Reduction of perchlorate ions by the sulfate-reducing bacteria
Desulfotomaculum sp. and Desulfovibrio desulfuricans

N. S. Verkholiak, T. B. Peretyatko, A. A. Halushka
Ivan Franko National University of Lviv, Lviv, Ukraine

Introduction

Problems of environment and food pollution by perchlorate ions are of worldwide relevance (Arbi et al., 2006). The perchlorate ion is a strong oxidizer which is mainly used as ammonium perchlorate (NH₄ClO₄) to produce solid rocket propellant, rockets and explosives, military ammunition, fireworks, airbags and paints. Presence of perchlorate ions in water supply systems and soil is also connected with the widespread usage of fertilizers, particularly, Chile saltpetre (Urbansky et al., 2001). Chlorine oxoniums can also be formed during the ozonation of chlorine treated drinking water (Sidiqqui, 1996). Perchlorate ions’ content in the water of centralized drinking water supply systems must not exceed 0.01 mg/dm³, according to DSTU 7525 from the year 2014. Perchlorate ions are also found in food, besides soil and the water environment (El Aribi et al., 2006). Perchlorate ions have a toxic effect on the human organism. Particularly, perchlorate ions competitively block iodine uptake by the thyroid gland and inhibit production of hormones, which can result in metabolic problems in adults and abnormal growth of children (Greer et al., 2002).

The redox potential of chlorine and perchlorate ions (E° (ClO₄⁻ /Cl⁻) = 1.03 V; E° (ClO₄⁻ /Cl⁻) = 1.287 V) gives them the possibility to be ideal electron acceptors for the metabolism of microorganisms. Ability to reduce perchlorate ions has been described for Vibrio dechlorati- lity to reduce perchlorate ions has been described for


The usage of microorganisms to clean the environment from xenobiotics, in particular chlorine-containing ones, is a promising method of detoxifying the contaminated environment. Sulfate-reducing bacteria Desulfotomaculum AR1, isolated from the Lviv sewage treatment system, are able to grow under conditions of environmental contamination by aromatic compounds and chlorine-containing substances. Due to their high redox potential, chlorine and perchlorate ions can be ideal electron acceptors for the metabolism of microorganisms. To test the growth of the tested microorganisms under the influence of perchlorate ions, bacteria were cultured in modified Postgate C medium with ClO₄⁻. Biomass was determined turbidimetrically, the content of sulfate ions and hydrogen sulfide – photoelectrocolorimetrically, the content of perchlorate ions – permanganometrically. The study of the ability of sulfate-reducing bacteria Desulfotomaculum AR1 and D. desulfuricans Ya-11 to grow in a medium with perchlorate ions as electron acceptors showed the inhibitory effect of ClO₄⁻ on sulfate ion reduction by bacteria. Bacteria Desulfotomaculum AR1 and D. desulfuricans Ya-11 are able to grow in environments with aromatic hydrocarbons, in particular tol- ene. The possibility of the growth of sulfate-reducing bacteria in the presence of toluene as an electron donor and perchlorate ions as an electron acceptor was investigated. The efficiency of perchlorate ion utilization by sulfate-reducing bacteria Desulfotomaculum AR1 and D. desulfuricans Ya-11 was about 90 %. The effect of molybdenum on the reduction of perchlorate ions by Desulfotomaculum AR1 is shown in the paper. Immobilization of bacteria Desulfotomaculum AR1 and D. desulfuricans Ya-11 was carried out in 3% agar and on wood chips. The ability of bacteria, immobilized on these media, to purify the aqueous medium from perchlorate ions was investigated. Reduction of perchlorate ions is more efficiently performed by cells of Desulfotomaculum AR1 and D. desulfuricans Ya-11 bacteria immobilized in agar than on wood chips. Sulfate-reducing bacteria Desulfotomaculum AR1 and D. desulfuricans Ya-11 are able to use perchlorate ions as electron acceptors, purifying the polluted aquatic environment from these pollutants.

Keywords: chlorine oxoniums; molybdenum; sulfidogenic activity; sulfate ion; perchlorate ion; sulfate-reducing bacteria; toluene.
Ya-11 and Desulfotomaculum AR1 bacteria.

