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REDUCTION OF Cr(VI) COMPOUNDS BY THE IMMOBILIZED CELLS OF SULFATE-REDUCING BACTERIA *DESULFOMICROBIUM* SP. CrR3

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Immobilization of microorganisms is an effective method of intensification of wastewater purification from chromate-containing compounds. This paper presents a method of sulfate-reducing bacteria immobilization in agar developed in order to determine the effectiveness of water purification from toxic hexavalent chromium compounds. Dependence of the influence of different factors on the reduction of hexavalent chromium compounds by immobilized cells of sulfate-reducing bacteria *Desulfomicrobium* sp. CrR3 has been studied. Both free and agar-immobilized *Desulfomicrobium* sp. CrR3 cells almost completely reduced Cr(VI) at the initial concentration of 1 mM. Immobilized cells reduced over 90 % of Cr(VI) in 4 days, and non-immobilized ones – in 6 days. Cr(III) content increased with the decrease of hexavalent chromium concentration. A possibility of multiple usage of agar-immobilized *Desulfomicrobium* sp. CrR3 cells for the purification of the model solution from Cr(VI) at the concentration of 0.5 mM and 1 mM during 60 h was confirmed. After three-time usage of the immobilized cells (1g/L), effectiveness of Cr(VI) reduction is 68 % at their initial concentration of 0.5 mM and 50 % – at 1 mM; at cells concentration 3 g/L – 87 % and 77 %; 5 g/L – 94 % and 92 %, and at cells concentration 8 g/L – 98 % and 96 %, respectively. As a result of the regression analysis of the influence of different factors on the purification of the model solution from Cr(VI) by agar-immobilized *Desulfomicrobium* sp. CrR3 cells a reliable dependence of the studied parameter change on time, initial content of hexavalent chromium and cells concentration was found. Determination indices have been calculated and the

equation of the dependence of change of hexavalent chromium concentration per unit of time on the three analysed factors has been derived. Immobilization of *Desulfomicrobium* sp. CrR3 cells is the promising way of water purification from hexavalent chromium compounds. Effectiveness and duration of the process of water purification from Cr(VI) by the immobilized *Desulfomicrobium* sp. CrR3 bacteria depend on the initial hexavalent chromium content and cell concentration.

Keywords: Cr(VI), purification, immobilized cells, sulfate-reducing bacteria, regression analysis

INTRODUCTION

Immobilized bacterial cells are increasingly used to purify the environment from both organic and inorganic pollutants. Activated charcoal, sand, porous glass, yellow saponite, wood chips, cotton fibers, corncobs, sunflower seed shells, etc. are the carriers for immobilization [1, 3, 16]. Incorporation of cells into different by structure natural (caraginan, agar, collagen, pectines) and synthetic (chitosan, silicate foam, polyurethane, polyacrylamide gel, photosensitive polymers) polymers is equally widespread [1, 17]. Biodegradable organic compounds (based on vinyl [2], alginates [1], and cotton fibers [3]) are used most often.

Compared to the traditional methods of biological purification of wastewater in aerated lagoons, immobilized microbiota has a number of advantages. First, such an “immobilized catalyzer” of the purification process is easy to remove from the reaction medium, which enables termination of the process at the right moment, while removal of active sludge from an aerated lagoon involves a long process of sedimentation and centrifugation. Besides, the carrier is suitable for multiple usage; purified water is not contaminated by microbial cells, so a secondary settler is not needed. Secondly, using the immobilized active sludge makes it possible to purify wastewater continuously and regulate the purification process by changing the flow velocity. Thirdly, immobilization of microbial biomass can increase the catalytic activity of enzymes [16].

Sulfate-reducing bacteria reduce sulfur oxoanions and oxidize organic compounds in anaerobic conditions in the process of sulfate respiration [5, 10]. Previous studies show that as electron acceptors they can also utilize nitrate ions, hexavalent chromium compounds and some heavy metal ions with variable valency [7, 13, 15].

In this study, we made an attempt to immobilize sulfate-reducing bacteria in order to use them for water purification from toxic compounds of hexavalent chromium.

