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INTERACTION OF OBLIGATE ANAEROBIC DESTROYER OF SOLID ORGANIC WASTE *Clostridium butyricum* GMP1 WITH SOLUBLE COMPOUNDS OF TOXIC METALS Cr(VI), Mo(VI) AND W(VI)

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Increasing pollution of environment by toxic metals is the urgent problem requiring effective solution worldwide. The goal of the work was to study the dynamics of the interaction of Cr(VI), Mo(VI), W(VI) compounds with obligate anaerobic microorganisms *Clostridium butyricum* GMP1, which ferment organic compounds with the synthesis of hydrogen. The standard methods were used to determine pH and redox potential (Eh), the gas composition, and the concentration of metals. The application *Clostridium butyricum* GMP1 was showed to be useful to investigate its interaction with toxic metals. The higher redox potential of metal provided the opportunity for its faster and more effective reduction. The patterns of the reduction of toxic metals Cr(VI), Mo(VI) and W(VI) by obligate anaerobic strain *Clostridium butyricum* GMP1 were obtained. The experimental data confirmed the thermodynamically calculated correlation between the redox potential of the metal reduction to insoluble form and effectiveness of its removal. Obtained results can serve as the basis for further optimization and development of environmental biotechnologies for wastewater treatment with the simultaneous destruction of solid organic waste and hydrogen synthesis.

Key words: thermodynamic prediction, toxic metals, organic waste, environmental biotechnologies, hydrogen fermentation.

Toxic metals in low concentrations (1–3 ppm) are natural components of the environment and are even necessary for microorganisms. But their irrational use causes the violation of their geochemical cycles and the biogeochemical balance. This leads to excessive accumulation of metals in soils, water reservoirs, etc. [1].

Constant contamination of soil and water with toxic metals is a serious problem for human health and the stability of natural ecosystems worldwide. Even the most toxic organic xenobiotics finally are degraded by microorganisms to non-toxic compounds (CO_2, CH_4). Unlike organic xenobiotics, metals cannot be destroyed to non-toxic compounds. Therefore, they can be accumulated in ecosystems in high concentrations and poison them for a long time [1].

Due to this, environment protection is one of the advanced directions of modern science. Industrial wastewater is one of the largest sources of toxic metal pollution [2]. The technologies currently applied are often inefficient, expensive, and cannot provide the required level of wastewater treatment from heavy metals in high concentrations [3, 4]. Most methods provide the removal of metals from

wastewater containing relatively high initial concentrations (for example, for Cr(VI), they are usually above 100 mg/l) [5]. However, according to the recommendations of the Environmental Protection Agency of the USA, the concentration of chromium compounds in water resources should not exceed 0.1 ppm [1, 5]. Thus, the development of new, more efficient technologies is absolutely necessary [3, 4].

The application of microorganisms for the treatment of metal-containing wastewater is one of the most promising areas of research at present. The selection of specific metal-resistant strains is conducted [6, 7].

Chromium is one of the most common toxic metals of man-made origin in the environment. Cr(VI) compounds have a wide range of industrial application, including electroplating, magnetic tapes, pigments, leather tanning, wood protection, chemical production, electrical and electronic equipment, etc. Annually, the global discharge of chromium into water reservoirs reaches up to 142,000 tons, and into the soil — 896,000 tons [5].

The International Agency for Research on Cancer (IARC) has classified Cr(VI) into the group 1 (carcinogenic to humans) and metallic chromium (Cr^0) and Cr(III) – into the group 3 (not to be carcinogenic to humans). Therefore, the removal of Cr(VI) from wastewater before the discharge into natural ecosystems is an extremely important problem that requires urgent solution [8].

Molybdenum is an important trace element that can become toxic due to excess intake to organism [9]. Excessive amounts of molybdenum in the human diet have been shown to cause an antagonistic decrease in the concentration of copper, another important cofactor of enzymes [10]. Molybdate (MoO_4^{2-}) is an inhibitor of sulfate metabolism at high concentrations. It can be toxic to sulfate reducing microorganisms [11]. Molybdate inhibits ATP-sulfurylase, the first enzyme in the activation of sulfate, causing energy depletion of cells [12].

The concentration of molybdenum is low in natural ecosystems, but industrial emissions can contain molybdate in high concentrations up to 100–200 ppm [9]. Administered orally into the organism molybdates are toxic at the doses of 400 to 800 ppm [10].

Together with chromium and molybdenum, tungsten (W) is the element of group VI of the periodic system. It is similar to molybdenum in its chemical properties [13]. Tungsten is used for a wide range of industrial and military purposes [14, 15]. The appearance of tungsten in groundwater as a result of mining activities and the potential danger to human health have required the increased control of tungsten content in the environment [16–18]. In addition, the use of fertilizers in agriculture may also be one of the possible ways of its spread in the environment [19]. For example, some phosphorus fertilizers contain tungsten in concentrations up to 100 mg/kg [14]. The disposal of countless light bulbs produced globally during the 20th century is another common source of environmental pollution [19]. Also, high concentrations of tungsten are detected in combat areas, at private and commercial shooting ranges, reaching concentrations up to 475–500 ppm [14].

Dissolved tungsten compounds at the concentration of 89 ppm reduce the yield of soil microbial biomass by 38%. The concentration of dissolved tungsten, which is 5–10 ppm, causes the decrease in the production of microbial biomass in soil by 8% [14].

Bacterial reduction of soluble toxic metals to insoluble compounds is a technology of great interest for wastewater purification. It is cheaper than existing chemical and physical methods and is environmentally friendly.

Today, there are the reports of the application of microbial reduction of Cr(VI) to insoluble Cr(III) for laboratory wastewater treatment [20]. *Escherichia coli*, *Pseudomonas putida*, *Desulfovibrio* sp. [21], *Ochrobactrum* sp., *Pseudomonas* sp., *Bacillus* sp., *Arthrobacter* sp., *Deinococcus* sp., *Enterobacter* sp., *Agrobacterium* sp., *Microbacterium* sp., *Shewanella* sp., *Desulfovibrio* sp., *Thermus* sp., *Thiobacillus tioparus*, etc. [22, 23] are able to reduce chromates.

