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INFLUENCE OF LITHIUM-COBALT MIXTURE ON BACTERIAL OXIDATION OF FERROUS IONS BY ACIDITHIOBACILLUS FERROOXIDANS JCM 3863

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Abstract: Iron oxidizing bacteria *Acidithiobacillus ferrooxidans* show high resistance to heavy and toxic metals such as Zn, Cd, Hg, As, Co, Cu etc. In recent years, the investigations are directed toward the possibility of reutilization of some heavy and precious metals by bioleaching from solid wastes of lithium-ions batteries and spent electronic devices. In this research, we evaluated the dynamics of biooxidation of ferrous iron by *Acidithiobacillus ferrooxidans* JCM 3863 in the presence of lithium as well as of a mixture of cobalt and lithium ions. The experiments were carried out in batch cell cultures, in shake flasks at 190 pm and temperature 28±0.5ºC. The original liquid medium 9K was used. Both metal ions were added in different concentrations from 0.5 to 3.25 g/l in a ratio 1:1 in the mixture.

The dynamics of bacterial oxidation of ferrous iron in presence of Li^+ and Co^{2+} have been investigated in comparison with this one without metal ions – biological control. The bacteria in suspended cell cultures haven't been adapted preliminarily to increasing concentrations of metal ions. It has been found that the combination of metal ions (Li^+ and Co^{2+}) completely inhibits the oxidation process at 3.25 g/l irreversibly. Lithium Li^+ ions in the range of concentration up to 3.25 g/l slow down the oxidation rate, but not inhibit bacterial activity. The results show that the bacteria *Acidithiobacillus ferrooxidans* JCM 3863 can be used in future investigations for bioleaching of lithium and cobalt from batteries for domestic electronic devices in the investigated range of concentrations.

INTRODUCTION

Lithium-ions batteries are widely distributed because they are the preferred energy source for small and portable devices. Difficult mining of lithium and mass consumption led to a sharp rise of its prices during the last years and imposes the necessity of development of effective and feasible recycling technologies (Kumar, 2011). Hydrometallurgical methods for recycling of Li-batteries require a large expenditure of energy and pollute the environment with harmful substances (Paulino, 2008). These results and the experience in biohydrometallurgy of other metals allow to assume that in the case of lithium and cobalt it is quite possible to implement the methods of bioleaching from exhausted batteries.

Iron oxidizing bacteria as *Acidithiobacillus ferrooxidans* produce ferric ions, which are strong oxidizers, ensuring the removal of many metals from low grade ores (Rawlings, 2005; Free, 2013) and secondary sources (Mishra et al., 2008; Wang et al., 2009; Ivănuş, 2010; Willner, 2012). Microorganisms *Acidithiobacillus ferrooxidans* are acidophilic chemolithoautotrophic bacteria that inhabit acid mine drainage waters. These bacteria possess tolerance to the presence of many metal ions such as Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+} (Imai, 1975; Velkova, 2011). Cobalt is a metal that has a strong impact on oxidative activity of *Acidithiobacillus ferrooxidans* (Velkova et al., 2011). As to the influence of lithium ions on the behaviour of bacteria – it is poorly studied.

The aim of this work is to investigate the influence of Li^{+} and mixture of Li^{+} and Co^{2+} on bacterial oxidation of ferrous ions by *Acidithiobacillus ferrooxidans* JCM 3863 in submerged cultures.

MATERIALS AND METHODS

Microorganisms. The experiments have been carried out with the strain *Acidithiobacillus ferrooxidans* JCM 3863 from Japan Collection of Microorganisms.

Medium. Bacteria have been cultivated in 9K liquid medium (Silverman and Lundgren, 1959). Lithium and cobalt ions have been added in liquid media with concentrations in the range 0.50 – 3.25 g/l as $Li_2SO_4.H_2O$ and $CoSO_4.7H_2O$ respectively. Lithium and cobalt ions in the mixtures have been in concentration ratio 1:1. Microorganisms were not pre-adapted to increasing concentrations of metal ions.

Experimental conditions. *Acidithiobacillus ferrooxidans* JCM 3863 were cultivated in shake flasks in batch at 190 rpm and 28±0.5°C. Inoculum 1% and metal salts containing lithium and cobalt were added in 100 ml original liquid medium 9K. The average oxidation rates were estimated and compared with a biological control cultivated without lithium and cobalt ions. The chemical oxidation without bacteria and metal ions has been used as chemical control. All experiments were carried out in triplicate.

Analytical methods. Concentrations of ferrous and ferric ions and total iron were determined spectrophotometrically (Karamanev et al., 2002).

Dynamics of At. ferrooxidans JCM 3863 oxidation activity. Average oxidation rates have been calculated as follows:

$$\mathbf{r = (P_t - P_o)/t,} \qquad (1)$$

where: **r** is the average oxidation rate [g/l.h], $\mathbf{P_o}$ – initial concentration of product Fe^{3+} [g/l], $\mathbf{P_t}$ – concentration of product Fe^{3+} at the time **t** [g/l], t – time from the start of process to the achievement of a defined degree of oxidation of Fe^{2+} [h].

Degrees of substrate oxidation have been calculated as follows:

$$\boldsymbol{\eta = (So - St).100/So \text{ or } \eta = (Pt - Po).100/So}, \quad (2)$$

where: η is the degree of oxidation of substrate Fe^{2+} [%], So – initial concentration of substrate Fe^{2+} [g/l].

Dynamics of both bacterial and chemical controls have been obtained under the same conditions as the experiments with metal ions.

Data processing. Final results have been obtained by means of Microsoft Excel 2010 and Origin 6.1.

