Reduction of sulfur and oxidized forms of nitrogen by bacteria of *Desulfuromonas* sp., isolated from Yavorivske Lake, under the influence of ferrum citrate

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Technogenic reservoirs mainly contain several possible electron acceptors of anaerobic respiration, many of which are dangerous to the environment. The succession of their reduction (and thus detoxification) by sulfur reducing bacteria is not yet sufficiently studied. We investigated the influence of ferrum (III) citrate, present in the cultivation medium, on the reduction of sulfur, nitrate and nitrite ions by sulfur reducing bacteria *Desulfuromonas acetoxidans* IMV B-7304, *Desulfuromonas* sp. Yavor-5 and *Desulfuromonas* sp. Yavor-7, isolated from Yavorivske Lake. It was established that ferrum (III) citrate inhibited the biomass accumulation and hydrogen sulfide production by bacteria of *Desulfuromonas* sp. after simultaneous addition to the medium of 3.47 mM S0 and 1.74–10.41 mM ferrum (III) citrate, as compared with growth and hydrogen sulfide production by bacteria in the medium with only sulfur. In the medium with the same initial content (3.47 mM) S0 and ferrum (III) citrate bacteria produced ferrum (II) ions at concentrations 3.5–3.9 times higher than that of hydrogen sulfide. Ferrum (III) citrate inhibits the biomass accumulation, the nitrate or nitrite ions reduction and the ammonium ions production by bacteria of *Desulfuromonas* sp. after simultaneous addition to the medium of 3.47 mM NaNO3 or NaNO2 and 1.74–10.41 mM ferrum (III) citrate. In the medium with the same initial content (3.47 mM) NaNO3 and ferrum (III) citrate, bacteria produced ammonium ions at concentrations 1.1 times higher than that of ferrum (II) ions. In the medium with the same initial content (3.47 mM) NaNO3 and ferrum (III) citrate, bacteria reduced 1.5–1.6 times more ferrum (III) than nitrite ions with production of ferrum (II) ions at concentrations 1.7 times higher than that of ammonium ions. The process of nitrate reduction carried out by bacteria of *Desulfuromonas* genus was less sensitive to the negative influence of ferrum (III) citrate, compared to the process of nitrite ions reduction. When the reduction of nitrate ions by bacteria in the presence of 1.74–10.41 mM ferrum (III) citrate decreased by 1.4–2.2 times, then the reduction of nitrite ions decreased by 1.8–3.2 times compared to their reduction in media with only NaNO3 or NaNO2, respectively. Although the reduction of ferrum (III) by cells in media with 3.47 mM S0, NaNO3 or NaNO2 and 1.74–10.41 mM ferrum (III) citrate decreased by 1.6–2.7, 1.6–2.7 and 1.1–2.2 times, respectively, compared to the reduction in medium with only ferrum (III) citrate, the investigated strains of bacteria were resistant to high concentrations of trivalent ferrum compounds and can therefore be used in technologies of complex purification of environments polluted by heavy metal and nitrogen compounds.

**Keywords**: sulfur reducing bacteria; electron acceptors; ferrum; sulfur; nitrates; nitrites.

**Introduction**

Sulfidogenic bacteria of the *Desulfuromonas* genus in the process of anaerobic respiration can use sulfur, oxidized forms of nitrogen or heavy metals, in particular, ferrum (III) as electron acceptors. Anthropogenically altered environments are most often contaminated with many organic and inorganic toxic compounds. Microorganisms are involved in their oxidative and reductive transformation.

The Yavoriv state mining and chemical enterprise “Sulfur” with an area of 74 km2 ceased industrial activity in 1993. The open-pit mining of sulfur ore in the 1970s led to the formation of one of the largest quarries in the world, covering an area of 1,080 hectares. Since 1998, a large area of the deposit, including the central well and the adjacent branches of quarry, has been flooded with water. As a result, Yavorivske Lake with an area of more than 700 hectares and a depth of more than 90 m was formed (Baran et al., 2003; Gudz et al., 2004; Gaidin & Zozulia, 2009). Because of the open-pit mining of sulfur, components of the natural evolutionarily formed biogeocenosis were destroyed. The technogenic landscape was formed, the recultivation of which led to the formation of a qualitatively new biogeocenosis, in particular, microbocenosis. Its components are constantly interacting with each other and with the rock brought to the surface from previously unavailable depths. New hydrogeological and hydrological conditions are formed, and a new grouping of microorganisms is created that does not have analogues in nature. A distinctive feature of the sulfur quarry is the presence of aquifers in the sulfate-carbonate layers of the Neogene, which contain large amounts of hydrogen sulfide (Gaidin & Zozulia, 2009). Therefore, the metabolic processes carried out by sulfidogenic bacteria determine the functioning of the microbocenosis of the lake area and are an essential factor in evaluation of the ecological status of the transformed biotope (Moroz et al., 2008; Tarabas et al., 2017).

