Biol. Stud. 2020: 14(1); 3–22 • DOI: https://doi.org/10.30970/sbi.1401.607
www.http://publications.lnu.edu.ua/journals/index.php/biology

UDC: 579.68+579.[266+222]+546.3

REDUCTION OF SULFATE, NITRATE AND NITRITE IONS BY *DESULFOVIBRIO* SP. UNDER THE INFLUENCE OF FERRUM (III) CITRATE

O. M. Moroz**, S. O. Hnatush, G. V. Yavorska

Ivan Franko National University of Lviv, 4 Hrushevskyi St., Lviv 79005, Ukraine

**Corresponding author: e-mail: oksana.moroz@lnu.edu.ua*

Moroz O.M., Hnatush S.O., Yavorska G.V. Reduction of sulfate, nitrate and nitrite ions by *Desulfovibrio* sp. under the influence of ferrum (III) citrate. **Studia Biologica**, 2020: 14(1); 3–22 • DOI: https://doi.org/10.30970/sbi.1401.607

The influence of ferrum (III) citrate added to the cultivation medium, on the reduction of sulfate, nitrate, and nitrite ions by sulfate-reducing bacteria *Desulfovibrio desulfuricans* IMV K-6, *Desulfovibrio* sp. Yav-6, and *Desulfovibrio* sp. Yav-8 isolated from Yavorivske Lake was studied. It was established that ferrum (III) citrate inhibits the biomass accumulation, SO_4^{2-} reduction, and H_2S production by the bacteria after addition of 1.74–3.47 mM $Na_2SO_4 \times 10\ H_2O$ and 1.74–10.41 mM $FeC_6H_5O_7$ to the medium, in comparison with the growth and level of the reduction of sulfate ions by bacteria in the medium supplemented with only $Na_2SO_4 \times 10\ H_2O$. At conditions of the bacteria cultivation in the presence of an equimolar amount (3.47 mM) of $Na_2SO_4 \times 10\ H_2O$ and $FeC_6H_5O_7$, they reduced 2.5−2.7 times more Fe(III) than SO_4^{2-} with Fe^{2+} production at a concentration 2.4−2.7 times greater than H_2S. $FeC_6H_5O_7$ inhibited growth, NO_3^- or NO_2^- reduction and NH_4^+ production by the bacteria in the presence of 1.74−3.47 mM $NaNO_3$ or $NaNO_2$ and 1.74−10.41 mM $FeC_6H_5O_7$ in the medium, compared to the growth and level of nitrate or nitrite ions reduction in the medium with only $NaNO_3$ or $NaNO_2$. In the medium with the same initial content of 3.47 mM $NaNO_3$ and 3.47 mM $FeC_6H_5O_7$, the bacteria reduced 1.4 times more NO_3^- than Fe(III), with NH_4^+ production at concentration 1.1 times higher than that of Fe^{2+}. In the medium with 3.47 mM $NaNO_2$ and 3.47 mM $FeC_6H_5O_7$, the cells reduced 1.4−1.6 times more Fe(III) than NO_2^-, with Fe^{2+} production at concentration 1.5−1.6 times higher than NH_4^+. Ferrum (III) citrate had more inhibitory effect on the dissimilatory reduction of sulfate by the bacteria than of nitrate and nitrite ions, since the SO_4^{2-} reduction by the bacteria at its presence in the medium decreased 2.0−4.7 times. The reduction of NO_3^- and NO_2^- decreased only 1.3−1.9 and 1.7−3.1 times, respectively, as compared with their reduction in the media with only $Na_2SO_4 \times 10\ H_2O$, $NaNO_3$ or $NaNO_2$. Despite the fact that the reduction by cells of 1.74−10.41 mM Fe(III) in the media

with $Na_2SO_4 \times 10\ H_2O$, $NaNO_3$ or $NaNO_2$ decreased 1.1−2.1, 1.6−2.7 and 1.1−2.5 times, respectively, compared with its reduction in the medium with only $FeC_6H_5O_7$. The investigated strains of bacteria were resistant to high concentrations of ferrum (III) citrate and, therefore, can be applied in the technologies of complex environment purification from pollution with ferrum, sulfur, and nitrogen compounds.

Keywords: *Desulfovibrio*, ferrum, sulfates, nitrates, nitrites

INTRODUCTION

In process of the anaerobic respiration, sulfidogenic bacteria of *Desulfovibrio, Desulfotomaculum, Desulfobacterium, Desulfobacter, Geobacter* etc. genera oxidize organic compounds using different electron acceptors [1, 2, 3, 16, 31, 37]. Sulfate-reducing bacteria produce H_2S, as a result of the dissimilatory reduction of sulfate ions that occurs in their cytoplasm with a formation of the adenosine-5′-phosphosulfate (APS), as an intermediate product. The stages of sulfate reduction are catalyzed by ATP sulfurylase, APS reductase, and a number of sulfite reductases [16]. These bacteria play an important role in regulating the level of not only compounds of sulfur and carbon, but also of nitrogen and metals in the environment [2, 23, 33, 38–40]. Pollution of media by heavy metals influences on the physiological and biochemical processes which are carried out by bacteria [10, 22, 24, 25, 33].

Facultative anaerobic bacteria carry out a 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^+ [16, 39]. Oxidized compounds of nitrogen are reduced by the microorganisms that synthesize nitrate and nitrite reductases [16]. Nitrate reductase NarGHI is an enzymatic complex that includes multi-heme *b*-type cytochrome, proteins with Fe-S clusters, and Mo-containing cofactor [13, 21]. Nitrate reduction with the formation of nitrite and its further reduction by complex of periplasmic dissimilatory nitrite reductases to NH_4^+ was described in *Desulfovibrio desulfuricans*, *Desulfotomaculum* sp., *Desulfobacter* sp., and *Wolinella succinogenes* [4, 11, 20, 25, 29, 39].

Sulfidogenic and metal-reducing bacteria occupy close ecological niches, providing various links in a cycle of chemical elements in nature [2, 40]. 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 (*Desulfovibrio, Geobacter, Desulfuromusa*, *Desulfuromonas, Desulfotomaculum*, *Shewanella, Wolinella*, *Pseudomonas* etc.) in process of the anaerobic respiration release electrons into the medium [5, 7, 31, 32, 34], due to which these bacteria are considered as anode biocatalysts in the microbial fuel cells [6, 10, 34].

In natural conditions most often there are several possible electron acceptors of anaerobic respiration, and bacteria, first of all, reduce acceptors with higher standard oxidation-reduction potential. Although a succession of reduction of electron acceptors by the microorganisms is determined by electrochemical laws, it is not sufficiently studied. In different microorganisms, the succession of the elements with a variable valence reduction is determined genetically and controlled by profound regulatory mechanisms [13, 16].

Ferrum enters the aquatic and soil environments with industrial, agricultural and household effluents, as well as due to natural processes of chemical weathering and

dissolution of rocks [14]. In the bacteria cells, ferrum is an essential trace element that participates in the processes of photosynthesis, N_2 fixation, methanogenesis, H_2 synthesis, respiration, regulation of gene expression and DNA biosynthesis [19]. The toxic effect of ferrum, as well as other metals, on the bacterial cell is its binding with the surface structures of the cell wall, the change in the electrophysiological properties of the cytoplasmic membrane, the blocking of transport systems, the replacement of the necessary ions from active centers of the enzymes, binding with functional groups of cell metabolites [27, 28]. Because the Fe(III)/Fe(II) pair at pH 7.0 has a very high oxidation-reduction potential (E_0' = +0.77 V), which, however, is highly dependent on the acidity of the medium [7, 8, 16], at high concentrations in the cytoplasm the Fe(III) is the catalyst of Fenton and Haber–Weiss reactions that result a formation of toxic forms of oxygen [10, 19, 27]. Therefore, an important mechanism for bacteria protecting from the toxic influence of heavy metals is their ability to extracellular metal reduction by a system of membrane-bound metal reductases (multi-heme *c*-type cytochromes) [5, 6, 31, 32, 34]. Another way of eliminating heavy metals by sulfidogenic bacteria from the natural cycle, is their immobilization in the form of sulfides formed as a result of interaction with H_2S [12–14, 17, 24].

A selection of resistant to pollutions strains of sulfate-reducing bacteria isolated from technogenically altered ecotopes, capable to reductive transformation of various nature pollutants, is especially actual task for the creation of biotechnologies for purification [3, 13, 14, 18, 37]. Previously we have shown that the bacteria of *Desulfovibrio* genus in addition to oxidized forms of sulfur or nitrogen can reduce oxidized forms of heavy metals, in particular, ferrum (III), transforming them into compounds less toxic for the living organisms [23, 25]. 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 ferrum (III) citrate to establish a succession of electron acceptors reduction by strains of sulfidogenic bacteria of *Desulfovibrio* genus, isolated by us from the Yavorivske Lake, and to evaluate an efficiency of their application in technologies of complex purification of environment pollution by ferrum, sulfur and nitrogen compounds.

MATERIALS AND METHODS

Sulfate-reducing bacteria *Desulfovibrio desulfuricans* IMV K-6, *Desulfovibrio* sp. Yav-6, *Desulfovibrio* sp. Yav-8 isolated from the Yavorivske Lake, were identified at the Department of Microbiology of Ivan Franko National University of Lviv. They are stored at the Depository of D. K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine and/or at the collection of the Department of Microbiology [26, 30].

Bacteria were grown for 10 days in Kravtsov–Sorokin medium [9] without SO_4^{2-} and without Mohr's salt of such composition (g/L): $NaH_2PO_4 \times 12\ H_2O$ (0.84), K_2HPO_4 (0.5), NH_4Cl (0.16), $MgCl_2 \times 6\ H_2O$ (0.1), sodium lactate ($NaC_3H_5O_3$) (2.0) or sodium citrate ($Na_3C_6H_5O_7$) (4.6), at a temperature of 30 °C in test tubes, volume 25 ml, completely topped up by the medium. Before bacteria seeding 0.05 ml of $Na_2S \times 9\ H_2O$ (1%) sterile solution was added to the medium. pH of the medium was 7.2. Bacteria were sown in medium to initial concentration of cells of 0.1 mg/ml. Solutions of sodium fumarate ($C_4H_3NaO_4$), $Na_2SO_4 \times 10\ H_2O$, $NaNO_3$, $NaNO_2$, $FeC_6H_5O_7$ were sterilized separately and placed into the medium before seeding of the cells. To media with $C_4H_3NaO_4$, $FeC_6H_5O_7$ and/or $NaNO_3$ or $NaNO_2$ cysteine (0.017 mM) was introduced to meet the assimilation needs of bacteria in sulfur [16]. To media supplemented with $NaNO_3$, $NaNO_2$ and $FeC_6H_5O_7$ or without it NH_4Cl was not added.

