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# SULFUR REDUCING BACTERIA FROM COAL PITS WASTE HEAPS OF CHERVONOGRAD MINING REGION

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Sulfur reducing bacteria from coal pits waste heaps of Chervonograd minig region were isolated and their seasonal number changes were established. Sulfur reducing bacteria number increases during cold season both at the end of vegetation season, depending on gangue humidity as well as on substrate temperature. The forty sulfur reducing bacteria isolates were selected. According to the highest biomass acumulation and hydrogen sulfide production for the following identification two strains were chosen. The morpho-physiological characteristics of isolated strains SV 30 and SV 35 were investigated. In accordance with obtained data, we assumed isolated strain SV 30 to be identified as genus *Desulfuromusa,* meanwhile SV 35 – *Geobacter*. After the seventh day of cultivation, the highest sulfur reducing activity of both strains was observed. Due to the metabolization ability of wide range of pollutants isolated sulfur reducing bacteria are perspective for application in environmental remediation technologies with biological methods.

***Keywords*:** sulfur reducing bacteria, *Geobacter, Desulfuromusa,* coal pits waste heaps.

## INTRODUCTION

Composited gangues from coal pits waste heaps consist of mudstone, siltstone, sandstone, coal, pyrite, sulfur [2]. Under the influence of high temperature and limited oxygen conditions pyrite decomposes to sulfur dioxide. Sulfuric acid and ferum-, manganese containing compounds are formed at the presence of water [38]. Obviously, in the sulfur compounds circulation including alternation of oxidation, reduction and transformation without changing the valence, microorganisms from gangues are involved [19]. Reduction of elemental sulfur occurs during sulfur respiration by sulfur reducing bacteria from waste heaps gangue. Molecular hydrogen or organic substrates are electron donors, while elemental sulfur, thiosulfate, tetrathionate, sulfite, polysulfide, heavy metals (HM) etc. are the terminal acceptors. Sulfur reducing bacteria produce hydrogen

sulfide during sulfur reduction dissimilation. Hydrogen sulfide reacts with HM ions, forming almost insoluble sulfides [14].

Dissimilatory sulfur reduction is carried out by meso- and thermophilic eubacteria of domain *Bacteria* and by hyperthermophilic bacteria of domain *Archaea*, that are widespread in anoxic water sediments, soils, hot springs. Sulfur respiration could be supported either by obligate anaerobic (genus *Desulfuromonas, Desulfurella, Desulfuromusa, Geobacter, Pelobacter*) or by microaerophilic and aerobic (genus *Wolinella, Shewanella, Campylobacter, Sulfospirillum, Alteromonas, Pseudomonas*) bacteria (Tab. 1) [16, 28, 30]. The most of representatives of *Desulfuromonaceae* and *Geobacteraceae* reduce sulfur compounds as well as some metals with variable valence [8, 21, 23, 25–27, 34] and support chlorine and nitrogen reduction [4, 15, 32].

Excessive HM accumulation in the substrate breaks the course of natural processes, slowing the natural reclamation of area. HM mobility regulation is possible due to fixing or increasing their solubility owing to interaction with gangue compounds. This leads to following organometallic or inorganic complexes formation: biogenic hydrogen sulfide, carbonate- and phosphate-ions [12, 18, 23, 24]. Therefore, isolation of sulfur reducing bacteria strains from waste heaps gangue with their following identification, research of particularities of its metabolism such as sulfur reducing activity are important for planning and developing waste heaps remediation.

The purpose of our research was isolation of sulfur reducing bacteria from coal pits waste heaps of Chervonograd mining region and investigation of their morpho-physiological characteristics.

## MATERIALS AND METHODS

20 gangue samples from 3 waste heaps (main heap of Central enrichment plant (CEP), waste heaps of coal pits “Vizejska” and “Nadija”) were taken with the aim of sulfur reducing bacteria isolation. We selected patterns of bare substrate (BS) and from areas under the mosses (UM) of black (still not overburn gangue) and red (overburn gangue) colors, that differ by pH value and chemical content [2]. Gangue sample with mass 3 g was put into 30 ml of sterile saline solution. Cultivation on Kravtsov-Sorokin medium without $SO_4^{2-}$ (pH 7.0–7.5) was carried out from the series of dilutions sowings. Bacteria were incubated in 25 ml tubes, which were tightly closed by rubber stoppers at thermostat under +28 $^0$C [42]. Bacteria were grown on Postgate C agar medium (pH 7.0–7.5) with sodium lactate (6 g/l) for investigation its physiological and biochemical properties. To identify and count colonies of sulfur reducing bacteria iron chloride was added to medium, that led to FeS formation and caused black color of colonies [36].