MATERIALS AND METHODS

Chromate-resistant sulfate-reducing bacteria *Desulfomicrobium* sp. CrR3, isolated from the wastewater of Lviv purification system, were used for this research [11].

Bacteria were grown in Postgate C medium [8] with potassium dichromate (1 mM of chromium) at 30 °C in 25 mL tubes. The choice of such a concentration of chromium was determined by the results of our previous study [12], according to which further increase of chromium content in the medium leads to the inhibition of bacterial growth. Water solution of potassium dichromate was added after sterilization. To achieve anaerobic conditions tubes were fully filled with the medium and stoppered with rubber corks. To confirm the anaerobic conditions, AnaerIndicator (bioMeriux, France) – an indicator of anaerobic conditions was used.

Biomass was measured turbidimetrically using the photoelectrocolorimeter KFK-3 ($\lambda = 340$ nm, cuvette 3 mm). Chromate content was measured spectrophotometrically ($\lambda = 540$ nm, cuvette 10 mm) by biphenyl carbaside method [6]. Chromasurrol S ($\lambda = 590$ nm, cuvette 10 mm) was used to measure Cr(III) content [4].

To immobilize cells of sulfate-reducing bacteria on the synthetic carrier "VIA", we grew them in the tube with synthetic fibres. After 14 days, "VIA" were added to the model solution. To obtain *Desulfomicrobium* sp. CrR3 cells immobilized in agar, the grown bacteria were centrifuged at 6000 g for 30 min at 4 °C. The cells were washed twice with the isotonic solution of sodium chloride and added to 2% agar solution cooled to 40...45 °C. The agarized solution with the cells was poured into Petri plates to form a uniform layer. After solidification, the agar was cut into 7–8×7–8×7–8 mm cubes and added to the model solution of potassium dichromate containing Cr(VI) with pH adjusted to 7.

Statistical analysis was performed in R medium (3.6.3) in RStudio envelope (1.2.5033) using ggplot2 library [9]. Method of multiple linear regression with different variable combinations in linear model was used to determine the significance of the dependence of chromium concentration on such parameters as initial chromium concentration, time of measuring after the addition of chromium and concentration of cells.

RESULTS AND DISCUSSION

This study was focuses on a possibility to immobilize sulfate-reducing bacteria *Desulfomicrobium* sp. CrR3 on the synthetic carrier "VIA", which is considered to be promising and readily available [14], and in agar. Effective growth of sulfate-reducing bacteria *Desulfomicrobium* sp. CrR3 in the Cr(VI) containing medium without sulfates and other terminal electron acceptors was reported previously [12]. After 14 days of *Desulfomicrobium* sp. CrR3 bacteria cultivation in Postgate C medium with "VIA" carriers, bacterial biomass accumulated, but no immobilization occurred. Bacterial biomass was equal in both culture liquids, with and without the synthetic fibres. Sedimentation of FeS, which stained the carrier in black colour, was observed in Postgate C medium with ferrous sulfate.

The synthetic carrier was substituted for agar, as immobilization on the synthetic carrier "VIA" was not successful. The pattern of chromate ions utilization by the sulfate-reducing bacteria *Desulfomicrobium* sp. CrR3 immobilized in agar proved to be similar to the pattern of Cr(VI) utilization by non-immobilized cells. The cells were immobilized in agar and their ability to reduce chromate ions was studied. We found that both immobilized and non-immobilized cells almost completely reduced Cr(VI) at the initial concentration of 1 mM (Fig. 1). The immobilized *Desulfomicrobium* sp. CrR3 cells reduced over 90% of Cr(VI) within 4 days, and the free ones – within 6 days. Cr(III) content increased with the decrease of chromate ions concentration. The concentration of chromate ions did not change in the control sample (without cells) (Fig. 1A).