Materials and methods

Object of research – sulfate-reducing bacteria Desulfovibrio desulfuricans Ya-11, isolated from Yavoriv Lake (Pereytak et al., 2006) and Desulfotomaculum AR1, isolated from Lviv wastewater purification system (Verkholiak & Pereytak, 2018). Bacteria were grown in Postgate C medium with the following content (g/L): potassium dihydrogen phosphate – 0.5; ammonium chloride – 1.0; sodium sulfate – 4.5; calcium chloride hexahydrate – 0.06; magnesium sulfate heptahydrate – 0.06; sodium lactate – 6; yeast extract – 1; ferrous sulfate heptahydrate – 0.004; sodium citrate dihydrate – 0.3; pH 7.6 (Postgate, 1984), modified Postgate C medium (without sulfate ions) and modified Postgate C medium with perchlorate ions to perform immobilization. Immobilization was achieved by barium chloride according to HOST 26426-85. Glycerol was used as the stabilizer of suspension. Hydrogen sulfide content was measured in culture liquid colorimetrically using p-aminodimethyl aniline (λ = 665 nm, 50 mm cuvette) (Sugiyama, 2002). Perchlorate ion was measured using permanganometry (by titration of Mohr’s salt residue with 0.1 n KMnO4 solution) (Petrashen, 1946).

Cultures of Desulfotomaculum AR1 and Desulfotomaculum AR1 microorganisms were previously grown in modified Postgate C medium with perchlorate ions to perform immobilization. Immobilization of the studied strains was performed in 3% agar. Immobilized bacteria were transferred to the column in the form of 1 × 1 cm bars and poured on system (Verkholiak & Peretyatko, 2018). Bacteria were grown in Postgate C media with sulfate ions (control), perchlorate ions, sulfate and perchlorate ions simultaneously for this purpose (Fig. 1).

Effectiveness of sulfate ion reduction during growth of bacteria in medium with toluene was approximately 16% in Desulfotomaculum AR1 and 20% in D. desulfuricans Ya-11 (Table 2).

Table 2

Effect of perchlorate ions on the amount of reduced sulfate ions and hydrogen sulfide accumulation by Desulfotomaculum AR1 and D. desulfuricans Ya-11 during the growth in sodium lactate medium

<table>
<thead>
<tr>
<th>Strain of bacteria</th>
<th>Cultivation medium</th>
<th>Concentration of sulfur-containing compounds, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toluene + SO42-</td>
<td>Toluene + SO42- + ClO4-</td>
</tr>
<tr>
<td></td>
<td>Lactate + SO42-</td>
<td>Lactate + SO42- + ClO4-</td>
</tr>
<tr>
<td></td>
<td>sulfate ion</td>
<td>hydrogen sulfide</td>
</tr>
<tr>
<td>Desulfotomaculum AR1</td>
<td>16.15 ± 0.26</td>
<td>16.74 ± 0.01</td>
</tr>
<tr>
<td>D. desulfuricans Ya-11</td>
<td>17.04 ± 0.19</td>
<td>13.58 ± 0.01</td>
</tr>
</tbody>
</table>

Note: initial sulfate ion concentration – 28 mM. * – P < 0.05, ** – P < 0.01 – probable changes of sulfate ion and hydrogen sulfide concentration (ANOVA).

Study of the ability of Desulfotomaculum AR1 and D. desulfuricans Ya-11 bacteria to grow using toluene (as electron donor) and perchlorate ions (as electron acceptor) showed the following results. Growth of Desulfotomaculum AR1 and D. desulfuricans Ya-11 bacteria in modified Postgate C media with toluene and sulfate ions, perchlorate ions, sulfate and perchlorate ions is almost equal in all media: D. desulfuricans Ya-11 biomass was approximately 1.3 g/L (Fig. 2b), Desulfotomaculum AR1 biomass – near 1 g/L (Fig. 2a).

Effective biomass accumulation by Desulfotomaculum AR1 (a) and D. desulfuricans Ya-11 (b) in media with sodium lactate and various electron acceptors (x ± m, n = 3); ** – P < 0.01, *** – P < 0.001 – probable biomass changes compared to control (Tukey test)

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Effect of perchlorate ions on the amount of reduced sulfate ions and hydrogen sulfide accumulation by Desulfotomaculum AR1 and D. desulfuricans Ya-11 during the growth in sodium lactate medium

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<td>sulfate ion</td>
<td>hydrogen sulfide</td>
</tr>
<tr>
<td>Desulfotomaculum AR1</td>
<td>4.79 ± 0.08</td>
<td>2.49 ± 0.20</td>
</tr>
<tr>
<td>D. desulfuricans Ya-11</td>
<td>5.79 ± 0.13</td>
<td>2.01 ± 0.10</td>
</tr>
</tbody>
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Note: initial sulfate ion concentration – 28 mM. * – P < 0.05, **– P < 0.01 – probable changes of sulfate ion and hydrogen sulfide concentration, compared to control (ANOVA).