Heavy metals, hydrogen sulfide, nitrates, nitrites and other oxidized nitrogen compounds are the most dangerous pollutants of the environment. A considerable share of them is not included in the natural cycle, accumulates in the biosphere and causes a negative influence on the structure of environment and man. In the water of Yavorivske Lake at all depths the concentrations of compounds of sulfur (SO42− up to 1529–1725 mg/L, H2S up to 34.0–47.2 mg/L), nitrogen (NO3– up to 2.5 mg/L, Fe(II) up to 0.243 mg/L, Co (II) up to 0.144 mg/L, Cu (II) up to 0.031 mg/L) and heavy metals (Mn (II) up to 0.243 mg/L, Cd (II) up to 0.031 mg/L, Cd (II) up to 0.031 mg/L, Cd (II) up to 0.031 mg/L, Cd (II) up to 0.031 mg/L, Cd (II) up to 0.031 mg/L, Cd (II) up to 0.031 mg/L, Cd (II) up to 0.031 mg/L) exceeded the maximum admissible norms. Chemical methods of purification of technogenically altered environments are ineffective and energy consuming. The development of new microbial biotechnologies directed at decreasing the content of inorganic toxicants in technogenic ecosystems is an especially relevant task. Strains of microorganisms, in particular, representatives of the sulfidogenic microbiota, isolated from contaminated environments, are adapted to stress factors, so they are most suitable.
Sulfur reducing bacteria of the Desulfuromonas genus are obligate anaerobes, they use elemental or polysulfide sulfur, nitrates, nitrates, L-malate, fumarate, tri- or tetrachlorehylene, oxidized forms of heavy metals as electron acceptors (Hedderich et al., 1999; Sung et al., 2003; Kuever et al., 2005; An & Picard, 2015); oxidizing at the same time a number of simple organic compounds to CO₂ (Maslovska & Hnutash, 2013; Vasyliv et al., 2015). Sulfidogenic bacteria attract the attention of researchers as potential agents for the purification of waters contami- nated with hydrogen sulfide and heavy metals. Bacteria of the Desulfu- romonas genus as a result of dissimilatory sulfur reduction produce the hydrogen sulfide that interacts with divalent metal ions with formation of insoluble sulfides, which are thus removed from the natural cycle. These bacteria oxidize organic substrates using metals with variable valence as electron acceptors (Moroz et al., 2014, 2016); reduce and transform them into forms non-toxic or less toxic for living organisms (Vasyliv et al., 2011; Bily et al., 2014; Maslovska et al., 2015). Bacteria of the Desulfuromonas genus contain a large number of various types of cytochromes (Rosenberg et al., 2014). In Desulfuromonas acetoxidans, tri-heme c-type cytochrome c7 is involved not only in sulfur reduction but also functions as Fe(III) reductase (Rodén & Lovley, 1993). Bacteria reduce nitrates and nitrates with the participation of NADH2, NADPH or reduced quinone to ammonium (carry out their ammonification) (Lenge- ler et al., 2005, Kozlova et al., 2008). NarGH nitrate reductase is an enzyme complex consisting of multi-heme b-type cytochrome, proteins with Fe-S clusters and Mo-containing cofactor (Morozkina & Zvyagilskaya, 2007; Kozlova et al., 2008). Nitrate reduction with the forma- tion of nitrates and their subsequent reduction by a complex of periplas- mic dissimilatory nitrite reductases to NH₄⁺ in Helonella succinogenes and Desulfuromonas sp. is described (Bokranz et al., 1983; Chyka & Pere- tyatko, 2018). Most representatives of sulfidogenic bacteria have a non- specific metal reductase enzyme system that allows them to use compo- nents of Fe(III), Mn(IV), U(VI), Cr(VI), Cu(I) and other metals as electron acceptors of anaerobic respiration (Kozlova et al., 2008). Solu- ble and insoluble metal compounds are reduced outside the cells 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, allowing these exoelectrogenic anaerobic bacteria to be used in the microbial fuel cells (MFC) as the high effective anode biocatalysts (Fitzgerald et al., 2013; Prokhorova et al., 2017; Simonet et al., 2017). Electric current generation by Desulfuromonas acetoxidans IMV B-7384 in a MFC was described (Bily et al., 2014; Vasyliv et al., 2015), and the interaction between Fe(III) reduction and exoelectrogenesis performed by this bacte- ria in the MFC was established (Vasyliv et al., 2016). Metal ions or oxoa- cids can be reduced by microorganisms not only on the cell surface but also in the internal compartments, in the periplasm and cytoplasm (Richter et al., 2012). In cells, they interact with intracellular redoxants (such as amino acids, nucleotides, sugars, organic acids, glutathione, flavon- enzymes, vitamins), generate chemical active intermediates, free radicals and can cause oxidative stress (Vit et al., 2012; Maslovska & Hnutash, 2015; Hnutash & Maslovska, 2018).

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). In various microor- ganisms, the succession of reduction of elements with variable valence is not always determined by their oxidation-reduction potential, but determined genetically and controlled by complex regulatory mechanisms (Lengeler et al., 2005; Rosenberg et al., 2014). Previously, we have shown that bacteria of the Desulfuromonas genus, isolated from Yavorivske La- ke, in addition to sulfur or oxidized forms of nitrogen, can use oxidized forms of heavy metals, in particular, ferrum (III) as terminal electron ac- ceptors (Moroz et al., 2014, 2016). The purpose of this work was to inves- tigate the regularities of sulfur, nitrate or nitrite ions usage by strains of Desulfuromonas sp. bacteria at conditions of simultaneous presence in the medium of another electron acceptor – ferrum (III), to establish the suc- cession of electron acceptors reduction and to determine the efficiency of their possible application in technologies for complex purification of the environment from inorganic toxicants.

Materials and methods

Sulfur reducing bacteria Desulfuromonas acetoxidans IMV B-7384, Desulfuromonas sp. Yavor-5 and Desulfuromonas sp. Yavor-7, isolated by us earlier from the Yavorivske Lake, were identified at the Microbiolo- gy Department of Ivan Franko National University of Lviv (Gudz et al., 2013; Moroz et al., 2013).