The reduction of soluble compounds of Mo(VI) to insoluble molybdenum blue by microorganisms, their metabolites with reducing properties is also the most promising direction for molybdate extraction [24].

Tungsten oxyanion (WO_4^{2-}) is the most common and stable form of tungsten compounds in aqueous solution. There is no information on the development of biotechnologies for its extraction from solutions to reduce the toxic effect on the environment [19].

Thus, the removal of toxic metals by microorganisms is promising for industrial application. Reduction of soluble metal compounds to insoluble by microorganisms is a quick and effective method. Despite the availability of experimental data on the effectiveness of microbial reduction of metals, there is still lack of the technologies

that could be applied in industry. This is due to the absence of the theoretical basis to determine the optimal pathways of interaction of microorganisms with metals, as well as a detailed study of the dynamics of the process.

Therefore, the goal of our work was to study the dynamics of the interaction of soluble Cr(VI), Mo(VI), W(VI) compounds in the forms of CrO_4^{2}, MoO_4^{2-} and WO_4^{2-} with obligate anaerobic microorganisms *Clostridium butyricum* GMP1, which provide fermentative conversion of organic compounds to produce hydrogen.

Materials and Methods

To study the patterns of microbial interaction with toxic metals (Cr(VI), Mo(VI), W(VI)) we used the strain of obligate anaerobic bacteria. It was isolated during the hydrogen fermentation of environmentally hazardous multicomponent organic waste via a granular microbial preparation (GMP) developed by us [25, 26]. According to the analysis of the sequence of 16S rRNA genes, it was classified as *Clostridium butyricum*.

Subsequently, the strain *Clostridium butyricum* GMP1 was used to study the efficiency of the reduction of Cr(VI), Mo(VI), W(VI) by obligate anaerobic microorganisms.

Theoretical substantiation of the patterns of the reduction of Cr(VI), Mo(VI), W(VI) compounds was conducted by determining the stability fields of metal compounds according to Pourbaix diagrams in pH-Eh coordinates [25, 27].

Experimental confirmation of the pathways of interaction of the strain *Clostridium butyricum* GMP1 with metals was performed under the following conditions. Cultivation of microorganisms was carried out in Nutrient Broth (NB) (HiMedia, India) with the addition of potatoes to create optimal growth conditions. Potato and nutrient broth simulated liquid and solid organic waste. For this purpose, 150 ml of NB, 50 g of grinded sterile raw potatoes and inoculum were added to the flasks with the volume of 250 ml. Sterilization of potatoes was performed as follows. Potatoes were prewashed from dirt with liquid detergent. Then, it was placed in a plastic container and filled with sterile hot distilled water (80–90 °C). To grind the potatoes, a ceramic board and a metal knife were sterilized in a burner flame. The potatoes were cut into cubes with a rib length of 0.5 cm and pasteurized in a boiling water bath for 10 min. The fermentation flasks were sealed with rubber stoppers and metal clamps. The cultivation was performed during 10 days at 30 °C.

At the first stage of research, hydrogen fermentation of the model organic substrate that simulated organic waste was performed. After 19 h of cultivation in the phase of active growth of microorganisms, toxic metals CrO_4^{2-}, MoO_4^{2-}, WO_4^{2-} were added to the flasks at the concentration of 100 ppm per cation of each of the metals. In addition, the influence of the metal concentration on the efficiency of its reduction was investigated on the example of chromate. For this purpose, we studied the patterns of reduction of Cr(VI) at the concentrations of 50, 100, 200 ppm.

The following parameters were controlled: pH and redox potential (Eh, mV) of the medium, volume and composition of the synthesized gas, concentration of soluble CrO_4^{2-}, MoO_4^{2-}, WO_4^{2-} in the medium.

The pH and Eh were determined using the universal ionometer EZODO MP-103 with remote electrodes Ezodo with BNC connectors — models PY41 and PO50, respectively.

The volume of gas was determined by the extruded piston of the syringe, which pierced the rubber stopper.

The gas composition was analyzed for the content of molecular hydrogen, oxygen, nitrogen and carbon dioxide according to the standard method on the gas chromatograph LHM-8-MD [28]. The sterile plastic syringes (2.5 ml volume) were used to sample the gas phase. The composition of the gas phase was calculated by the square of the peaks of its components.

The chromatograph is equipped with two steel columns — one (I) for the analysis of H_2, O_2, N_2 and CH_4, the second (II) - for the analysis of CO_2. Column parameters: I — l = 3 m, d = 3 mm, with molecular sieve 13X (NaX); II – l = 2 m, d = 3 mm, with Porapak-Q carrier; column temperature +60 °C, evaporator temperature +75 °C, detector temperature (catharometer) +60 °C, detector current — 50 mA. Carrier gas — argon, gas flow rate – 30 cm^3/min.

The concentration of Cr(VI) was determined by a qualitative reaction with diphenylcarbazide (DPC) [29]. For this, 3 mL of distilled water, 0.5 ml of concentrated nitric acid and 0.5 mL of DPC (0.5%) were added to 1.0 ml of the test sample. In the presence of chromate, the solution was colored in purple. The values of optical density were determined on a photoelectrocolorimeter, λ = 540 nm, optical step 3 mm.

Mo(VI) and W(VI) concentrations were determined by the reaction with pyrocatechol [30].

The alkaline solution of pyrocatechol (10.0 g/l) was used to determine the concentration of Mo(VI) in the solution. To prepare it, 2.0 g of NaOH was dissolved in 500 ml of distilled water. Then 15.0 g of $Na_2S_2O_5$ and 10.0 g of pyrocatechol crystals were added to the solution. After complete dissolution of all components, the solution was transferred to the measuring flask (1.0 l) and the volume was established. The solution can be stored in a tightly closed vial for 3 months. The addition of sodium bisulfate prevented the oxidation of pyrocatechol by oxygen.

To determine the concentration of Mo(VI) 5.0 ml of pyrocatechol solution (10 g/l) was added to 1 ml of the test sample. In the presence of molybdate, the solution turned into yellow. The values of optical density were determined on the photoelectrocolorimeter, λ = 400 nm, optical step 3.0 mm.