Biomass was determined by colorimetric method (with application of photoelectric colorimeter KFK-3, wavelenght 340 nm, cuvette with 3 mm optical way) by using the calibration curve. For investigation by electron microscopy cells were twice washed by distilled water, precipitated by centrifugation for 10000 rev./min during 15 minutes. Cells were fixed by 1.5% $OsO_4$ solution in cacodylate buffer (pH 7.2) during 90 minutes at $0^0$C. Fixed cells were washed and rehydrated in solutions with increasing concentrations of ethanol and propylene oxide. Samples were transferred into epoxy resin Epon 812. Cells slices were prepared with ultramickrotome UMTP-6 and then contrasted with lead citrate by Reynolds [33]. Viewing and photographing of samples was performed with transmission electron microscope PEM-100 at 75 kV accelerating voltage. Sulfur reducing bacteria identification was carried out according to morpho-physiological characteristics [17]. Gram staining conducted according to the method [8].

*Table 1*. **Sulfur reducing bacteria of *Desulfuromonadales* order**

*Таблиця 1.* **Сірковідновлювальні бактерії порядку *Desulfuromonadales***

| Genus | Species | References |
|---|---|---|
| | Family *Desulfuromonadaceae* | |
| *Desulfuromonas* | *D. acetexigens* | Finster et al., 1994 |
| | *D. acetoxidans* | Pfennig and Biebl, 1976 |
| | *D. carbonis* | Thuy et al, 2015 |
| | *D. chloroethenica* | Krumholz, 1997 |
| | *D. michiganensis* | Sung et al., 2003 |
| | *D. palmitatis* | Coates et al., 1995 |
| | *D. soudanensis.* | Badalamenti et al., 2016 |
| | *D. svalbardensis* | Vandieken et al., 2006 |
| | *D. thiophila* | Finster et al., 1997 |
| *Desulfuromusa* | *D. bakii* | Liesack and Finster 1994 |
| | *D. ferrireducens* | Vandieken et al., 2006 |
| | *D. kysingii* | Liesack and Finster, 1994 |
| | *D. succinoxidans* | Liesack and Finster, 1994 |
| | Family *Geobacteraceae* | |
| *Geobacter* | *G. anodireducens* | Sun et al., 2014 |
| | *G. argillaceus* | Shelobolina *et al.,* 2007 |
| | *G. bemidjiensis* | Nevin et al., 2005 |
| | *G. bremensis* | Straub and Buchholz-Cleven,2001 |
| | *G. chapellei* | Coates et al., 2001 |
| | *G. daltonii* | Prakash et al., 2010 |
| | *G. grbiciae* | Coates et al., 2001 |
| | *G. hydrogenophilus* | Coates et al., 2001 |
| | *G. lovleyi* | Sung et al., 2009 |
| | *G. luticola* | Viulu et al., 2013 |
| | *G. metallireducens* | Lovley et al., 1995 |
| | *G. pelophilus* | Straub and Buchholz-Cleven,2001 |
| | *G. pickeringii* | Shelobolina et al., 2007 |
| | *G. psychrophilus* | Nevin et al., 2005 |
| | *G. soli* | Zhou et al., 2014 |
| | *G. sulfurreducens* | Caccavo et al., 1995 |
| | *G. sulfurreducens* subsp. *ethanolicus* | Viulu et al., 2014 |
| | *G. sulfurreducens* subsp. *Sulfurreducens* | Viulu et al,. 2014 |
| | *G. thiogenes* | (De Wever et al., 2001) Nevin et al., 2007 |
| | *G. toluenoxydans* | Kunapuli et al., 2010 |
| | *G. uraniireducens* | Shelobolina et al., 2008 |
| *Geoalkalibacter* | *G. ferrihydriticus* | Zavarzina et al., 2006 |
| | *G. subterraneus* | Greene et al., 2009 |
| *Geopsychrobacter* | *G. electrodiphilus* | Holmes et al., 2004 |
| *Geothermobacter* | *G. ehrlichii* | Kashefi et al. 2005 |
| | Family *Pelobacteraceae* | |
| *Malonomonas* | *M. rubra* | Dehning and Schink, 1990 |
| *Pelobacter* | *P. acetylenicus* | Schink, 1986 |
| | *P. acidigallici* | Schink and Pfennig, 1983 |
| | *P. carbinolicus* | Schink, 1984 |
| | *P. massiliensis* | Schnell et al., 1991 |
| | *P. propionicus* | Schink, 1984 |
| | *P. seleniigenes* | Narasingarao and Häggblom, 2007 |
| | *P. venetianus* | Schink and Stieb, 1984 |

In order to establish the usage of different carbon sources and electron donors the range of substances at concentration 53.57 mM were added into Postgate C medium with sulfur and lack of sulfate-ions. There are sodium lactate (control), sodium acetate, sodium citrate, sodium piruvate, ascorbic, acetic, aspartic, benzoic, fumaric, malonic, nicotinic, stearic, succinic, palmitic and propionic acids, butanol, ethanol, fenol, mannitol, glucose, sucrose, fructose, alanine, glycine, urea.