Bacteria were grown in Krvats-Sorokin medium (Gudz et al., 2014) without SO₄²⁻ and without Mohr’s salt of such composition (g/L): NaH₂PO₄·12H₂O (0.84), K₂HPO₄ (0.5), NH₄Cl (0.16), MgCl₂·6H₂O (0.10), sodium lactate (NaC₆H₅O₇·2H₂O) (2.0) or sodium citrate (NaC₆H₅O₇) (4.6). Before bacteria seeding 0.05 mL of Na₂SO₄·9H₂O (1%) sterile solu- tion 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 medium to initial cells concentration of 0.1 mg/mL. The sulfur was sterilized sepa- rately (0.5 atm) and placed in medium as weighted quantities at concentra- tion of 3.47 mM (concentration of SO₂⁻ in medium of standard composition). 1 M solutions of CH₃NO₂, NaNO₃, NaNO₂ and FeC₆H₅O₇ were sterilized separately and placed into the medium before seeding of the cells. Into media with CH₃NO₂, NaNO₃ and FeC₆H₅O₇ or without it the NH₄Cl was not added. Bacteria were grown for 10 days in test tubes (25 mL), compli- totally topped up by the medium, at a temperature of 30°C.

To determine the efficiency of sulfur, nitrate, nitrite ions or Fe(III) reduction at simultaneous presence in the medium of two electron ac- ceptors (S², NO₃⁻ or NO₂⁻ and Fe(III)), 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. They were sown in the medium with sodium citrate (17.86 mM), to which weighted quantities of insoluble in water sterile S² and sterile solutions of NaNO₃ or NaNO₂ were added to their final concentration in the medium of 3.47 mM and different volu- mes of the sterile FeC₆H₅O₇ solution to its final concentrations of 1.74, 3.47, 5.21, 6.94 and 10.41 mM, which differs by 0.5, 1.0, 1.5, 2.0 and 3.0 times from the standard electron acceptor content in Krvats-Sorok- kin medium. The cells were also sown in a medium with sodium citrate, to which were added weighted quantities of S², solutions of NaNO₃, NaNO₂ or FeC₆H₅O₇ to their final concentration in the medium of 3.47 mM, to test the bacteria growth in media with sulfur, nitrate, nitrite ions or Fe(III) as the sole electron acceptor (control). Into the medium without cells the weighted quantities of S², solutions of NaNO₃, NaNO₂ or FeC₆H₅O₇ were added at concentration of 3.47 mM to verify their spontaneous reduction. Biomass, the concentrations of nitrate or nitrite, ferrum (II) ions, Fe(III), hydrogen sulfide or ammonium ions in cultural liquid were determined on 10 day of growth. To determine the concentra- tions of ferrum (II) ions and hydrogen sulfide, the precipitate of FeS, which formed during bacterial evolution in medium with S² and FeC₆H₅O₇, was dissolved after interaction with HCl according to the equation: FeS + 2HCl → FeCl₂ + H₂S (the HCl concentration exceeded twice the concen- tration of S² in the medium and was 6.94 mM). By the difference between the initial and residual content of electron acceptors in the medium the efficiency (%) of their reduction by bacteria was calculated, based on the ratio of molar concentrations of reduced by bacteria nitrate, nitrite ions or Fe(III) in the process of anaerobic respiration and their concentrations at the beginning of cultivation, which were taken as 100%.

Biomass was determined by the turbidimetric method by the optical density of the cell suspension by measuring it at a wavelength of 540 nm in a cuvette with an optical way of 3 mm and calculated using the formula: C, g/L = (E₅₄₀ + n)K, where E₅₄₀ = extinction (λ = 540 nm); n – dilution factor; K – coefficient of recalculating, obtained from the calibration curve of the dependence of extinction from the mass of dry cells, determined by the weight method, and equal to 0.72 (Gudz et al., 2014). In a cultural liquid, separated from the cells by centrifugation (4025 g, 15 min), we determined the concentrations of nitrate ions (after their reduction to ni-
trites in the presence of ZnMnSO4 (1:100 powder as a reducing agent) and nitrite ions by spectrophotometric method which relies on a diazotiza-

Fig. 1. Efficiency of Fe(III) reduction by Desulfuramos sp. after 10 days of growth in media with S0 or Fe3C6H5O7 (M ± m, n = 3); designations on the horizontal axis: control – 3.47 mM Fe(III); 1 – 3.47 mM S0 and 1.74 mM Fe(III); 2 – 3.47 mM S0 and 3.47 mM Fe(III); 3 – 3.47 mM S0 and 5.21 mM Fe(III); 4 – 3.47 mM S0 and 6.94 mM Fe(III); 5 – 3.47 mM S0 and 10.41 mM Fe(III); 6 – 3.47 mM Fe(III) (without bacteria); * – P < 0.05

Experiments were repeated three times with three parallel formulations for each variant of experimental and control conditions. The reliability of the difference was evaluated using ANOVA. Differences between the samples were considered reliable at P < 0.05.