To study the unfluence of ferrum (III) citrate on the kinetics of sulfate or nitrate ions usage by bacteria, the cells, previously grown in medium with sodium fumarate (3.47 mM), as an electron acceptor, and sodium lactate (17.86 mM), as an electron donor, to middle of exponential growth phase, were sown in medium with sodium citrate ($Na_3C_6H_5O_7$) as an electron donor (17.86 mM) to which sterile 1 M solutions of $Na_2SO_4 \times 10\ H_2O$ or $NaNO_3$ and $FeC_6H_5O_7$ were added at concentrations of 1.74 mM to obtain the total content of electron acceptors in medium of 3.47 mM (SO_4^{2-} concentration in the medium of standard composition). The cells were also sown in media with only 3.47 mM $Na_2SO_4 \times 10\ H_2O$, $NaNO_3$ or $FeC_6H_5O_7$ to verify their growth in media with SO_4^{2-}, NO_3^- or Fe(III) as the sole electron acceptor (control). As control the spontaneous reduction of sulfate, nitrate ions and ferrum (III) in media without cells with $Na_2SO_4 \times 10\ H_2O$, $NaNO_3$ and/or $FeC_6H_5O_7$ at concentrations of 1.74 or 3.47 mM was investigated. The biomass, and the concentrations of sulfate or nitrate, ferrum (II) ions, ferrum (III), hydrogen sulfide or ammonium ions in cultural liquid were determined on 2, 4, 6, 8 and 10 days of growth.

To determine the efficiency of reduction of sulfate, nitrate, or nitrite ions at simultaneous presence in the medium of ferrum (III) citrate, cells were previously cultivated in medium with sodium fumarate (3.47 mM) and sodium lactate (17.86 mM) to the middle of the exponential growth phase. They were sown in the medium with sodium citrate (17.86 mM), to which 1 M solutions of $Na_2SO_4 \times 10\ H_2O$, $NaNO_3$ or $NaNO_2$ were added to final concentration in the medium of 3.47 mM and different volumes of sterile $FeC_6H_5O_7$ solution in its final concentrations of 1.74; 3.47; 5.21; 6.94; 10.41 mM that differ 0.5; 1; 1.5; 2 and 3 times from standard electron acceptor content in Kravtsov–Sorokin medium. The cells were also sown in a medium with sodium citrate (17.86 mM) supplemented with sterile 1 M solutions of $Na_2SO_4 \times 10\ H_2O$, $NaNO_3$, $NaNO_2$ or $FeC_6H_5O_7$ in a final concentration in the medium of 3.47 mM, to test the bacteria growth in media with sulfate, nitrate, nitrite ions or ferrum (III) as the sole electron acceptor (control). Into the media without cells, solutions of $Na_2SO_4 \times 10\ H_2O$, $NaNO_3$, $NaNO_2$ or $FeC_6H_5O_7$ were added at concentration of 3.47 mM to verify the reduction of SO_4^{2-}, NO_3^-, NO_2^- or Fe(III) (control). The biomass, the concentrations of sulfate, nitrate or nitrite, ferrum (II) ions, ferrum (III), hydrogen sulfide or ammonium ions in cultural liquid were determined on 10 day of growth. According to difference between the initial and residual content of electron acceptors in the medium, the efficiency (%) of their reduction by bacteria, based on the ratio of molar concentrations of reduced by bacteria sulfate, nitrate, nitrite ions or ferrum (III) in the process of anaerobic respiration and their concentrations at the beginning of cultivation taken as 100 % was calculated.

To determine the concentrations of ferrum (II) ions and hydrogen sulfide, the precipitate of FeS formed after bacteria cultivation in medium with $Na_2SO_4 \times 10\ H_2O$ and $FeC_6H_5O_7$ was dissolved after interaction with HCl according to the equation: $FeS + 2HCl \rightleftarrows FeCl_2 + H_2S$ (the HCl concentration exceeded twice the concentration of SO_4^{2-} in the medium and was 3.5 mM or 7.0 mM).

The biomass was determined by the turbidimetric method using optical density of the cell suspension measured at 340 nm wavelength 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 upon the mass of dry cells, determined by the weight method, and equal 0.19 [9]. In a cultural liquid separated from the cells by centrifugation (6,000 rpm, 15 min), the concentrations of sulfate ions by turbidimetric method for the formation of barium sulfate were determined after sulfates precipitation by barium

chloride. Concentrations of nitrate ions were measured after their reduction to nitrites in the presence of Zn:$MnSO_4$ (1:100) powder, as a reducing agent, while concentrations of nitrite ions were measured by spectrophotometric method in the reaction with Griss reagent (*n*-(1-naphthyl)ethylenediamine dihydrochloride, sulfanil and acetic acid). Concentrations of ferrum (III) were determined after its reduction to ferrum (II) ions in the acidic medium by interaction with hydroxylamine, ferrum (II) ions – by spectrophotometric method in reaction with *o*-phenanthroline, hydrogen sulfide – by spectrophotometric method of the formation of the methylene blue, and the ammonium ions – by the colorimetric method of the formation of the indophenol [9].

Experiments were repeated three times with three parallel staging for each variant of the experimental and control conditions. The obtained data were processed using the Microsoft Excel 2010 program. To evaluate the certainty of the difference between the statistical characteristics of the two alternative sets of data, Student's coefficient *t* was counted. The difference was considered to be significant at $P<0.05$ [15].

RESULTS AND DISCUSSION

The intensity of the anaerobic respiration of microorganisms in the contaminated ecotopes is determined by the level of their adaptation to unfavorable environmental conditions, in particular, increased content of metal compounds [18, 34, 35, 37, 38]. In the technogenic reservoir that arose on the place of Yavoriv sulfur deposit open pit, high concentrations of toxic to living organisms compounds of sulfur, nitrogen and heavy metals were detected [24, 36]. The concentrations of Fe(III) were significantly higher than maximum permissible concentrations (MPC) (0.1−0.3 mg/L [14]) at a depths of 50−70 m and reached to 0.28−0.34 mg/L, while the content of Fe(II) at no depth exceeded the MPC [24]. The influence of Fe(III) at concentrations significantly higher, than in the reservoir, on sulfate-, nitrate- and nitrite-reducing activity of *Desulfovibrio* genus bacteria, isolated from Yavorivske Lake, was studied. Earlier we have shown that these bacteria in the process of the anaerobic destruction of the organic compounds, except of sulfate, nitrate or nitrite ions, can use oxidized forms of heavy metals with a variable valence, in particular, Fe(III) as electron acceptors [23, 25].

To study the influence of ferrum (III) citrate on the sulfate ions reduction by sulfate-reducing bacteria, they were sown in a medium with sodium citrate as an electron donor, to which 1.74 mM $Na_2SO_4\times10\ H_2O$ and 1.74 mM $FeC_6H_5O_7$ were added to obtain total content of electron acceptors in the medium of 3.47 mM. The cultures were also sown in the medium with sodium citrate and 3.47 mM $Na_2SO_4\times10\ H_2O$ or 3.47 mM $FeC_6H_5O_7$ (Fig. 1, Table 1). The biomass of bacteria in medium with $Na_2SO_4\times10\ H_2O$ did not differ from the biomass accumulated by cells in medium with $FeC_6H_5O_7$. After a simultaneous introduction of $Na_2SO_4\times10\ H_2O$ and $FeC_6H_5O_7$ to the cultivation medium, a slight inhibition of biomass accumulation by bacteria, compared with their growth in the media with one electron acceptor was observed. In the medium with $Na_2SO_4\times10\ H_2O$ and $FeC_6H_5O_7$ a 3.4−4.6 times decrease in the efficiency of sulfate ions reduction by cells (which did not exceed 21−28 %), as compared with their reduction in the medium only with $Na_2SO_4\times10\ H_2O$ (96−97 %) was revealed. In that medium cells produced 0.36−0.46 mM of hydrogen sulfide. The efficiency of ferrum (III) reduction by cells in the medium with $Na_2SO_4\times10\ H_2O$ and $FeC_6H_5O_7$ (81−84 %) did not significantly differ from those in the medium with ferrum (III) citrate (90−94 %). In a medium with $Na_2SO_4\times10\ H_2O$ and $FeC_6H_5O_7$, the cells produced 1.23−1.39 mM of ferrum (II) ions. In the media with

$Na_2SO_4 \times 10\ H_2O$ and/or $FeC_6H_5O_7$ without bacteria, the efficiency of the reduction of sulfate ions and Fe(III) was insignificant and did not exceed 1.2−2.3 and 4.0−6.3 %, respectively (Table 1). It was established that in medium with the same initial content (1.74 mM) $Na_2SO_4 \times 10\ H_2O$ and $FeC_6H_5O_7$ bacteria reduced 2.9−3.9 times more Fe(III) than sulfate ions with ferrum (II) ions production at concentration 2.2−3.6 times higher than that of the hydrogen sulfide.

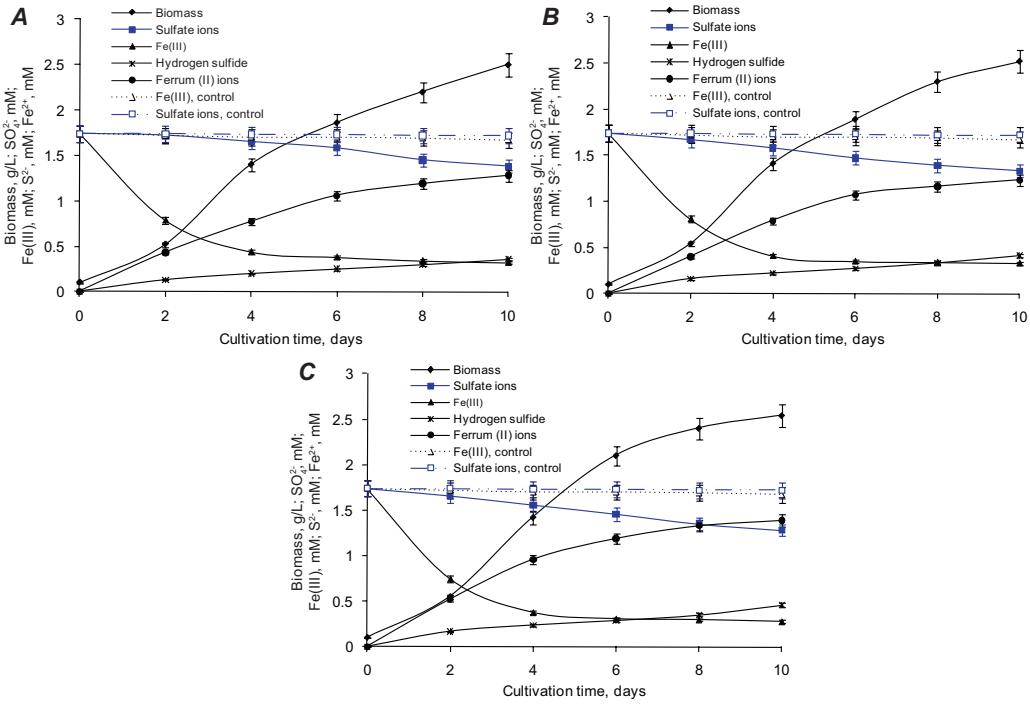

Fig. 1. The biomass accumulation, the content of sulfate, ferrum (II) ions, Fe(III) and hydrogen sulfide in the cultural liquid during the growth of *D. desulfuricans* IMV K-6 (***A***), *Desulfovibrio* sp. Yav-6 (***B***) and *Desulfovibrio* sp. Yav-8 (***C***) in the medium with 1.74 mM $Na_2SO_4 \times 10\ H_2O$ and 1.74 mM $FeC_6H_5O_7$. The medium with 1.74 mM $Na_2SO_4 \times 10\ H_2O$ and 1.74 mM $FeC_6H_5O_7$ without cells was control (M±m, n = 3)