To investigate the ability of bacteria to utilize different electron acceptors, fumaric (control) and malonic acids, sodium sulfate, sodium nitrate and sodium nitrite as well as sodium thiosulfate and sodium dithionite, cysteine, different metals compounds (Cr (VI), Mn(VII), Fe(II), Cu(II)) were added into the medium at concentration 32.29 mM.

The presence of acetate-ion in the medium was established according to the described method [1].

For the determination of influence of temperature and pH on bacterial sulfur reducing activity bacteria cultivated at temperatures +4, +16, +30, +37, +60 °C and pH 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5.

To establish sulfur reductase activity bacteria cells were separated from the medium by centrifugation at 8000 rev. per./min during 20 min. Enzymatic activity was measured by amount of hydrogen sulfide, produced during reaction [39]. The composition of reaction mixture was the following: potassium phosphate buffer (pH 7.5) – 440 μl; $S^0$ – 40 mg; 10 mM $NADH^+$ – 120 μl; 10 mM EDTA – 120 μl; glycerin – 120 μl; culture fluid – 400 μl. Reaction mixture was transferred into the tubes filled by argon. Incubation period was 10 min. The reaction was started by adding 120 μl $NADH^+$ and finished by adding 200 μl of 2 M NaOH. Hydrogen sulfide was determined by the method of methylene blue formation [40].

Statistical data processing was performed with “Microsoft Excel 2007”. Student coefficient was calculated for estimation the validity of differences between statistical characteristics of alternative data sets. The difference was claimed to be valid under the index of validity $p \leq 0.05$ [20].

## RESULTS

Opened freshly deposited gangues are almost devoid of microorganisms. Nevertheless during the first year intensive gangue inoculation by microorganisms occur. Investigated waste heaps were deposited during years, thus they differs also by remediation levels. We observed changes of sulfur reducing bacteria number in December, April, July and October during 2014–2015.

In December, maximal rate of sulfur reducing bacteria was established in gangues from coal pit “Nadija” waste heap, minimal – from CEP waste heap (Tab. 2). In April the number of this group of microorganisms increase significantly in all gangue samples. After the analysis of sulfur reducing bacteria quantity in July, it was noticed, that only seven samples contained microorganisms. Moreover, all samples from CEP had no sulfur reducing bacteria. The probable causes were gangues self-heating and continued lack of precipitation, resulting in substrate drying. In October, the number of sulfur reducing bacteria continued to decrease: it was not higher than 110 CFU/g in absolutely dry gangue (ADG) in 15 samples. Therefore, al gangue samples contain sulfur reducing bacteria with their highest number during the vegetation season.

*Table 2.* **Changes in number of sulfur reducing bacteria on coal pits waste heaps depending on the season, [CFU/g ADG]**

*Таблиця 2.* **Зміна чисельності сірковідновлювальних бактерій породних відвалів вугільних шахт залежно від сезону, [КУО/ г АСП]**

| Place of sampling | December | April | July | October |
|---|---|---|---|---|
| CEP waste heap | | | | |
| Terrace, black gangue, UM *Ceratodon* sp. | 319±15 | 2041±98 | 0 | 19561±635 |
| Terrace, black gangue, BS | 10±1 | 40000±1700 | 0 | 16±1 |
| Main dump, black gangue, BS | 11±1 | 1099±53 | 0 | 6±1 |
| Main dump, red gangue, BS | 319±17 | 16495±817 | 0 | 6±1 |
| Freshly deposited gangue[1], BS | 204±8 | 108±4 | 0 | 50±8 |
| Coal pit “Vizejska” waste heap | | | | |
| Top, red gangue, UM *Polytrichum* sp. | 91±5 | 244±12 | 25641±1314 | 18±3 |
| Top, red gangue, BS | 296±13 | 69231±321 | 2564±98 | 52±11 |
| Terrace, black gangue, UM *Brachitecium* sp. | 2041±102 | 8602±370 | 2809±146 | 55±4 |
| Tepaca, black gangue, BS | 1776±86 | 2353±122 | 0 | 6±1 |
| Base, black gangue, UM *Ceratodon* sp. | 581±27 | 112±7 | 0 | 46±3 |
| Base, black gangue, UM *Polytrichum* sp. | 10±1 | 8696±449 | 575±29 | 11824±546 |
| Base, black gangue, BS | 133±6 | 238±8 | 0 | 14752±717 |
| Coal pit “Nadija” waste heap | | | | |
| Top, black gangue, UM *Polytrichum* sp. | 112±6 | 3409±163 | 0 | 108±4 |
| Top, black gangue, BS | 505±26 | 27551±814 | 6030±255 | 109±3 |
| Top, red gangue, UM *Ceratodon* sp. | 1392±70 | 0 | 0 | 17±1 |
| Top, red gangue, BS | 1146±56 | 34091±159 | 0 | 16548±662 |
| Terrace, black gangue, UM, *Ceratodon* sp. | 34895±1691 | 116±2 | 20833±927 | 17887±812 |
| Terrace, black gangue, BS | 11±1 | 0 | 0 | 27±2 |
| Base, black gangue, UM *Ceratodon* sp. | 543±27 | 24742±141 | 54348±2739 | 12±1 |
| Base, black gangue, BS | 115±5 | 41237±2001 | 0 | 23±2 |

**Comments:** CFU – colony forming units, BS – bare substrate, UM – under the mosses. [1] – gangue, deposited in 2013 year

**Примітки:** CFU – колонієутворювальні одиниці, BS – оголений субстрат, UM – під мохом. [1] – порода, насипана у 2013 р.

