media without NH_4Cl, to which $NaNO_2$ and $K_2Cr_2O_7$ were added to equal NO_2^- and Cr(VI) concentrations in the medium of 3.47 mM. The bacteria were also sown in the media with $NaNO_2$ or $K_2Cr_2O_7$ to final NO_2^- or Cr(VI) concentration in the medium of 3.47 mM, to test the bacteria growth in the media with nitrite ions or hexavalent chromium as the sole electron acceptor (Fig. 9–11). On day 10 of growth the biomass of bacteria in the medium with $NaNO_2$ was revealed to be up to 2.2 times higher than in the medium with $K_2Cr_2O_7$. In the medium with $NaNO_2$ and $K_2Cr_2O_7$ the biomass of bacteria was almost the same as in the medium with only $NaNO_2$, but 2.1 times higher than in the medium with $K_2Cr_2O_7$ as the sole electron acceptor (Fig. 9). In the medium with $NaNO_2$ and $K_2Cr_2O_7$ there was a 1.6 times decrease in the efficiency of nitrite ions' reduction by cells, compared with their reduction in the medium with only $NaNO_2$ (93.1–96.5%, Fig. 10a, 10b). In the medium with $NaNO_2$ and $K_2Cr_2O_7$ bacteria produced 1.96–2.01 mM of ammonium ions (control – 2.59–2.71 mM, Fig. 11a, 11b, 11c). The efficiency of Cr(VI) reduction by cells in the medium with $NaNO_2$ and $K_2Cr_2O_7$ was revealed to be from 1.2 to 1.3 times lower than its reduction in the medium only with $K_2Cr_2O_7$ (69.7–71.2%, Fig. 10c, 10d). In the medium with $NaNO_2$ and $K_2Cr_2O_7$ bacteria produced 1.86–1.93 mM of Cr(III) (control – 2.36–2.43 mM, Fig. 11d, 11e, 11f). In the medium with $NaNO_2$ and $K_2Cr_2O_7$ bacteria reduced up to 1.1 times more nitrite ions than Cr(VI) with the production of NH_4^+ at concentrations in the same number times higher than that of Cr(III) (Fig. 11). In a media with $NaNO_2$ or $K_2Cr_2O_7$ without bacteria the efficiency of NO_2^- and Cr(VI) reduction did not exceed 3.2–3.8 and 4.0–4.3%, respectively (Fig. 10). Thus, it has been shown that $K_2Cr_2O_7$ inhibits the biomass accumulation, the nitrite ions reduction and the ammonium ions production by bacteria of *Desulfovibrio* sp. after simultaneous addition into the medium of 3.47 mM NO_2^- and 3.47 mM Cr(VI).

