ISSN : 0974 - 7435 Volume 11 Issue 11

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 11(11), 2015 [436-440]

Influence of ions of different metals on growth and development of acidithiobacillusferrooxidans

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ABSTRACT

Cultures capable to active growth at presence of 30 g/l and higher of Fe^{3+} in solution were obtained by method of consequent adaptation. It was established that AcidithiobacillusferrooxidansK-1is not inhibited by high concentrations of cobalt (20.3 mg/l), zinc (348 mg/l), nickel (58.25 mg/l), but silver at concentration 100 mg/l and gold at 2.5 mg/l totally inhibit growth and development of this culture and its oxidizing activity. Influence of uranium ions on growth and development of A. ferrooxidansK-1 was studied. Comparative characterization of IR-spectra of A. ferrooxidans K-1 cells cultivated at different modes of cultivation (on medium with 100 mg/l of uranium and without it) was conducted.

KEYWORDS

Acidithiobacillusferrooxidans;
Leaching;
Gold;
Silver;
Uranium.

INTRODUCTION

Important role in processes of bioleaching belongs to iron oxidizing bacteria A.ferrooxidans, which are used in the process of leaching of gold-sulphide, copper-molybdenum, uraniferous and other ores[1,2].In natural conditions, these microorganisms participate at the process of metals' leaching from sulphide ores and rocks. These microorganisms possess different resistance towards ions of heavy metals and toxic elements;,they oxidize the same substrate with different velocity at the same time they cover wide range of physical and chemical parameters of medium (pH range from 0.7 to 3.5; temperature pH from 5°to 80°C)[3,4,5]. Biological nature of such diversity of strains is not studied well. But, its relationship with concrete life conditions of strains in different ecological niches, where process of strains microevolution was accompanied with changes in nucleotide sequence of chromosome DNA, is obvious. Peculiarities of structure of chromosome DNA at different strains are so stable that can be used in identification of the new strains, in strains monitoring in natural conditions and in biotechnological processes at study of experimental variability[6]. Analysis of restriction profiles of chromosome DNA revealed that each type of ore or concentrate has its own dominating strain adapted to concrete factors of medium[7]. Use of wide range of oxidizing substrates (from hydrogen to multicomponent sulphide ores) as an energy source, resistance to ions of heavy metals and high level of variability

in extreme conditions of the medium stipulate the leading role of A. ferrooxidansin bacterial and chemical processes of gold extraction or leaching of non-ferrous metals. The selection of highly effective, resistant to extreme factors of the environment, possessing high regulatory potential bacterial strains is very important, which is particularly essential in technological processes in conditions of permanent changes of substrate's characteristics, pH and temperature regimes, concentration of ions of metals and toxic elements in liquid phase[8]. Subsequent adaptation to complex of technological factors increases competitiveness of such strains and secures high efficiency in substrate oxidation.

MATERIALSANDMETHODS

Cultivation of micro organisms was conducted on medium 9K−Silverman-Lundgren[9]. Quantitative count of viable cells was done using method of subsequent ten-fold dilutions[10]. pH and redox potential of the medium was measured electrometrically. Quantity of Fe^{2+} and $Fe^{3+}$was determined by complexometric method[11], with use of EDTA as titrant. Solutions with different content of studied metals were prepared on basis of industrial solutions.

To analyze changes taking place in cells of A.ferrooxidansunder action of uranium ions in solution the method of oscillatory spectroscopy was used, which is applied at study of complex biological systems and at comparative study of cells of microorganisms[11,12].

RESULTS AND DISCUSSION

Microorganisms A.ferrooxidansare the most stable towards high content of iron in solution. But, at the leaching of concentrates the content of iron ions may considerably exceed concentrations acceptable for normal vital functioning of these cultures. To overcome this obstacle cultures capable to active growth at concentration of Fe^{3+} to 30 g/l and higher in solution were received by method of consistent adaptation. It was established that at cultivation of different strains of A. ferrooxidanson medium 9Kthe complete oxidation of iron takes place already on 4^{th} day and number of cells reaches $2.5x10^7$cells/ml Figure 1.