Рис. 1. Нагромадження біомаси, вміст йонів сульфату, феруму (II), Fe(III) та гідроген сульфіду в культуральній рідині під час росту *D. desulfuricans* IMB K-6 (***A***), *Desulfovibrio* sp. Yav-6 (***B***) та *Desulfovibrio* sp. Yav-8 (***C***) у середовищі з 1,74 мМ $Na_2SO_4 \times 10\ H_2O$ та 1,74 мМ $FeC_6H_5O_7$. Контроль − середовище з 1,74 мМ $Na_2SO_4 \times 10\ H_2O$ та 1,74 мМ $FeC_6H_5O_7$ без клітин (M±m, n = 3)

The efficiency of the biological methods for purifying the environment from the pollutants depends not only upon the metabolic activity of the selected strains of bacteria, but primarily upon their resistance to metal compounds [2, 7, 10, 14, 40]. Therefore, we studied the ability of these bacteria to reduce in the process of the anaerobic respiration of sulfate, nitrate or nitrite ions at a simultaneous presence in the medium of 1.74−10.41 mM $FeC_6H_5O_7$.

The bacteria were grown in the medium with sodium citrate supplemented with 3.47 mM $Na_2SO_4 \times 10\ H_2O$ and $FeC_6H_5O_7$ at different concentrations. The bacteria were also sown in the medium with sodium citrate and 3.47 mM $Na_2SO_4 \times 10\ H_2O$ or 3.47 mM $FeC_6H_5O_7$ (Table 2). After 10 days of growth, the biomass of bacteria in the medium with

Table 1. **SO_4^{2-} and Fe(III) reduction by the bacteria after 10 days of growth in the media with $Na_2SO_4 \times 10\ H_2O$ and/or $FeC_6H_5O_7$ (M±m, n = 3)***

Таблиця 1. **Відновлення бактеріями SO_4^{2-} і Fe(III) після 10 діб росту в середовищах з $Na_2SO_4 \times 10\ H_2O$ та/або $FeC_6H_5O_7$ (M±m, n = 3)***

Strain	Electron acceptors of anaerobic respiration	Residual content in cultural liquid, mM		Reduction, %		Biomass, g/L
		SO_4^{2-}	Fe(III)	SO_4^{2-}	Fe(III)	
D. desulfuricans IMV K-6	SO_4^{2-}	0.14±0.03	0	96.0	0	2.58±0.03
	SO_4^{2-} (c)	3.39±0.01	0	2.3	0	0
	SO_4^{2-} and Fe(III)	1.38±0.06	0.32±0.02	20.7	81.6	2.50±0.03
	SO_4^{2-} and Fe(III) (c)	1.72±0.04	1.67±0.02	1.2	4.0	0
	Fe(III)	0	0.35±0.03	0	89.9	2.81±0.02
	Fe(III) (c)	0	3.25±0.04	0	6.3	0
Desulfovibrio sp. Yav-6	SO_4^{2-}	0.11±0.02	0	96.8	0	2.60±0.01
	SO_4^{2-} (c)	3.39±0.01	0	2.3	0	0
	SO_4^{2-} and Fe(III)	1.33±0.04	0.33±0.02	28.1	81.0	2.52±0.05
	SO_4^{2-} and Fe(III) (c)	1.72±0.04	1.67±0.02	1.2	4.0	0
	Fe(III)	0	0.33±0.05	0	90.5	2.82±0.03
	Fe(III) (c)	0	3.25±0.04	0	6.3	0
Desulfovibrio sp. Yav-8	SO_4^{2-}	0.09±0.01	0	97.4	0	2.62±0.06
	SO_4^{2-} (c)	3.39±0.01	0	2.3	0	0
	SO_4^{2-} and Fe(III)	1.28±0.04	0.28±0.04	26.4	83.9	2.54±0.05
	SO_4^{2-} and Fe(III) (c)	1.72±0.04	1.67±0.02	1.2	4.0	0
	Fe(III)	0	0.21±0.03	0	94.0	2.84±0.03
	Fe(III) (c)	0	3.25±0.04	0	6.3	0

Comments: * The initial concentration of $Na_2SO_4 \times 10\ H_2O$ or $FeC_6H_5O_7$ in the medium equal 3.47 mM, at the presence of $Na_2SO_4 \times 10\ H_2O$ and $FeC_6H_5O_7$ in the medium – 1.74 mM; (c) – control: the medium without cells

Примітки: * − вихідна концентрація $Na_2SO_4 \times 10\ H_2O$ або $FeC_6H_5O_7$ у середовищі – 3,47 мМ, за наявності у середовищі $Na_2SO_4 \times 10\ H_2O$ і $FeC_6H_5O_7$ – 1,74 мМ; (с) – контроль: середовище без клітин

$Na_2SO_4 \times 10\ H_2O$ was not significantly lower than in the medium with $FeC_6H_5O_7$. After a simultaneous addition of $Na_2SO_4 \times 10\ H_2O$ and $FeC_6H_5O_7$ to the cultivation medium with growing of ferrum (III) citrate concentrations, a gradual decrease in the biomass accumulation by bacteria, compared to growth in the media with $Na_2SO_4 \times 10\ H_2O$ or $FeC_6H_5O_7$ was observed. In a medium with $Na_2SO_4 \times 10\ H_2O$ and 10.41 mM $FeC_6H_5O_7$, the growth of bacteria was decreased by about half, compared with growth in the media with only $Na_2SO_4 \times 10\ H_2O$ or $FeC_6H_5O_7$. In the media with $Na_2SO_4 \times 10\ H_2O$ and $FeC_6H_5O_7$ with increasing of ferrum (III) citrate concentrations, a gradual (from 2.0 to 4.7 fold) decrease in the efficiency of sulfate ions reduction by bacteria as compared with their reduction in the medium with only $Na_2SO_4 \times 10\ H_2O$ (95−98 %) was detected (Fig. 2, *A*). In that medium, the cells produced 0.65−1.32 mM of hydrogen sulfide (control: 2.54−2.76 mM) (Table 2). The efficiency of Fe(III) reduction by the bacteria in media with $Na_2SO_4 \times 10\ H_2O$ and 1.74−3.47 mM $FeC_6H_5O_7$ practically did not differ from their reduction in the medium with ferrum (III) citrate (92−94 %). It was found to be 1.1−2.1 times lower at $FeC_6H_5O_7$ concentrations in the medium of 5.21−10.41 mM (Fig. 2, *B*). In the media with Na-

$_2SO_4 \times 10\ H_2O$ and $FeC_6H_5O_7$, the bacteria produced 1.52−4.76 mM of ferrum (II) ions (control: 2.90−3.19 mM) (Table 2). In the medium with $Na_2SO_4 \times 10\ H_2O$ and $FeC_6H_5O_7$ without bacteria, the efficiency of sulfate ions and Fe(III) reduction was insignificant and did not exceed 1.7 and 5.8 %, respectively (Fig. 2). Thus, it has been established that ferrum (III) citrate inhibits the biomass accumulation, sulfate ions reduction and hydrogen sulfide production by *Desulfovibrio* sp. bacteria after simultaneous introducing into the medium of 3.47 mM $Na_2SO_4 \times 10\ H_2O$ and $FeC_6H_5O_7$ (1.74−10.41 mM). In the medium with the same initial content (3.47 mM) $Na_2SO_4 \times 10\ H_2O$ and $FeC_6H_5O_7$, the bacteria reduced 2.5−2.7 times more Fe(III) than sulfate ions with ferrum (II) ions production at a concentration 2.4−2.7 times higher than the hydrogen sulfide.

Table 2. **SO_4^{2-} and Fe(III) reduction by *Desulfovibrio* sp. after 10 days of growth in the media supplemented with $Na_2SO_4 \times 10\ H_2O$ and/or $FeC_6H_5O_7$ (M±m, n = 3)**

Таблиця 2. **Відновлення SO_4^{2-} і Fe(III) *Desulfovibrio* sp. після 10 діб росту в середовищах з $Na_2SO_4 \times 10\ H_2O$ та/або $FeC_6H_5O_7$ (M±m, n = 3)**

Strain	Electron acceptors of anaerobic respiration	Residual content in cultural liquid, mM		Fe^{2+}, mM	S^{2-}, mM	Biomass, g/L
		SO_4^{2-}	Fe(III)			
D. desulfuricans IMV K-6	3.47 mM SO_4^{2-}	0.16±0.01	0	0	2.54±0.02	2.50±0.03
	3.47 mM SO_4^{2-} (c)	3.41±0.03	0	0	0.05±0.01	0
	3.47 mM SO_4^{2-} and 1.74 mM Fe(III)	2.16±0.08	0.12±0.01	1.53±0.01	1.22±0.04	2.50±0.02
	3.47 mM SO_4^{2-} and 3.47 mM Fe(III)	2.24±0.06	0.32±0.01	2.94±0.02	1.07±0.07	2.26±0.07
	3.47 mM SO_4^{2-} and 5.21 mM Fe(III)	2.10±0.01	0.86±0.06	4.21±0.02	0.89±0.02	1.99±0.02
	3.47 mM SO_4^{2-} and 6.94 mM Fe(III)	2.48±0.09	3.51±0.02	3.35±0.06	0.74±0.03	1.52±0.08
	3.47 mM SO_4^{2-} and 10.41 mM Fe(III)	2.55±0.08	5.87±0.04	4.41±0.04	0.65±0.07	1.23±0.01
	3.47 mM Fe(III)	0	0.28±0.04	2.90±0.04	0	2.58±0.02
	3.47 mM Fe(III) (c)	0	3.27±0.03	0.15±0.01	0	0
Desulfovibrio sp. Yav-6	3.47 mM SO_4^{2-}	0.14±0.02	0	0	2.67±0.06	2.61±0.02
	3.47 mM SO_4^{2-} (c)	3.41±0.03	0	0	0.05±0.01	0
	3.47 mM SO_4^{2-} and 1.74 mM Fe(III)	1.76±0.09	0.10±0.01	1.58±0.01	1.31±0.01	2.55±0.01
	3.47 mM SO_4^{2-} and 3.47 mM Fe(III)	2.20±0.05	0.28±0.08	3.09±0.08	1.13±0.05	2.34±0.06
	3.47 mM SO_4^{2-} and 5.21 mM Fe(III)	2.36±0.09	0.74±0.01	4.35±0.04	0.94±0.04	2.13±0.07
	3.47 mM SO_4^{2-} and 6.94 mM Fe(III)	2.46±0.01	3.39±0.03	3.42±0.04	0.83±0.01	1.55±0.02
	3.47 mM SO_4^{2-} and 10.41 mM Fe(III)	2.49±0.03	5.54±0.07	4.76±0.09	0.75±0.02	1.30±0.07
	3.47 mM Fe(III)	0	0.26±0.09	3.16±0.02	0	2.69±0.08
	3.47 mM Fe(III) (c)	0	3.27±0.03	0.15±0.01	0	0
Desulfovibrio sp. Yav-8	3.47 mM SO_4^{2-}	0.06±0.02	0	0	2.76±0.07	2.62±0.06
	3.47 mM SO_4^{2-} (c)	3.41±0.03	0	0	0.05±0.01	0
	3.47 mM SO_4^{2-} and 1.74 mM Fe(III)	2.09±0.04	0.16±0.01	1.52±0.02	1.32±0.04	2.60±0.02
	3.47 mM SO_4^{2-} and 3.47 mM Fe(III)	2.40±0.09	0.61±0.05	2.88±0.09	1.21±0.06	2.44±0.03
	3.47 mM SO_4^{2-} and 5.21 mM Fe(III)	2.35±0.05	1.12±0.01	4.01±0.04	1.05±0.09	2.23±0.02
	3.47 mM SO_4^{2-} and 6.94 mM Fe(III)	2.42±0.03	3.32±0.09	3.54±0.05	0.96±0.01	1.57±0.09
	3.47 mM SO_4^{2-} and 10.41 mM Fe(III)	2.75±0.07	5.68±0.03	4.68±0.06	0.70±0.02	1.39±0.04
	3.47 mM Fe(III)	0	0.22±0.06	3.19±0.08	0	2.77±0.08
	3.47 mM Fe(III) (c)	0	3.27±0.03	0.15±0.01	0	0