To determine the concentration of W(VI) several crystals of pyrocatechol were added to 1.0 ml of the sample. The solution was shaked for 3–5 min until the crystals were completely dissolved and then 5.0 mL of distilled water was added. The solution turned pale yellow at the concentration of 100 ppm W(VI). The values of optical density were determined on the photoelectrocolorimeter, λ = 400 nm, optical step 3.0 mm.

The experiment was carried out in triplicate. Statistical analysis was carried out using Excel and Origin 8.5.1. software determining the standard deviation of the data.

Results and Discussion

The consideration the optimal pathways of microbial interaction with toxic metals is the first stage required for effective biotechnologies development. It allows avoiding the long term experimental testing of the efficiency of each pathway. Instead of this, the preferable conditions and the pathway of microbial metabolism can be effectively calculated applying thermodynamic prediction. The detailed analysis of microbial interaction with Cr(VI), Mo(VI), W(VI) compounds was described previously [3, 31].

Here is the general overview of the most promising reactions of metals removal (Fig. 1) and the following experimental confirmation of the theoretical considerations.

As it was established, dissimilative microbial metabolism takes place in the zone of water thermodynamic stability. It is limited by the values of the redox potential from −414 mV to +814 mV at pH 7.0.

The reactions aimed to remove toxic metals have to provide the formation of insoluble compounds. Thus, removal of soluble Cr(VI) from the solution is provided by the reaction:

$$CrO_4^{2-} + (n\text{-}1)H_2O + 5H^+ + 3e = Cr(OH)_3{\cdot}nH_2O$$

$$Eh = 1.244 - 0.0985pH + 0.0197\lg\{CrO_4^{2-}\};\ E_{o\ (pH = 7)} = +555\ mV$$

At the pH > 6.45 insoluble nontoxic Cr(III) hydroxide is formed [3, 27, 31].

According to the thermodynamic calculations, the preferable reactions for the removal of soluble Mo(VI) at the pH >3.7 are the following:

$$MoO_4^{2-} + 4H^+ + 2e = MoO_2 + 2H_2O$$

$$Eh = 0.606 - 0.1182pH + 0.0295\lg\{MoO_4^{2-}\};\ E_{o\ (pH = 7)} = -221\ mV$$ [27, 31].

However, in natural ecosystems Mo(IV) occurs mostly in the form of complex compounds. The most probable product of MoO_4^{2-} reduction is the formation of complex compounds of Mo(V) — molybdenum blue ($Mo_3O_8{\cdot}nH_2O$) [27, 32, 33].

At pH > 6.5 molybdenum blue precipitates in the form of brown molybdenyl hydroxide ($MoO(OH)_3$) [27, 32, 34]. Reduction of molybdates by microorganisms is described as follows:

$$Mo_6O_{21}^{6-} + 3H_2 = 6MoO_{2.5} + 6OH^-.$$

The redox potential of reduction of Mo(VI) to Mo(V) is −320 mV pH = 7.0 [31, 34, 35].

The reduction of W(VI) by microorganisms is thermodynamically prohibited, since the reaction is located below the lower limit of water thermodynamic stability:

$$2WO_4^{2-} + 6H^+ + 2e = W_2O_5 + 3H_2O$$

$$Eh = 0.801 - 0.1773pH + 0.0591\ \lg\{WO_4^{2-}\};\ E_{o\ (pH = 7)} = -440\ mV$$ [31].

In this regard microbial reduction cannot be applied for removal of W(VI).

The thermodynamic considerations were successfully confirmed experimentally. The efficiency and the rate of metal reduction were studied applying the obligate anaerobic strain *Clostridium butyricum* GMP1. Dark hydrogen fermentation of organic compounds (especially glucose containing) can provides the redox potential of the medium up to −414 mV:

$$Starch\ [C_6H_{12}O_6]_n \rightarrow nC_6H_{12}O_6 \rightarrow$$

$$\rightarrow 2H^+ + 2e = H^2$$ [25, 26, 31].

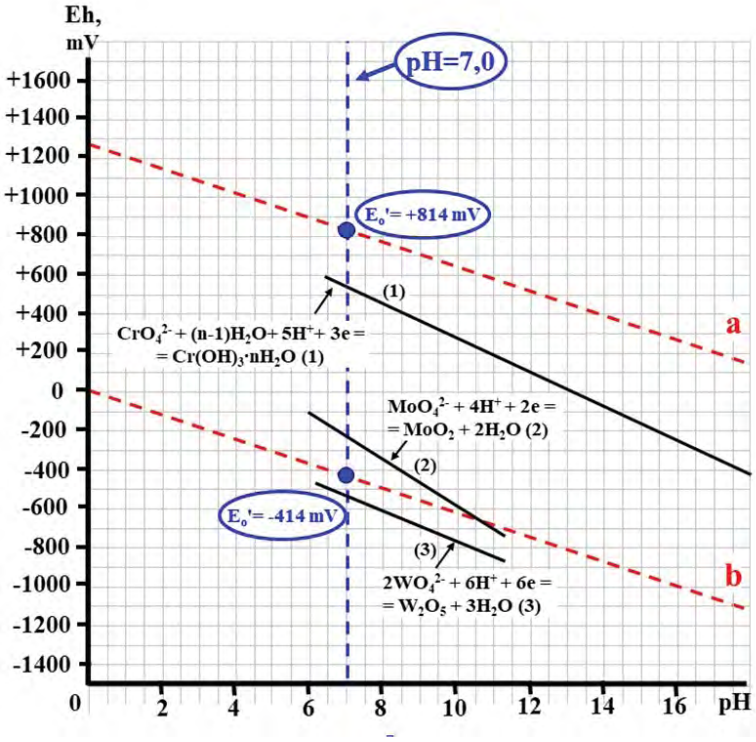

Fig. 1. **Redox-states of metals: *a* (top) and *b* (lower) – limit of thermodynamic stability of water:** *a* — is described by the equation: $O_2+4H^+=2H_2O$ and Eh =1.228–0.0591·pH – 0.0295·lgPH_2; *b* — is described by the equation: $2H^+ + 2e=H_2$ and Eh = 0.000 – 0.0591·pH – 0.0591·lgPH_2

Since the redox potential difference between hydrogenases and Cr(VI) is the largest 969 mV its reduction was the fastest and the most effective. The difference with Mo(VI) was only 94 mV the process was long termed. The reduction of W(VI) was not observed since microbial reduction was thermodynamically prohibited.