Isolation and selection of sulfur reducing bacteria have been conducted according to the intensity of the colonies color on agar medium as a result of ferum sulfide formation. Among 40 isolates 6 were selected. Then according to the highest biomass accumulation and the amount of hydrogen sulfide production two strains (SV 30 and SV 35) were chosen for following identification by morpho-physiological characteristics.

The cells of both isolates are elongated rods, motile, non-spore-forming, gram-negative, support completely oxidation of organic substrates (to $CO_2$) (Fig. 1).

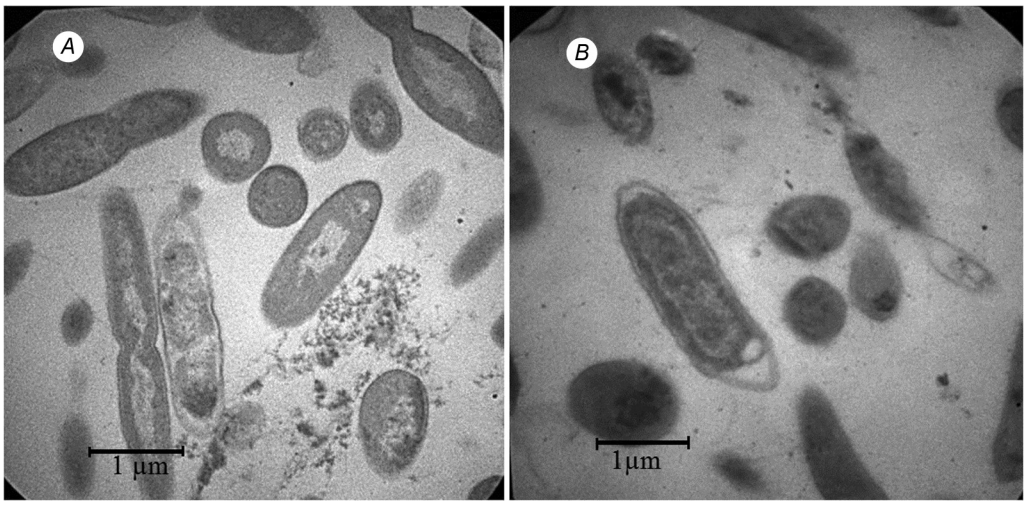


**Fig. 1.** Cells of sulfur reducing bacteria, that were isolated: *A* – SV 30; *B* – SV 35 (electron microscopy, ×10 000)

**Рис. 1.** Клітини виділених штамів сірковідновлювальних бактерій: *A* – СВ 30; *B* – СВ 35 (електронна мікроскопія, ×10 000)

Strain SV 30 accumulates the highest biomass at 25 °C and pH 7.0, meanwhile SV 35 – at 28 °C and pH 8.0. Thus both isolated strains are mesophilous, SV 30 is neutrophilous and SV 35 – moderate alkaliphilic.

The biomass accumulation and hydrogen sulfide production were studied to determine the ability of different electron donors and acceptors utilization by isolated SV 30 and SV 35 strains (Fig. 2). The highest biomass was accumulated on the seventh day. SV 30 strain produced the highest amount of hydrogen sulfide after two weeks of cultivation (1.02±0.02 mM), and SV 35 strain – on the tenth day (0.90±0.03 mM).

Bacterial utilization of different organic compounds as carbon sources and electron donors were investigated. SV 30 strain accumulated 0.73±0.04 g/l biomass, producing 1.04±0.06 mM of hydrogen sulfide and SV 35 strain – 0.69±0.02 g/l with 0.82±0.04 mM of hydrogen sulfide on the control medium (electron donor – lactate). Similar to control biomass were accumulated on the mediums with sodium acetate (0.70±0.08 and 0.67±0.03 g/l respectively) and sodium citrate (0.73±0.07 and 0.61±0.01 g/l respectively). SV 35 strain produced in 1.35 time higher amount of hydrogen sulfide in the case of citrate utilization. Utilization of fumaric acid as carbon source leaded to production in 1.28 and 1.49 times more hydrogen sulfide, compared to lactate usage. The usage of sodium pyruvate, acetic, ascorbic, malonic, stearic acids and glucose, alanine, mannitol, urea caused less biomass accumulation, compared to the control. Bacteria growth was not observed at presence of benzoic, nicotinic palmitic, propionic and succinic acids the same as butanol, ethanol. However ethanol usage caused the accumulation of 0.81±0.04 mM of hydrogen sulfide by SV 30 strain. The fructose and sucrose usage (but without sulfur reducing) by SV 35 strain as well as glycine, aspartic acid and fenol by SV 30 strain made the significant differences between two isolated strains.