Perchlorate ions were practically absent in the medium during growth of sulfate-reducing bacteria Desulfotomaculum AR1 and D. desulfuricans Ya-11 in Postgate C medium with ClO4- (Fig. 3). Sulfate-reducing bac-
teria Desulfotomaculum AR1 were cultivated in sulfate-free Postgate C medium with 0.1, 1.0 and 5.0 mM of MoO₄²⁻ to study effects of molybdenum on their growth. Initial concentration of perchlorate ions was 1 mM. Medium without MoO₄⁻ was used as a control. The process of perchlorate ion reduction increased by almost 15% at MoO₄²⁻ concentration 0.1 and 1.0 mM (Fig. 4). Bacteria reduced less ClO₄⁻ at molybdenum concentration 5 mM, than in control.

We have checked the ability of the studied agar-immobilized bacteria to purify polluted water from perchlorate ions. Full removal of perchlorate ions by Desulfotomaculum AR1 bacteria at concentration 1 g/L was found in 24 h, and in 76 h at initial concentration of cells 0.1 g/L (Fig. 5a). D. desulfuricans Ya-11 bacteria at concentration 1 g/L fully removed perchlorate ions from solution in 48 h of cultivation. Purification from perchlorate ions by cells concentration 0.1 g/L to 0.02 mM occurred in 48 h and perchlorate ion concentration did not further decrease (Fig. 5b).
In Figure 6, the results are presented of experiments on reduction of ClO₄⁻ by immobilized on wooden chips cells of bacteria Desulfotomaculum AR1 and D. desulfuricans Ya-11. ClO₄⁻ concentration decreased from 0.07 to 0.02 mM in 30 h and did not further decrease in the case of incubation of 0.1 g/L of Desulfotomaculum AR1 cells, and to 0.03 mM in 30 h and to 0.02 mM in 74 h in the case of incubation of D. desulfuricans Ya-11 cells (Fig. 6a). Desulfotomaculum AR1 bacteria reduced 0.09 mM of ClO₄⁻ at concentration of cells 0.4 g/L, whereas D. desulfuricans Ya-11 bacteria – 0.06 mM (Fig. 6b). The studied cultures of microorganisms reduced 0.03 mM of perchlorate ions more at concentration 1.0 g/L than at 0.4 g/L.

In Figure 7, the results are presented of experiments on reduction of perchlorate ions by immobilized on wooden chips cells of bacteria Desulfotomaculum AR1, D. desulfuricans Ya-11 and AR1 and D. desulfuricans Ya-11 at a concentration 0.1 g/L (a) and 0.4–1.0 g/L (b); * – P < 0.05, ** – P < 0.01 – significant changes in ClO₄⁻ concentration compared to control (ANOVA).

Effectiveness of perchlorate ion utilization by agar-immobilized Desulfotomaculum AR1 cells (0.1 g/L) was 81% in 24 h, while by suspension cells (initial biomass – 0.1, 0.2 g/L in 24 h) – 17% (Fig. 7a).

Perchlorate ion content also decreased by 81% in 24 h with the usage of agar-immobilized D. desulfuricans Ya-11 bacteria (biomass – 0.1 g/L) and by 67% by suspension cells, but the amount of cells increased three times (0.1–0.3 g/L) in one day in this case (Fig. 7b). Such effectiveness of perchlorate ion utilization was not found in the case of immobilization of bacteria on wooden chips. Process of ClO₄⁻ reduction was slower and, as a consequence, less effective at these conditions.