Table 1

| Strain | Electron acceptors of anaerobic respiration | Residual content of Fe(III) in cultural liquid, mM | Fe(II), mM | S0, mM | Biomass, g/L |
|--------|------------------------------------------|-----------------------------------------------|------------|--------|-------------|
| 3.47 mM S0                          | 0                                         | 1.60 ± 0.03                                  | 2.37 ± 0.02 |
| 3.47 mM S0 (wb)                      | 0                                         | 0.04 ± 0.01                                  | 0          |
| D. acetoxidans IMB-7384               | 3.47 mM S0 and 1.74 mM Fe(III)             | 1.51 ± 0.02                                  | 2.55 ± 0.01 |
| 3.47 mM S0 and 3.47 mM Fe(III)       | 0.08 ± 0.01                               | 2.90 ± 0.02                                  | 2.36 ± 0.09 |
| 3.47 mM S0 and 5.21 mM Fe(III)       | 0.38 ± 0.09                               | 3.98 ± 0.04                                  | 2.11 ± 0.04 |
| 3.47 mM S0 and 6.94 mM Fe(III)       | 0.93 ± 0.07                               | 3.15 ± 0.03                                  | 1.43 ± 0.07 |
| 3.47 mM S0 and 10.41 mM Fe(III)      | 3.74 ± 0.03                               | 3.43 ± 0.01                                  | 1.24 ± 0.02 |
| 3.47 mM S0 and 10.41 mM Fe(III)      | 0.28 ± 0.01                               | 2.94 ± 0.04                                  | 2.50 ± 0.02 |
| 3.47 mM Fe(III)                      | 3.29 ± 0.01                               | 0.17 ± 0.04                                  | 0          |
| 3.47 mM S0 (wb)                      | 0                                         | 1.68 ± 0.08                                  | 2.20 ± 0.09 |
| 3.47 mM S0 (wb)                      | 0                                         | 0.04 ± 0.01                                  | 0          |
| Desulfuramos sp. Yavor-5             | 3.47 mM S0 and 1.74 mM Fe(III)             | 1.57 ± 0.03                                  | 2.31 ± 0.02 |
| 3.47 mM S0 and 3.47 mM Fe(III)       | 0.11 ± 0.01                               | 2.89 ± 0.04                                  | 2.23 ± 0.08 |
| 3.47 mM S0 and 5.21 mM Fe(III)       | 0.50 ± 0.03                               | 3.89 ± 0.03                                  | 1.90 ± 0.01 |
| 3.47 mM S0 and 6.94 mM Fe(III)       | 1.12 ± 0.06                               | 3.89 ± 0.03                                  | 1.90 ± 0.01 |
| 3.47 mM S0 and 10.41 mM Fe(III)      | 3.68 ± 0.04                               | 3.10 ± 0.08                                  | 1.33 ± 0.09 |
| 3.47 mM S0 and 10.41 mM Fe(III)      | 5.88 ± 0.09                               | 4.40 ± 0.01                                  | 1.22 ± 0.06 |
| 3.47 mM Fe(III)                      | 0.39 ± 0.08                               | 2.93 ± 0.03                                  | 2.43 ± 0.06 |
| 3.47 mM Fe(III) (wb)                 | 3.29 ± 0.07                               | 0.17 ± 0.04                                  | 0          |
| 3.47 mM S0 (wb)                      | 0                                         | 1.84 ± 0.08                                  | 2.32 ± 0.03 |
| 3.47 mM S0 (wb)                      | 0                                         | 0.04 ± 0.01                                  | 0          |
| Desulfuramos sp. Yavor-7             | 3.47 mM S0 and 1.74 mM Fe(III)             | 1.64 ± 0.02                                  | 2.35 ± 0.09 |
| 3.47 mM S0 and 3.47 mM Fe(III)       | 0.02 ± 0.06                               | 2.99 ± 0.09                                  | 2.30 ± 0.06 |
| 3.47 mM S0 and 5.21 mM Fe(III)       | 0.22 ± 0.05                               | 3.49 ± 0.04                                  | 2.00 ± 0.03 |
| 3.47 mM S0 and 6.94 mM Fe(III)       | 1.61 ± 0.09                               | 3.49 ± 0.04                                  | 2.00 ± 0.03 |
| 3.47 mM S0 and 10.41 mM Fe(III)      | 3.14 ± 0.08                               | 3.62 ± 0.05                                  | 1.46 ± 0.01 |
| 3.47 mM S0 and 10.41 mM Fe(III)      | 6.91 ± 0.04                               | 3.41 ± 0.06                                  | 1.32 ± 0.05 |
| 3.47 mM S0 and 10.41 mM Fe(III)      | 0.42 ± 0.02                               | 2.87 ± 0.08                                  | 2.45 ± 0.07 |
| 3.47 mM Fe(III)                      | 3.29 ± 0.07                               | 0.17 ± 0.04                                  | 0          |
| 3.47 mM Fe(III) (wb)                 | 0                                         | 0          |

Note: (wb) – the medium without bacteria.

Results

To study the influence of ferrum (III) citrate on the sulfur reduction by sulfur reducing bacteria, they were grown in the medium with sodium citrate, to which 3.47 mM S0 and FeC6H5O7 at different concentrations were added. The bacteria were also grown in media with sodium citrate and 3.47 mM S0 or 3.47 mM FeC6H5O7 to test the use by bacteria of sulfur or ferrum (III) as the sole electron acceptor (Table 1). After 10 days of growth the biomass of bacteria in the medium with S0 was not significantly lower than in the medium with FeC6H5O7. After the simultaneous addition of S0 and Fe(III) to the cultivation medium with growth of ferrum (III) citrate concentrations, a gradual decreasing in the biomass accumulation by bacteria was observed, compared to growth in media with only S0 or FeC6H5O7. In the medium with S0 and 10.41 mM FeC6H5O7 the growth of bacteria decreased 1.8–2.0 times, compared with growth in the medium with only S0 or FeC6H5O7. In the medium with S0 and FeC6H5O7 with increasing of ferrum (III) citrate concentrations, a gradual decrease was observed in concentrations of hydrogen sulfide produced by bacteria, as compared with its production in the medium with only S0. In media with S0 and FeC6H5O7, the cells produced 0.45–0.94 mM hydrogen sulfide (control: 1.60–1.84 mM). The efficiency of Fe(III) reduction by bacteria in the medium with S0 and 1.74–5.21 mM FeC6H5O7 practically did not differ from their reduction in the medium with ferrum (III) citrate as the sole electron acceptor (87.9–91.9%), but was found to be 1.6–2.7 times lower at FeC6H5O7 concentrations in the medium of 6.94–10.41 mM (Fig. 1). In media with sulfur and ferrum (III) citrate, bacteria produced 1.51–4.40 mM of ferrum (II) ions (control: 2.87–2.94 mM) (Table 1). In the medium with S0 or FeC6H5O7 without bacteria, the efficiency of spontaneous sulfur and Fe(III) reduction was found to be insignificant and did not exceed 1.2 (calculated according to the produced H2S) and 5.2% respectively (Table 1, Fig. 2). Thus, it has been established that ferrum (III) citrate inhibits the biomass accumulation and hydrogen sulfide production by bacteria of Desulfuramos sp. after simultaneous addition into the medium of 3.47 mM S0 and 1.74–10.41 mM FeC6H5O7. In the medium with the same initial content (3.47 mM) S0 and FeC6H5O7, bacteria produced ferrum (II) ions at concentrations 3.5–3.9 times higher than that of hydrogen sulfide.