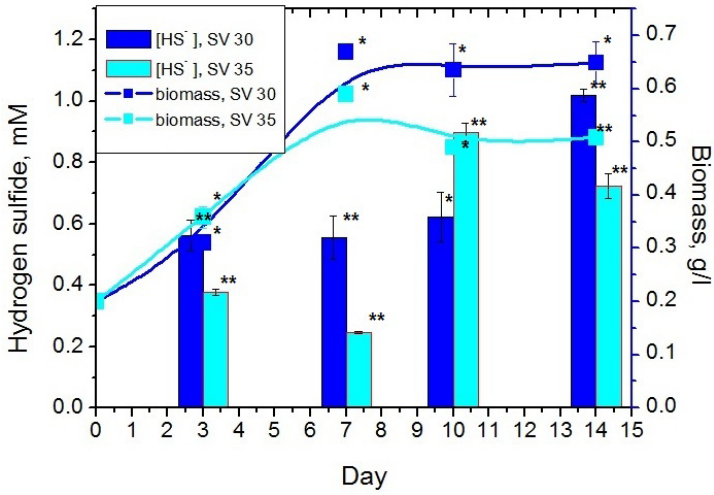


**Fig. 2.** Biomass growth and hydrogen sulfide production by SV 30 and SV 35 strains on the medium with sodium lactate and elemental sulfur during 14 days of cultivation. * − p ≤ 0.05, n = 4; ** − p ≤ 0.01, n = 4 – significant biomass and hydrogen sulfide changes compared to an appropriate controls (0.2 g/l – initial biomass, 0 мМ – initial hydrogen sulfide concentration).

**Рис. 2.** Нагромадження біомаси й утворення гідроген сульфіду штамами СВ 30 і СВ 35 у середовищі з натрій лактатом і елементною сіркою упродовж 14 діб культивування. * − p ≤ 0,05, n = 4; ** − p ≤ 0,01, n = 4 – вірогідні зміни біомаси та гідроген сульфіду порівняно з відповідними контролями (0,2 г/л – біомаса засіву, 0 мМ – початкова концентрація гідроген сульфіду)

Sulfur reducing activity was not observed during strains cultivation on Postgate C medium with sodium sulfate, sodium thiosulfate and sodium dithionite or determined in small amounts on medium with cycteine. Since isolated strains could not use these compounds as terminal electron acceptors, therefore we can not identify them as genus *Desulfomicrobium* or *Desulfovibrio.* We reject genus *Malomonas*, because its representatives do not use inorganic substances as terminal electron acceptors [17].

The growth of both strains on medium with fumatare that simultaneously served as electrons donor and acceptor without elemental sulfur, was established (Fig. 3).

Bacteria are able of nitrate- and nitrite as electron acceptors reduction (Fig. 3). The applying of 32 mM sodium nitrite on the tenth day of cultivation leaded to biomass lowering: in 2.40 (SV 30) and in 2.23 (SV 35) times less than during fumarate reduction. After the usage of sodium nitrate strains accumulated in 1.5 and 1.2 times smaller biomass compared to fumarate.

Isolated strains utilize heavy metals ions as electron acceptors (Fig. 4). The limit metals concentrations were following: chrome – 1 mM, manganese and ferum – 5 mM.

Absolutely inhibiting of biomass accumulation by strain SV 35 was observed after adding of 1 mM of potassium dichromate applying. This also leaded to biomass lowering of SV 30 in 4.81 times comparatively with a control (fumarate). In the same time increasing the potassium permanganate content till 5 mM caused the biomass growth of strain SV 30 in 1.31 times comparatively to the control. Ferric citrate applying leaded to noticeable biomass decrease: in 1.97 and 1.85 times – after 0,5 mM ferric citrate applying and in 5.42 and 10.76 times – after 5 mM.

The impact of temperature and pH value on biomass accumulation and hydrogen sulfide production by strain SV 30 was investigated (Fig. 5). Cells were grown at tem-

perature from +4 till +60 °C and pH value from 3.5 till 9.5. The highest biomass was accumulated at temperature from +16 till +30 °C and pH 6.5.The highest amount of hydrogen sulfide was determined after 6 days of cultivation at +37 °C and pH value from 7.5 till 9.5 as well as at +30 °C and pH 7.5.

