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THE EFFECT OF HIGH CONCENTRATION OF MOLIBDENIUM AND ZINC ON THE GROWTH OF THE STRAIN THERMODESULFOVIBRIO SP. V2 FROM THE DEEP TERRESTRIAL BIOSPHERE

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ВЛИЯНИЕ ВЫСОКИХ КОНЦЕНТРАЦИЙ МОЛИБДЕНА И ЦИНКА НА РОСТ ШТАММА THERMODESULFOVIBRIO SP. V2 ИЗ ГЛУБИННОЙ БИОСФЕРЫ

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Аннотация. *В свете увеличения темпов развития нефте-, газо-, горнодобывающей и перерабатывающей промышленности тяжелые металлы стали занимать особое место среди загрязняющих веществ по масштабам и воздействию на биологические объекты. Отходы производств, отвалы бедных руд, шлаки и шламы металлургического производства, а также промышленные стоки представляют с каждым годом всё большую опасность. Вследствие этого, актуальным становятся изучение методов очистки воды от тяжелых металлов, в том числе биоремедиации. Целью исследования является определение влияния ионов тяжелых металлов на рост клеточной биомассы штамма термофильных сульфатредуцирующих бактерий Thermodesulfovibrio sp. V2 взятых из глубинной биосферы. Найдены предельные концентрации, при которых возможен рост данного штамма для молибдена и цинка, а также построены кривые роста для обоих металлов.*

Introduction. The application of biotechnology in water purification is not new. In order to treat copper-containing wastewater effectively using sulfate reducing bacteria (SRB), iron (Fe^0) was added to enhance the activity of SRB. Mine water with high concentrations of protons and metals is formed in the course of biological and chemical oxidation of residual metal sulfides. Microbial sulfidogenesis plays an important part in natural self-purification of mine wastes. SRB produce considerable amounts of sulfide, precipitate metals as sulfides, and increase pH due to proton consumption. This process is used in biotechnologies for treatment of metal contaminated water in bioreactors [1]. Industrial wastewater, such as wastewater originating from mining and metallurgical industries, is typically characterized by a significant content of sulphate and soluble metals, such as Fe, Zn, Cu, Ni, Pb and Cd. Biological treatment of such wastewater, based on SRB, is a viable option due to lower cost and better sludge qualities compared to conventional chemical treatment [2]. Removal of sulphates by chemical treatment is very costly, and therefore the biological method of sulphate reduction in wastewater and sludge can be very effective and economically viable.

SRB are commonly found in sediments and groundwater, especially in the vicinity of organic pollutants. Their effect on the ecosystem is limited to anaerobic conditions, the activity of these microorganisms conditioned by supply of organic substrates and sulphates depending mainly on the quantitative relationship between organic contaminants are prone to degradation in the process of metabolism, and sulphates (or sulfites, tiosulphites) reduced to hydrogen sulphide or sulphide [3]. Due to these properties, these bacteria are used in wastewater and sludge treatment, industrial waste treatment, bioremediation of soil, and in other activities used in the environmental protection of the environment.

Materials and Methods. Groundwater was sampled from a deep artesian borehole, drilled in 1957. The borehole is designated 5^{kp} and is located near the Chazhemto village in the Tomsk region (concentration of Mo and Zn shown in Table 1 was taken from [4]). Samples were stored in headspace-free bottles until analyzing. Enrichment cultures were established from positive most probable number (MPN) series in lactate-sulfate medium prepared from the wastewater samples. The medium contained (per liter) 1 g NaCl, 0,4 g $MgCl_2 \cdot 6H_2O$, 0,15 g $CaCl_2 \cdot 2H_2O$, 4,0 g Na_2SO_4, 0,25 g NH_4Cl, 0,2 g KH_2PO_4, 0,5 g KCl, 1 ml trace element solution, 1 ml vitamin solution, and 1 ml selenite-tungstate solution (Widdel and Bak 1992). The medium was supplemented with 1,6 ml of 50% (5,7 M) lactic acid solution. *Thermodesulfovibrio sp.* V2 was grown with lactate as electron donor. The initial pH was adjusted to 7,0-7,2 with NaHCO3 solution. Sulfide, added as $Na_2S \cdot 9H_2O$, was used as a reducing agent for enrichment and stock cultures. During inoculation Zn^{2+} was added as $ZnSO_4 \cdot 7H_2O$ to a final concentration of 10, 25, 50, 75 and 100 mg/liter. Mo^{2+} was added as Na_2MoO_3 to a final concentration of 10, 30, 40, 50, 60, 70, 75 mg/liter. Iron paper clips were placed in culture tubes as a source of Fe^{2+}. The sealed culture tubes were headspace-free and incubated under static dark conditions at 65 C°. Observation of cells using light microscopy was also routinely used to determine the growth of SRB. In the growth medium the lag period was 7-9 days for zinc and molybdenum.