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After simultaneous addition of NaNO3 and FeC6H5O7. Strain Desulfuromonas sp. had a higher NO3−/Fe(II) ratio (3:47 mM Fe(ІІІ) and 3:47 mM NO3−, respectively) compared to its reduction in media with NaNO3 or FeC6H5O7 (M ± m, n = 3) (Fig. 2). Thus, the NO3−/Fe(II) ratio of Desulfuromonas sp. Yavor-5 was 1.6–2.7 times higher than their reduction by bacteria was also observed, compared with their reduction in the medium with only NaNO3 (92.7–94.8%) (Fig. 2a). In the medium with NaNO3 and FeC6H5O7, bacteria produced 0.71–2.09 mM of ammonia ions (control: 1.90–2.03 mM, Table 2). The efficiency of Fe(III) reduction by cells with increasing its concentrations in media with NaNO3 and FeC6H5O7 was revealed to be 1.6–2.7 times lower than their reduction in medium with only FeC6H5O7 (93.4–96.8%) (Fig. 2b). In contrast, in the medium with NaNO3 and FeC6H5O7, bacteria produced 0.32–3.28 mM of ferrum (II) ions (control: 2.33–2.38 mM, Table 2). In the medium with NaNO3 or FeC6H5O7 without bacteria, the efficiency of NO3− and Fe(III) reduction did not exceed 4.8% and 4.0%, respectively (Fig. 2). Thus, it has been shown that ferrum (III) citrate inhibits the biomass accumulation, the nitrate ions reduction and the ammonium ions production by bacteria of Desulfuromonas sp. after simultaneous addition into the medium of 3:47 mM NaNO3 and 1:74 mM FeC6H5O7. In the medium with the same initial content (3:47 mM) of NaNO3 and FeC6H5O7 bacteria produced ammonium ions at concentrations 1.1 times higher than that of ferrum (II) ions.

Table 2
Reduction of NO3− and Fe(III) by Desulfuromonas sp. after 10 days of growth in media with NaNO3 or FeC6H5O7 (M ± m, n = 3)

| Strain                   | Electron acceptors of anaerobic respiration | Residual content in cultural liquid, mM | NO3− | Fe(II) | NH4+, mM | Biomass, g/L |
|--------------------------|--------------------------------------------|----------------------------------------|------|--------|----------|-------------|
|                          |                                            |                                        |      |        |          |             |
| Desulfuromonas sp.       |                                            |                                        |      |        |          |             |
| Yavor-5                  | 3:47 mM NO3− and 1:74 mM Fe(III)           | 0.25 ± 0.06                            | 0    | 0      | 1.99 ± 0.02 | 2.49 ± 0.01 |
|                          | 3:47 mM NO3− and 3:47 mM Fe(III)           | 3.30 ± 0.03                            | 0    | 0      | 1.15 ± 0.01 | 0           |
|                          | 3:47 mM NO3− and 1:74 mM Fe(III)           | 1.22 ± 0.04                            | 0.74 ± 0.03 | 0.94 ± 0.01 | 2.09 ± 0.05 | 2.23 ± 0.03 |
|                          | 3:47 mM NO3− and 3:47 mM Fe(III)           | 1.31 ± 0.08                            | 1.45 ± 0.02 | 1.90 ± 0.02 | 2.03 ± 0.01 | 1.96 ± 0.05 |
|                          | 3:47 mM NO3− and 5:21 mM Fe(III)           | 1.54 ± 0.01                            | 2.50 ± 0.05 | 2.61 ± 0.02 | 1.24 ± 0.08 | 1.74 ± 0.01 |
|                          | 3:47 mM NO3− and 6:94 mM Fe(III)           | 1.73 ± 0.02                            | 4.01 ± 0.08 | 2.87 ± 0.06 | 1.02 ± 0.01 | 1.51 ± 0.01 |
|                          | 3:47 mM NO3− and 10:41 mM Fe(III)          | 1.89 ± 0.03                            | 6.09 ± 0.01 | 3.60 ± 0.04 | 0.71 ± 0.03 | 1.36 ± 0.02 |
|                          | 3:47 mM Fe(III)                            | 0                                      | 0.15 ± 0.02 | 3.25 ± 0.04 | 0          | 2.65 ± 0.05 |
|                          | 3:47 mM Fe(III)                            | 0                                      | 3.33 ± 0.06 | 0.14 ± 0.01 | 0          | 0           |
| D. acetoxidans IMV B-7384|                                            |                                        |      |        |          |             |
|                          | 3:47 mM NO3−                               | 0.22 ± 0.01                            | 0    | 0      | 2.03 ± 0.06 | 2.41 ± 0.03 |
|                          | 3:47 mM NO3−                               | 3.30 ± 0.01                            | 0    | 0      | 1.15 ± 0.01 | 0           |
|                          | 3:47 mM NO3− and 1:74 mM Fe(III)           | 1.19 ± 0.03                            | 0.71 ± 0.08 | 1.00 ± 0.01 | 1.91 ± 0.08 | 2.32 ± 0.07 |
|                          | 3:47 mM NO3− and 3:47 mM Fe(III)           | 1.31 ± 0.08                            | 1.48 ± 0.02 | 1.54 ± 0.08 | 1.89 ± 0.09 | 1.96 ± 0.08 |
|                          | 3:47 mM NO3− and 5:21 mM Fe(III)           | 1.49 ± 0.05                            | 2.30 ± 0.03 | 2.82 ± 0.04 | 1.31 ± 0.07 | 1.63 ± 0.01 |
|                          | 3:47 mM NO3− and 6:94 mM Fe(III)           | 1.62 ± 0.02                            | 3.71 ± 0.06 | 3.07 ± 0.04 | 1.08 ± 0.02 | 1.45 ± 0.03 |
|                          | 3:47 mM NO3− and 10:41 mM Fe(III)          | 1.88 ± 0.04                            | 5.99 ± 0.08 | 4.34 ± 0.09 | 0.82 ± 0.03 | 1.30 ± 0.01 |
|                          | 3:47 mM Fe(III)                            | 0                                      | 0.23 ± 0.04 | 3.23 ± 0.02 | 0          | 2.73 ± 0.04 |
|                          | 3:47 mM Fe(III)                            | 0                                      | 3.33 ± 0.05 | 0.14 ± 0.01 | 0          | 0           |