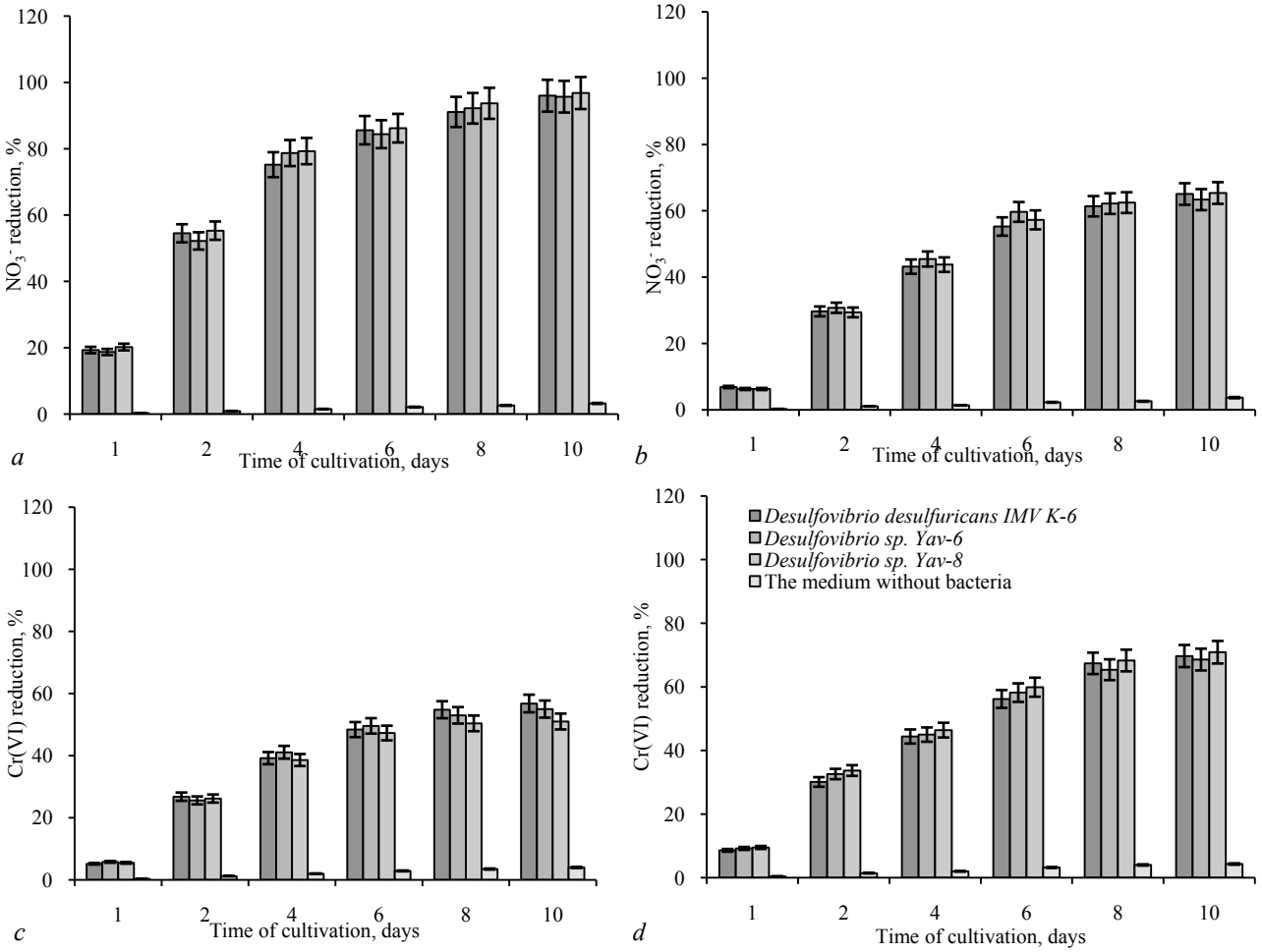

Fig. 7. Efficiency of 3.47 mM NO_3^- (*a*, *b*) or 3.47 mM Cr(VI) (*c*, *d*) reduction by *Desulfovibrio* sp. after 10 days of growth in the media with $NaNO_3$ (*a*), $NaNO_3$ and $K_2Cr_2O_7$ (*b*, *c*), $K_2Cr_2O_7$ (*d*) and in the same media without bacteria (x ± SD, n = 3)

Discussion

Studies of metabolic processes carried out by sulfidogenic bacteria of contaminated ecosystems are important because they determine the functioning of microbocenoses of these areas and are an important factor in evaluation of ecological status of transformed biotopes. Sulfate reducing bacteria actively grow in water or other environments with low redox potential, they have a unique metabolic property – the ability to transfer hydrogen from organic substrates to sulfate ions as the terminal electron acceptor and reduce them to H_2S, which is a dangerous factor of environmental pollution. Hydrogen sulfide inhibits the processes of anaerobic respiration in microorganisms, damages the structure of metalloproteins and sulfur-containing proteins, disrupts the mitochondrial respiration in eukaryotes due to depolarization of mitochondrial membranes or inhibition of cytochrome oxidase – a key enzyme in the respiratory chain (Kuznetsov et al., 2015; Basniwal et al., 2017). Sulfate reducing bacteria play an important role in the regulation the level not only of sulfur and carbon compounds, but also of nitrogen and metals in the environment. However, contamination of the environment with heavy metals negatively affects the physiological and biochemical processes carried out by these bacteria (Kozlova et al., 2008; Moroz et al., 2016). The efficiency of biological methods of environmental purification depends not only on the metabolic activity of selected strains of bacteria, but also on their resistance to contaminants.