Fig. 2 shows positive correlation between the rate of Cr(VI) utilization and the amount of immobilized cells. Multiple uses of immobilized sulfate-reducing bacteria cells for the purification of a medium from Cr(VI) are possible. Cr(VI) content in the model solution decreased from 0.5 mM to 0.26 mM in 17 hours after the addition of 1 g/L of *Desulfomicrobium* sp. CrR3 cells immobilized in agar. After the second addition of 0.5 mM of Cr(VI), bacteria reduced 0.17 mM in 17 h. With the next addition of 0.5 mM of Cr(VI) bacteria utilized only 0.06 mM of Cr(VI) (Fig. 2A).

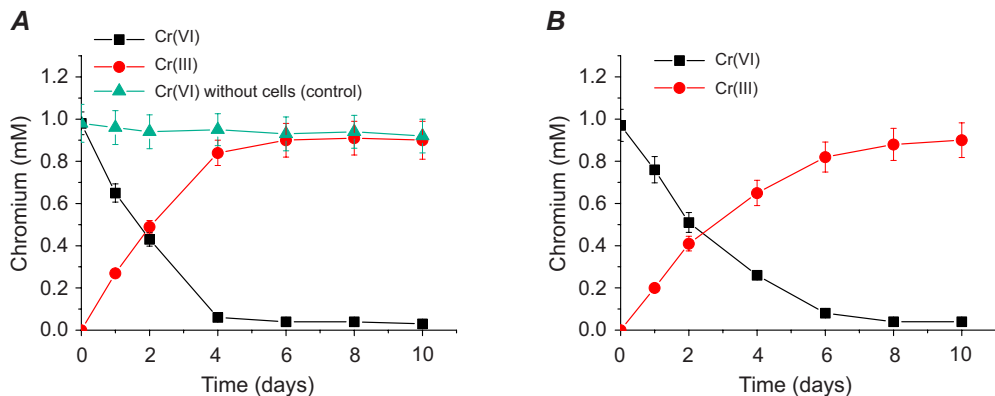


Fig. 1. Reduction of Cr(VI) by agar-immobilized (A) and free (B) *Desulfomicrobium* sp. CrR3 cells. Initial concentration of chromate ions – 1 mM; of cells – 0.1 g/L

Рис. 1. Відновлення Cr (VI) іммобілізованими в агар (A) та неіммобілізованими (B) клітинами *Desulfomicrobium* sp. CrR3. Вихідна концентрація хромат-йонів – 1 mM, клітин – 0,1 г/л