Results. The results have shown that the growth of isolate *Thermodesulfovibrio sp.* V2 could survive and grow in the presence of molybdenum and zinc. For both metals maximum concentrations of molybdenum and zinc permissible to growth in the liquid medium 75 and 100 mg/liter for isolate *Thermodesulfovibrio sp.* V2, respectively were determined. The concentration of metal ions, in which the growth is possible, is much higher than in the borehole 5^{kp} (Table 1).

Table 1

The metal content in the borehole 5^{kp} and in the Widdel medium

Ion of heavy metal	The metal content in the borehole 5^{kp} (mg / dm3)	The metal content in the Widdel medium (mg / dm3)
Mo^{2+}	0,21	75
Zn^{2+}	0,05-0,06	100

The growth rate of strain V2 was 0.53×10^6 cells/mL and $1,13 \times 10^6$ cells/mL when the initial molybdenum and zinc concentration in culture medium was 10 mg/liter, respectively.

Molibdenium at 30 mg/liter in the growth medium decreased the growth rate to $0,21 \times 10^6$ cells/mL. Further increase in molibdenium concentration in V2 cultures was gradual and the maximum number of cells in molybdenum was observed for concentration of 60 mg/liter, $0,49 \times 10^6$ cells/mL. The number of cells in the final concentration of Mo was $0,14 \times 10^6$ cells/mL.

The isolate was resistant to zinc more than to molibdenium. At first the growth increased from 10 to 50 mg/liter. The maximum number of cells in Zn was observed for concentration of 50 mg/liter, $3,39 \times 10^6$ cells/mL. At a concentration of 60 to 100 ml/liter the growth was abruptly reduced. The number of cells in the final concentration of Zn was $0{,}11\times10^6$ cells/mL (Fig. 1).

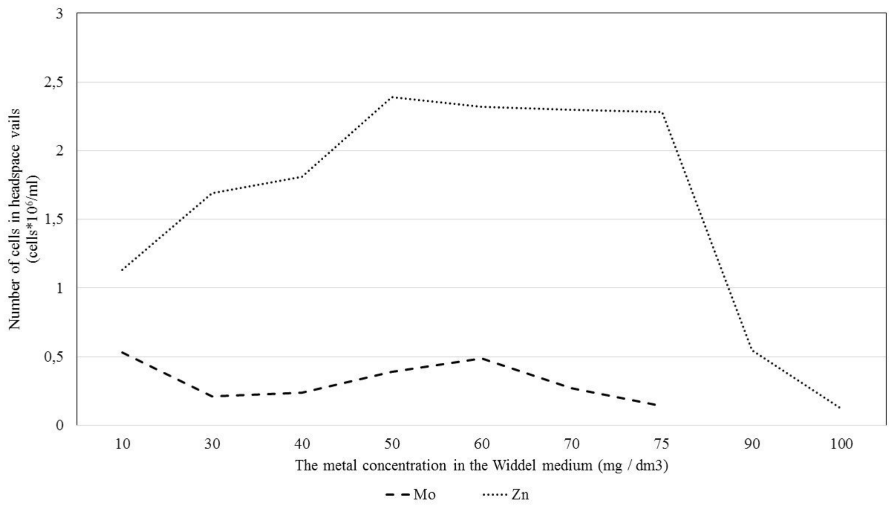

Fig. 1. The effect of molybdenum and zinc on the growth of the strain Thermodesulfovibrio sp. V2

Conclusion. Molibdenium and zinc pollution is not uncommon in mine waters and metallurgical effluents. Zn-sulfide formation is important in zinc-polluted environments, because it represents biologically unavailable form of the potentially toxic metal.

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