The strain *Clostridium butyricum* GMP1 was inoculated into the nutrient broth with addition of grinded potatoes to reach the lowest possible redox potential during fermentation aiming to provide further microbial reduction of metals. It showed the high efficiency of organic compounds fermentation (Fig. 2).

After 24 hours of cultivation, the pH values decreased from 7.25 to 5.5. The redox potential was also sharply reduced from +311 mV to −285 mV providing the conditions for hydrogen synthesis. The synthesis of H_2 occurred after 5 hours of fermentation. Its concentration reached 16.4%. The maximum H_2 concentration (38.1%) appeared after 19 hours of fermentation. Obtained fermentation conditions were optimal for the investigation of the efficiency of metals reduction.

Toxic metals were added separately in each flask. The studied concentration of Cr(VI), Mo(VI), W(VI) was 100 ppm.

The reduction of Cr(VI) was showed to be the most effective as it was thermodynamically predicted (Fig. 3). The solution of chromate was injected after 19 hours of fermentation in the middle phase of the process. As soon as Cr(VI) was added, the redox potential sharply increased from −285 mV to +200 mV. The pH values were not affected.

It took only 20 min to reduce 80% of chromate from 100 ppm to 20 ppm of Cr(VI). The complete removal of soluble chromate was observed after 10 hours. The effect of Cr(VI) on the synthesis of hydrogen was not significant. Immediately after addition of chromate, it decreased only by 1.4% (from 38.4 to 37% in the gas phase). The decrease of the concentration of H_2 after 48 hours (from 38.4 to 28%) of fermentation is assumed to be related to the total inhibition of the process due to the completion of digestion of organics.

As it was thermodynamically predicted, the efficiency of Mo(VI) reduction was much lower (Fig. 4). The prompt reduction of 10% of soluble molybdate occurred during 1 hour after its addition (from 100 ppm to 90 ppm). It is considered to be due to the presence of excessive reducing microbial exometabolites.

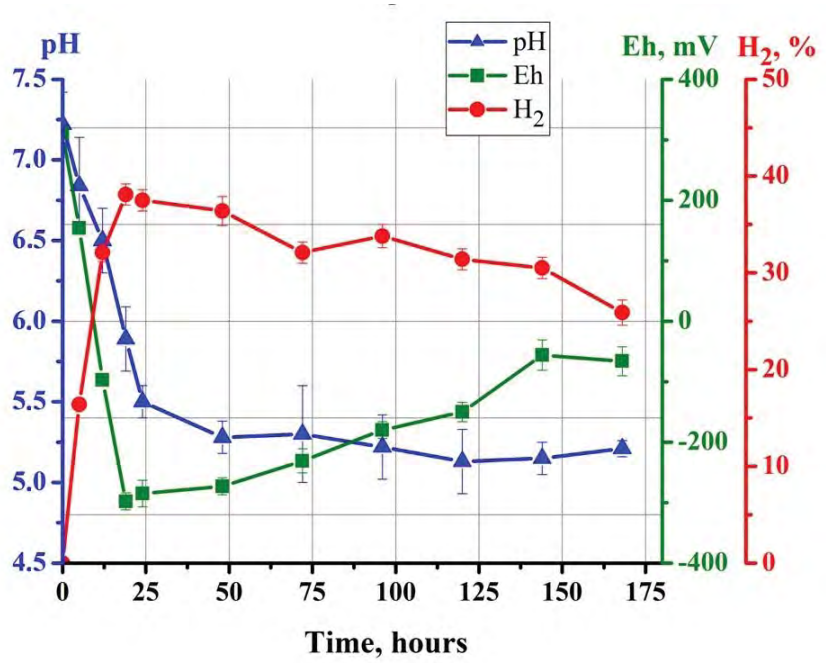

Fig. 2. **The dynamics of organic compounds fermentation by the strain Clostridium butyricum GMP1**

Nevertheless, the total efficiency of Mo(VI) removal reached only 25% after 6 days of cultivation (form 100 ppm to 75 ppm). The addition of Mo(VI) at the concentration of 100 ppm did not result in the significant change of the values of pH, Eh and H_2 concentration.

The compounds of W(VI) were shown to be stable during the whole term of incubation as calculated thermodynamically (Fig. 5). Tungstate was not reduced by microorganisms. Its concentration remained stable at 100 ppm. The activity of microorganisms was not changed due to the presence of W(VI).

Cr(VI) is the most affected by microorganisms. Moreover, it is the most toxic to them due to its high redox potential. In this regard, the patterns of its interaction with *Clostridium butyricum* GMP1 at the concentration 50 ppm (Fig. 6) and 200 ppm (Fig. 7) were additionally investigated.

Thus, the addition of Cr(VI) at the 50 ppm concentration did not inhibit microbial growth, change of hydrogen concentration in the gas phase or the pH values of culture medium (Fig. 6). It caused the predicted sharp increase of the Eh values from −300 mV to +30 mV. The efficiency of 86 % of chromate reduction from 50 ppm to 7 ppm occurred after 15 min of Cr(VI) addition. The complete removal of chromate took place in 4 hours of incubation.

At the concentration of 200 ppm, Cr(VI) did not inhibit the synthesis of H_2 or change of pH (Fig. 7). But the addition of chromate increased the Eh value from −280 mV to +250 mV with its following decrease to −130 mV due to Cr(VI) reduction.

It took 1 hour to reduce 81% of chromate from 197 ppm to 37 ppm. To obtain the complete removal of soluble chromate, 7 days of incubation were required. Such patterns can be explained by accumulation of reducing microbial exometabolites that provided the first effect of fast chromate removal. The remaining part of Cr(VI) could be reduced further due to the following growth of bacteria.