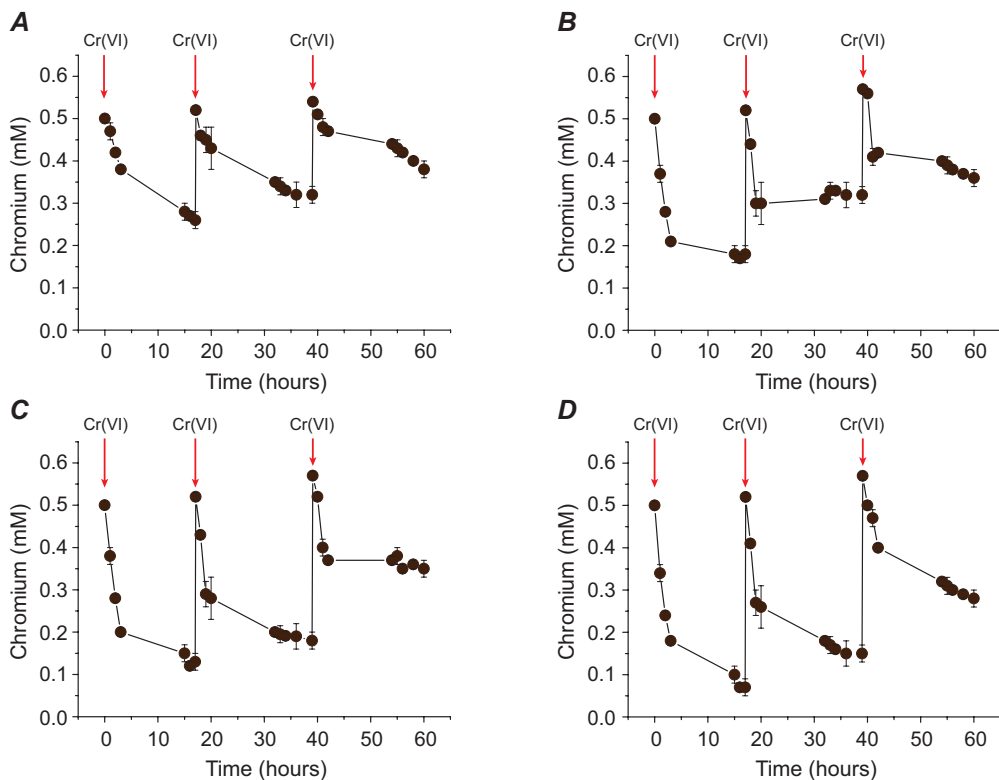


Fig. 2. Utilization of Cr(VI) (0.5 mM) by the agar-immobilized cells of *Desulfomicrobium* sp. CrR3 bacteria at different concentration (A – 1 g/L; B – 3 g/L; C – 5 g/L; D – 8 g/L) during 60 h. Arrows indicate the addition of potassium dichromate into the model solution

Рис. 2. Використання Cr(VI) (0,5 mM) іммобілізованими в агар клітинами бактерій *Desulfomicrobium* sp. CrR3 за різних концентрацій (A – 1 г/л; B – 3 г/л; C – 5 г/л; D – 8 г/л) упродовж 60 год. Стрілками показано внесення калій біхромату в модельний розчин

3 hours after the addition of 3 g/L of agar-immobilized cells into the model solution, which contained 0.5 mM of Cr(VI), the utilization of 0.3 mM of Cr(VI). Cr(VI) concentration was 0.17 mM after 17 h was observed. Repeated addition of 0.5 mM of Cr(VI) resulted in the two-fold decrease of Cr(VI) utilization by bacterial cells. With the next addition of 0.5 mM of Cr(VI) bacteria utilized only 0.15 mM of Cr(VI) (Fig. 2B).

The increase in concentration of the added cells to 5 g/L accelerates the process of Cr(VI) reduction. Cr(VI) concentration in the medium reached 0.11 mM after 17 h. We observed an insignificant deceleration of Cr(VI) reduction in the case of a repeated addition of 0.5 mM. Cr(VI) concentration was 0.19 mM after 34 h. With the next addition of 0.5 mM of Cr(VI), its utilization decelerated (Fig. 2C). 0.06 mM of Cr(VI) was found in the medium 17 h after the addition of 8 g/L of agar-immobilized cells into the model solution with Cr(VI) concentration of 0.5 mM. Its concentration was 0.16 mM in 17 h (on the 34th hour) after the repeated addition of 0.5 mM of Cr(VI) (Fig. 2D).

A two-fold increase in the initial Cr(VI) concentration in the model solution results in different extent of its reduction by the agar-immobilized *Desulfomicrobium* sp. CrR3 cells depending on their concentration (Fig. 3).