Nitrates, nitrites and other oxidized nitrogen compounds (dinitrogen and nitrogen oxides, nitrogen dioxide, peroxyacetyl nitrates) are among the most dangerous pollutants of the environment. The main toxic effect of nitrates and nitrites on eukaryotes is the conversion of hemoglobin to

methemoglobin, which is unable to carry oxygen. In addition, nitrites in eu- and prokaryotic cells cause changes in extracellular and intracellular levels of Cl^- and K^+, which causes a strong electrolyte imbalance, as well as the formation of N-nitroso compounds (nitrosamines), which are mutagenic and carcinogenic (Camargo & Alonso, 2006; Kuypers et al., 2018).

Although at simultaneous presence in medium of SO_4^{2-} and oxidized form of chromium bacteria were used to a greater extent than $K_2Cr_2O_7$, at all investigated concentrations it displays on microorganisms more or less expressive toxic action, which was shown in inhibition of their dissimilatory sulfate reduction. Despite the fact that the reduction of metal oxidants by membrane-bound metal reductases is mainly carried out outside the cell (Gescher & Kappler, 2012; Simonte et al., 2017), with increasing the concentration of soluble $K_2Cr_2O_7$ in the medium increase in the degree of Cr(VI) penetration through the cytoplasmic membrane of bacteria into the cytoplasm, where its interaction with intracellular metabolites occurs, oxygen radicals are formed, Cr(III) accumulates as a reduced end product, which causes inhibition of bacteria growth and their metabolic activity (Richter et al., 2012; Viti et al., 2014).

