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Detoxication of toxic chromate at fermentation of ecologically dangerous food waste using soil microorganisms

Goal. Determine the quantitative indices of the stability of the natural microbial grouping to chromate anion and the ability to restore it to Sr (III). Methods. Periodic cultivation, gas chromatographic and photocolorimetric analyzes. Results The microbial association effectively destroys waste, reducing its mass by 6 times in 9 - 12 days. The metabolically active association quickly and efficiently restores chromosomes. An increase in the concentration of chromates leads to an increase in the time of their recovery. Conclusions. The obtained data can be used to create the latest nature conservation biotechnologies and predict natural remediation of the environment contaminated with chromates.

Key words: spore association, chromate renewal, biotechnology, bioremediation, solid food waste.

An element of chrome is essential for the valuable life of humans and animals [6]. Its lack may lead to a disruption of lipid and glucose metabolism in humans and mammals. However, the supply of chromium in high concentrations in the body can be a threat to health and even life. Thus, LD_{50} oral toxicity Cr (VI) for rats is 50 - 100 mg / kg. The most toxic are 6-valent chromium compounds (CrO_4^{2-}, $Cr_2 O_7^-$). They are mutagens and carcinogens.

Cr (VI) compounds are widely used in many industries, for example, in the production cycles of electroplating coatings [5-7]. Therefore, their disposal is an important area of preservation of the environment.

At present in biotechnologies the disposal of chromium-containing wastewater is used specially selected chromatose-containing microorganisms that restore Cr (VI) to insoluble, and therefore nontoxic, Cr (III) [6]. Obtaining industrially important strains requires a lot of effort and time. Additional difficulties arise from the need to build "biomass" "special" stacks, store them, transport them to the place of implementation and ensure the operation of the industrial plant. The approach we propose is devoid of this disadvantage. The fundamental novelty of our approach is the combination of degradation processes of environmentally hazardous solid food waste and the removal of Cr (VI) compounds from solutions of microorganisms unadapted to toxic chromium III. We believe that destructors of solid food waste can be non-specific recover and deposit chromium. This technology relies on the following provisions:

• thermodynamic prediction, that is, the selection of optimal conditions for the restoration of chromate by microorganisms;

• determination of quantitative indices of homeostasis of a natural soil association. Under homeostasis, we mean: the ability to maintain a stable functioning at the maximum permissible concentration of chromate and the ability to recover Cr (VI) to insoluble Cr $(OH)_3$.

The purpose of research - quantitative determination of these parameters stability (homeostasis) pryrod-tion of microbial communities to chromate ani- one and the ability to restore to Cr (III).

Spore bacteria were chosen because they are very common in the environment, resistant to adverse factors through the formation of endospores. In addition, due to thermodynamic calculations, they create optimal conditions for the restoration of chromates. Thus, bacilli hydrolyze starch and create the anaerobic conditions required for clostridia. The effective recovery of chromate occurs at the maximum potential difference between the donor system (microorganisms) and the acceptor (chromate). The standard potential (E_0') of the Cr (VI) reduction to the insoluble Cr (III) hydroxide is +555 mV (Figure 1, reaction 5), and clostridia are capable of creating the lowest potential of the medium - from - 200 to - 400 mV. Therefore, the potential difference between donor and actuator systems at 700-900 mV should ensure fast and complete recovery chromate anion (CrO_4^{2-}) to insoluble, and hence, nontoxic Cr $(OH)_3$.

According to our goal, it is determined: quantitative characteristics of the metabolic activity of the microbial association during periodic cultivation in the absence of Cr (VI); influence of Cr (VI) on metabolic indexes of association; lethal and maximum allowable concentrations (MDC) Cr (VI) for this association; Quantitative parameters of the Cr (VI) reduction process depending on its concentrations in the medium.

Materials and methods. Research objects: chopped potatoes as a substrate, bacilli-clostridial natural microbial grouping and chromate anion (CrO_4^{2-}). This substrate has been chosen because starch is one of the most common natural polymers from which food waste is formed. In addition, the high efficiency of its use for anaerobic cultivation of spore-forming soil microbial associations was previously demonstrated [3, 10].

Cultivation: In vials of 250 ml, 50 g of potatoes (1 cubic cubic meters) and inoculum (1 ml) were added. Substrate and inoculum preheated to 100 ° C. for 5 minutes. The ratio of volumes of gas and liquid phases was 1: 1. The volume of excess gas was measured with a syringe of 50 ml once a day, pH and Eh - by potentiometric method. PH-meter-millivoltmeter "pH-150 MA" with the measuring electrode ESK-10603/4 was used for determination of pH. Redox potential was measured with pH meter-millivoltmeter "pH-150 MA" with measuring electrode EPV-1. Electrode comparison was a chloride electrode EVL-1M3.

The concentration of hydrogen in the gas phase was determined according to the standard method on the gas chromatograph LHM-8-MD [1]. 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.