It is known that a number of elements at bacterial leaching of gold-containing ores transits to solution and at reaching of certain concentration of these metals there is a chance for inhibition of growth of microorganisms. Study of bioleaching of gold-containing ores of Kokpatas deposit revealed that a number of elements accumulates in composition of liquid phase or leaching floating solutions, and some of them reach considerably high concentrations (cobalt 20.3 mg/l, zinc 348 mg/l, nickel 58.25 mg/l, silver 37 mg/l). Analysis of microorganisms' development in these solutions revealed that resistant strain A.ferrooxidansK-1is not inhibited by such concentrations of these metals. But, at higher concentrations of silver there is possibility for suppression of bacterial growth. In these regards, it was interesting to study influence of ions of gold and silver at

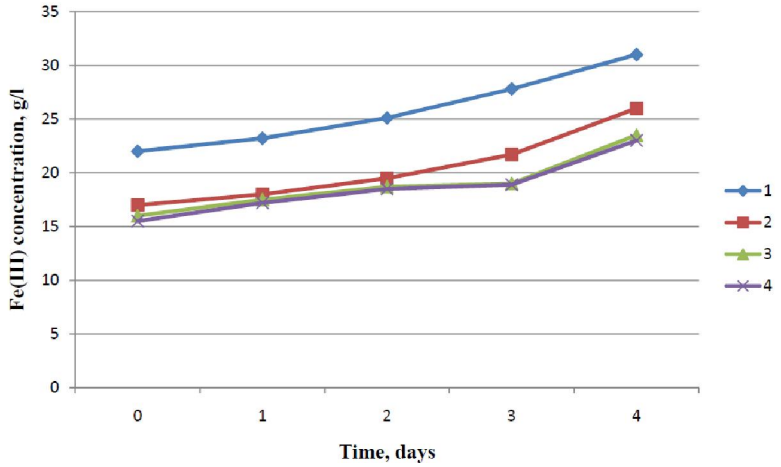

Figure 1 : Iron oxidation by different cultures of A. ferrooxidans(1 – K-1; 2 – KSB;3 – 3-9M; 4 – B-12) after preservation on higher concentrations of iron

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TABLE 1 : Influence of ions of gold (2.5 mg/l)and silver (100 mg/l) on growth and development of A.ferrooxidansK-1

№	Sample	Time, h	pH	Concentration of Fe, g/l			Quantity of microorganisms, cells/ml
				Fe^{3+}	Fe^{2+}	Fe_{total}	
1	Control	0	2.20	2.7	5.8	8.5	$2.5x10^4$
	Test	0	2.00	2.6	4.7	6.3	$2.5x10^4$
2	Control	24	2.20	4.0	4.2	8.2	$6.0x10^5$
	Test	24	2.10	2.8	4.5	7.3	-
3	Control	48	2.25	6.7	1.6	8.3	$6.0x10^6$
	Test	48	1.98	2.8	5.1	7.9	-
4	Control	96	2.60	7.8	0.5	8.3	$6.0x10^7$
	Test	96	1.98	3.0	5.6	8.6	-

increasing concentrations on growth and development of industrial strain A.ferrooxidansK-1. As result of conducted study it was determined that mixture of solutions of gold (2.5 mg/l) and silver (100 mg/l) completely inhibit growth and development of microorganisms and their oxidizing activity as well TABLE 1.

It is known that uranium in small quantities promotes to growth of microorganisms and increase of its concentration in medium suppresses growth of natural forms of heterotrophic microorganisms such as Bacillus megateriumisolated from soils with low content of uranium. Adaptation to certain level of uranium content in natural medium containing $1.5x10^{-3}$ % of uranium allows to receive stable forms of microorganisms[13,14,15].

Study of development of A.ferrooxidans K-1on medium 9Kcontaining 60 mg/l of uranium revealed that this concentration does not suppress growth and development of bacteria and even stimulates it to certain extent TABLE 2.

Further increase of uranium content to 100 mg/l in medium expresses negative influence on growth of A.ferrooxidansK-1. On third day of cultivation the number of cells in test variant decreases to $6.0x10^4$ cells/ml and only on 6th day the number of cells reaches the value corresponding to number of microorganisms grown in control variant. Similar changes are observed on oxidative activity of bacteria. Iron oxidation on medium with uranium runs less intensive, which correlates with change in number of bacterial cells in solution (Figure 2).

(1–Fe^{3+}concentration in control; 2–Fe^{3+} concentration in medium with uranium; 3– number of microbial cells in control; 4– number of microbial cells in medium with uranium)

Study of IR-spectra of Afer rooxidansK-1cellscultivatednnutrient medium 9 Kallowedtodetect following region sofabsorption (Figure 3):

3600 – 3000 cm^{-1} – valent fluctuations linked with hydrogen link NH (peptides), of OH (carbohydrates, water) – groups;

3000 – 2700 cm^{-1} – valent fluctuationof CH, CH_2, CH_3 – groups;

1700 – 1500 cm^{-1} – valent fluctuation of C=O (Amide I) and mixed deformative fluctuations (δ) NH and valent C=O (Amide II) of peptide group.