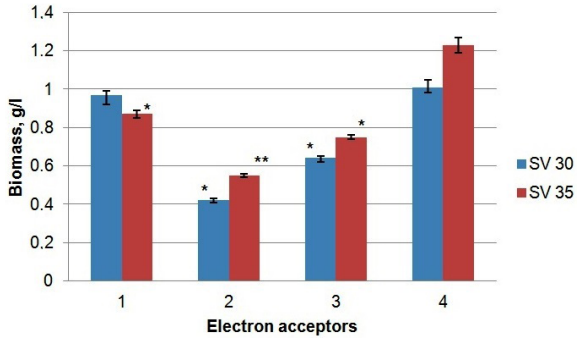


**Fig. 3.** Different electron acceptors utilization by sulfur reducing bacteria: 1 – malate, 2 – $NO_2^-$; 3 – $NO_3^-$; 4 – fumarate (control). (* – $p \le 0.05$, n = 3; ** – $p \le 0.01$, n = 3 – significant changes compared to the control)

**Рис. 3.** Використання сірковідновлювальними бактеріями різних акцепторів електронів: 1 – малат; 2 – $NO_2^-$; 3 – $NO_3^-$; 4 – фумарат (контроль). (* – $p \le 0,05$, n = 3; ** – $p \le 0,01$, n = 3 – вірогідні зміни порівняно з контролем)

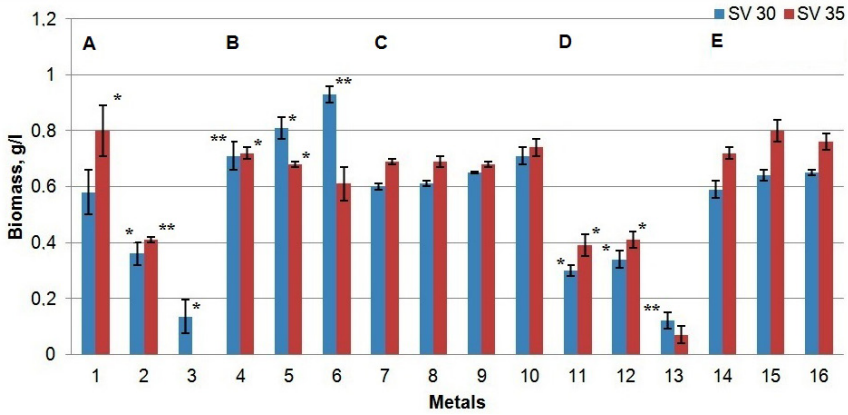


**Fig. 4.** Utilization of metal compounds as electron acceptors by sulfur reducing bacteria (*A* – Cr (VI), *B* – Mn (IV), *D* – Fe (III); *C* – control 1: electron donor – sodium lactate, acceptor – fumarate; E – control 2: electron donor – sodium citrate, acceptor – fumarate). 1–3 – Cr (VI) concentration: 0,1 mM (1); 0,5 mM (2); 1 mM (3). 4–6 – Mn (VI) concentration: 0,5 mM (4); 1 mM (5); 5 mM (6). 7–10 – fumarate (donor – sodium lactate) concentration: 0,1 mM (7); 0,5 mM (8); 1 mM (9); 5 mM (10). 11–13 – Fe (III) concentration: 0,5 mM (11); 1 mM (12); 5 mM (13). 14–16 – fumarate (donor – sodium citrate) concentration: 0,5 mM (14); 1 mM (15); 5 mM (16), * – $p \le 0.05$, n = 3; ** – $p \le 0.01$, n = 3 – significant changes of indices *A* and *B* compared to control *C* and *D* indices – to control *E*

**Рис. 4.** Використання сірковідновлювальними бактеріями сполук металів як акцепторів електронів (*A* – Cr (VI), *B* – Mn (IV), *D* – Fe (III); *C* – контроль 1: донор електронів – натрій лактат, акцептор – фумарат; E – контроль 2: донор електронів натрій цитрат, акцептор – фумарат). 1–3 – Cr (VI) у концентраціях: 0,1 мМ (1); 0,5 мМ (2); 1 мМ (3). 4–6 – Mn (VI) у концентраціях: 0,5 мМ (4); 1 мМ (5); 5 мМ (6). 7-10 – фумарат (донор – Na лактат) у концентраціях: 0,1 мМ (7); 0,5 мМ (8); 1 мМ (9); 5 мМ (10). 11-13 – Fe (III) у концентраціях: 0,5 мМ (11); 1 мМ (12); 5 мМ (13). 14-16 – фумарат (донор – Na цитрат) у концентраціях: 0,5 мМ (14); 1 мМ (15); 5 мМ (16). * – $p \le 0,05$, n = 3; ** – $p \le 0,01$, n = 3 – вірогідні зміни показників *A* і *B* порівняно з контролем *C* та показників *D* – із контролем *E* відповідно

Therefore, according to the temperature optimum and the ability to organic compounds complete oxidation (Tab. 3), we reject genus *Desulfurella* [17]. Since *Pelobacter*

grows only by fermentation of a limited range of substrates that are relatively rare in most anaerobic environments (acetylene, acetoin, ethylene glycol, butanediol, ethanol, 2,3-butanediol and acetate) and do not uses saccharides as carbon sources and electron donors, consequently isolated strains could not belong to this genus [17, 25]. *Geoalkalibacter, Geopsychrobacter* and *Geothermobacter* were eliminated because of highly specific growth conditions (high *pH* value, NaCl need, temperature optimums respectively) [6, 9, 13, 45]. Isolated bacteria were not determined as genus *Desulfuromonas* due to morphological and physiological differences [17, 31].

