Effect of abiotic factors on sulfidogenic activity of bacteria Desulfuromonas sp.

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Introduction

Microorganisms play an important role in the biosphere, in particular, in the processes of biotransformation of organic and inorganic substances, biogeochemical cycles of metallic and non-metallic elements, transformation of metals, minerals, soil and sediment formation (Bertrand et al., 2014; Li et al., 2018; Crane, 2019). Sulfur-reducing bacteria attract the attention of researchers as potential agents for the treatment of wastewater contaminated with sulfur and sulfur compounds, ions of heavy metals and organic compounds. By performing dissimilatory sulfur oxidation, Desulfuromonas bacteria oxidize various organic substrates using metals of variable valency as electron acceptors and turn them into forms which are non-toxic or less toxic for living organisms (Smith & Gadd, 1993). Sulfur-reducing bacteria resistant to high concentrations of heavy metal ions are involved in the reduction deposition of toxic metal ions. Usage of these microorganisms can neutralize the toxicity of the hydrogen sulfide produced by them and heavy metal ions due to their binding to produce insoluble sulfides (Lovley, 2004; Quiet et al., 2017; Suna et al., 2020). A number of studies have been conducted to investigate sulfidogenic systems based on sulfate-reducing bacteria, which were used for the purification of wastewaters from heavy metal ions; their effectiveness compared to chemical methods is confirmed. However, it is found that sulfidogenic processes caused by sulfur-reducing bacteria are more cost effective in wastewater treatment than conventional systems of sulfate ion treatment (Qui et al., 2017; Suna et al., 2020). It is suggested that elemental sulfur is a good alternative to sulfate ions since only two electrons are required for sulfur reduction, as distinct from the eight-electron process of sulfate ion reduction (Suna et al., 2020). Sulfur-reducing bacteria are highly efficient biocatalysts of microbial anodic fuel cells, which provide the electric current generation during oxidation of organic compounds (Logan & Regan, 2006). Carbohydrates (glucose, sucrose, cellulose, starch), volatile fatty acids (formate, acetate, butyrate), alcohols (ethanol, methanol), amino acids, proteins and even inorganic components (Alves et al., 2011; Richter et al., 2012; Vasyliv et al., 2015; Knoche et al., 2016) are used to generate the electric current in the microbial anodic fuel cells.

Sulfur-reducing bacteria attract the attention of researchers as potential agents for the purification of wastewater from the compounds of sulfur, heavy metal ions and organic compounds. However, the role of sulfur-reducing bacteria for the purification of wastewaters from heavy metal ions has been little investigated. Hydrogen sulfide accumulation efficiency is influenced by the various environmental factors, such as pH, hydrogen sulfide, presence of various donors and acceptors of electrons, concentration of heavy metal ions etc. (An & Picardal, 2015; Moroz et al., 2018). The objective of this work consisted in the evaluation of the effect of abiotic factors of the medium on sulfidogenic activity of bacteria Desulfuromonas sp.

Materials and methods

Sulfur-reducing bacteria Desulfuromonas sp. YSDDS-3 isolated from soil of Yazivsky sulfur deposit (Yavorivsky district, L’viv region, Ukraine).
Results

The largest biomass and the highest content of hydrogen sulfide during cultivation of bacteria *Desulfuromonas* sp. YSDS-3 in the medium with polysulfide sulfur was found at pH 7.5. At acid pH values (5 and 4) sulfidogenic activity decreased 3 and 7 times, accordingly (Fig. 1). The medium was inoculated with a suspension of cells at the concentration of 0.2 g/L and cultured at 30 ºC during 5–14 days. Generators GEN-boxanaer (France) were used to absorb oxygen. The conditions of anaerobiosis were confirmed with the use of anaerobic conditions’ indicator AnaerIndicator (bioMeriux, France). Cell biomass was determined by turbidimetric method on the photoelectric colorimeter КФК – 3 (λ = 340 nm, cuvette length – 3 mm). Content of hydrogen sulfide was found by photoelectric colorimetry method on the formation of methylene blue (Sugiyama, 2002). Content of Cr (VI), Fe (III), Mn (IV) and NO3- in the culture liquid was determined using qualitative measurement (Harris, 2003). The initial concentration of cells in all experiments was equal to 2003). The medium was sterilized at 0.75 atm during 30 min.