Notes: (wb) – the medium without bacteria; to the media with NO3− and Fe(III) or without if the NH4Cl was not added.
Reduction of NO₂⁻ and Fe(III) by Desulfuromonas sp. after 10 days of growth in media with NaNO₂ or FeC₆H₅O₇ (M ± m, n = 3): designation on the horizontal axis: control – 3.47 mM nitrite ions (a), 3.47 mM Fe(III) (b); 1 – 3.47 mM nitrite ions and 1.74 mM Fe(III); 2 – 3.47 mM nitrite ions and 3.47 mM Fe(III); 3 – 3.47 mM nitrite ions and 5.21 mM Fe(III); 4 – 3.47 mM nitrite ions and 6.94 mM Fe(III); 5 – 3.47 mM nitrite ions and 10.41 mM Fe(III); 6 – 3.47 mM nitrite ions (without bacteria) (a), 3.47 mM Fe(III) (without bacteria) (b); * – P < 0.05

Table 3
Reduction of NO₂⁻ and Fe(III) by Desulfuromonas sp. after 10 days of growth in media with NaNO₂ or FeC₆H₅O₇ (M ± m, n = 3)

| Strain                      | Electron acceptors of anaerobic respiration | Residual content in cultural liquid, mM NO₂⁻ | Fe²⁺, mM | NH₄⁺, mM | Biomass, g/L |
|-----------------------------|---------------------------------------------|---------------------------------------------|----------|----------|--------------|
|                             | D. acetoxidans IMV B-7384                    |                                             |          |          |              |
|                             | 3.47 mM NO₂⁻                                  | 0.08 ± 0.03                                 | 0         | 0.02 ± 0.03 | 3.22 ± 0.02  |
|                             | 3.47 mM NO₂⁻ (wb)                             | 3.35 ± 0.07                                 | 0         | 0.09 ± 0.05 | 0            |
|                             | 3.47 mM NO₂⁻ and 1.74 mM Fe(III)              | 1.60 ± 0.09                                 | 0.17 ± 0.01 | 1.46 ± 0.02 | 1.74 ± 0.04  |
|                             | 3.47 mM NO₂⁻ and 3.47 mM Fe(III)              | 1.71 ± 0.01                                 | 0.61 ± 0.03 | 2.77 ± 0.01 | 1.60 ± 0.01  |
|                             | 3.47 mM NO₂⁻ and 5.21 mM Fe(III)              | 1.87 ± 0.02                                 | 1.37 ± 0.07 | 3.71 ± 0.03 | 1.01 ± 0.08  |
|                             | 3.47 mM NO₂⁻ and 6.94 mM Fe(III)              | 2.25 ± 0.03                                 | 3.48 ± 0.01 | 3.34 ± 0.05 | 0.83 ± 0.02  |
|                             | 3.47 mM NO₂⁻ and 10.41 mM Fe(III)             | 2.36 ± 0.07                                 | 5.79 ± 0.02 | 4.58 ± 0.04 | 0.55 ± 0.03  |
|                             | D. acetoxidans IMV B-7384 (wb)                 | 0                                          | 0.24 ± 0.03 | 3.17 ± 0.07 | 0.08 ± 0.02  |
|                             | Desulfuromonas sp. Yavor-5                    | 3.47 mM NO₂⁻                                  | 0         | 3.40 ± 0.09 | 0.07 ± 0.04  |
|                             |                                            | 3.35 ± 0.07                                 | 0         | 0         |              |
|                             | 3.47 mM NO₂⁻                                  | 1.58 ± 0.04                                 | 0.20 ± 0.01 | 1.38 ± 0.06 | 1.70 ± 0.02  |
|                             | 3.47 mM NO₂⁻ and 3.47 mM Fe(III)              | 1.64 ± 0.06                                 | 0.68 ± 0.03 | 2.60 ± 0.04 | 1.54 ± 0.01  |
|                             |                                            | 1.75 ± 0.01                                 | 1.51 ± 0.01 | 3.60 ± 0.01 | 1.00 ± 0.03  |
|                             | 3.47 mM NO₂⁻ and 5.21 mM Fe(III)              | 2.31 ± 0.02                                 | 3.78 ± 0.04 | 3.02 ± 0.02 | 0.87 ± 0.01  |
|                             |                                            | 2.04 ± 0.04                                 | 5.86 ± 0.05 | 4.48 ± 0.01 | 0.58 ± 0.02  |
|                             |                                            | 0                                          | 0.17 ± 0.09 | 3.24 ± 0.03 | 0            |
|                             | 3.47 mM Fe(III)                               |                                            | 0         | 3.40 ± 0.09 | 0.07 ± 0.04  |
|                             | 3.47 mM Fe(III) (wb)                          | 0                                          | 0         | 0         |              |
|                             | Desulfuromonas sp. Yavor-7                    | 3.47 mM NO₂⁻                                  | 0.08 ± 0.08 | 0         | 2.05 ± 0.01  |
|                             |                                            | 3.35 ± 0.07                                 | 0         | 0         |              |
|                             |                                            | 1.60 ± 0.02                                 | 0.16 ± 0.07 | 1.40 ± 0.07 | 1.71 ± 0.01  |
|                             |                                            | 1.79 ± 0.01                                 | 1.30 ± 0.04 | 3.78 ± 0.02 | 1.02 ± 0.01  |
|                             |                                            | 2.13 ± 0.04                                 | 3.64 ± 0.01 | 3.26 ± 0.01 | 0.80 ± 0.02  |
|                             |                                            | 2.25 ± 0.04                                 | 5.58 ± 0.03 | 4.69 ± 0.03 | 0.54 ± 0.01  |
|                             |                                            | 0                                          | 0.12 ± 0.01 | 3.25 ± 0.07 | 0            |
|                             |                                            | 3.47 mM Fe(III) (wb)                         | 0         | 3.40 ± 0.09 | 0.07 ± 0.04  |
| Notes: (wb) – the medium without bacteria; to the media with NO₂⁻ and Fe(III) or without it the NH₄Cl was not added.