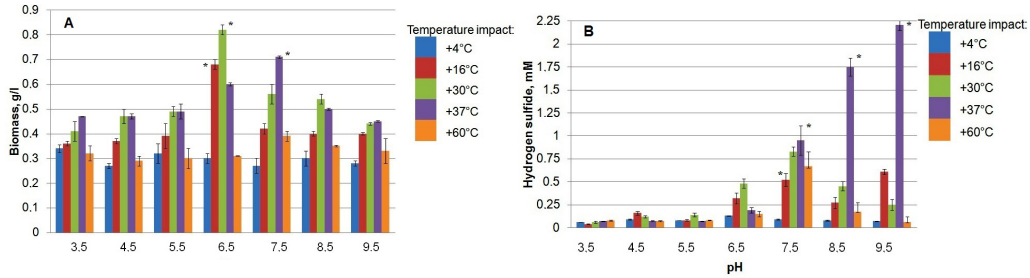


**Fig. 5.** Impact of temperature and pH value of medium on biomass growth (*A*) and hydrogen sulfide production (*B*) by SV 30 strain on Postgate C medium with sodium lactate and elemental sulfur on the 6th day of cultivation. * − $p \leq 0.05$, n = 3 – significant changes compared to the control (at 30 °C and pH 7.5)

**Рис. 5.** Вплив температури та pH середовища на нагромадження біомаси (*A*) й утворення гідроген сульфіду (*B*) штамом СВ 30 у середовищі Постгейта С з натрій лактатом і елементною сіркою на 6 добу культивування. * − $p \leq 0{,}05$, n = 3 – вірогідні зміни порівняно з контролем (за температури 30 °С та pH 7,5)

*Table 3.* **Morpho-physiological characteristics of isolated SV 30 and SV 35 strains**

*Таблиця 3.* **Морфофізіологічні характеристики виділених штамів СВ 30 і СВ 35**

| Characteristic | CB 30 | CB 35 | *Desulfuromusa* | *Geobacter* |
|---|---|---|---|---|
| Source | Gangue | Gangue | Anoxic marine water or estuarine muds | Soil and water sediments |
| Morphology | Curved rods | Rods | Bananashaped | Curved rods |
| Cell size, μm | 0.4–0.6 × 1.5–2.4 | 0.5–0.9 × 1.7–2.5 | 0.4–0.8 × 1–6 | 0.3–0.5 × 1.3–1.7 |
| Motility | + | + | + | + |
| Spore forming ability | – | – | – | – |
| Oxidation of organic compounds | Complete | Complete | Complete | Complete |
| Temperature optimum, °C | 25 | 28 | 35 | 35 |
| pH optimum | 7.0–7.5 | 7.5–8.0 | 6.5–7.0 | 6.5–7.0 |
| Sulfurreductase | + | + | + | + |
| Electron acceptors | | | | |
| S° | + | + | + | + |
| Malonic acid | + | + | + | + |

*The end of the Table 3*

| | | | | |
|---|---|---|---|---|
| Fumaric acid | + | + | + | + |
| Cysteine | - | - | - | NR |
| $NO_2^-$ | + | + | - | NR |
| $NO_3^-$ | + | + | +/- | + |
| Cr (VI) | + | + | NR | + |
| Mn(VII) | + | + | NR | + |
| Fe(III) | + | + | + | + |
| Cu(II) | + | + | NR | NR |
| $SO_4^{2-}$ | - | - | - | - |
| $S_2O_4^{2-}$ | - | - | - | NR |
| $S_2O_3^{2-}$ | - | - | - | NR |
| Electron donors and carbon sources | | | | |
| Sodium acetate | + | + | NR | NR |
| Sodium citrate | + | + | + | + (ferric citrate) |
| Sodium lactate | + | + | + | + |
| Sodium pyruvate | + | + | + | + |
| Acetic acid | + | + | + | + |
| Ascorbic acid | + | + | NR | NR |
| Aspartic acid | + | - | + | NR |
| Benzoic acid | - | - | NR | + |
| Fumaric acid | + | + | + | + |
| Malonic acid | + | + | + | + |
| Nicotinic acid | - | - | NR | NR |
| Palmitic acid | - | - | NR | NR |
| Propionic acid | - | - | NR | NR |
| Stearic acid | + | + | NR | NR |
| Succinic acid | - | - | + | - |
| Butanol | - | - | - | NR |
| Ethanol | + | - | - | + |
| Fenol | + | - | - | + |
| Glucose | + | + | - | + |
| Sucrose | - | + (not reduces $S^0$) | - | NR |
| Fructose | - | + (not reduces $S^0$) | - | NR |
| Alanine | + | + | + | NR |
| Glycine | + | - | + | NR |
| Mannitol | + | + | - | + |
| Urea | + +(not reduces $S^0$) | + (not reduces $S^0$) | NR | NR |