During the cultivation of bacteria *Desulfuromonas* sp. YSDS-3 in the medium with elemental sulfur, the hydrogen sulfide concentration increased with the growth of elemental sulfur concentration (Fig. 2); maximum hydrogen sulfide concentration is 5.4 mM. At the concentration of elemental sulfur of 1000 mM and above, biomass of bacteria halved. The most effective growth of bacteria was observed at the concentration of elemental sulfur of 10–100 mM. In order to study the effect of hydrogen sulfide on the accumulation of biomass of bacteria *Desulfuromonas* sp. YSDS-3, they were cultured in Postgate C medium with fumarate without elemental sulfur; hydrogen sulfide was introduced in concentrations of 3, 5, 10, 15, 20, 25, 30, 35 mM. Hydrogen sulfide in the concentration of 3 mM did not inhibit the growth of bacteria *Desulfuromonas* sp. YSDS-3. Hydrogen sulfide concentrations of 5–20 mM were observed to inhibit the growth 1.5 and 3 times, respectively (Fig. 3). No growth of bacteria occurred at the hydrogen sulfide concentration of 25 mM and more (Fig. 4). In the presence of MnO2, iron (III) citrate and potassium bichromate, bacteria *Desulfuromonas* sp. YSDS-3 accumulated the same biomass as in the test medium. Nevertheless, under such conditions, bacteria accumulated 1.5–2.0 times less hydrogen sulfide than in the test medium. Cr (VI), Fe (III), Mn (IV), NO3- were not detected in the culture medium.

**Fig. 1.** The effect of pH to accumulation of hydrogen sulfide by bacteria *Desulfuromonas* sp. YSDS-3 in the presence of polysulfide (x ± SD, n = 5)

**Fig. 2.** The accumulation of hydrogen sulfide by bacteria *Desulfuromonas* sp. YSDS-3 in the presence of elemental sulfur: x ± SD, n = 5

**Fig. 3.** The effect of hydrogen sulfide by bacteria *Desulfuromonas* sp. YSDS-3: ** – P < 0.01, *** – P < 0.001 significant changes of biomass compared to control (with Bonferroni correction); x ± SD, n = 5

**Fig. 4.** Effect of different electron acceptors for the accumulation of biomass and hydrogen sulfide by bacteria *Desulfuromonas* sp. YSDS-3: * – P < 0.05, ** – P < 0.01 significant changes of biomass and hydrogen sulfide compared to control (with Bonferroni correction); x ± SD, n = 5
C/S ratio (mg of oxidized organic carbon to mg of sulfide formed) for bacteria *Desulfuromonas* sp. YSDS-3 and sulfate-reducing bacteria *Desulfotomaculum* AR1 in the presence of elemental sulfur is 0.212–0.241, which is 2.5 times less than for bacteria *Desulfomicrobium* sp. CrR3 and *D. desulfuricans* Ya-11.

**Table 1**
The ratio of C/S after cultivation of sulfate and sulfur-reducing bacteria in media with sodium lactate (x ± SD, n = 5)

| Bacteria                      | Sulfur source | Biomass, g/L | Concentration of hydrogen sulfide, mM | C/S* ratio |
|-------------------------------|---------------|--------------|---------------------------------------|------------|
| *Desulfotomaculum* AR1        | S0            | 3.21±0.15    | 16.12±0.71                            | 0.241      |
| *Desulfomicrobium* sp. CrR3   | SO42−         | 3.01±0.11    | 14.11±0.64                            | 0.763      |
| *D. desulfuricans* Ya-11      | SO42−         | 2.41±0.12    | 4.01±0.19                             | 0.582      |
| *Desulfuromonas* sp. YSDS-3   | SO42−         | 1.12±0.05    | 14.67±0.73                            | 0.665      |

*Note: *C/S ratios – mg of oxidized organic carbon to mg of produced sulfide.*

The effect of elemental sulfur at the concentration of 32, 64 and 96 mM and the initial cell concentration (0.1, 0.5, 1.0 and 3.0 g/L) on the sulfidogenic activity of bacteria *Desulfuromonas* sp. YSDS-3 was evaluated during 16 days of cultivation using two-way analysis. Six series of two-way analysis of variance were performed, with the results presented in Figure 5. As the concentration of elemental sulfur and cell density increases, sulfidogenic activity of the bacteria grows.