Thus, the thermodynamic calculations were experimentally confirmed. As it was thermodynamically predicted, the higher redox potential of metal provided the opportunity for its more effective reduction. At the concentration of 100 ppm, highly potential Cr(VI) (+555 mV at pH = 7.0) was completely removed after 10 hours of incubation. On the other hand, low potential Mo(VI) (−320 mV at pH = 7.0) revealed much lower efficiency of its reduction. It took 6 days to reduce 25% of Mo(VI). The tungstate was confirmed to be resistant to microbial reduction due to its redox potential out of the zone of water thermodynamic stability (−440 mV at pH = 7.0). The lower concentration of Cr(VI) (50 ppm) was shown to be effectively removed from solution during only 4 hours without inhibition of microbial growth. The concentration of 200 ppm of Cr(VI) did not cause the death of microorganisms. They could also remove Cr(VI) by reducing it, but it took

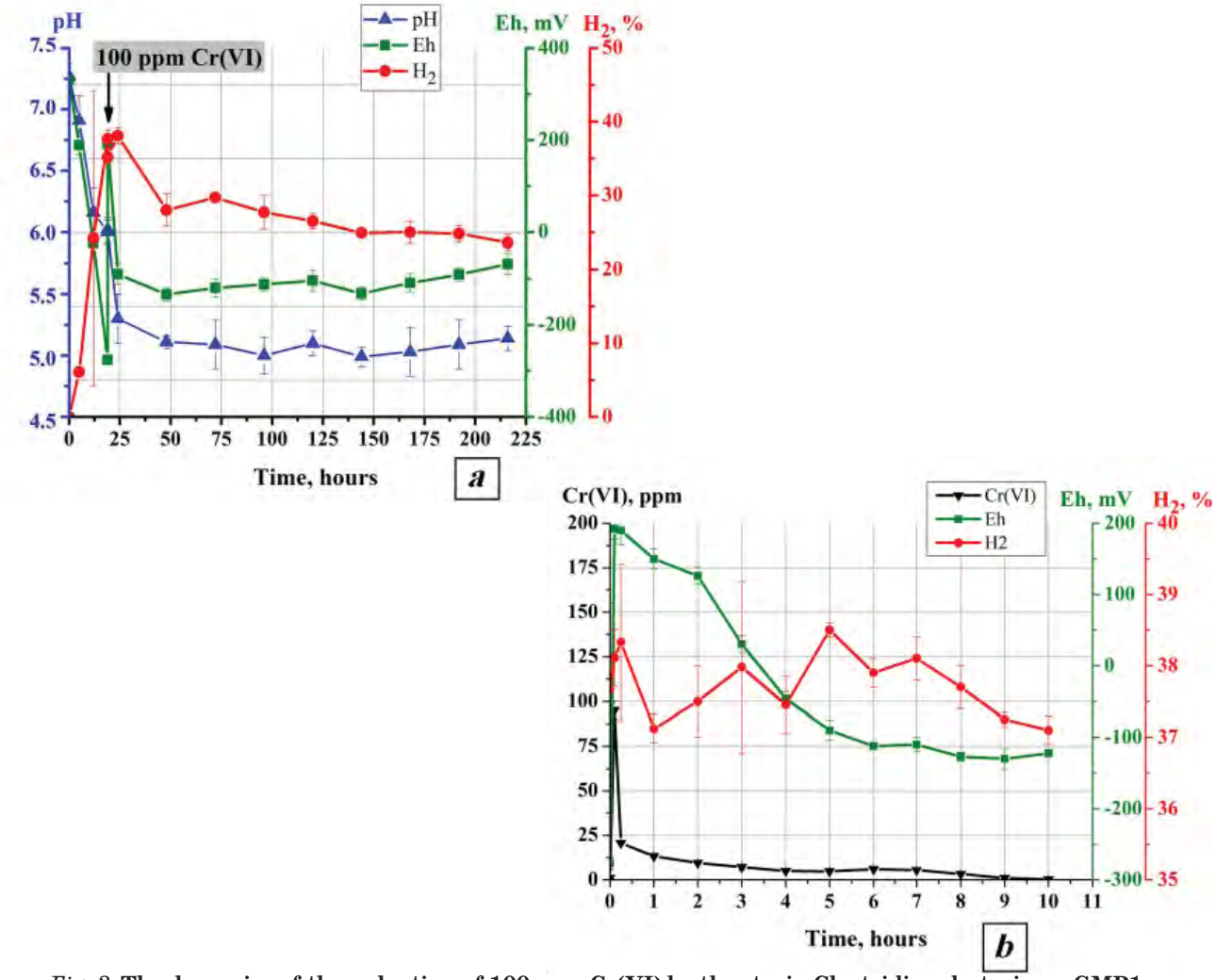

Fig. 3. **The dynamics of the reduction of 100 ppm Cr(VI) by the strain Clostridium butyricum GMP1:** *a* — the overall dynamics of the process after addition of Cr(VI); *b* — the detailed dynamics of Cr(VI) reduction

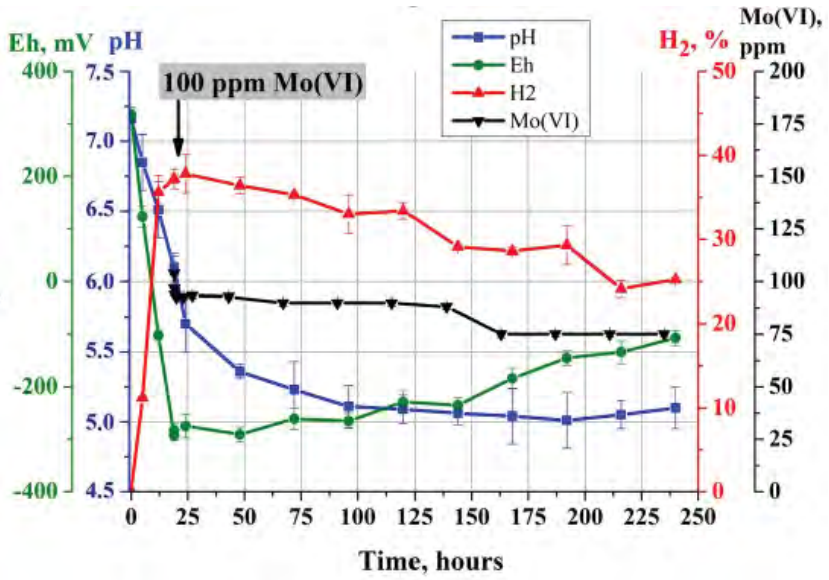

Fig. 4. **The dynamics of the reduction of 100 ppm Mo(VI) by the strain *Clostridium butyricum* GMP1**