**Comments:** NR – not reported in literature; “+” – utilized; “-“– not utilized
**Примітки:** NR – не повідомлено у першоджерелах; “+” – утилізують, “-” – не утилізують

## CONCLUSION

Therefore, there are sulfur reducing bacteria in waste heaps gangues. Their number increases during cold season both at the end of vegetation season, depending on gangue humidity as well as on substrate temperature. Isolated strains cells are rod-shaped, non-spore-forming, gram-negative, motile, provide completely oxidation of organic substrates, do not utilize sulfate-, thiosulfate- and dithionite-ions as electron acceptors, reduce only elemental sulfur. Another possible acceptors are malate, fumarate, nitrite- and nitrate-ions both Cr (VI), Mn (VII) and Fe (III) compounds. However strains differ by the ability of usage of organic substrates as carbon sources and electron donors. Unlike strain SV 30, SV 35 utilizes fructose and sucrose, but neither aspartate and glycine, nor fenol and ethanol could be used by it. According to data obtained we assume the isolated strain SV 30 belongs to genus *Desulfuromusa,* meanwhile SV 35 – to *Geobacter*. The highest sulfur reducing activity of isolated strains was observed after the seventh day of cultivation: strain SV 30 produces maximum hydrogen sulfide up to 1.02±0.02 mM, and SV 35 – 0.90±0.03 mM. Due to the metabolization ability of wide range of pollutants isolated sulfur reducing bacteria are perspective for usage in environmental remediation technologies with biological methods.


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## СІРКОВІДНОВЛЮВАЛЬНІ БАКТЕРІЇ ПОРОДНИХ ВІДВАЛІВ ВУГІЛЬНИХ ШАХТ ЧЕРВОНОГРАДСЬКОГО ГІРНИЧОПРОМИСЛОВОГО РАЙОНУ

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Із порід відвалів вугільних шахт Червоноградського гірничопромислового району виділені сірковідновлювальні бактерії та досліджено сезонні зміни їхньої чи-

сельності. Встановлено, що кількість сірковідновлювальних бактерій зростає у холодні пори року та по закінченні вегетаційного сезону і залежить від вологості породи й температури субстрату. Виділено 40 культур сірковідновлювальних бактерій. Відповідно до накопичення найвищої біомаси та продукції гідроген сульфіду для ідентифікації відібрано 2 штами. Досліджено морфофізіологічні характеристики виділених штамів СВ 30 та СВ 35. Відповідно до отриманих даних припускаємо, що виділений штам СВ 30 належить до роду *Desulfuromusa,* а СВ 35 – *Geobacter*. Найвищу сульфідогенну активність виділених культур виявили після сьомої доби культивування. За здатністю метаболізувати широкий спектр полютантів виділені сірковідновлювальні бактерії, перспективні для використання у технологіях ремедіації середовищ біологічними методами.

***Ключові слова:*** сірковідновлювальні бактерії, *Geobacter*, *Desulfuromusa,* породні відвали вугільних шахт.

## СЕРОВОССТАНАВЛИВАЮЩИЕ БАКТЕРИИ С ПОРОД ОТВАЛОВ УГОЛЬНЫХ ШАХТ ЧЕРВОНОГРАДСКОГО ГОРНОПРОМЫШЛЕННОГО РЕГИОНА

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С пород отвалов угольных шахт Червоноградского горнопромышленного района выделены серовосстанавливающие бактерии и исследованы изменения их численности. Установлено, что количество серовосстанавливающих бактерий возрастает в холодное время года и по окончании вегетационного сезона и зависит от влажности породы и температуры субстрата. Выделены 40 культур серовосстанавливающих бактерий. В соответствии с накоплением самой высокой биомассы и продукции гидроген сульфида для идентификации выбраны 2 штамма. Исследованы морфофизиологические характеристики виделенных штаммов СВ 30 и СВ 35. В соответствии с полученными данными выделенный штамм СВ 30 определен до рода *Desulfuromusa, а* СВ 35 – *Geobacter*. Самую высокую сульфидогенную активность выделенных культур обнаружили после седьмых суток культивирования. По способности метаболизировать широкий спектр поллютантов виделенные серовосстанавливающие бактерии являются перспективными для использования в технологиях ремедиации среды с помощью биологических методов.

***Ключевые слова:*** серовосстанавливающие бактерии, *Geobacter, Desulfuromusa,* породные отвалы угольных шахт

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