Comment: (c) – control: the medium without cells

Примітка: (c) – контроль: середовище без клітин

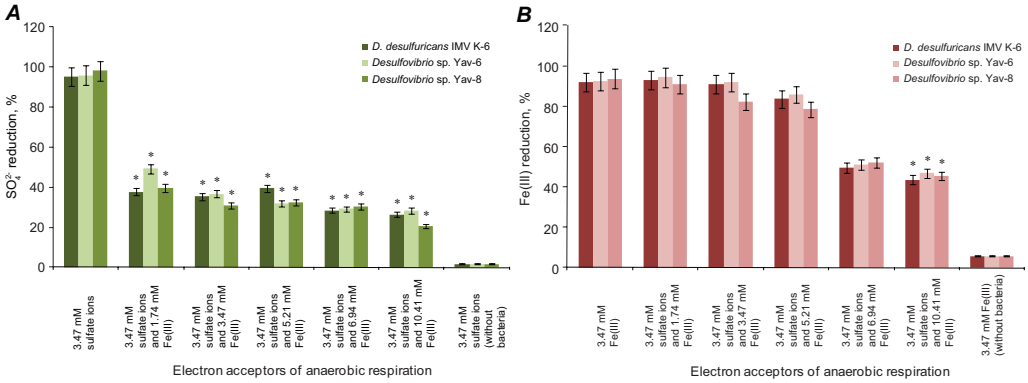

Fig. 2. Efficiency of SO_4^{2-} (***A***) and Fe(III) (***B***) reduction by *Desulfovibrio* sp. after 10 days of growth in the media with $Na_2SO_4 \times 10\ H_2O$ and/or $FeC_6H_5O_7$ (M±m, n = 3). * − P <0.05 (vs control)

Рис. 2. Ефективність відновлення SO_4^{2-} (***A***) і Fe(III) (***B***) *Desulfovibrio* sp. після 10 діб росту в середовищах з $Na_2SO_4 \times 10\ H_2O$ та/або $FeC_6H_5O_7$ (M±m, n = 3). * − P < 0,05 (vs контроль)

Although at simultaneous presence SO_4^{2-} and oxidized form of ferrum in medium the bacteria were used to a greater extent $FeC_6H_5O_7$, at all investigated concentrations its effect on the microorganisms was more or less toxic. That was confirmed by inhibition of dissimilatory reduction of the sulfate ions. Despite the fact that the reduction of metal oxidants by membrane-bound metal reductases is mainly carried out outside the cell [7, 32, 34], with an increase in the concentration of soluble $FeC_6H_5O_7$ in the medium increase in the degree of Fe(III) penetration through the cytoplasmic membrane of bacteria into the cytoplasm. Here its interaction with intracellular metabolites occured, oxygen radicals are formed, and ferrum (II) ions accumulated as a reduced end product, that caused inhibition of bacteria growth and their metabolic activity [32, 38].

To study the influence of ferrum (III) citrate on the nitrate ions usage by bacteria, they were seeded in the medium without NH_4Cl with sodium citrate to which 1.74 mM $NaNO_3$ and 1.74 mM $FeC_6H_5O_7$ were added to obtain the total content of electron acceptors in the medium of 3.47 mM. The bacteria were also seeded in a medium without NH_4Cl with sodium citrate to which 3.47 mM $NaNO_3$ or 3.47 mM $FeC_6H_5O_7$ was added (Fig. 3, Table 3). After simultaneous addition of $NaNO_3$ and $FeC_6H_5O_7$ into the cultivation medium, the bacteria were accumulated the biomass 1.2 times higher than in the medium with $NaNO_3$. That appeared 1.2 times lower than it was in the medium with $FeC_6H_5O_7$. In the medium with $NaNO_3$ and $FeC_6H_5O_7$, 1.3 times decrease in the efficiency of the nitrate ions reduction by cells (which did not exceed 75−78 %), compared with their reduction in the medium only with nitrate ions (98−99 %) was observed. In this medium, the bacteria produced 1.21−1.31 mM of ammonium ions. The efficiency of the Fe(III) reduction by the bacteria in the medium with $NaNO_3$ and $FeC_6H_5O_7$ (55−60 %) was revealed 1.5−1.7 times lower than in the medium with $FeC_6H_5O_7$ (90−92 %). In the medium with $NaNO_3$ and $FeC_6H_5O_7$, the efficiency of reduction of nitrate ions by the bacteria exceeded 1.3−1.4 times the efficiency of the Fe(III) reduction. In the medium with $NaNO_3$ and $FeC_6H_5O_7$, the bacteria produced only 0.96−1.05 mM of ferrum (II) ions. In the media with $NaNO_3$ and/or $FeC_6H_5O_7$ without bacteria, the efficiency of reduction of nitrate ions and Fe(III) was negligible and it did not exceed 2.9−4.6 and 4.6−5.8 %, respectively (Table 3). It was shown that in the medium with an equimolar initial content (1.74 mM) of $NaNO_3$

and $FeC_6H_5O_7$ bacteria of the *Desulfovibrio* genus were reduced 1.3−1.4 times more nitrate ions than the Fe(III) with the production of the ammonium ions at concentration 1.3 times higher than that of the ferrum (II) ions.

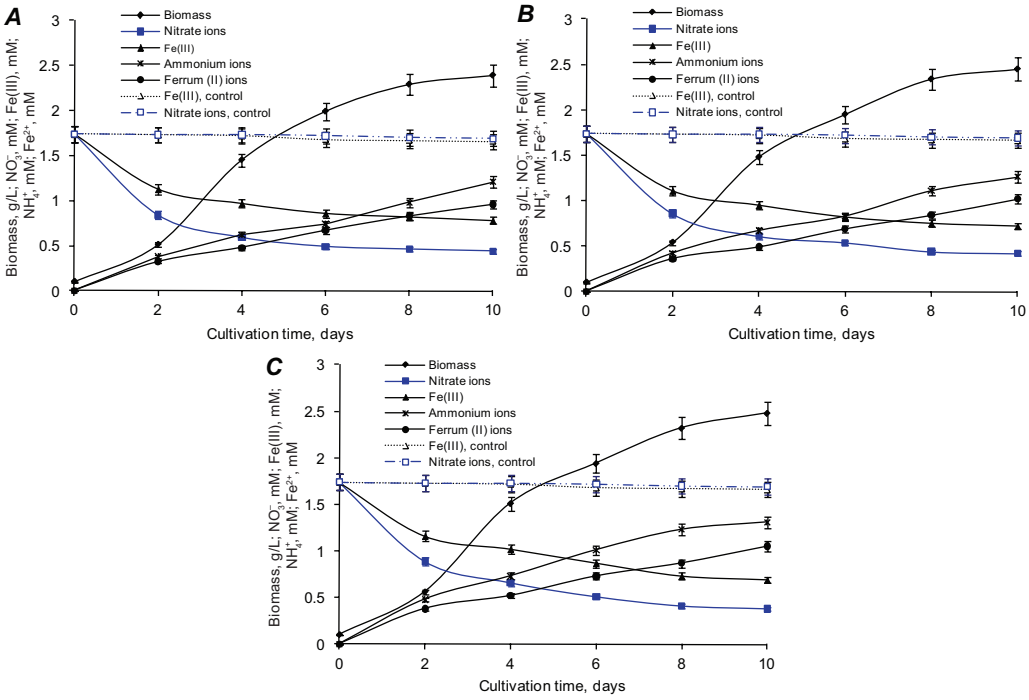

Fig. 3. The biomass accumulation, the content of nitrate, ammonium, ferrum (II) ions and Fe(III) in the cultural liquid during the growth of *D. desulfuricans* IMV K-6 (***A***), *Desulfovibrio* sp. Yav-6 (***B***) and *Desulfovibrio* sp. Yav-8 (***C***) in the medium with 1.74 mM $NaNO_3$ and 1.74 mM $FeC_6H_5O_7$. Control – the medium with 1.74 mM $NaNO_3$ and 1.74 mM $FeC_6H_5O_7$ without cells (M±m, n = 3)

Рис. 3. Нагромадження біомаси, вміст йонів нітрату, амонію, феруму (II) та Fe(III) у культуральній рідині під час росту *D. desulfuricans* IMB K-6 (***A***), *Desulfovibrio* sp. Yav-6 (***B***) та *Desulfovibrio* sp. Yav-8 (***C***) у середовищі з 1,74 мМ $NaNO_3$ та 1,74 мМ $FeC_6H_5O_7$. Контроль − середовище з 1,74 мМ $NaNO_3$ та 1,74 мМ $FeC_6H_5O_7$ без клітин (M±m, n = 3)