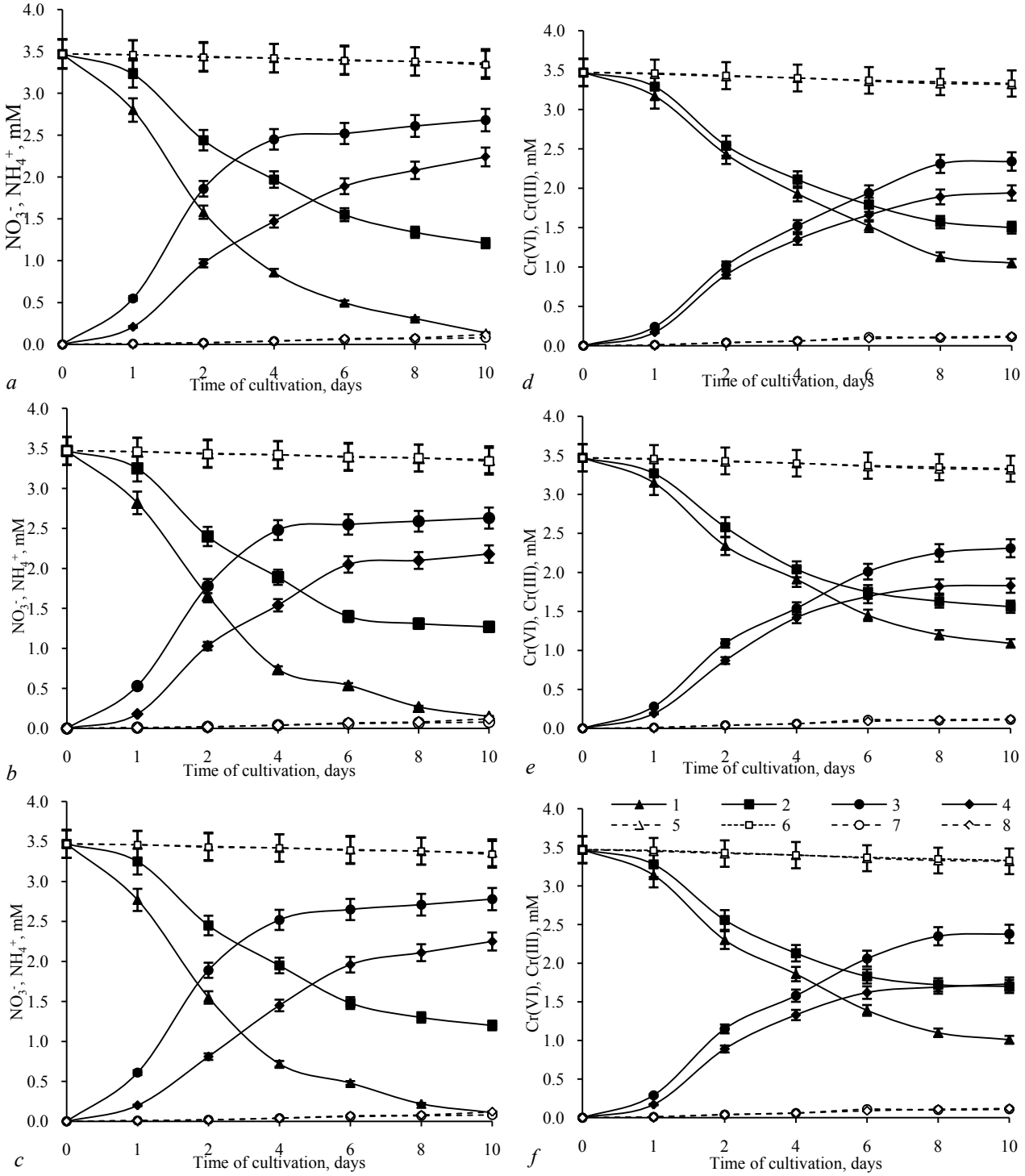

Fig. 8. Residual content of NO_3^- or Cr(VI) (*1*, *2*, *5*, *6*) and concentrations of NH_4^+ or Cr(III) (*3*, *4*, *7*, *8*) in cultural liquid during growth *Desulfovibrio desulfuricans* IMV K-6 (*a*, *d*), *Desulfovibrio* sp. Yav-6 (*b*, *e*) and *Desulfovibrio* sp. Yav-8 (*c*, *f*) in the media with $NaNO_3$ or $K_2Cr_2O_7$ (*1*, *3*), $NaNO_3$ and $K_2Cr_2O_7$ (*2*, *4*) and in the media with $NaNO_3$ or $K_2Cr_2O_7$ (*5*, *7*), $NaNO_3$ and $K_2Cr_2O_7$ (*6*, *8*) without bacteria (x ± SD, n = 3)