much longer time. In this regard, the approach to predict thermodynamically the pathways of microbial interaction with toxic metals was showed to be effective tool for the development of environmental biotechnologies. It should be mentioned that the addition of metal soluble compounds to the sterile nutrient medium did not cause any changes in their concentration. Thus, the decrease in the concentration of metal compounds takes place due to metabolic activity of microorganisms. Further, the more precise study on the influence of microbial cells or their exometabolites on the removal of metal compounds will be conducted.

The literature data show that metal extraction technologies are based on different approaches, such as adsorption, filtration, precipitation, ion exchange, reverse osmosis, electrochemical treatment, evaporation, etc. [36]. But all these methods have significant disadvantages, including incomplete removal, high energy consumption, obtaining of toxic secondary waste [21].

Therefore, alternative, environmentally friendly and cost-effective technologies have begun to gain popularity. The application of microorganisms to remove toxic metals from industrial wastewater has gained the interest in the recent years due to increased production, availability and low cost [36–38]. Microbial treatment technologies require fewer reagents and generate less sludge, increasing cost-effectiveness [37].

Reduction of Cr(VI) to insoluble Cr(III) is possible with the application of both pure cultures and microbial communities [39]. For example, some species of bacteria are able to remove Cr(VI) with high efficiency: *Bacillus* sp. JDM-2-1 removed 86% at the initial concentration of 100 ppm Cr(VI); *Staphylococcus capitis* 89% [38]. The efficiency of Cr(VI) removal (at the initial concentration of 50 ppm) by 90% due to its reduction by a community of sulfate-reducing bacteria of anaerobic sediments was shown. Bacterial associations (*Acinetobacter junii, Escherichia coli, Bacillus subtilis*) were also applied to remove Cr(VI) at the concentration of 100 ppm. The efficiency of the process was 55–65% [40]. The use of activated sludge for the treatment of chromate-containing wastewater at the concentration of Cr(VI) of 0.5–5 ppm is shown. The efficiency of the process was about 80% [39]. Soil microbial communities also provided high efficiency of Cr(VI) removal in laboratory reactors with the initial chromate concentration of 200 ppm. The efficiency of the process was 95–98% [20]. It has been shown that sulfate-reducing microbial groups were able to remove up to 100% Cr(VI) via the reduction by sulfide, as well as direct enzymatic reduction of chromates in the UASB reactor (Upflow Anaerobic Sludge Blanket). However, due to the toxicity of Cr(VI), its maximum permissible concentrations in the

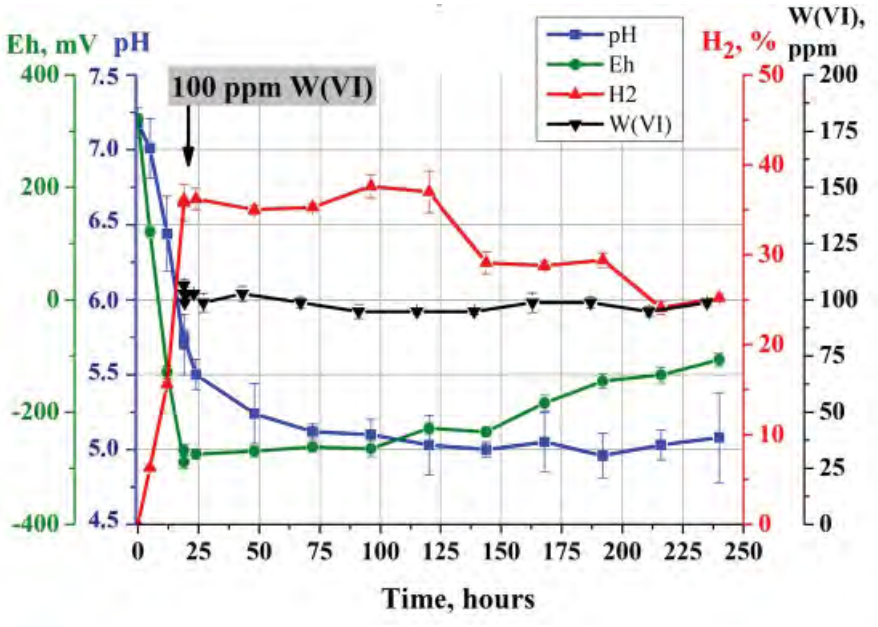

Fig. 5. **The dynamics of the reduction of 100 ppm W(VI) by the strain *Clostridium butyricum* GMP1**

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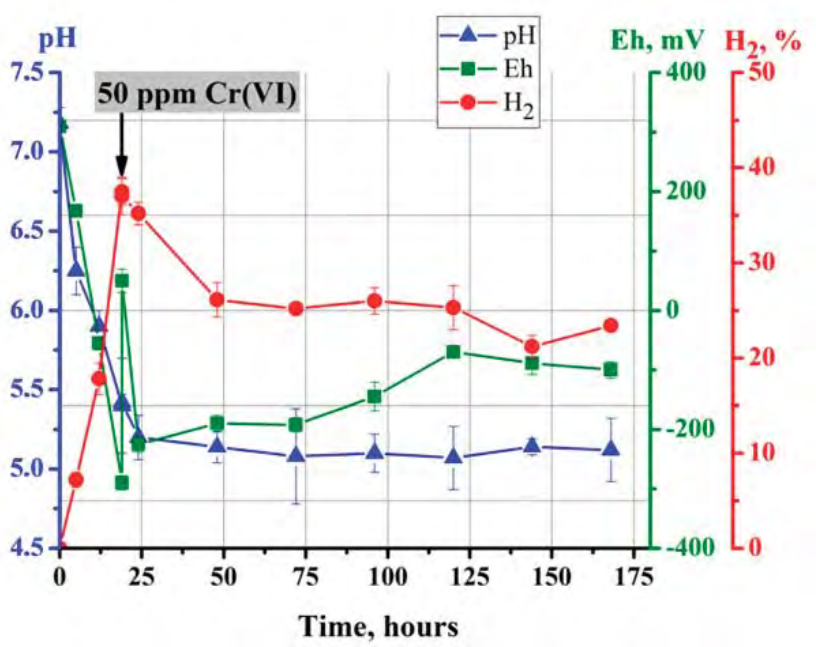