The bacteria were cultivated in the medium without NH_4Cl with sodium citrate to which 3.47 mM $NaNO_3$ and $FeC_6H_5O_7$ at different concentrations were added. The bacteria were also sown in the medium with sodium citrate and 3.47 mM $NaNO_3$ or 3.47 mM $FeC_6H_5O_7$ (Table 4). After 10 days of growth, the biomass of bacteria in the medium with $NaNO_3$ revealed the same one as in the medium with $FeC_6H_5O_7$. After simultaneous introduction into the medium of $NaNO_3$ and $FeC_6H_5O_7$ with increasing of ferrum (III) citrate concentrations a gradual repression of bacteria growth was observed, compared with growth in the medium with $NaNO_3$ or $FeC_6H_5O_7$. In the medium with $NaNO_3$ and 10.41 mM $FeC_6H_5O_7$, the growth of bacteria decreased 1.7−1.9 times, compared with the growth in the media only with $NaNO_3$ or $FeC_6H_5O_7$. In the media with $NaNO_3$ and $FeC_6H_5O_7$ with increasing of ferrum (III) citrate concentrations, there was also a gradual (1.3−1.9 times) decrease in the efficiency of nitrate ions reduction by cells, compared with their reduction in the medium only with $NaNO_3$ (96−97 %) (Fig. 4, *A*). In the media

Table 3. **NO_3^- and Fe(III) reduction by bacteria after 10 days of growth in the media with $NaNO_3$ and/or $FeC_6H_5O_7$ (M±m, n = 3)***

Таблиця 3. **Відновлення бактеріями NO_3^- і Fe(III) після 10 діб росту в середовищах з $NaNO_3$ та/або $FeC_6H_5O_7$ (M±m, n = 3)***

Strain	Electron acceptors of anaerobic respiration	Residual content in cultural liquid, mM		Reduction, %		Biomass, g/L
		NO_3^-	Fe(III)	NO_3^-	Fe(III)	
D. desulfuricans IMV K-6	NO_3^-**	0.05±0.01	0	98.6	0	1.95±0.05
	NO_3^-** (c)	3.31±0.03	0	4.6	0	0
	NO_3^- and Fe(III)**	0.44±0.05	0.78±0.06	74.7	55.2	2.39±0.03
	NO_3^- and Fe(III)** (c)	1.69±0.05	1.66±0.04	2.9	4.6	0
	Fe(III)	0	0.31±0.03	0	91.1	2.85±0.02
	Fe(III) (c)	0	3.27±0.02	0	5.8	0
Desulfovibrio sp. Yav-6	NO_3^-**	0.06±0.01	0	98.3	0	2.04±0.04
	NO_3^-** (c)	3.31±0.03	0	4.6	0	0
	NO_3^- and Fe(III)**	0.42±0.03	0.72±0.01	75.9	58.6	2.45±0.04
	NO_3^- and Fe(III)** (c)	1.69±0.05	1.66±0.04	2.9	4.6	0
	Fe(III)	0	0.36±0.05	0	89.6	2.81±0.03
	Fe(III) (c)	0	3.27±0.02	0	5.8	0
Desulfovibrio sp. Yav-8	NO_3^-**	0.03±0.01	0	99.1	0	2.14±0.07
	NO_3^-** (c)	3.31±0.03	0	4.6	0	0
	NO_3^- and Fe(III)**	0.38±0.03	0.69±0.02	78.2	60.4	2.48±0.02
	NO_3^- and Fe(III)** (c)	1.69±0.05	1.66±0.04	2.9	4.6	0
	Fe(III)	0	0.27±0.03	0	92.2	2.79±0.03
	Fe(III) (c)	0	3.27±0.02	0	5.8	0

Comments: * The initial concentration of $NaNO_3$ or $FeC_6H_5O_7$ in the medium was 3.47 mM, at the presence of $NaNO_3$ and $FeC_6H_5O_7$ in the medium was 1.74 mM; ** the media with $NaNO_3$ and $FeC_6H_5O_7$ or without it were not included the NH_4Cl; (c) – control: the medium without cells

Примітки: * − вихідна концентрація $NaNO_3$ або $FeC_6H_5O_7$ у середовищі – 3,47 мМ, за наявності у середовищі $NaNO_3$ і $FeC_6H_5O_7$ – 1,74 мМ; ** − до середовищ із $NaNO_3$ і $FeC_6H_5O_7$ або без не додавали NH_4Cl; (c) – контроль: середовище без клітин

with $NaNO_3$ and $FeC_6H_5O_7$, the bacteria produced 0.78−1.91 mM of ammonium ions (control: 2.00−2.10 mM) (Table 4). The efficiency of Fe(III) reduction by cells with increasing its concentrations in the media with $NaNO_3$ and $FeC_6H_5O_7$ was revealed from 1.6 to 2.7 times lower than its reduction in the medium with only $FeC_6H_5O_7$ (89−92 %) (Fig. 4, *B*). In the media with $NaNO_3$ and $FeC_6H_5O_7$, the bacteria were produced 0.92−3.61 mM of ferrum (II) ions (control: 3.05−3.18 mM) (Table 4). In a medium with $NaNO_3$ and $FeC_6H_5O_7$ without bacteria, the efficiency of NO_3^- and Fe(III) reduction did not exceed 4.3 and 4.6 %, respectively (Fig. 4). It was shown that ferrum (III) citrate inhibits the biomass accumulation, the nitrate ions reduction and the ammonium ions production by the bacteria of *Desulfovibrio* sp. after simultaneous addition into medium of $NaNO_3$ and $FeC_6H_5O_7$ (1.74−10.41 mM). In medium with the same initial content (3.47 mM) of $NaNO_3$ and $FeC_6H_5O_7$, the bacteria reduced 1.4 times more nitrate ions than Fe(III) with the production of ammonium ions at concentration 1.1 times higher than that of ferrum (II) ions.

Table 4. **NO_3^- and Fe(III) reduction by *Desulfovibrio* sp. after 10 days of growth in the media with $NaNO_3$ and/or $FeC_6H_5O_7$ (M±m, n = 3)**

Таблиця 4. **Відновлення NO_3^- і Fe(III) *Desulfovibrio* sp. після 10 діб росту в середовищах з $NaNO_3$ та/або $FeC_6H_5O_7$ (M±m, n = 3)**

Strain	Electron acceptors of anaerobic respiration	Residual content in cultural liquid, mM		Fe^{2+}, mM	NH_4^+, mM	Biomass, g/L
		NO_3^-	Fe(III)			
D. desulfuricans IMV K-6	3.47 mM NO_3^-	0.13±0.03	0	0	2.00±0.01	2.58±0.01
	3.47 mM NO_3^-(c)	3.32±0.04	0	0	0.14±0.01	0
	3.47 mM NO_3^- and 1.74 mM Fe(III)	0.92±0.02	0.80±0.01	0.92±0.01	1.88±0.05	2.34±0.06
	3.47 mM NO_3^- and 3.47 mM Fe(III)	0.91±0.06	1.69±0.01	1.74±0.02	1.85±0.01	2.26±0.04
	3.47 mM NO_3^- and 5.21 mM Fe(III)	1.26±0.07	2.58±0.06	2.61±0.02	1.29±0.03	1.79±0.01
	3.47 mM NO_3^- and 6.94 mM Fe(III)	1.59±0.08	3.97±0.02	2.95±0.06	1.06±0.04	1.62±0.05
	3.47 mM NO_3^- and 10.41 mM Fe(III)	1.72±0.04	6.75±0.04	3.61±0.04	0.86±0.03	1.43±0.04
	3.47 mM Fe(III)	0	0.37±0.03	3.05±0.04	0	2.78±0.06
	3.47 mM Fe(III) (c)	0	3.31±0.04	0.15±0.01	0	0
Desulfovibrio sp. Yav-6	3.47 mM NO_3^-	0.11±0.02	0	0	2.05±0.08	2.61±0.02
	3.47 mM NO_3^-(c)	3.32±0.04	0	0	0.14±0.01	0
	3.47 mM NO_3^- and 1.74 mM Fe(III)	0.85±0.09	0.78±0.01	0.94±0.01	1.85±0.06	2.53±0.02
	3.47 mM NO_3^- and 3.47 mM Fe(III)	0.99±0.05	1.72±0.08	1.86±0.08	1.83±0.02	2.44±0.01
	3.47 mM NO_3^- and 5.21 mM Fe(III)	1.31±0.09	2.89±0.01	2.33±0.04	1.30±0.01	2.03±0.02
	3.47 mM NO_3^- and 6.94 mM Fe(III)	1.54±0.01	3.99±0.03	2.94±0.04	1.18±0.03	1.95±0.06
	3.47 mM NO_3^- and 10.41 mM Fe(III)	1.69±0.03	6.80±0.07	3.59±0.09	0.78±0.08	1.50±0.05
	3.47 mM Fe(III)	0	0.27±0.09	3.18±0.02	0	2.69±0.03
	3.47 mM Fe(III) (c)	0	3.31±0.04	0.15±0.01	0	0
Desulfovibrio sp. Yav-8	3.47 mM NO_3^-	0.09±0.02	0	0	2.10±0.02	2.56±0.03
	3.47 mM NO_3^-(c)	3.32±0.04	0	0	0.14±0.01	0
	3.47 mM NO_3^- and 1.74 mM Fe(III)	0.82±0.04	0.74±0.09	1.03±0.02	1.91±0.06	2.50±0.06
	3.47 mM NO_3^- and 3.47 mM Fe(III)	0.87±0.09	1.66±0.05	1.77±0.09	1.85±0.01	2.34±0.04
	3.47 mM NO_3^- and 5.21 mM Fe(III)	1.20±0.05	2.50±0.01	2.72±0.04	1.27±0.01	1.73±0.01
	3.47 mM NO_3^- and 6.94 mM Fe(III)	1.34±0.03	3.63±0.09	3.28±0.05	1.03±0.02	1.57±0.01
	3.47 mM NO_3^- and 10.41 mM Fe(III)	1.52±0.07	6.87±0.03	3.51±0.06	0.88±0.05	1.39±0.06
	3.47 mM Fe(III)	0	0.30±0.06	3.15±0.08	0	2.57±0.05
	3.47 mM Fe(III) (c)	0	3.31±0.04	0.15±0.01	0	0

Comments: (c) – control: the medium without cells; the media supplemented with $NaNO_3$ and $FeC_6H_5O_7$ or without it did not include the NH_4Cl

Примітки: (c) – контроль: середовище без бактерій; до середовищ з $NaNO_3$ і $FeC_6H_5O_7$ або без не додавали NH_4Cl