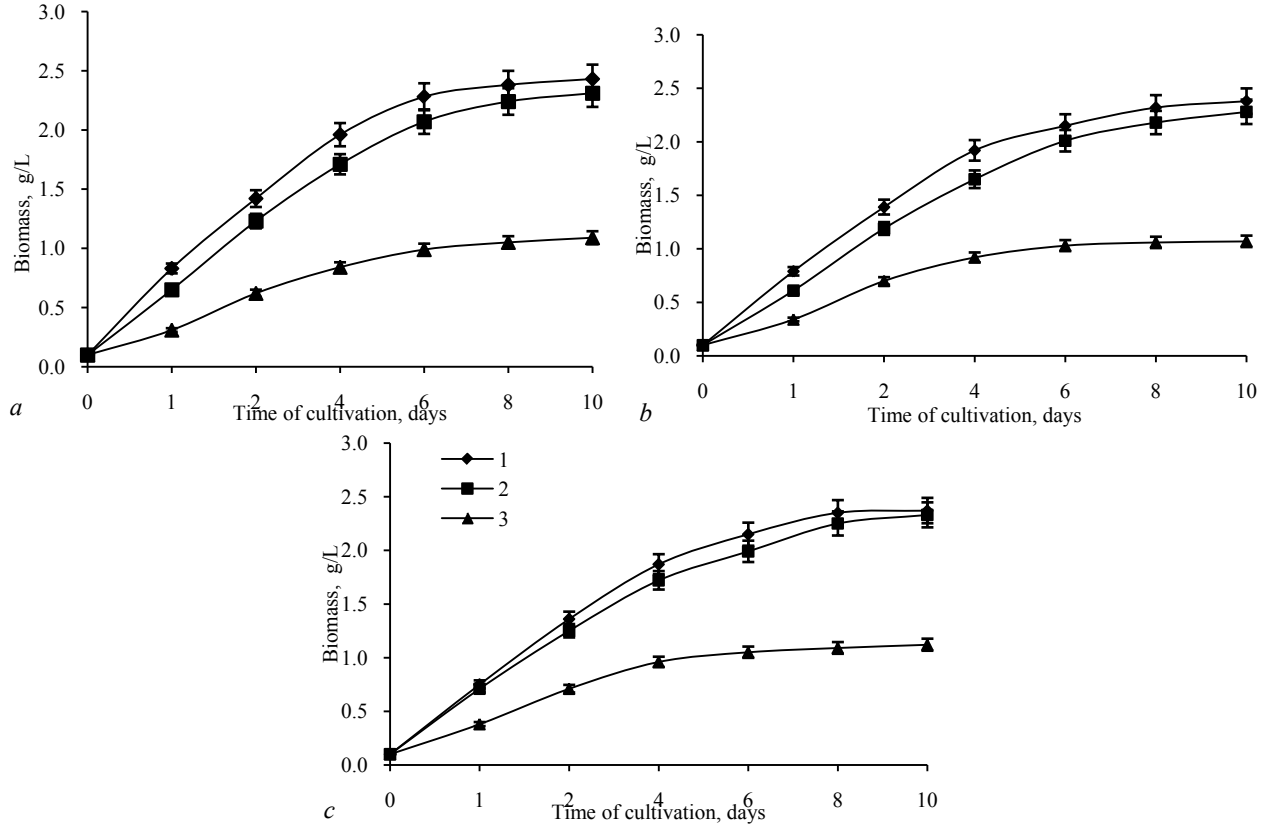

Fig. 9. Biomass accumulation by *Desulfovibrio desulfuricans* IMV K-6 (*a*), *Desulfovibrio* sp. Yav-6 (*b*) and *Desulfovibrio* sp. Yav-8 (*c*) during growth in media with $NaNO_2$ (*1*), $NaNO_2$ and $K_2Cr_2O_7$ (*2*), $K_2Cr_2O_7$ (*3*) (x ± SD, n = 3)