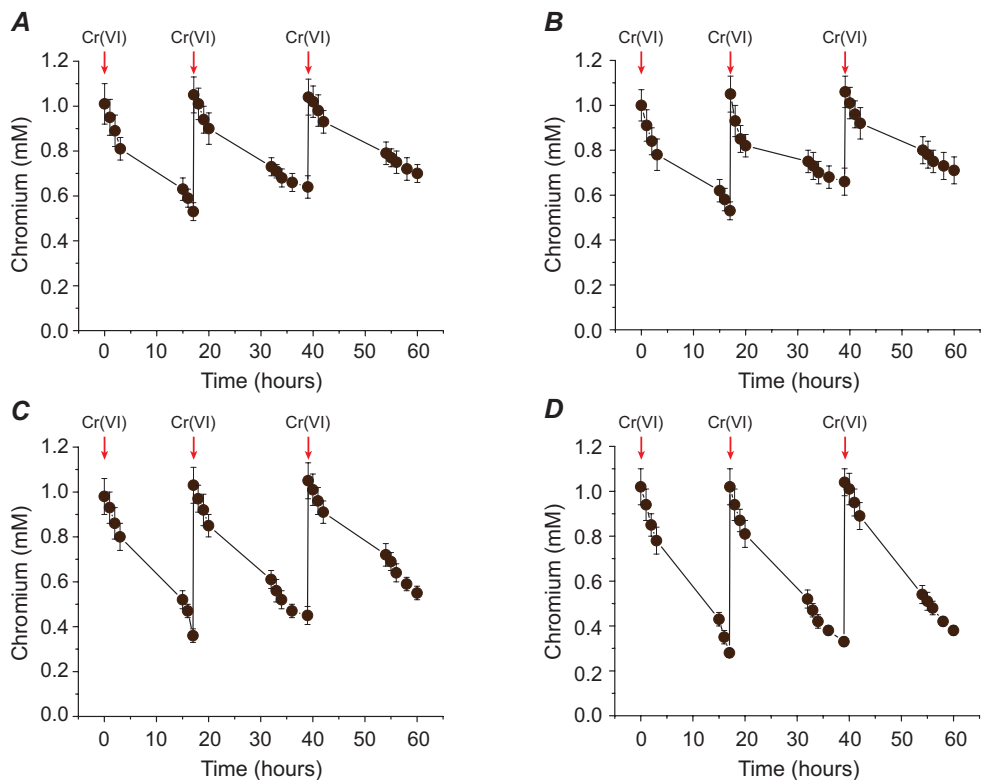


Fig. 3. Utilization of Cr(VI) (1 mM) by agar-immobilized cells of *Desulfomicrobium* sp. CrR3 bacteria at different concentrations (**A** – 1 g/L; **B** – 3 g/L; **C** – 5 g/L; **D** – 8 g/L) during 60 h. Arrows indicate the addition of potassium dichromate into the model solution

Рис. 3. Використання Cr (VI) (1 мМ) іммобілізованими в агар клітинами бактерій *Desulfomicrobium* sp. CrR3 за різних концентрацій клітин (**A** – 1 г/л; **B** – 3 г/л; **C** – 5 г/л; **D** – 8 г/л) упродовж 60 год. Стрілками показано внесення калій бихромату в модельний розчин

Cr(VI) content in the model solution was decreased from 1.0 to 0.56 mM within 17 hours after the addition of 1 g/L of *Desulfomicrobium* sp. CrR3 cells immobilized in agar. After the repeated addition of 1.0 mM of Cr(VI), bacteria reduced 0.32 mM in 17 h. With the next addition of 1.0 mM of Cr(VI), bacteria utilized only 0.27 mM of Cr(VI) (Fig. 3A).

After the addition of 3 g/L of agar-immobilized cells into the model solution, which contained 1 mM of Cr(VI), the utilization of 0.23 mM of Cr(VI) after 3 h was observed. Cr(VI) concentration was 0.53 mM after 17 hours. A repeated addition of 1 mM of Cr(VI) resulted in the 1.6-fold deceleration of CrO_4^{2-} utilization by cells. With the next addition of 1 mM of Cr(VI) bacteria utilized only 0.29 mM of Cr(VI) (Fig. 3B).

The increase of cell concentration to 5 g/L accelerates the process of Cr(VI) reduction. Cr(VI) concentration reached 0.36 mM after 17 h. Its utilization decelerated in the case of a repeated addition of 1 mM of Cr(VI). After 34 h, Cr(VI) concentration was 0.52 mM. With the next addition of 1 mM of Cr(VI), its utilization decelerated (Fig. 3C).

After the addition of 8 g/L of agar-immobilized cells into the model solution with 1 mM of Cr(VI) 0.28 mM of Cr(VI) was found in the medium 17 h. 17 h after the repeated addition of 1 mM of Cr(VI) (in 34 hours), its concentration was 0.42 mM. With the next addition of 1 mM, utilization of Cr(VI) decelerated (Fig. 3D).