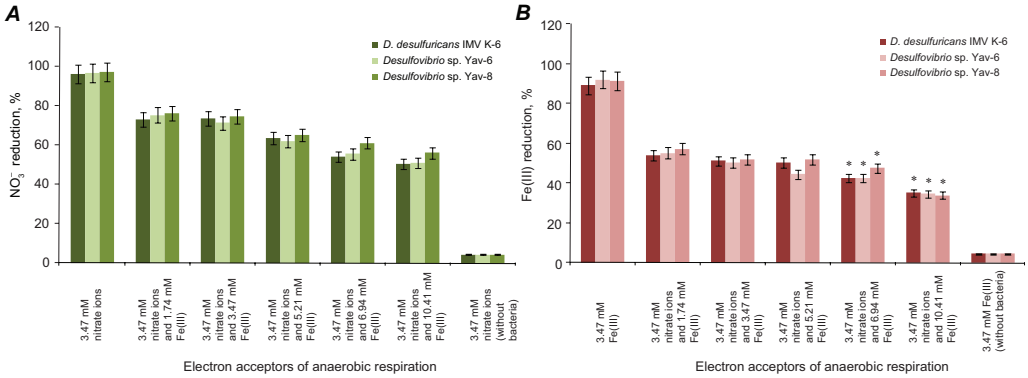

Fig. 4. Efficiency of NO_3^- (***A***) and Fe(III) (***B***) reduction by *Desulfovibrio* sp. after 10 days of growth in the media with $NaNO_3$ and/or $FeC_6H_5O_7$ (M±m, n = 3). * − P <0.05 (vs control)

Рис. 4. Ефективність відновлення NO_3^- (***A***) і Fe(III) (***B***) *Desulfovibrio* sp. після 10 діб росту в середовищах з $NaNO_3$ та/або $FeC_6H_5O_7$ (M±m, n = 3). * − P < 0,05 (vs контроль)

The bacteria were grown in the medium without NH_4Cl with sodium citrate to which 3.47 mM $NaNO_2$ and $FeC_6H_5O_7$ at different concentrations were added. The bacteria were also sown in the medium with sodium citrate and 3.47 mM $NaNO_2$ or 3.47 mM $FeC_6H_5O_7$ (Table 5). The biomass of bacteria in the medium with $NaNO_2$ was revealed 1.2 times lower than that in the medium with $FeC_6H_5O_7$. After simultaneous addition into the medium of $NaNO_2$ and $FeC_6H_5O_7$ with increasing concentrations of the ferrum (III) citrate there was a decreasing in the bacteria growth, compared with growth in a medium with $NaNO_2$ or $FeC_6H_5O_7$. In the medium with $NaNO_2$ and 10.41 mM $FeC_6H_5O_7$, the growth of bacteria was decreased 2.6−3.1 times, compared with growth in media only with $NaNO_2$ or $FeC_6H_5O_7$. In the media with $NaNO_2$ and $FeC_6H_5O_7$ with increasing concentrations of the ferrum (III) citrate, there was a gradual (1.7−3.1 times) decrease in the efficiency of nitrite ions reduction by the bacteria, as compared with their reduction in the medium with $NaNO_2$ (96−97 %) (Fig. 5, *A*). In the media containing $NaNO_2$ and $FeC_6H_5O_7$, the cells produced 0.52−1.77 mM of ammonium ions (control: 1.82−1.88 mM) (Table 5). The efficiency of Fe(III) reduction by the bacteria with increasing its concentration in the media with $NaNO_2$ and $FeC_6H_5O_7$ was revealed from 1.1 to 2.5 times lower than its reduction in the medium with $FeC_6H_5O_7$ (92−93 %) (Fig. 5, *B*). In the media with $NaNO_2$ and $FeC_6H_5O_7$, cells produced 1.30−4.16 mM of the ferrum (II) ions (control: 2.88−2.97 mM) (Table 5). In the media with $NaNO_2$ and $FeC_6H_5O_7$ without bacteria, the reduction of NO_2^- and Fe(III) did not exceed 4.0 and 3.5 %, respectively (Fig. 5). Thus, it was established that ferrum (III) citrate inhibits the biomass accumulation, the nitrite ions reduction, and the ammonium ions production by the bacteria of *Desulfovibrio* sp. after simultaneous addition into the medium of $NaNO_2$ and $FeC_6H_5O_7$ (1.74−10.41 mM). In the medium with the same initial content (3.47 mM) $NaNO_2$ and $FeC_6H_5O_7$, the bacteria reduced 1.4−1.6 times more Fe(III) than the nitrite ions with production of ferrum (II) ions at concentration 1.5−1.6 times higher than that of the ammonium ions.

Table 5. **NO_2^- and Fe(III) reduction by *Desulfovibrio* sp. after 10 days of growth in the media with $NaNO_2$ and/or $FeC_6H_5O_7$ (M±m, n = 3)**

Таблиця 5. **Відновлення NO_2^- і Fe(III) *Desulfovibrio* sp. після 10 діб росту в середовищах з $NaNO_2$ та/або $FeC_6H_5O_7$ (M±m, n = 3)**

Strain	Electron acceptors of anaerobic respiration	Residual content in cultural liquid, mM		Fe^{2+}, mM	NH_4^+, mM	Biomass, g/L
		NO_2^-	Fe(III)			
D. desulfuricans IMV K-6	3.47 mM NO_2^-	0.15±0.01	0	0	1.85±0.08	2.40±0.07
	3.47 mM NO_2^- (c)	3.33±0.09	0	0	0.08±0.01	0
	3.47 mM NO_2^- and 1.74 mM Fe(III)	1.64±0.03	0.29±0.02	1.38±0.02	1.77±0.04	2.38±0.09
	3.47 mM NO_2^- and 3.47 mM Fe(III)	1.71±0.04	0.74±0.03	2.51±0.08	1.59±0.07	2.23±0.04
	3.47 mM NO_2^- and 5.21 mM Fe(III)	1.84±0.01	1.25±0.04	3.80±0.04	1.10±0.07	1.66±0.02
	3.47 mM NO_2^- and 6.94 mM Fe(III)	2.29±0.07	4.01±0.09	2.73±0.05	0.84±0.09	1.43±0.03
	3.47 mM NO_2^- and 10.41 mM Fe(III)	2.40±0.03	6.42±0.01	3.83±0.01	0.61±0.08	0.94±0.08
	3.47 mM Fe(III)	0	0.29±0.02	2.88±0.01	0	2.82±0.01
	3.47 mM Fe(III) (c)	0	3.35±0.07	0.12±0.03	0	0
Desulfovibrio sp. Yav-6	3.47 mM NO_2^-	0.12±0.05	0	0	1.88±0.07	2.43±0.09
	3.47 mM NO_2^- (c)	3.33±0.09	0	0	0.08±0.01	0
	3.47 mM NO_2^- and 1.74 mM Fe(III)	1.67±0.09	0.34±0.09	1.30±0.02	1.61±0.01	2.40±0.01
	3.47 mM NO_2^- and 3.47 mM Fe(III)	1.65±0.07	0.86±0.01	2.59±0.04	1.68±0.05	2.20±0.03
	3.47 mM NO_2^- and 5.21 mM Fe(III)	1.90±0.06	1.42±0.02	3.62±0.09	1.04±0.08	1.56±0.04
	3.47 mM NO_2^- and 6.94 mM Fe(III)	2.28±0.06	3.50±0.09	3.38±0.02	0.77±0.06	1.41±0.01
	3.47 mM NO_2^- and 10.41 mM Fe(III)	2.37±0.03	6.51±0.02	3.85±0.02	0.58±0.08	0.93±0.02
	3.47 mM Fe(III)	0	0.24±0.01	2.97±0.01	0	2.88±0.04
	3.47 mM Fe(III) (c)	0	3.35±0.07	0.12±0.03	0	0
Desulfovibrio sp. Yav-8	3.47 mM NO_2^-	0.13±0.08	0	0	1.82±0.01	2.45±0.01
	3.47 mM NO_2^- (c)	3.33±0.09	0	0	0.08±0.01	0
	3.47 mM NO_2^- and 1.74 mM Fe(III)	1.52±0.02	0.23±0.01	1.39±0.07	1.72±0.03	2.38±0.04
	3.47 mM NO_2^- and 3.47 mM Fe(III)	1.68±0.01	0.79±0.04	2.58±0.03	1.66±0.08	2.22±0.03
	3.47 mM NO_2^- and 5.21 mM Fe(III)	1.95±0.03	1.46±0.07	3.53±0.05	1.18±0.04	1.62±0.02
	3.47 mM NO_2^- and 6.94 mM Fe(III)	2.23±0.04	3.36±0.03	3.48±0.01	0.71±0.02	1.40±0.01
	3.47 mM NO_2^- and 10.41 mM Fe(III)	2.30±0.04	6.08±0.03	4.16±0.02	0.52±0.04	0.94±0.01
	3.47 mM Fe(III)	0	0.28±0.01	2.94±0.02	0	2.87±0.03
	3.47 mM Fe(III) (c)	0	3.35±0.07	0.12±0.03	0	0

Comments: (c) – control: the medium without cells; the media with $NaNO_2$ and $FeC_6H_5O_7$ or without it did not include the NH_4Cl

Примітки: (c) – контроль: середовище без бактерій; до середовищ з $NaNO_2$ і $FeC_6H_5O_7$ або без не додавали NH_4Cl

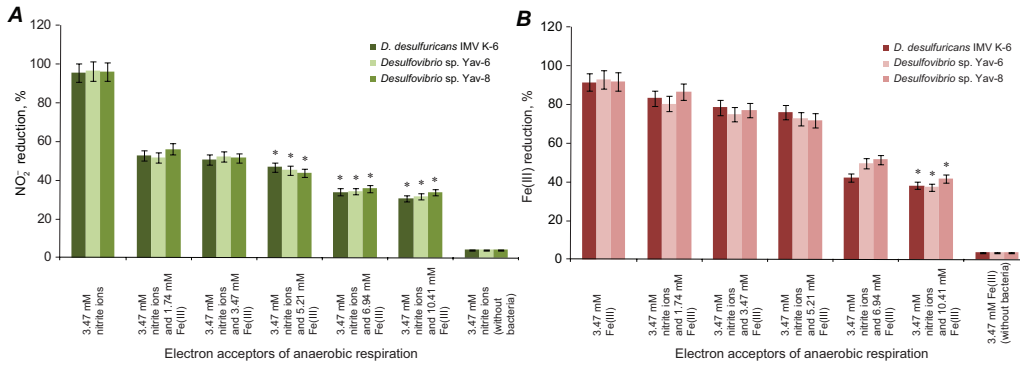

Fig. 5. Efficiency of NO_2^- (***A***) and Fe(III) (***B***) reduction by *Desulfovibrio* sp. after 10 days of growth in media with $NaNO_2$ and/or $FeC_6H_5O_7$ (M±m, n=3). * − P <0.05 (vs control)

Рис. 5. Ефективність відновлення NO_2^- (***A***) і Fe(III) (***B***) *Desulfovibrio* sp. після 10 діб росту в середовищах з $NaNO_2$ та/або $FeC_6H_5O_7$ (M±m, n=3). * − P < 0,05 (vs контроль)

In the medium with the same initial content (3.47 mM) of $NaNO_3$ and $FeC_6H_5O_7$, the bacteria reduced 1.4 times more nitrate ions than of Fe(III), and in medium with the same content (3.47 mM) of $NaNO_2$ and $FeC_6H_5O_7$ strains reduced 1.4−1.6 times more Fe(III) than the nitrite ions. Nevertheless, $FeC_6H_5O_7$ at all concentrations in the medium showed an inhibitory action on nitrate and nitrite reduction, that was carried out investigated strains of the bacteria. Negative influence of Fe(III) on the activity of molybdenum-containing membrane-bound respiratory and/or dissimilatory nitrate reductase [21], as well as periplasmic nitrite reductase, containing siro heme as a prosthetic group [16, 20], in the bacteria of *Desulfovibrio* genus can be due to a damage of the cytoplasmic membrane structure or modification of active conformation and denaturation of protein molecule, as a result of the replacement of the necessary metal ion by the ferrum in the active center of the enzyme. Although at pH 7.0 the standard oxidation-reduction potential of the Fe(III)/Fe(II) pair (E_0'= +0.77V) is lower than that of the oxidation-reduction NO_3^-/NO_2^- pair (E_0' = +0.78V), but higher than that of NO_2^-/NH_4^+ pair (E_0' = +0.34V) [16, 32], the efficiency of electron acceptor reduction by the microorganisms is primarily determined by the difference between the donor and electron acceptor potentials, that depend on the pH of the medium and change during cultivation of the bacteria [8]. Sulfate-reducing bacteria of the *Desulfovibrio* genus oxidize organic substrates only to acetic acid. Among them are species able to ferment, except fumarate, lactate, pyruvate or other organic acids [16]. Therefore, energy supply of cells in the process of the anaerobic respiration depends not only on the oxidation-reduction potential of present in the medium electron acceptor, but also on the ways of ATP synthesis in process of electron donor oxidation – by substrate or electron transport phosphorylation.