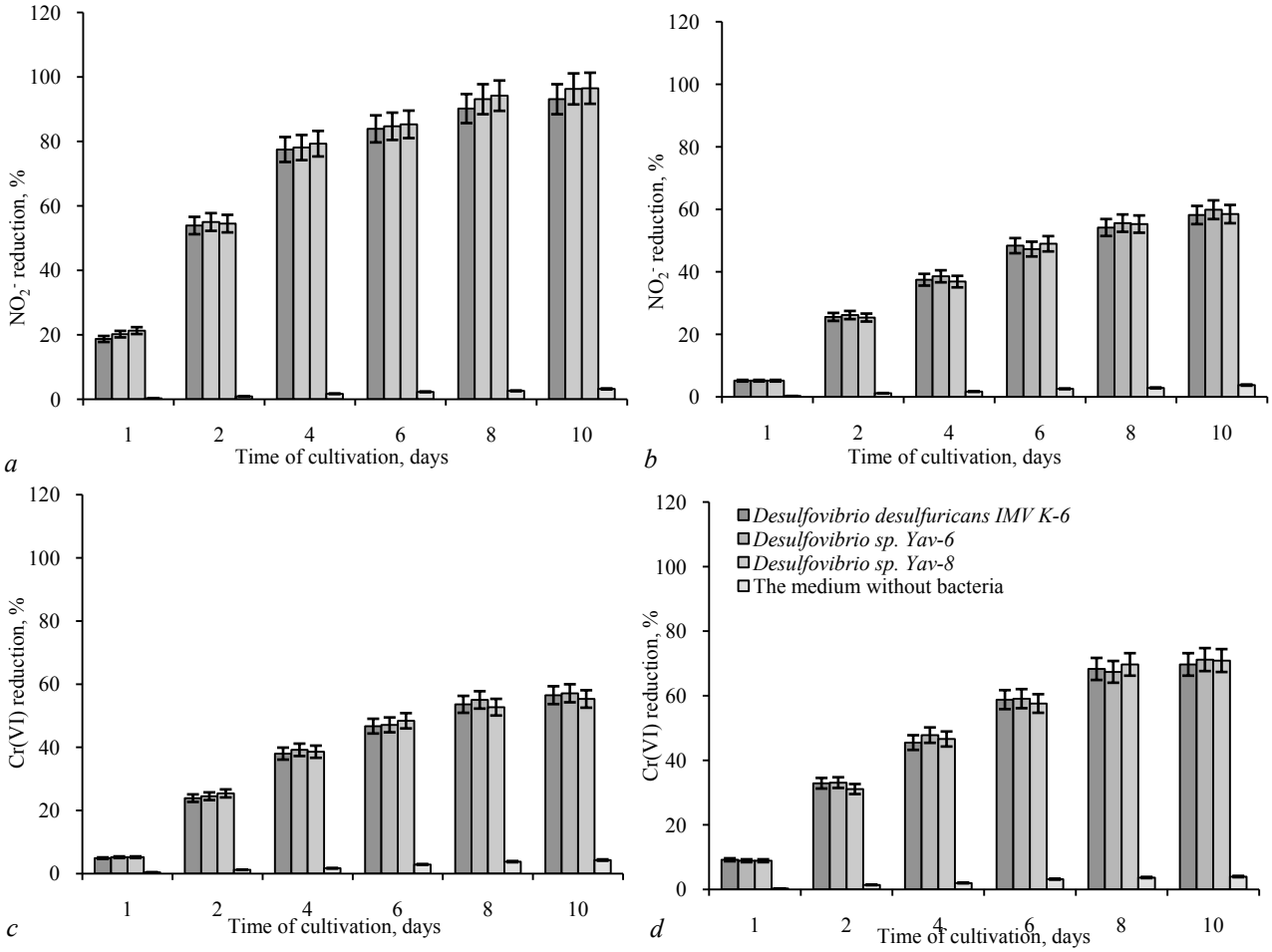

Fig. 10. Efficiency of 3.47 mM NO_2^- (*a*, *b*) or 3.47 mM Cr(VI) (*c*, *d*) reduction by *Desulfovibrio* sp. after 10 days of growth in the media with $NaNO_2$ (*a*), $NaNO_2$ and $K_2Cr_2O_7$ (*b*, *c*), $K_2Cr_2O_7$ (*d*) and in the same media without bacteria (x ± SD, n = 3)