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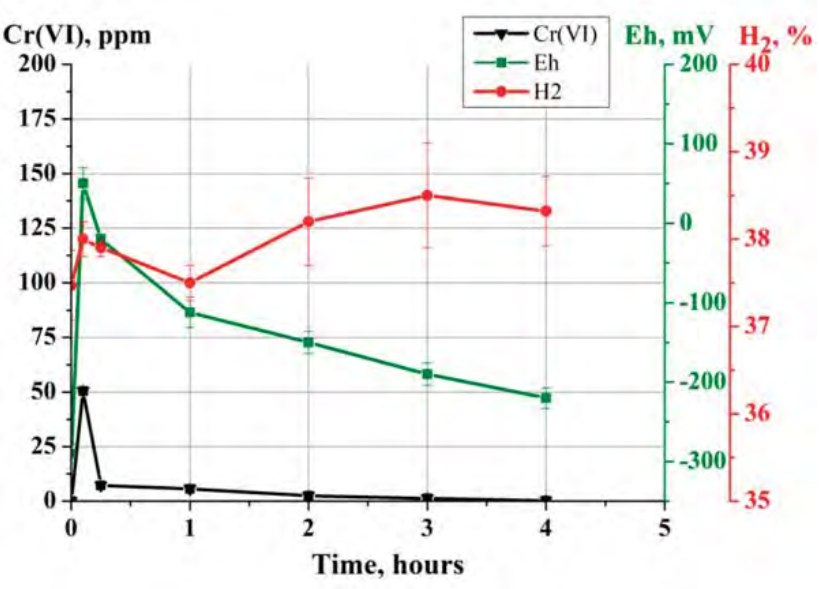

Fig. 6. **The dynamics of the reduction of 50 ppm Cr(VI) by the strain *Clostridium butyricum* GMP1:**
a —the overall dynamics of the process after addition of Cr(VI); *b* — the detailed dynamics of Cr(VI) reduction

reactor did not exceed 25–50 ppm [41]. In addition, the application of sulfate-reducing microorganisms on an industrial scale is dangerous due to the formation of H_2S, which is a neuroparalytic poison.

Three mechanisms of Cr(VI) reduction were summarized. First, chromate reduction occurs with the help of soluble chromate reductases associated with bacterial respiration. Second, Cr(VI) is used as an electron acceptor under anaerobic conditions (*Desulfotomaculum reducens*). Third, biologically mediated reduction of Cr(VI) by such exometabolites as nucleotides, vitamins, etc. [22].

Due to the toxicity of molybdenum compounds and its growing spreading in environmental pollution, biotechnological approaches as a promising alternative to existing methods of wastewater treatment and environmental remediation are attractive tools. The reduction of molybdate to insoluble molybdenum blue is a promising method for the development of environmental biotechnology to remove it [24, 42].

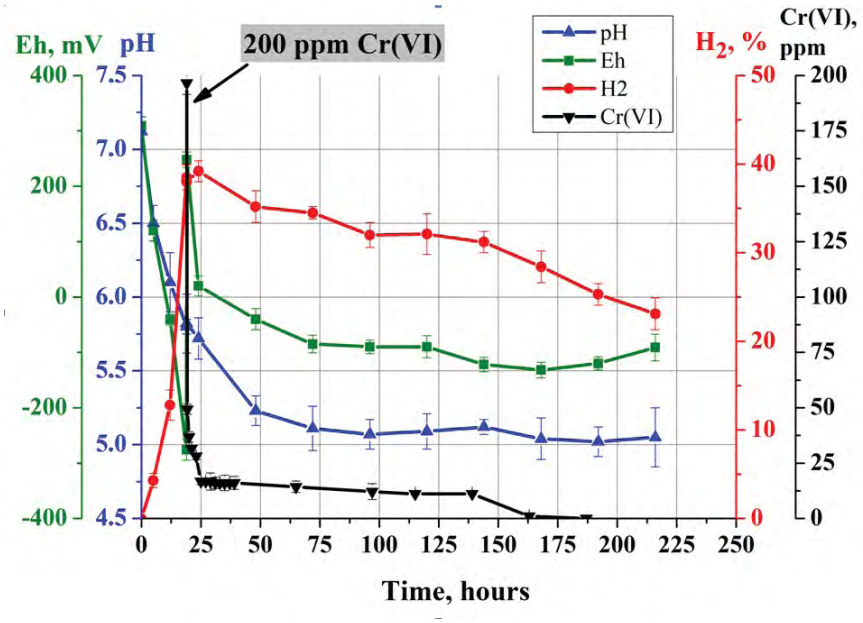

Fig. 7. **The dynamics of the reduction of 200 ppm Cr(VI) by the strain *Clostridium butyricum* GMP1**

Microbial reduction of molybdate to molybdenum blue was first reported in 1896 [43, 44]. Since then, the study of this process has been conducted. Detailed studies of molybdate reduction were started only in 1985 with *E. coli* K12 [10, 45, 46]. In 1988, the reduction of molybdate to molybdenum blue by the strain *Thiobacillus ferrooxidans* AP19-3 (now the strain Acidithiobacillus ferrooxidans AP19-3) was reported.