The processes of nitrate and nitride reduction carried out by the bacteria of the *Desulfovibrio* genus was less sensitive to negative influence of ferrum (III) citrate, as compared with the process of sulfate ions reduction. When sulfate ions reduction by the bacteria in the presence of 1.74−10.41 mM $FeC_6H_5O_7$ decreased 2.0−4.7 times, the nitrate ions reduction − 1.3−1.9 times, nitrite ions − 1.7−3.1 times, in comparison with their reduction in the media with only $Na_2SO_4 \times 10\ H_2O$, $NaNO_3$ or $NaNO_2$ respectively. It is possible that at growth in the medium with $FeC_6H_5O_7$ and sulfate, nitrate or nitrite ions,

nitrate and nitrite reductases of the investigated strains are less sensitive to negative influence of ferrum (III) citrate than the cytoplasmic enzymes involved in sulfate respiration of these bacteria – ATP sulfurylase, pyrophosphatase, APS reductase, sulfite reductase, as described [16, 21, 27]. This can be explained by the fact that at high concentrations in the medium Fe(III) can interact not only with functional groups of a number of bacteria cellular metabolites, but also cause an oxidative stress [10, 28].

Despite the fact that the reduction of 1.74−10.41 mM Fe(III) by cells in the media with $Na_2SO_4 \times 10\ H_2O$, $NaNO_3$ or $NaNO_2$ decreased by 1.1−2.1, 1.6−2.7 and 1.1−2.5 times, respectively, compared with its reduction in the medium with only $FeC_6H_5O_7$, the obtained results suggest that the investigated strains of bacteria are adapted to high concentrations of trivalent ferrum compounds (up to 10.41 mM) and, therefore, can survive in environments contaminated by heavy metals. The isolated strains are perspective for application in the technologies of complex purification of the environment from heavy metals, sulfur and nitrogen compounds, since they are capable of active reductive transformation of these pollutants.

CONCLUSIONS

Sulfate-reducing bacteria, oxidizing organic compounds, beside sulfates, can use other electron acceptors in process of the anaerobic respiration. These are oxidized metal forms, in particular, ferrum, nitrates or nitrites that are dangerous for biological organisms. In the media with $Na_2SO_4 \times 10\ H_2O$ or $NaNO_2$ and $FeC_6H_5O_7$ at all tested concentrations, the bacteria reduced more Fe(III) than SO_4^{2-} or NO_2^-. In the media with $NaNO_3$ and $FeC_6H_5O_7$ at all concentrations, the bacteria reduced more NO_3^-, than of Fe(III). Nevertheless, at all concentrations in the medium $FeC_6H_5O_7$ showed a toxic effect on dissimilatory sulfate-, nitrate- and nitrite reduction, carried out by the bacteria. Due to the exoelectrogenic properties, the investigated strains of *Desulfovibrio* sp., demonstrated high metal-reducing activity even in the media with two various electron acceptors, can be applied as the anode biocatalysts in the microbial fuel cells for the formation of electric current during the oxidation of the organic matter. A resistance of strains of *Desulfovibrio* genus, isolated from Yavorivske Lake, to different pollutants can be a basis for their application in different biotechnologies, aimed at bioremediation of the polluted soils and waters.

ACKNOWLEDGMENTS

This work was financially supported by the Ministry of Education and Science of Ukraine, grant Bm-25F “Functioning of microbocenoses of technogenically altered territories and their participation in transformation of heavy metal and sulfur compounds”, No 0116U001534.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Rights: This article does not contain any studies with animal subjects performed by the any of the authors.

1. *Abdulina D.R. Purish L.M., Iutynska G.O.* Microbial communities and sulfate-reducing bacteria in soils near main-gas pipeline. **Microbiol. Zhurn.**, 2018; 80(5): 3–14. [DOI: https://doi.org/10.15407/microbiolj80.05.003; Google Scholar]
2. *Barton L.L., Tomei-Torres F.A., Xu H., Zocco T.* Metabolism of metals and metalloids by the sulfate-reducing bacteria. In: D. Saffarini (Ed.) **Bacteria-metal interactions**. Switzerland: Springer International Publishing, 2015. P. 57−83. [Google Scholar]
3. *Basniwal R.K., Singh N. J., Kumar M., Kumar V., Tuteja N., Varma A., Goyal P.* Biotransformation of xenobiotic compounds: microbial approach. In: Hashmi M., Kumar V., Varma A. (Eds) **Xenobiotics in the Soil Environment: Monitoring, Toxicity and Management**. Springer: Cham, 2017; 49: 335−345. [DOI: https://doi.org/10.1007/978-3-319-47744-2_22; Google Scholar]
4. *Bokranz M.J., Katz J., Schröder I., Roberton A.M., Kröger A.* Energy metabolism and biosynthesis of *Vibrio succinogenes* growing with nitrate or nitrite as terminal electron acceptor. **Arch. Microbiol.**, 1983; 135: 36–41. [Google Scholar]
5. *Breuer M., Rosso K.M., Blumberger J., Butt J.N.* Multi-haem cytochromes in *Shewanella oneidensis* MR-1: structures, functions and opportunities. **J. R. Soc. Interface**, 2015; 12(102): 20141117. [DOI: https://doi.org/10.1098/rsif.2014.1117; Google Scholar]
6. *Fitzgerald L.A., Petersen E.R., Leary D.H., Nadeau L.J., Soto C.M., Ray R.I., Little B.J., Ringeisen B.R., Johnson G.R., Vora G.J., Biffinger J.C. Shewanella frigidimarina* microbial fuel cells and the influence of divalent cations on current output. **Biosens. & Bioel.**, 2013; 40(1): 102–109. [DOI: https://doi.org/10.1016/j.bios.2012.06.039; Google Scholar]
7. *Gescher J., Kappler A.* **Microbial metal respiration: from geochemistry to potential applications.** Berlin, Heidelberg: Springer-Verlag, 2012; 8. 236 p. [DOI: https://doi.org/10.1007/978-3-642-32867-1_2; Google Scholar]
8. *Govorukha V.M., Havrylyuk O.A., Tashyrev O.B.* Regularities of quantitative distribution for Fe(III)-reducing bacteria in natural ecosystems. **Biotechnologia Acta**, 2015; 8(3): 123−128. [DOI: https://doi.org/10.15407/biotech8.03.123; Google Scholar]
9. *Gudz S.P., Hnatush S.O., Yavorska G.V., Bilinska I.S., Borsukevych B.M.* **Workshop on microbiology: textbook.** Lviv: Ivan Franko Nac. Univ. of Lviv, 2014. 436 p. (In Ukrainian)
10. *Hnatush S., Maslovska O.* Sulfur-reducing bacteria *Desulfuromonas acetoxidans* IMV B-7384 under the influence of heavy metal ions. In: **The Development of Natural Sciences.** Riga, Latvia, Izdevnieciba: Baltija Publishing, 2018. P. 98−122.
11. *Keith S.M., Herbert R.A.* Dissimilatory nitrate reduction by a strain of *Desulfovibrio desulfuricans*. **FEMS Microbiol. Lett.**, 1983; 18(1–2): 55–59. [Google Scholar]
12. *Kiran M.G., Pakshirajan K., Das G.* Heavy metal removal from multicomponent system by sulfate reducing bacteria: Mechanism and cell surface characterization. **Journal of Hazardous Materials**, 2017; 324(PtA): 62−70. [DOI: https://doi.org/10.1016/j.jhazmat.2015.12.042; Google Scholar]
13. *Kozlova I.P., Radchenko O.S., Stepura L.G., Kondratiuk T.O., Pilyashenko-Novochatnyi A.I.* **Geochemical activity of microorganisms and it practical aspects: textbook.** Kyiv: Naukova Dumka, 2008. 526 p. (In Ukrainian)
14. *Kuznetsov A., Gradova N., Lushnikov S., Éngelkhart M., Vaysser T., Chebotareva M.* **Applied Ecobiotechnology.** Moscow: Laboratoria znanij, BINOM, 2015; 2. 492 p. (In Russian)
15. *Lakyn G.F.* **Biometrics.** Moscow: Vysshaya Shkola, 1990. 352 p. (In Russian)
16. *Lengeler J., Drevs G., Shlegel G.* (Ed.) **Contemporary Microbiology. Prokaryotes.** Moscow: Mir, 2005; 1. 654 p. (In Russian)