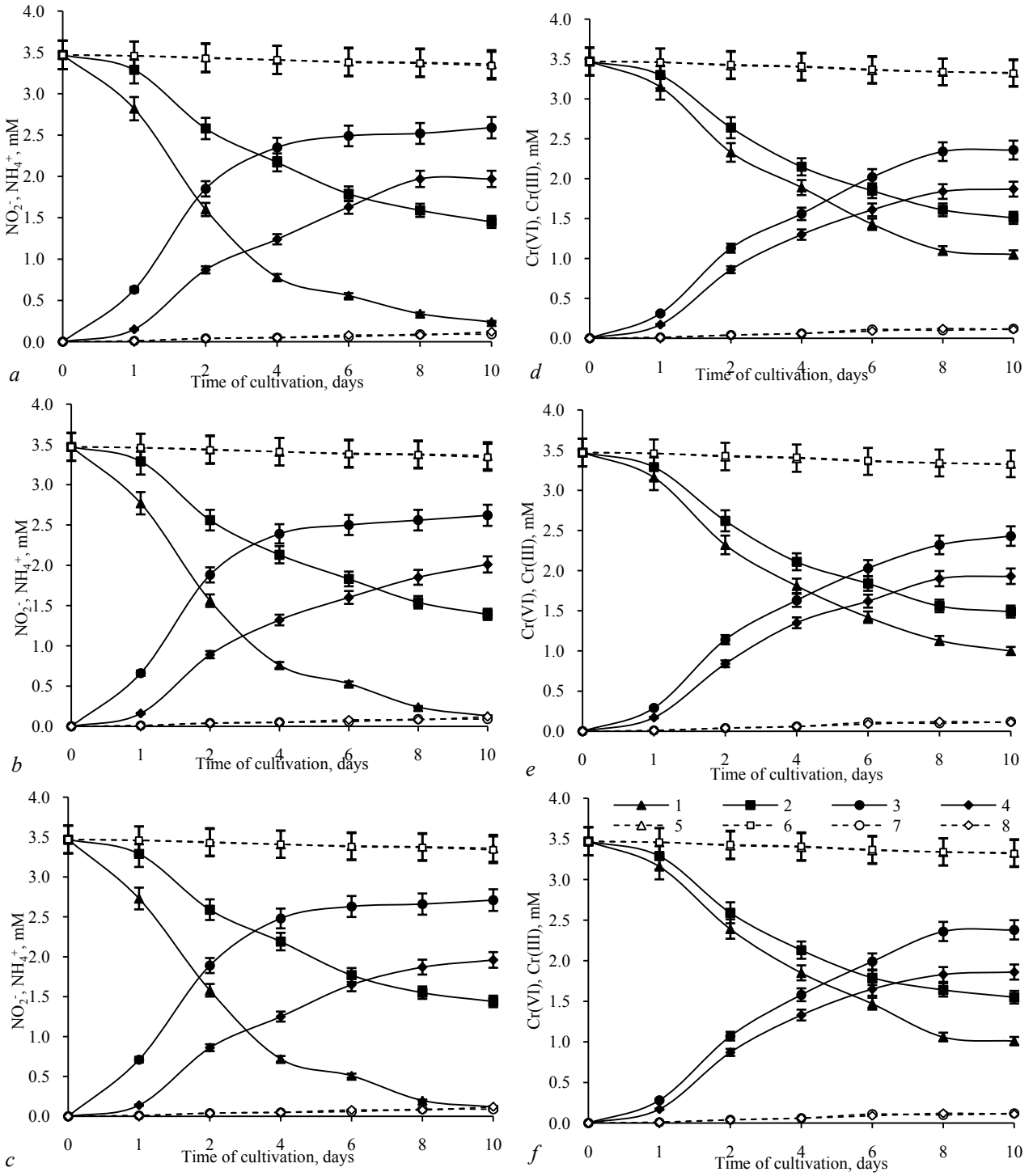

Fig. 11. Residual content of NO_2^- or Cr(VI) (*1*, *2*, *5*, *6*) and concentrations of NH_4^+ or Cr(III) (*3*, *4*, *7*, *8*) in cultural liquid during growth *Desulfovibrio desulfuricans* IMV K-6 (*a*, *d*), *Desulfovibrio* sp. Yav-6 (*b*, *e*) and *Desulfovibrio* sp. Yav-8 (*c*, *f*) in the media with $NaNO_2$ or $K_2Cr_2O_7$ (*1*, *3*), $NaNO_2$ and $K_2Cr_2O_7$ (*2*, *4*) and in the media with $NaNO_2$ or $K_2Cr_2O_7$ (*5*, *7*), $NaNO_2$ and $K_2Cr_2O_7$ (*6*, *8*) without bacteria (x ± SD, n = 3)

In the medium with the same initial content (3.47 mM) of NO_3^- and Cr(VI) bacteria reduced 1.2–1.3 times more nitrate ions than Cr(VI), and in the medium with the same content (3.47 mM) of NO_2^- and Cr(VI) strains reduced up to 1.1 times more nitrite ions than Cr(VI). Nevertheless, $K_2Cr_2O_7$ at all concentrations in the medium showed an inhibitory action on nitrate and nitrite reduction, which was carried out by the investigated strains of bacteria. The negative influence of Cr(VI) on the activity of molybdenum-containing membrane-bound respiratory or dissimilatory nitrate reductase (Morozkina & Zvyagilskaya, 2007), as well as periplasmic nitrite reductase, containing siro heme as prosthetic group (Keith & Herbert, 1983; Xia et al., 2018), in bacteria of the *Desulfovibrio* genus can be due to damage to the cytoplasmic membrane structure or modification of active conformation and denaturation of the protein molecule as a result of the replacement of the necessary metal ion by the chromium in the active center of the enzyme. Although at pH 7.0 the standard oxidation-reduction potential of the Cr(VI)/Cr(III) pair (E_0' = +1.33V) is higher than that of the oxidation-reduction NO_3^-/NO_2^- pair (E_0'=+0.78 V), and higher than that of NO_2^-/NH_4^+ pair (E_0'=+0.34 V) (Lengeler et al., 2005; Richter et al., 2012), the efficiency of electron acceptor reduction by microorganisms is primarily determined by the difference between the donor and electron acceptor potentials, which depend on the pH of the medium and change during cultivation of bacteria (Gescher & Kappler, 2012). Sulfate reducing bacteria of the *Desulfovibrio* genus oxidize organic substrates only to acetate; among them are species able to use lactate, pyruvate, malate, fumarate, citrate, ethanol, butanol, glucose, molecular hydrogen, and formate as electron donors (Lengeler et al., 2005). Therefore, the energy supply of

cells in the process of anaerobic respiration depends not only on the oxidation-reduction potential of the electron acceptor present in the medium, but also on the ways of ATP synthesis in the process of electron donor oxidation: by substrate level or oxidative phosphorylation (Lengeler et al., 2005; Rosenberg et al., 2014; McKinlay et al., 2020).