**Discussion**

Usage of biological methods of water purification from perchlorate ions is most often connected with the ability of microorganisms to reduce ClO₄⁻ to CO₂ using electron donors – different organic substrates – alcohols, carboxic acids, simple fatty acids, etc. (Nerenberg et al., 2002; Nerenberg et al., 2006; Prata, 2007). We have checked the ability to purify polluted water from perchlorate ions using Desulfotomaculum AR1 and D. desulfuricans Ya-11 bacteria. Similar research was conducted by Smirnova (2010) using bacteria, isolated from different ecological niches, most of which had low oxygen concentration, particularly from places of natural enrichment (wastewater of plants and dumps), etc. The best growth of studied bacterial strains was found in control medium, whereas Desulfotomaculum AR1 bacteria grew a little bit worse in the medium with perchlorate ion than D. desulfuricans Ya-11 (Fig. 1a, b). Presence of both electron acceptors – sulfate and perchlorate ions resulted in accumulation of approximately 2.5 g/L of biomass, which is twice as high as biomass of Desulfotomaculum AR1 bacteria in the medium with ClO₄⁻. D. desulfuricans Ya-11 grew equally in media with ClO₄⁻ and both SO₄²⁻ and ClO₄⁻. Presence of perchlorate ions in the medium somewhat inhibited sulfate ion reduction by Desulfotomaculum AR1 and D. desulfuricans Ya-11 bacteria (Table 1).
reductase showed that its expression is regulated by the presence of atmospheric oxygen (Bender et al., 2005). It is known that reduction of perchlorate ions depends on the availability of molybdenum compounds. Molecular studies of genetic systems, connected with the reduction of perchlorate ions, indicate the presence of molybdenum dependent chaperone, connected with genes, coding synthesis of chlorite dismutase (EC 1.13.11.49) and perchlorate reductase (EC 1.97.1.1) – enzymes of perchlorate ion reduction in Desulfomonas aromatica RC and Pseudomonas sp. PK (Bender et al., 2002). MoO₂⁺ at concentrations 0.1 and 1.0 mM had a positive effect on the process of perchlorate ion reduction, whereas some inhibitory effect on the reduction of ClO₄⁻ in Desulfitomaculum AR1 bacteria was found at molybdenum concentration 5 mM (Fig. 4).

Immobilized cells of microorganisms have a number of advantages over suspension cells, particularly, higher activity and stability, and also economic effectiveness. Immobilization enables the creation of non-stop automated processes, durable functioning of polynzyme systems, independent from exogenic factors (Starovoitova, 2012). That is why we decided to check the ability of agar-immobilized Desulfitomaculum AR1 and D. desulfuricans Ya-11 bacteria to utilize perchlorate ions (Fig. 5). A high amount of waste, which can be further used, particularly, with the aim of biological purification, is produced in the process of wood treatment. Immobilization of microorganisms’ cells on the wood chips was used to obtain vinegar more than 150 years ago (Melnyk et al., 2019). We have shown the suitability of usage of wooden chips-immobilized sulfate-reducing bacteria to purify water, polluted by perchlorate ions (Fig. 6). We can conclude from the obtained results that perchlorate ion reduction is performed somewhat faster by agar-immobilized Desulfitomaculum AR1 and D. desulfuricans Ya-11 bacteria cells, than by such cells immobilized by wooden chips, but agar is a less economically profitable substrate than wooden chips. Reduction of perchlorate ion by wooden chips-immobilized Desulfitomaculum AR1 bacteria is performed a little better than by D. desulfuricans Ya-11. Results of comparison of effectiveness of perchlorate ion reduction by suspension, agar- and wooden chips-immobilized Desulfitomaculum AR1 and D. desulfuricans Ya-11 bacteria cells are shown in Figure 7. We can conclude according to the obtained results of the study of perchlorate ion utilization by sulfate-reducing bacteria that immobilized Desulfitomaculum AR1 and D. desulfuricans Ya-11 bacteria cells purified perchlorate-containing water from ClO₄⁻ more effectively than suspension ones.

Environmental pollution by oxygen-containing chlorine compounds is a problem that requires solution. Sulfate-reducing bacteria Desulfitomaculum AR1 and D. desulfuricans Ya-11 are able to utilize perchlorate ions as electron acceptors in their metabolism, decreasing their content in polluted water. Immobilization of Desulfitomaculum AR1 and D. desulfuricans Ya-11 bacteria cells shows the suitability of their usage in methods of purification of water polluted by perchlorate ions.

Conclusions

The high redox potential of the perchlorate ion causes its utilization as an electron acceptor by microorganisms. Usage of anaerobic bacteria with the aim of purification of water polluted by perchlorate ions is more suitable than that of aerobic ones because oxygen inhibits perchlorate reduction in bacteria. Environmental factors have influence on the biological reduction of perchlorate ions, particularly, molybdenum, which had a positive effect on the process of perchlorate reduction in Desulfitomaculum AR1 bacteria at concentrations 0.1–1.0 mM. Organic compounds, particularly, aromatic, for example, toluene, are present in water, besides inorganic ones. Desulfitomaculum AR1 and D. desulfuricans Ya-11 bacteria are able to grow in media, utilizing aromatic compounds as the sole energy source and electron donor and perchlorate ions as electron acceptors. Effectiveness of perchlorate ions utilization was near 90 %. Biological purification of environments polluted by toluene, sulfate and perchlorate ions using sulfate-reducing bacteria is safe and promising method of bioremediation. Immobilization of sulfate-reducing bacteria strains is suitable in the conditions of purification of polluted environments from perchlorate ions.

References