Parameters of columns: I - l = 3 m, d = 3 mm, with a molecular sieve 13X (NaX); II - l = = 2 m, d = 3 mm, with Porapak-Q carrier; column temperature - + 60 ° C, evaporator - + 75 ° C, detector - + 60 ° C, detector current - 50 mA. Gas carrier - argon, flow rate of gas - 30 cm3 / min. Volume of gas samples: on the 1st column - 2.5 cm^3, on II-1 cm^3.

The content of H_2 in the gas mixture (in%) was calculated according to the standard method.

The concentration of Cr (VI) was determined by a colorimetric method with diphenylcarbazide (DFK) [2]. The method is based on the ability of the DPC to form colored complexes with chromate anion and to measure optical density.

Results and discussion. The obtained results correspond to thermodynamic calculations. Thus, the disputing association effectively destroys potatoes and reduces redox potential to levels close to the theoretically predicted, that is, optimal for the reduction of toxic CrO_4^{2-}.

The initial potential of 460 mV for 13 hours reduced to - 270 mV and vidbuvalo- be acidification of the medium (pH change from 6.92 to 5.51) (Fig. 2 and 3). Next, the synthesis of hydrogen began at a rate of approximately 104 ml per 1 kg of potato for 1 hour. During the first 22 hours of culturing biomass growth was negligible (optical density does not exceed 0.4 per initial figure 0.2). Only after the reduction of Eh to - 260 mV started the active growth of microorganisms. This is evidence of the domination of obligated anaerobic microorganisms in accordance with Jacob's concept [9].

The maximum biomass increase (up to 1.9 ounces of optical density) was observed after 70 h. The complete destruction of the potato by the association took place on the 9th-12th day of cultivation. The weight of the substrate was reduced by 6 times, that is, the rate of destruction (K_D) was 6.

Therefore, we proved that the association rapidly and efficiently destroys the model food waste and reduces the redox-potential to the minimum values that are most suitable for the recovery of chromates.

The obtained results indicate that it is expedient to introduce chromosomes in the phase of the lowest value of the redox potential and in the middle of the logarithmic phase of growth, since these two factors determine the effective recovery of chromates. That is why we introduced chromate into a wandering culture after 40 years of cultivation. At this time, the redox potential was already stable and stood at 210 ... 240 mV, and the optical density was 0.8 units.

The efficiency of chromate reduction by association was investigated in the concentration gradient Cr (VI) 100, 200 and 500 mg / l. The goal was to determine the MDC and to establish the ability of microorganisms to grow and restore chromate (homeostasis).

The introduction of chromate at a concentration of 100 mg / L resulted in a leap-like increase in Eh and a very rapid full recovery of chromates for 30 minutes (Fig. 4). Along with the restoration of chromates, the redox potential decreased to the original value. The rate of reduction of Cr (VI) was so high that when it was introduced at a concentration of 50 mg / l, we did not have time to track the potential jump up and returning it to the original value. Just as lightning was happening and full recovery of chromate.

The return of the redox potential to the original values and the renewal of hydrogen synthesis unambiguously proves the ability to associate with the active metabolism in the presence of chromate. The dynamics of chromate recovery indicates that the concentration of Cr (VI) can be significantly increased. Therefore, the next step was to add Cr (VI) to the active culture at double the concentration - 200 mg / liter. As it was supposed, in this concentration of chromate restoration was slowed down (Fig. 5). A further slowdown was observed for the introduction of 6-valent chromium at a concentration of 500 mg / l. The time of complete recovery of Cr (VI) in the medium depended on the initial concentration and was 30 minutes for

100 mg / l, 3 days for 200 mg / l and 7 days for 500 mg / l. For making 6-valent chromium in the medium at a concentration of 1000 mg / L occurred complete inhibition of the metabolic activity of the association. Consequently, the lethal concentration of Cr (VI) was 1000 mg / l.

The rate of reduction of chromates was maximum immediately after application and decreased in accordance with the reduction of concentrations of 6-valent chromium (Fig. 6).

The main application aspect of the results obtained is the determination of concentration 6-valent chromium in 100 mg / l, suitable for biotechnological purification. Under these conditions, the recovery rate of Cr (VI) to Cr (III) will be 100 mg / h.

The concentration of Cr (VI) in a concentration of 500 mg / l apparently is not acceptable. However, the importance of the results obtained is that natural disruptors are capable of spontaneous remediation during the accidental discharges of large volumes of Cr 6+ into the environment. In accordance with the thermodynamic calculations achieved.

Conclusions

In accordance with the thermodynamic calculations, the effective restoration of chromites by soil microorganisms that have not adapted to them has been achieved. The spore natural clostridium and bacillus groups effectively destroy environmentally hazardous solid food waste and restore chromates to form non-toxic hydroxide Cr (III). The efficiency and speed of removing chromates are very high, so these associations are very promising for the further development of the latest environmental biotechnologies. The obtained quantitative indices of homeostasis are also the basis for predicting the involvement of chromium compounds in biogeochemical cycles and obtaining quantitative indices of natural remediation.

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