The processes of nitrate and nitride reduction, carried out by the bacteria of the *Desulfovibrio* genus, were revealed to be less sensitive to the negative influence of sodium dichromate, as compared with the process of sulfate ions reduction. When the sulfate ions' reduction by bacteria in the presence of 1.74–10.41 mM Cr(VI) decreased by 3.2–4.6 times, then the nitrate ions' reduction was by 1.2–1.9 times, nitrite ions was by 1.5–2.7 times, in comparison with their reduction in the media only with $Na_2SO_4\times 10H_2O$, $NaNO_3$ or $NaNO_2$ respectively. It is possible that under conditions of growth in the medium with hexavalent chromium and sulfate, nitrate or nitrite ions, nitrate and nitrite reductases of the investigated strains are less sensitive to the negative influence of Cr(VI) than the cytoplasmic enzymes involved in the sulfate respiration of these bacteria: ATP sulfurylase, pyrophosphatase, APS reductase, sulfite reductase as described (Lengeler et al., 2005; Morozkina & Zvyagilskaya, 2007; Sobol & Schiestl, 2012). This can be explained by the fact that at high concentrations in the medium Cr(VI) can interact not only with functional groups of a number of bacteria cellular metabolites, but also cause oxidative stress (Viti et al., 2014).

During anaerobic growth in medium with SO_4^{2-} and Cr(VI), bacteria to a greater extent use a powerful oxidizing agent, such as hexavalent chromium, in preference to sulfates. This could confirm the assumption that when more than one potential source for energy is available, bacterial regulatory mechanisms ensure that the thermodynamically most favourable electron acceptors are used first (Cadby et al., 2017). But bacteria, normally associated with environments that have, in addition to oxidized forms of metals, less-powerful electron acceptors, such as nitrates or nitrites, use a thermodynamically more favourable electron acceptor, such as hexavalent chromium, to a lesser extent, possibly due to its high toxicity.

Despite the fact that the reduction of 1.74–10.41 mM Cr(VI) by cells in media with $Na_2SO_4\times 10H_2O$, $NaNO_3$ or $NaNO_2$ decreased by 1.2–2.2, 1.1–2.2 and 1.2–2.2 times, respectively, compared with its reduction in the medium only with $K_2Cr_2O_7$, the results obtained by us suggest that the investigated strains of bacteria are adapted to high concentrations of hexavalent chromium compounds (up to 10.41 mM) and therefore can survive in environments contaminated by heavy metals. Due to the ability to carry out reductive transformation of chromium, sulfur and nitrogen compounds, isolated strains are promising for application in technologies of complex purification of environments from dangerous toxicants.

Conclusion

Sulfate reducing bacteria, oxidizing organic compounds, besides sulfates, can use other electron acceptors in the process of anaerobic respiration. They are oxidized metal forms, in particular, chromium, nitrates or nitrites, which are harmful for organisms. In the media with $Na_2SO_4\times 10H_2O$ and $K_2Cr_2O_7$ at all tested concentrations the bacteria reduced more Cr(VI) than SO_4^{2-}. In the media with $NaNO_3$ or $NaNO_2$ and $K_2Cr_2O_7$ at all concentrations the bacteria reduced more NO_3^- or NO_2^-, than Cr(VI). Nevertheless, $K_2Cr_2O_7$ at all concentrations in the medium showed a toxic effect on dissimilatory sulfate, nitrate and nitrite reduction, carried out by bacteria. Due to the exoelectrogenic properties the investigated strains of *Desulfovibrio* sp., which demonstrated the high metal reducing activity even in the media with two various electron acceptors, can be applied as the anode biocatalysts in microbial fuel cells for the formation of electric current during the oxidation of organic matter. The resistance of the strains of *Desulfovibrio* genus, isolated from the Yavorivske Lake, to different pollutants can be the basis for their application in bioremediation technologies.

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