The relative proportions of the effect of different concentrations of elemental sulfur, different cell density and other unaccounted factors on the sulfidogenic activity of bacteria *Desulfuromonas* sp. YSDS-3 are shown in (Fig. 6).

After 12–24 hours of cultivation, the most significant effect on sulfidogenic activity was observed on the part of different concentrations of elemental sulfur. The percent of the effect is 73% after 12 h and 70% after 24 h of cultivation. However, within 3–16 days of cultivation, the percentage of effect of elemental sulfur concentration decreased to 31% and the percentage of effect of cell density increased from 17% to 57%. The effect of other unaccounted factors was 3–13% depending on duration of the bacterial cultivation.

**Discussion**

Sulfur-reducing bacteria are involved in the reduction deposition of highly toxic ions of metals in the process of reduction of elemental sulfur to hydrogen sulfide, oxidizing various organic compounds as well (An & Picardal, 2015; Kefeni et al., 2017). In the previous studies, we found that bacteria *Desulfuromonas* sp. YSDS-3 in the presence of elemental sulfur use ethanol, propanol, butanol, sodium pyruvate, sodium acetate, sodium lactate, propionic, succinic, fumaric, malic acids, glucose, alanine, and casein as a carbon source. In the absence of elemental sulfur *Desulfuromonas* sp. YSDS-3 may use Fe (III), Mn (IV), Cr (VI), NO3−, cysteine and malate as acceptors of electrons. Fumarate is used by *Desulfuromonas* sp. YSDS-3 as a donor and acceptor of electrons (Chayka et al., 2016; Chayka et al., 2018).

Elemental sulfur is poorly soluble in water (5 mg/L at 25 ºC); therefore, low sulfur bioavailability for sulfur-reducing bacteria may reduce the cost effectiveness of the sulfur reduction process for wastewater treatment (Zhang et al., 2018; Suna et al., 2020). The level of pH has a significant effect on the rate of hydrogen sulfide formation by sulfur-reducing bacteria (Suna et al., 2020). At the neutral pH value (7.0–7.5), elemental sulfur becomes bio-available; it dissolves in aqueous solution of hydrogen sulfide and goes into polysulfide (soluble form). At the acid pH values (less than 6.0) formation of polysulfide becomes limited; low solubility of elemental sulfur in water restricts its bioavailability and leads to the decre-
ase in the rate of hydrogen sulfide formation (Suna et al., 2020). A sharp decrease in the sulfidogenic activity of bacteria *Desulfuromonas* sp. YSDDS-3 at low pH values (5 and 4) can be possibly related to the fact that polysulfide saturation is reduced, and soluble sulfide goes into the elemental form (Fig. 1). The elemental sulfur concentration of up to 20 mM is optimal for sulfur reduction by the bacteria *Desulfuromonas* sp. YSDDS-3 (Fig. 2).

![Fig. 6. Results of a two-way analysis of variance of the effect of different starting concentrations of elemental sulfur, different cell density and other unaccounted factors on the sulfidogenic activity of *Desulfuromonas* sp. YSDDS-3 during 16 days of cultivation](image)

Hydrogen sulfide is one of the main factors that can limit growth of sulfur-reducing bacteria (Reis et al., 1992). According to the literature data, cyanobacteria are the most sensitive to hydrogen sulfide. The inhibiting effect of hydrogen sulfide was already manifested at its concentration from 13 to 60 μM (Miller & Bébout, 2004). Growth of sulfate-reducing bacteria *D. desulfuricans* Ya-11 was completely inhibited in the presence of 6 mM sulfide in the medium. Addition of sulfide in the concentrations of 6.3 and 9.4 mM to the medium of cultivation of bacteria being in the logarithmic phase of growth led to complete termination of the culture growth (Verkholiak & Peretyatko, 2018). The most resistant among microorganisms to action of hydrogen sulfide are the archaea *Methanocaldococus jannaschii* and *Archaeoglobus profundus*, which grow well at the optimum temperature of growth (82 °C) at high enough concentrations of hydrogen sulfide – 80 and 60 mM, accordingly (Lloyd et al., 2005). The bacteria *Desulfuromonas* sp. YSDDS-3 under study are resistant to higher concentrations of hydrogen sulfide compared to sulfate-reducing bacteria. Growth inhibition was observed at hydrogen sulfide concentrations of 5–20 mM. The bacteria did not grow at a hydrogen sulfide concentration of 25 mM and more (Fig. 3).