17. *Li X., Dai L., Zhang C., Zeng G., Liu Y., Zhou C., Xu W., Wu Y., Tang X., Liu W., Lan S.* Enhanced biological stabilization of heavy metals in sediment using immobilized sulfate reducing bacteria beads with inner cohesive nutrient. **J. Hazard. Mater.**, 2017; 324: 340–347. [DOI: https://doi.org/10.1016/j.jhazmat.2016.10.067; Google Scholar]
18. *Li X., Lan S.M., Zhu Z.P., Zhang C., Zeng G.M., Liu Y.G., Cao W.C., Song B., Yang H., Wang S.F., Wu S.H.* The bioenergetics mechanisms and applications of sulfate-reducing bacteria in remediation of pollutants in drainage: a review. **Ecotoxicol. Environ. Saf.**, 2018; 158: 162–170. [DOI: https://doi.org/10.1016/j.ecoenv.2018.04.025; Google Scholar]
19. *Lucana D., Wedderhoff I., Groves M.* ROS-mediated signalling in bacteria: zinc-containing Cys-X-X-Cys redox centres and iron-based oxidative stress. **J. Signal Transduct.**, 2012; 2012: 1–9. [DOI: https://doi.org/10.1155/2012/605905; Google Scholar]
20. *Mitchell G.J., Jones J.G., Cole J.A.* Distribution and regulation of nitrate and nitrite reduction by *Desulfovibrio* and *Desulfotomaculum* species. **Arch. Microbiol.**, 1986; 144 (1): 35–40. [Google Scholar]
21. *Morozkina E.V., Zvyagilskaya R.A.* Nitrate reductases: structure, functions, and effect of stress factors. **Biochemistry**, 2007; 72(10): 1151–1161. [Google Scholar]
22. *Moroz O.M., Hnatush S.O., Bohoslavets Ch.I., Hrytsun' T.M., Borsukevych B.M.* The influence of potassium dichromate on dissimilatory reduction of sulfate and nitrate ions by bacteria *Desulfovibrio* sp. **Ecol. & Noospherol.**, 2017; 28(1−2): 84−95. (In Ukrainian) [DOI: https://doi.org/10.15421/031708; Google Scholar].
23. *Moroz O., Hnatush S., Bohoslavets Ch., Yavorska G., Borsukevych B.* Sulfate, nitrate and nitrite ions reduction by bacteria of *Desulfovibrio* genus under the influence of ferrum (III), chrome (VI) and manganese (IV) compounds. **Visn. Lviv. Univ. Ser. Biol.**, 2016; 71: 190−205. (In Ukrainian) [Google Scholar]
24. *Moroz O.M., Hnatush S.O., Tarabas O.V., Bohoslavets Ch.I., Yavorska G.V., Borsukevych B.M.* Sulfidogenic activity of sulfate and sulfur reducing bacteria under the influence of metal compounds. **Biosystems Diversity,** 2018; 26(1): 3–10. (In Ukrainian) [DOI: https://doi.org/10.15421/011801; Google Scholar]
25. *Moroz O.M.* Metal salts influence on nitrates reduction by sulfate reducing bacteria. **Studia Biologica**, 2013; 7(2): 47–56. (In Ukrainian) [DOI: https://doi.org/10.30970/sbi.0702.287; Google Scholar]
26. *Moroz O.M.* Regularities of hydrogen sulfide production by sulfate reducing bacteria from water of Yavoriv sulfur deposit open pit. **Nauk. Visn. Uzhgorod. Univ. Ser. Biol.**, 2010; 27: 56–63. (In Ukrainian)
27. *Papanikolaou G., Pantopoulos K.* Iron metabolism and toxicity. **Toxicol. Appl. Pharm.**, 2005; 202: 199–211. [Google Scholar]
28. *Peretyatko T.B., Halushka A.A., Gudz S.P.* Usage of metals as the terminal electron acceptors by the sulfate-reducing bacteria. **Studia Biologica**, 2009; 3(3): 141–158. [DOI: https://doi.org/10.30970/sbi.0303.048; Google Scholar]
29. *Peretyatko T.B., Gudz S.P.* The ability of sulfate reducing bacteria *Desulfovibrio desulfuricans* Ya-11 and *Desulfobacter* sp. to use nitrate as electron acceptor. **Studia Biologica**, 2011; 5(2): 51–60. (In Ukrainian) [DOI: https://doi.org/10.30970/sbi.0502.150; Google Scholar]
30. *Peretyatko T.B., Halushka A.A., Gudz S.P., Hnatush S.O.* Certificate of deposition of bacteria Ya-11 (*Desulfovibrio desulfuricans* Ya-11 and *Pseudomonas* sp.) in the Depository of D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine with appropriation of registration number IMV K-6. 2009. (In Ukrainian)

31. *Prokhorova A., Sturm-Richter K., Doetsch A., Gescher J.* Resilience, dynamics and interactions within a multi-species exoelectrogenic model biofilm community. **Appl. Environ. Microbiol.**, 2017; 83(6): e03033–e03016.
[DOI: https://doi.org/10.1128/AEM.03033-16; Google Scholar]
32. *Richter K., Schicklberger M., Gescher J.* Dissimilatory reduction of extracellular electron acceptors in anaerobic respiration. **Appl. Environ. Microbiol.**, 2012; 78(4): 913–921.
[DOI: https://doi.org/10.1128/AEM.06803-11; PMID: 22179232; Google Scholar]
33. *Sholiak K.V., Peretyatko T.B., Verkholyak N.S., Gudz S.P.* Utilization of sulfate and nitrate by sulfate reducing bacteria *Desulfomicrobium* sp. CrR3 in the presence of bichromate in the medium. **Studia Biologica**, 2013; 7(3): 153–160. (In Ukrainian)
[DOI: https://doi.org/10.30970/sbi.0703.300; Google Scholar]
34. *Simonte F., Sturm G., Gescher J., Sturm-Richter K.* Extracellular electron transfer and biosensors. In: **Advances in Biochemical Engineering / Biotechnology.** Berlin, Heidelberg: Springer, 2017: 1–24.
[DOI: https://doi.org/10.1007/10_2017_34; Google Scholar]
35. *Si Y., Zou Y., Liu X., Si X., Mao J.* Mercury methylation coupled to iron reduction by dissimilatory iron-reducing bacteria. **Chemosphere**, 2015; 122: 206–212.
[DOI: https://doi.org/10.1016/j.chemosphere.2014.11.054; Google Scholar]
36. *Tarabas O., Moroz O., Hnatush S., Yavorska G., Zvir G., Kovalchuk M.* Ecological trophic groups of microorganisms of Yavorivske Lake water. **Visn. Lviv. Univ. Ser. Biol.**, 2017; 76: 166−178. (In Ukrainian)
[Google Scholar]
37. *Teng Y., Xu Y., Wang X., Christie P.* Function of biohydrogen metabolism and related microbial communities in environmental bioremediation. **Front. Microbiol.**, 2019; 10 (106): 1–14.
[DOI: https://doi.org/10.3389/fmicb.2019.00106; Google Scholar]
38. *Viti C., Marchi E., Decorosi F., Giovannetti L.* Molecular mechanisms of Cr (VI) resistance in bacteria and fungi. **FEMS Microbiol. Rev.**, 2014; 38(4): 633–659.
[DOI: https://doi.org/10.1111/1574-6976.12051; Google Scholar]
39. *Xia X., Zhang S., Li S., Zhang L., Wang G., Zhang L., Wang J., Li Z.* The cycle of nitrogen in river systems: sources, transformation, and flux. **Environ. Sci.: Processes Impacts**, 2018; 20: 863−891.
[DOI: https://doi.org/10.1039/C8EM00042E; Google Scholar]
40. *Yan J., Ye W., Jian Z., Xie J., Zhong K., Wang S., Hu H., Chen Z., Wen H., Zhang H.* Enhanced sulfate and metal removal by reduced graphene oxide self-assembled *Enterococcus avium* sulfate-reducing bacteria particles. **Bioresource Technology**, 2018; 266: 447−453.
[DOI: https://doi.org/10.1016/j.biortech.2018.07.012; Google Scholar]

ВІДНОВЛЕННЯ ЙОНІВ СУЛЬФАТУ, НІТРАТУ Й НІТРИТУ *DESULFOVIBRIO* SP. ЗА ВПЛИВУ ФЕРУМ (III) ЦИТРАТУ

О. М. Мороз*, С. О. Гнатуш, Г. В. Яворська
Львівський національний університет імені Івана Франка
вул. Грушевського, 4, Львів 79005, Україна
**Кореспондуючий автор: e-mail: oksana.moroz@lnu.edu.ua*

Досліджено вплив ферум (III) цитрату за наявності його в середовищі культивування на відновлення сульфат-, нітрат- і нітрит-йонів сульфатвідновлювальними бактеріями *Desulfovibrio desulfuricans* IMB K-6, *Desulfovibrio* sp. Yav-6 і *Desulfovibrio* sp. Yav-8, виділеними з озера Яворівське. Встановлено, що ферум (III) цитрат пригнічує нагромадження біомаси, відновлення SO_4^{2-} та утворення бактеріями

H_2S за внесення у середовище 1,74–3,47 мМ $Na_2SO_4 \times 10\ H_2O$ та 1,74–10,41 мМ $FeC_6H_5O_7$, порівняно з ростом і рівнем відновлення йонів сульфату в середовищі лише з $Na_2SO_4 \times 10\ H_2O$. За умов культивування бактерій у наявності еквімолярної кількості (3,47 мМ) $Na_2SO_4 \times 10\ H_2O$ і $FeC_6H_5O_7$ вони відновлювали у 2,5−2,7 разу більше Fe(III), ніж SO_4^{2-} з утворенням Fe^{2+} за концентрації у 2,4−2,7 разу більшої, ніж H_2S. $FeC_6H_5O_7$ пригнічував ріст, відновлення NO_3^- або NO_2^- й утворення NH_4^+ бактеріями за внесення у середовище 1,74–3,47 мМ $NaNO_3$ чи $NaNO_2$ та 1,74–10,41 мМ $FeC_6H_5O_7$, порівняно з ростом і рівнем відновлення йонів нітрату або нітриту в середовищі лише з $NaNO_3$ або $NaNO_2$. У середовищі з однаковим початковим вмістом 3,47 мМ $NaNO_3$ та 3,47 мМ $FeC_6H_5O_7$ бактерії відновлювали в 1,4 разу більше NO_3^-, ніж Fe(III) з утворенням NH_4^+ за концентрації в 1,1 разу більшої, ніж Fe^{2+}. У середовищі з 3,47 мМ $NaNO_2$ та 3,47 мМ $FeC_6H_5O_7$ клітини відновлювали в 1,4−1,6 разу більше Fe(III), ніж NO_2^- з утворенням Fe^{2+} за концентрації в 1,5−1,6 разу більшої, ніж NH_4^+. Ферум (III) цитрат більш пригнічував дисиміляційне відновлення бактеріями йонів сульфату, ніж нітрату й нітриту, оскільки відновлення бактеріями SO_4^{2-} за його наявності в середовищі знижувалося у 2,0−4,7 разу, а відновлення NO_3^- та NO_2^- – лише в 1,3−1,9 і 1,7−3,1 разу відповідно, порівняно з їхнім відновленням у середовищах тільки з $Na_2SO_4 \times 10\ H_2O$, $NaNO_3$ або $NaNO_2$. Незважаючи на те, що відновлення 1,74–10,41 мМ Fe(III) клітинами в середовищах з $Na_2SO_4 \times 10\ H_2O$, $NaNO_3$ або $NaNO_2$ знижувалося в 1,1−2,1, 1,6−2,7 та 1,1−2,5 разу відповідно, порівняно з його відновленням у середовищі лише з $FeC_6H_5O_7$, досліджені штами бактерій є стійкими до високих концентрацій ферум (III) цитрату і тому можуть бути застосовані у технологіях комплексного очищення довкілля від сполук важких металів, сульфуру та нітрогену.

Ключові слова: *Desulfovibrio*, ферум, сульфати, нітрати, нітрити

Received / Одержано 20 January 2020 | Accepted / Прийнято 11 March 2020 | Published / Опубліковано 5 May 2020