To investigate the influence of ferrum (III) citrate on the nitrite ions reduction by sulfur reducing bacteria, they were grown in the medium without NH₄Cl with sodium citrate to which 3.47 mM NaNO₂ and FeC₆H₅O₇ at different concentrations were added. The bacteria were also sown in media with sodium citrate and 3.47 mM NaNO₂ or 3.47 mM FeC₆H₅O₇ to study the usage by bacteria of nitrite ions or Fe(III) as the sole electron acceptor (Table 3). Biomass of bacteria in the medium with only NaNO₂ was revealed to be 1.2 times lower than in the medium with only FeC₆H₅O₇. After simultaneous addition into the medium of NaNO₂ and FeC₆H₅O₇ with increasing concentrations of the ferrum (III) citrate, a decrease in the bacteria growth was observed, compared with growth in the medium with only NaNO₂ or FeC₆H₅O₇. In the medium with NaNO₂ and 10.41 mM FeC₆H₅O₇, the growth of bacteria was decreased 2.0–2.5 times, compared with growth in the medium with NaNO₂ or FeC₆H₅O₇ as the sole electron acceptor. In media with NaNO₂ and FeC₆H₅O₇ with increasing concentrations of the ferrum (III) citrate there was a gradual decrease (1.8–3.2 times) of the efficiency of nitrite ions reduction by bacteria, as compared with their reduction in the medium with only NaNO₂ (97.7–98.0%, Fig. 3a). In media, containing NaNO₂ and FeC₆H₅O₇, the cells produced 0.54–1.74 mM of ammonium ions (control: 2.00–2.05 mM, Table 3). The efficiency of the Fe(III) reduction by bacteria increased in its concentration in media with NaNO₂ and FeC₆H₅O₇ and was revealed to be 1.1–2.2 times lower than its reduction in medium with only FeC₆H₅O₇ (93.1–96.9%, Fig. 3b). In the media with NaNO₂ and FeC₆H₅O₇ cells produced 1.38–4.69 mM of the ferrum (II) ions (control: 3.17–3.25 mM, Table 3). In the medium with NaNO₂ or FeC₆H₅O₇ without bacteria the reduction of NO₂⁻ and Fe(III) did not exceed 3.5% and 2.0%, respectively (Fig. 3). Thus, it has been established that ferrum (III) citrate inhibits the biomass accumulation, the nitrite ions reduction and the ammonium ions production by bacteria of Desulfuromonas sp. after simultaneous addition into the medium of 3.47 mM NaNO₂ and 1.74–10.41 mM FeC₆H₅O₇. In the medium with the same initial content (3.47 mM) NaNO₂ and FeC₆H₅O₇ bacteria reduced 1.5–1.6 times more Fe(III) than nitrite ions with production of ferrum (II) ions at a concentration 1.7 times higher than that of ammonium ions.

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Discussion

The efficiency of reduction of electron acceptors by microorganisms in ecotopes with complex contamination is determined by the level of their adaptation to unfavourable environmental conditions, in particular, the increased content of metal compounds (Teng et al., 2019). Therefore, the ability of bacteria of Desulfuromonas sp. to reduce sulfur, nitrate or nitrite ions in the process of anaerobic respiration at a simultaneous presence in the medium of FeC₆H₅O₇ at concentrations up to 10.41 mM, which exceed the maximum admissible norms, was studied (Kuznetsova et al., 2015).

The efficiency of electron acceptor reduction by bacteria is primarily determined by the difference between the oxidation-reduction potential of the donor (in this work, sodium citrate) and the electron acceptor, which depends on the pH of the medium and changes during cultivation of bacteria (Govorkova et al., 2015). Sulfur-reducing bacteria of the Desulfuromonas genus oxidize organic substrates, in particular, acetate, pyruvate, ethanol, butanol, propional, lactate, citrate, propionate, glutamate, higher fatty acids completely to CO₂ and H₂O in the tricarboxylic acid cycle or in the acetyl-CoA/CO-dehydrogenase pathway (Sang et al., 2003; An & Picarda, 2015), among them are species able to ferment L-malate or fumarate with or without acetate by action of fumarate reductase (or succinate dehydrogenase) (Gebhardt et al., 1985; Lemos et al., 2002; Lengeler et al., 2005). Although the succession of electron acceptors reduction by microorganisms at their simultaneous presence in the medium is determined by their standard oxidation-reduction potential (pH 7.0), the energy supply of cells during anaerobic respiration depends on the ways of ATP synthesis in the process of electron donor oxidation.