Thus, it is not only the concentration of cells, but also Cr(VI) concentration that influences the reduction of chromate ions by the immobilized *Desulfomicrobium* sp. CrR3 cells. After a three-time use of the immobilized *Desulfomicrobium* sp. CrR3 cells (1g/L), the effectiveness of chromate ions reduction is 68% at their initial concentration of 0.5 mM and 50% – at 1 mM; at cells concentration of 3 g/L – 87% and 77%; 5 g/L – 94% and 92%, and at cells concentration of 8 g/L – 98% and 96%, respectively (see Table).

Effectiveness of chromate ions reduction by immobilized *Desulfomicrobium* sp. CrR3 cells

Ефективність відновлення хромат-іонів іммобілізованими клітинами *Desulfomicrobium* sp. CrR3

| Chromate ions concentration (mM) | Concentration of cells (g/L) | | | |
|----------------------------------|--------------------------------|----|----|----|
| | 1 | 3 | 5 | 8 |
| | Effectiveness of reduction (%) | | | |
| 0.5 | 68 | 87 | 94 | 98 |
| 1.0 | 50 | 77 | 92 | 96 |

Thus, agar-immobilized cells of sulfate-reducing bacteria *Desulfomicrobium* sp. CrR3 are capable of full detoxification of 0.5 mM of Cr(VI) in 17 h. The increase of cells concentration accelerates the process of chromate utilization in the model solution. *Desulfomicrobium* sp. CrR3 bacteria have a potential for anaerobic purification of wastewater from hexavalent chromium compounds.

The study demonstrated that *Desulfomicrobium* sp. CrR3 cells immobilized in agar have an ability to reduce Cr(VI) at the concentration of 0.5 mM in the model solution. The dependence of the concentration of hexavalent chromium on time (in hours) is described by the equation

$$[\text{Cr}] = 0.429 - 0.009 \cdot t, \quad (1)$$

where $[\text{Cr}]$ – concentration of hexavalent chromium, mM; t – time after the moment of Cr(VI) addition to the medium, hours.

The dependence of chromium concentration on time is significant ($p < 0.001$), but the determination coefficients that indicate the part of variation of the dependent variable, which can be explained by the variation of predictors, are low in this model ($R^2 = 0.3234$, $R^2_{adj} = 0.320$). It can be seen from equation 1 that Cr(VI) concentration changes by 0.009 mmol per hour.

At the initial Cr(VI) concentration of 1 mM in the model solution, the dependence of hexavalent chromium concentration on time is described by the equation

$$[Cr] = 0.971 - 0.022 \cdot t, \quad (2)$$

where $[Cr]$ – concentration of hexavalent chromium, mM; t – time after the moment of Cr(VI) addition to the medium, hours.

The dependence of chromium concentration on time is significant ($p < 0.001$) and the determination coefficients are high ($R^2 = 0.6873$, $R^2_{adj} = 0.6857$). Cr(VI) concentration decreases by 0.022 mmol per hour (equation 2).

According to the regression analysis, the changes in Cr(VI) concentration when using agar-immobilized *Desulfomicrobium* sp. CrR3 cells conform with the interpretation of the visualization presented at Fig. 4.