1400 – 1500 cm^{-1} – deformative fluctuations of $CH(CH_2-CH_3)$ – groups;

1000 –1200 cm^{-1} – C–links of pyron cycle and valent fluctuations of P=phosphate groups;

1000 – 400 cm^{-1} – out-plane fluctuations of $CH(CH_2-CH_3)$, OH– groups,

Overtones of hydrogen links OH...(X=O, N S ит.д)[16].

Examination of spectral curves reveals that strengthening of intensity in the band of asymmetric valent fluctuations CH in CH_3 – groups and symmetric valent fluctuations CH in CH_3– and CH in CH_2– group (2850 cm^{-1}) is recorded in bacterial cells grown on medium with uranium ions.

Different value of frequency corresponding to valent fluctuations C=O (1623 – 1600 cm^{-1}, Amide I) and mixed deformative fluctuations of NH with valent C=O (1500 cm^{-1}, Amide II), peptides is observed in spectra of these cells. The decrease of intensity for bacterial cells on medium with uranium

TABLE 2 : Influence of uranium ions (60 mg/l)on growth and development of A.ferrooxidansK-1

Period of cultivation, h	pH		Concentration of Fe^{3+}, g/l		Quantity of microorganisms, cells/ml	
	Control	Test	Control	Test	Control	Test
Initial	2.2	2.2	2.7	2.7	$2.5x10^4$	$2.5x10^4$
24	2.2	2.1	4.0	4.0	$6.0x10^5$	$2.5x10^5$
48	2.25	2.2	6.7	6.5	$6.0x10^6$	$2.5x10^6$
72	2.6	2.35	7.8	7.3	$6.0x10^7$	$2.5x10^7$

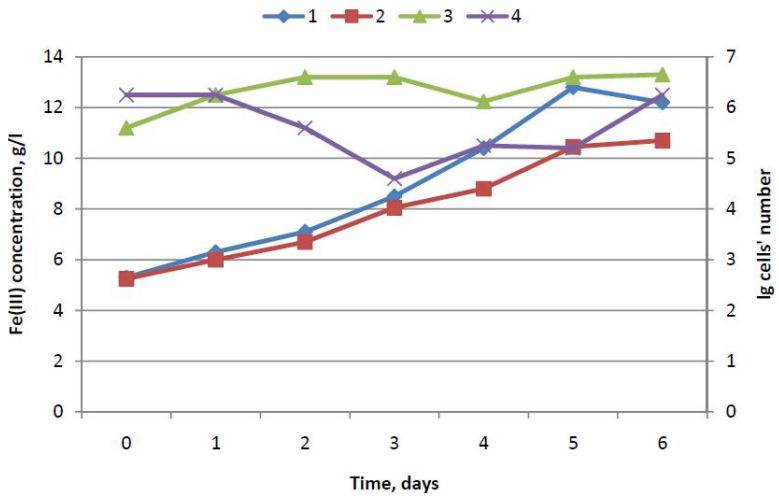

Figure 2 : Geochemical activity of A.ferrooxidans K-1on medium with uranium (100 mg/l)

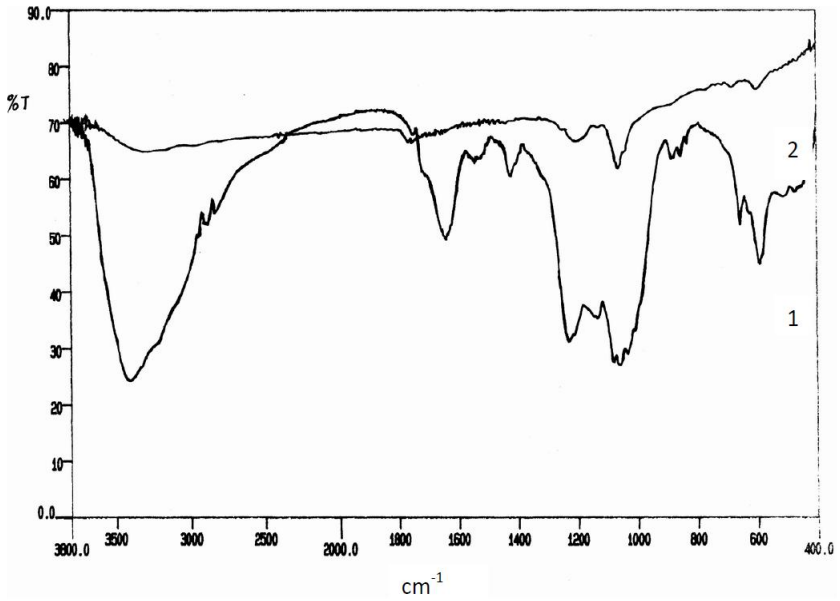

Figure 3 : IR-spectra of bacterial cells A.ferrooxidansK-1 on medium 9K (1) and on medium with uranium (100 mg/l) (2)

in the region 1130 – 1070 $cm^{-1}$is observed, which is possibly testifies the decrease of polyphosphates level.