Among the most common environmental pollutants are compounds of hexavalent chromium, ferrum, nitrate and nitrite ions, etc. (Lovley et al., 2004; Owld, et al., 2009; Esther et al., 2015). It is known that a sulfur-reducing bacteria in the absence of elemental sulfur reduce the metals of variable valency as electron acceptors and turn them into forms which are non-toxic or less toxic for living organisms (Lovley et al., 2004; An et al., 2015; Chayka et al., 2018). In the natural environments, where several potential electron acceptors are usually present at the same time, microorganisms initially use those that have the higher redox potential (Bertrand et al., 2014). It was established that presence of two acceptors of electrons (S and Cr (VI), S and MnO₂, S and Fe (III)) had no effect on the accumulation of biomass by the bacteria *Desulfuromonas* sp. YSDDS-3 (Fig. 4). However, bacteria under such conditions accumulated 1.5–2.5 times less hydrogen sulfide than in the test medium. Cr (VI), Fe (III), Mn (IV), NO₃⁻ were not detected in the culture medium. The simultaneous introduction of two electron acceptors – elemental sulfur with MnO₂, KNO₃, iron (III) citrate and potassium dichromate – led to inhibition of sulfur reduction.

The effectiveness of microbiological deposition of metal ions by hydrogen sulfide, production of sulfur-reducing bacteria, depends on the concentration of H₂S (Gudz et al., 2011). It is known that the deposition of metal ions by hydrogen sulfide formed by sulfur-reducing bacteria is more cost effective in wastewater treatment compared to systems of sulfate ion treatment (Quet et al., 2017; Zhang et al., 2018; Suna et al., 2020). For evaluation of the efficiency of hydrogen sulfide production, the calculation of C/S ratio (mg of oxidized organic carbon to mg of sulfide formed) was made for sulfur-reducing bacteria *Desulfuromonas* sp. YSDDS-3 and sulfate-reducing bacteria taken from the literature data (Dorosh et al., 2015; Quet et al., 2017; Verkholiak & Peretyatko, 2018; Verkholiak & Peretyatko, 2019). Low C/S ratio indicates the lower energy consumption for electron transfer from donor to elemental sulfur and formation of hydrogen sulfide (Table 1). In the presence of elemental sulfur, the C/S ratio is 0.212–0.241, which is 2.5 times lower compared to sulfate-reducing bacteria *Desulfomicrobium* sp. CrR3, *D. desulfuricans* Ya-11. Therefore, hydrogen sulfide formation during sulfur reduction is a more energy efficient process than sulfite reduction for the purification of wastewater from heavy metal ions.

**Conclusions**

Different concentration of elemental sulfur has its effect on sulfidogenic activity and growth of bacteria *Desulfuromonas* sp. YSDDS-3. The content of hydrogen sulfide grows with the increase in elemental sulfur concentration; maximum hydrogen sulfide concentration is 5.4 nm. In the medium with polysulfide at the neutral pH value (7.5), bacteria produce hydrogen sulfide and accumulate biomass in the most effective way. When pH decreases to 5.0 and 4.0, sulfidogenic activity of bacteria is inhibited 3 and 7 times, accordingly.

Hydrogen sulfide at the concentration of 3 mM does not inhibit the growth of bacteria; at the concentration of 5–20 mM it inhibits the accumulation of biomass 1.5 and 3.0 times, respectively. Bacteria did not grow at hydrogen sulfide concentration of 25 mM and above. In the presence of elemental sulfur, the C/S ratio is 2.5 times lower compared to sulfite-reducing bacteria (*Desulfomicrobium* sp. CrR3, *D. desulfuricans* Ya-11). The elemental sulfur and initial cell concentration has the most significant effect on sulfidogenic activity of bacteria *Desulfuromonas* sp. YSDDS-3.

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