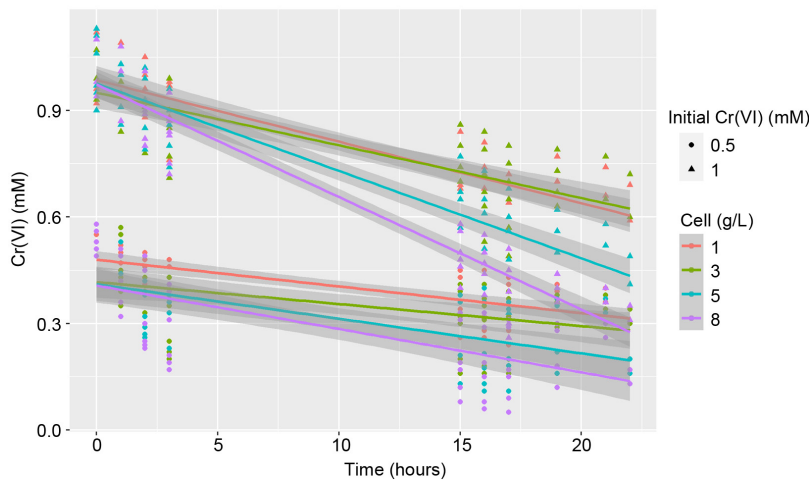


Fig. 4. Changes in Cr(VI) concentration by agar-immobilized *Desulfomicrobium* sp. CrR3 cells taking into account the factors of time and initial chromium content during 23 hours

Рис. 4. Зміни концентрації Cr(VI) іммобілізованими в агар клітинами *Desulfomicrobium* sp. CrR3 з урахуванням чинника часу і початкового вмісту хрому протягом 23 год

The multiple linear regression, performed for the dependent index – chromium concentration, and the independent indexes (predictors) – time and initial chromium concentration, shows that the change of Cr(VI) concentration is described by the equation

$$[Cr] = -0.114 + 0.004 \cdot t + 1.085 \cdot [Cr_0] - 0.026 \cdot t \cdot [Cr_0], \quad (3)$$

where $[Cr_0]$ – initial concentration of hexavalent chromium, mM.

In this case, the p -level of significance of dependence of Cr(VI) concentration on time is low ($p = 0.0544$), and the coefficient of this predictor is positive, which indicates that only time in this model is not a significant parameter. However, the p -level of significance of dependence of hexavalent chromium concentration on the initial Cr(VI) concentration is notable ($p < 0.001$) in the same manner as for the interaction/combination of these factors (the extent of expression of measuring time factor significantly depends on

the gradation of the initial chromium concentration, $p < 0.001$). Determination coefficients for such a model indicate that a significant part of the chromium concentration variability is described by the variability of these two parameters ($R^2 = 0.829$, $R^2_{\text{adj}} = 0.827$).

Thus, the influence of the factor of time depends significantly on other parameters, particularly, the initial concentration of Cr(VI), which by itself is a significant predictor.

The multiple linear regression, performed for the dependent index of chromium concentration and for the independent indices of time, *Desulfomicrobium* sp. CrR3 cells concentration and the initial Cr(VI) concentration, shows that the change of hexavalent chromium concentration is described by the equation

$$[Cr] = -0.037 + 1.010 \cdot [Cr_0] - 0.018 \cdot [C] - 0.013 \cdot t \cdot [C] - 0.003 \cdot t \cdot [Cr_0] \cdot [C], \quad (4)$$

where $[C]$ – concentration of cells, g/L.

The most significant predictor is the initial Cr(VI) concentration ($p < 0.001$) and an aggregate of all three factors (expression of time as a factor depends on the initial chromium concentration factor gradation and cells concentration, $p \approx 0.001$). Time in relation to the gradation of initial chromium concentration ($p = 0.003$) and concentration of cells ($p = 0.046$) are significant factors as well. Determination coefficients of the model indicate its conformity with the data variation ($R^2 = 0.883$, $R^2_{\text{adj}} = 0.881$).

Changes in Cr(VI) concentration when using agar-immobilized *Desulfomicrobium* sp. CrR3 cells taking into consideration the factors of time, initial chromium content and concentration of cells conform with the interpretation of the visualization presented in Fig. 5.

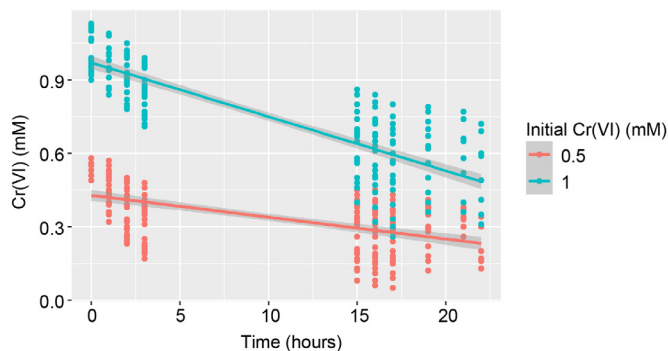


Fig. 5. Changes in Cr(VI) concentration by the agar-immobilized *Desulfomicrobium* sp. CrR3 cells taking into consideration the factors of time, initial chromium content and concentration of cells