The noted differences in IR-spectra express influence of uranium ions on bacterial cells and reveal changes taking place in them under action of metals and to higher extend it is obviously linked with cell wall. Gulyaeva et al. established by analysis of IR-spectra of yeasts cell that obtained regions of absorption are determined mainly by those components, which composes cell wall[17]. It is explained by the fact that contribution of intracellular compo-

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nents in total IR spectrum of whole cell is relatively small or is absent compared to the contribution of the whole cell. Analysis of absorption spectra testifies presence of proteins, carbohydrates and lipids in bacterial biomass. Changes occurring in structure of peptides and in level of accumulation of polyphosphates shows influence of uranium on bacteria.

CONCLUSION

Thus, conducted study revealed that selected the most active bacterial culture A.ferrooxidans K-1 possessing high velocity of iron oxidation possesses high resistance to increased content of ions of different metals in solution as well. At the same time, even in such unfavorable conditions the oxidizing activity of this culture does not decrease, which is favorable factor for application of A.ferrooxidansK-1 in industrial conditions. Obtained results of physical and chemical study prove our hypothetical assumptions about character of changes taking place in bacterial cells under action of uranium ions and allow reliably estimate occurring changes.

REFERENCES

[1] G.I.Karavayko; Micro organisms and the irrolein biotechnology of metals, in G.I.Karavayko et al., "Biogeotechnology of metals: practical guide", Center for international projects GKNT, Moscow, 11-50 **(1989)**.

[2] G.J.Olson, J.A.Brierley, C.L.Brierley; Appl.Microbiol.Biotechnol, **63**, 249-257 **(2003)**.

[3] S.N.Grudev; Differences between strains Thiobacillusferrooxidansby ability to oxidize sulphide minerals, in G.I.Karavayko, S.N.Grudev, "Biogeotechnology of metals", Center for international projects GKNT, Moscow, 85-89 **(1985)**.

[4] C.L.Brierley, J.A.Brierley, P.R.Norris, D.P.Kelly; Metal-tolerant microorganisms of hot, Acidic environments, in G.W.Gould, J.E.L.Corry; "Microbiological growth and survival in extremes of environment", Academic Press, London, **(1980)**.

[5] T.V.Kovalenko, G.I.Karavayko, V.P.Piskunov; Microbiology, **51**, 1 **(1982)**.

[6] S.N.Ageeva, T.F.Kondrat'eva, G.I.Karavayko; Microbiology, **70**, **2 (2001)**.

[7] T.F.Kondrat'eva, S.N.Ageeva, T.A.Pivovarova, G.I.Karavayko; Microbiology, **71**, 4 **(2002)**.

[8] D.E.Rawlings; Pure Appl.Chem., **76**, 4 **(2004)**.

[9] M.P.Silverman, D.G.Lundgren; J.Bacteriol., **77(5)**, (1959).

[10] A.I.Netrusov; "Microbiology guide", Akademiya, Moscow, **(2005)**.

[11] Lur'eYu.Yu.; "Analytical chemistry of industrialse wages",Khimiya, Moscow, **(1984)**.

[12] Zaslavskiy B.Yu, N.D.Gulyaeva, S.V.Rogozhin; Microbiology, **3(XLVI)**, **(1977)**.

[13] H.Morikawa, K.Tanizawa; Biol.Chem., **38**, **(1974)**.

[14] S.V.Letunova, V.V.Kovalskiy; "Geochemical ecology of microorganisms", Nauka, Moscow, **(1978)**.

[15] G.Basnakova, E.R.Stephens, M.C.Thaller, G.M.Rossolini, L.E.Macaskie; J.ApplMicrobiolBiotechnol., **2(50)**, **(1998)**.

[16] M.R.Mirzaeva, R.Rakhmanberdyeva, E.L.Kristallovich, D.A.Rakhimov, N.L.Shtonda; Journal Chemistry of Natural Compounds, 6, **(1998)**.

[17] N.D.Gulyaeva, M.B.Yakovleva, B.ZaslavskiyYu, S.V.Rogozhin, L.G.Loginova; Microbiology, **1(XLIX)**, **(1980)**.