At simultaneous presence in the cultivation medium of sulfur and Fe(III) citrate, bacteria reduce more Fe(III) than S⁰ at (pH 7.0) the standard oxidation-reduction potential (E⁰’ = +0.77 V) of the Fe(III)/Fe(II) pair is considerably higher than that (E⁰’ = −0.27 V) of S⁰/HS⁻ pair (Lengeler et al., 2005; An & Picarda, 2015). The evidence of this is the fact that in the medium with the same initial concentration (3.47 mM) of S⁰ and FeC₆H₅O₇, the content of H₂S was 3.5–3.9 times lower than the content of ferrum (II) ions, produced by bacteria. In the sulfur respiration of bacteria of Desulfuromonas genus sulfur reductase and polysulfide reductase are involved, which are located in the cytoplasmic membrane and bonded with hydrogenase by cytochromes or quinones (Lengeler et al., 2005). Ferrum (III) citrate at all tested concentrations in the medium represses the dissimilatory sulfur reduction carried out by bacteria, as evidenced by the decrease in the concentrations of hydrogen sulfide produced by bacteria in the media with S⁰ and FeC₆H₅O₇. Under these conditions of growth, ferrum (III) cations, chelated by citrate, may damage the structure of the cytoplasmic membrane of bacteria and thus influence the activity of membrane bound enzymes (Maslovka & Hnatush, 2013; Maslovka et al., 2014). Although the reduction of metals–oxidents by membrane-bound metal reductases is mainly carried out outside the cell (Gescher & Kappler, 2012; Richter et al., 2012; Simone et al., 2017), with increase in the concentration of soluble FeC₆H₅O₇ in the medium the degree of Fe(III) penetration through the cytoplasmic membrane of bacteria into the cytoplasm increases, where its interaction with intracellular metabolites occurs, oxygen radicals are formed, which causes the inhibition of growth and metabolic activity of bacteria (Hnatush & Maslovka, 2018).

At pH 7.0 the standard oxidation-reduction potential of the Fe(III)/Fe(II) pair (E⁰’ = +0.77 V) is lower than that of NO₃⁻/NO₂⁻ pair (E⁰’ = +0.77 V), but higher than that of NO₃⁻/NH₄⁺ pair (E⁰’ = +0.34 V) (Lengeler et al., 2005; Richter et al., 2012). In the medium with the same initial concentration (3.47 mM) of NaNO₃ and FeC₆H₅O₇, bacteria did not reduce many more nitrate ions than Fe(III), and in the medium with the same content (3.47 mM) of NaNO₃ and FeC₆H₅O₇ the strains reduced 1.5–1.6 times more Fe(II) than nitrite ions. Nevertheless, FeC₆H₅O₇ at all concentrations in the medium inhibited the nitrate and nitrite reduction, which was carried out by the investigated strains of bacteria. The process of nitrate reduction carried out by bacteria of Desulfuromonas genus was less sensitive to the negative influence of FeC₆H₅O₇, compared to the process of nitrite ions reduction. When the reduction of nitrate ions by bacteria in the presence of 1.74–10.41 mM FeC₆H₅O₇ decreased 1.4–2.2 times, then the reduction of nitrite ions decreased 1.8–3.2 times, compared to their reduction in the media with only NaNO₃ or NaNO₂, respectively. The negative influence of ferrum (III) citrate on the activity of molybdenum-containing membrane-bound respiratory or dissimilatory nitrate reductase (Morozkina & Zvyagilskaya, 2007), as well as periplasmic nitrite reductase, containing siroheme as a prosthetic group (Lengeler et al., 2005), in bacteria of Desul- furomonas genus can be caused by damage of the cytoplasmic membrane structure or modification of the active conformation and denaturation of the protein molecule as a result of the replacement by ferrum of the necessary metal ion in the active centre of the enzyme.

Despite the fact that the reduction of Fe(III) by cells in media with 1.74–10.41 mM FeC₆H₅O₇ and 3.47 mM S⁰, NaNO₃ or NaNO₂ decreased 1.6–2.7, 1.6–2.7 and 1.1–2.2 times, respectively, compared with its reduction in medium with only FeC₆H₅O₇ as the sole electron acceptor, the results obtained by us suggest that the investigated strains of bacteria are resistant to high concentrations of irrevocable ferrum compounds (up to 10.41 mM) and therefore can survive in environments contaminated by heavy metals. The studied strains are promising for use in technologies of complex purification of the environment polluted by ferrum and nitrate compounds due to their ability to effect reductive transformation of these toxicants.

Conclusion

Because of the long-term entry into the environment of various chemical contaminants at critical concentrations, the metabolism of bacteria slows down, the species composition of microorganisms changes, and in the process of natural selection few resistant strains of microorganisms survive. Sulfidogenic bacteria of the Desulfuromonas genus, isolated by us from the technogenically formed Yavorivske Lake, in the process of organic compounds oxidation use, besides sulfur, oxidized forms of nitrogen and heavy metals, in particular, ferrum (III) as electron acceptors of anaerobic respiration. In media with elemental sulfur or sodium nitrate (3.47 mM) and ferrum (III) citrate (1.74–10.41 mM) bacteria reduced more Fe(III) than S⁰ or NO₂⁻. In media with 3.47 mM sodium nitrate and 1.74–10.41 mM ferrum (III) citrate bacteria reduced more NO₃⁻ than Fe(III). Despite this, ferrum (III) citrate at all concentrations in the medium inhibited the dissimilatory sulfur, nitrate and nitrite reduction carried out by bacteria. Due to the high plasticity of the processes of metabolism adapted to survival conditions, bacteria of Desul- furomonas sp. can be the basis of the new developments in the field of ecobiotechnology.

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