Рис. 5. Зміни концентрації Cr(VI) іммобілізованими в агар *Desulfomicrobium* sp. CrR3 з урахуванням чинників часу, початкового вмісту хрому і концентрації клітин

Thus, the regression analysis of the influence of various factors on the of model solution purification from Cr(VI) by the agar-immobilized *Desulfomicrobium* sp. CrR3 cells showed a significant dependence of the changes in the parameter under study on time, initial content of hexavalent chromium and concentration of cells.

CONCLUSIONS

Immobilization of *Desulfomicrobium* sp. CrR3 cells is a promising way of water purification from hexavalent chromium compounds, which can be used to develop technologies for bioremediation of the environment from these pollutants. Effectiveness and duration of the process of water purification from Cr(VI) by the immobilized *Desulfomic-*

robium sp. CrR3 bacteria depend on the initial hexavalent chromium content and the concentration of cells.

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.

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ВІДНОВЛЕННЯ СПОЛУК Cr(VI) ІММОБІЛІЗОВАНИМИ КЛІТИНАМИ СУЛЬФАТВІДНОВЛЮВАЛЬНИХ БАКТЕРІЙ *DESULFOMICROBIUM SP. CrR3*

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Імобілізація мікроорганізмів є ефективним методом інтенсифікації процесу очищення стічних вод від хроматовмісних сполук. У цій статті розроблено спосіб іммобілізації в агар сульфатвідновлювальних бактерій з метою з'ясування ефективності очищення води від токсичних сполук Cr(VI). Досліджено залежність впливу різних чинників на відновлення сполук Cr(VI) іммобілізованими клітинами сульфатвідновлювальних бактерій *Desulfomicrobium sp. CrR3*. Іммобілізовані в агар та неіммобілізовані клітини *Desulfomicrobium sp. CrR3* практично повністю відновили Cr(VI) за вихідної концентрації 1 мМ. Іммобілізовані клітини відновили понад 90 % Cr(VI) протягом чотирьох діб, а неіммобілізовані – протягом шести діб. Зі зменшенням концентрації Cr(VI) зростав вміст Cr(III). Встановлено можливість кількаретового використання іммобілізованих в агар клітин *Desulfomicrobium sp. CrR3* для очищення модельного розчину від Cr(VI) у концентрації 0,5 та 1 мМ протягом 60 год. За триразового використання іммобілізованих клітин ефективність відновлення Cr(VI) становить 68 % за вихідної їхньої концентрації 0,5 мМ та 50 % – за 1 мМ; за концентрації клітин 3 г/л – 87 та 77 %; 5 г/л – 94 та 92 % і за концентрації клітин 8 г/л – 98 і 96 %, відповідно. Унаслідок проведеного регресійного аналізу щодо впливу різних чинників на очищення модельного розчину від Cr(VI) іммобілізованими в агар клітинами *Desulfomicrobium sp. CrR3*, встановлено достовірну залежність зміни досліджуваного параметра від часу, початкового вмісту Cr(VI) та концентрації клітин. Розраховано показники детермінації та виведено рівняння залежності зміни концентрації Cr(VI) за одиницю часу від двох–трьох проаналізованих чинників. Імобілізація в агар клітин *Desulfomicrobium sp. CrR3* є перспективним способом очищення води від сполук Cr(VI). Ефективність і тривалість процесу очищення водного середовища від Cr(VI) іммобілізованими бактеріями *Desulfomicrobium sp. CrR3* залежать від початкового вмісту Cr(VI) та концентрації клітин.

Ключові слова: Cr(VI), очищення, іммобілізовані клітини, сульфатвідновлювальні бактерії, регресійний аналіз