Recently, the possibility of Mo(VI) reduction by microorganisms belonging to various taxa was shown. They include *Serratia marcescens* Dr.Y6, *Serratia* sp. DRY5, *Enterobacter* sp. DRY13, *Serratia* sp. Dr.Y8, *S. marcescens* DRY9, *Acinetobacter calcoaceticus* and *Pseudomonas* sp. DRY2, *Enterobacter cloacae* [10, 24, 47–49]. Also, several Gram-positive Mo(VI)-reducing bacteria, such as *Staphylococcus* sp. and *Bacillus* sp. were selected [10]. It was shown that the optimal concentration of molybdate was from 50 to 60 mM. Concentrations of molybdate above 80 mM strongly inhibited its microbial reduction [43].

Tungsten is present in the environment in biologically significant concentrations. The development of industry leads to the spread of tungsten in natural ecosystems. Hexavalent tungsten in the form of tungstate is readily soluble in water at neutral and alkaline pH. Tungsten is not a highly reactive chemical compound, so no information on its reduction has been found [19].

Despite the success of laboratory research, the gap between laboratory and industrial scale is very large. The lack of the theoretical basis, deficit of working experience and associated economic risks are the main reasons for the currently limited application of the biotechnology in industry. It does not allow quantifying, forecasting and providing effective wastewater treatment. However, there is some information about the scaling of laboratory systems. The key problem that limits the widespread application of such biotechnologies is the need to improve and optimize the following items, such as the kinetics of the process, the choice of microorganisms used, cultivation conditions, etc.

The obtained results reveal the opportunity to predict the efficiency of Cr(VI), Mo(VI) and W(VI) removal from solution due to the redox potential of its reduction to insoluble compounds. As the highest potential, Cr(VI) was effectively (100%) removed from the solution at the concentration 50 ppm and 100ppm during several hours (4–10 hours) while 200 ppm required longer period of time. The soluble compounds of Mo(VI) were removed only by 25% during 6 days, since the redox potential of its reduction is close to the lower limit of water stability. The compounds of W(VI) cannot be removed from solution via its reduction, because of the Eh out of water stability zone.

The patterns of the reduction of toxic metals Cr(VI), Mo(VI) and W(VI) by obligate anaerobic strain *Clostridium butyricum* GMP1 were obtained. The experimental data confirmed the thermodynamically calculated correlation between the redox potential of the metal reduction to insoluble form and effectiveness of its removal. Obtained results can serve as the basis for further optimization and development of environmental biotechnologies for wastewater treatment with the simultaneous destruction of solid organic waste and hydrogen synthesis.

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ВЗАЄМОДІЯ ОБЛІГАТНО АНАЕРОБНОГО ДЕСТРУКТОРА ТВЕРДИХ ОРГАНІЧНИХ ВІДХОДІВ *Clostridium butyricum* GMP1 З РОЗЧИННИМИ СПОЛУКАМИ ТОКСИЧНИХ МЕТАЛІВ Cr(VI), Mo(VI) ТА W(VI)

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Збільшення забруднення навколишнього середовища токсичними металами є актуальною проблемою у всьому світі, що потребує ефективного вирішення. Метою роботи було вивчити динаміку взаємодії сполук Cr(VI), Mo(VI), W(VI) з облігатно-анаеробними мікроорганізмами *Clostridium butyricum* GMP1, які ферментують органічні сполуки, синтезуючи водень. Використовували стандартні методи для визначення pH і Eh, складу газу та концентрації металів. Застосування *Clostridium butyricum* GMP1 виявилось корисним для дослідження його взаємодії з токсичними металами. Вищий окисно-відновний потенціал металу забезпечував можливість його швидшого та ефективнішого відновлення. Встановлено закономірності відновлення токсичних металів Cr(VI), Mo(VI) та W(VI) за допомогою облігатно-анаеробного штаму *Clostridium butyricum* GMP1. Експериментальні дані підтвердили термодинамічно розраховану кореляцію між окисно-відновним потенціалом відновлення металу до нерозчинної форми та ефективністю його видалення. Отримані результати можуть слугувати основою подальшої оптимізації та розвитку екологічних біотехнологій для очищення стічних вод з одночасною деструкцією твердих органічних відходів та синтезом водню.

Ключові слова: термодинамічне прогнозування, токсичні метали, органічні відходи, екологічні біотехнології, водневе зброджування.

ВЗАИМОДЕЙСТВИЕ ОБЛИГАТНО-АНАЭРОБНОГО ДЕСТРУКТОРА ТВЕРДЫХ ОРГАНИЧЕСКИХ ОТХОДОВ *Clostridium butyricum* GMP1 С РАСТВОРИМЫМИ СОЕДИНЕНИЯМИ ТОКСИЧНЫХ МЕТАЛЛОВ Cr(VI), Mo(VI) И W(VI)

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Увеличение загрязнения окружающей среды токсичными металлами является актуальной проблемой во всем мире, требующей эффективного решения. Целью работы было изучение динамики взаимодействия соединений Cr(VI), Mo(VI), W(VI) с облигатно-анаэробными микроорганизмами *Clostridium butyricum* GMP1, которые ферментируют органические соединения, синтезируя водород. Использовали стандартные методы для определения pH и Eh, состава газа и концентрации металлов. Применение *Clostridium butyricum* GMP1 оказалось полезным для исследования его взаимодействия с токсичными металлами. Более высокий окислительно-восстановительный потенциал металла обеспечивал возможность его более быстрого и эффективного восстановления. Установлены закономерности восстановления токсичных металлов Cr(VI), Mo(VI) и W(VI) с помощью облигатно-анаэробного штамма *Clostridium butyricum* GMP1. Экспериментальные данные подтвердили термодинамически рассчитанную корреляцию между окислительно-восстановительным потенциалом восстановления металла до нерастворимой формы и эффективностью его удаления. Полученные результаты могут служить основой дальнейшей оптимизации и развития экологических биотехнологий для очистки сточных вод с одновременной деструкцией твердых органических отходов и синтезом водорода.

Ключевые слова: термодинамическое прогнозирование, токсичные металлы, органические отходы, экологические биотехнологии, водородное сбраживание.