RESULTS AND DISCUSSION

Dynamics of ferrous ions oxidation in presence of lithium ions. The experiments with submerged cultures of *Acidithiobacillus ferrooxidans* JCM 3863 had shown that at the concentration of lithium ions in the range 2.00 – 3.25 g/l dynamics of bacterial oxidation of ferrous ions were different (fig. 2). It can be seen that the increase of lithium ions concentration led to elongation of lag phase, slowing down the dynamics of the oxidation process. In the range 0.50 – 3.25 g/l Li^{+} is not a dynamics inhibitor.

We have determined the average rate of ferrous ions oxidation at the degree of oxidation η = 60% (fig. 2.). This degree has been reached in all experiments with lithium ions, and it has been sufficient to assess oxidation activity of *Acidithiobacillus ferrooxidans* JCM 3863.

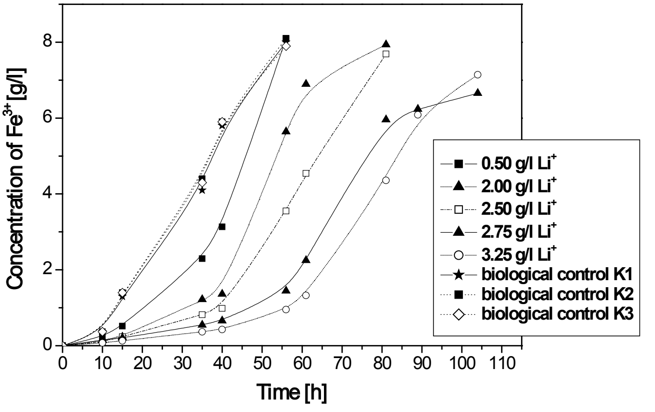

Figure 1. Dynamics of bacterial oxidation of Fe^{2+} in presence of Li^{+}.

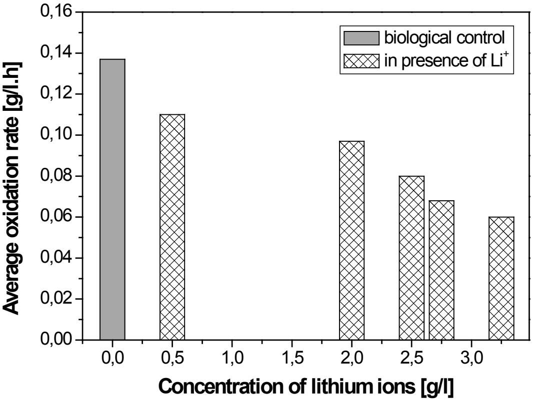

Figure 2. Average rate of oxidation in presence of Li^{+}.

It could be seen that the increase of concentration of lithium ions led to reduction of average rate of oxidation. These results have been confirmed by ANOVA Single factor (Microsoft Excel 2010).

Comparison of these results with those obtained by Velkova et al. (2011) showed that *Acidithiobacillus ferrooxidans* JCM 3863 is more sensitive to influence of cobalt ions – fig. 3. Complete process inhibition has occurred at 2.5 g/l Co^{2+}.

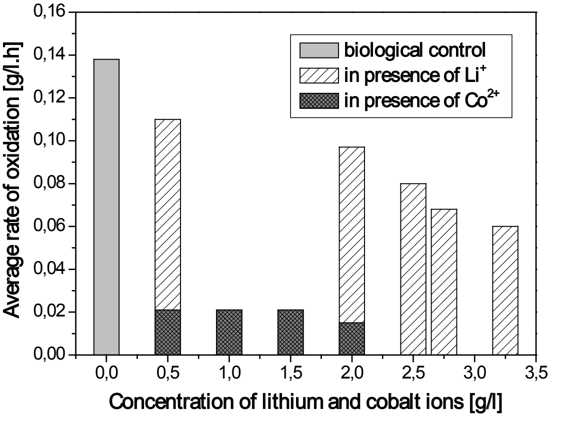

Figure 3. Comparison of average rates of oxidation in the presence of lithium and cobalt ions, added separately in medium 9K (η 60%).

Dynamics of ferrous ion oxidation in presence of mixture of lithium and cobalt ions. The investigations with submerged cultures of *Acidithiobacillus ferrooxidans* JCM 3863 had been carried out in presence of mixture of lithium and cobalt ions in the range of concentrations 0.50 – 3.25 g/l. Both types of ions have been added in nutrient media in the ratio 1:1. The dynamics of the oxidative process are presented in fig. 4.

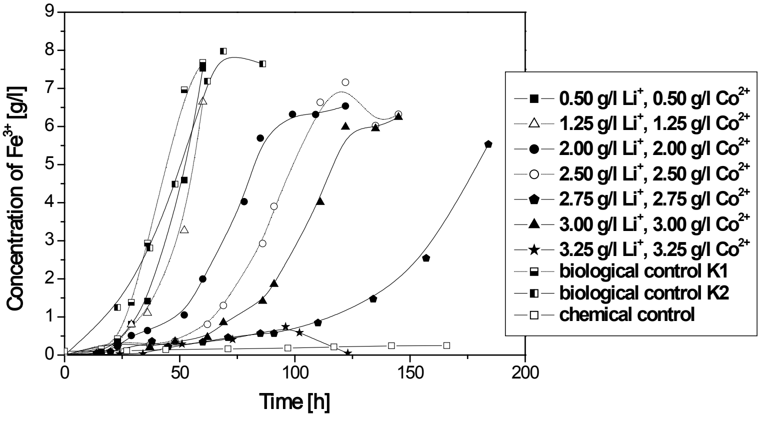

Figure 4. Dynamics of bacterial oxidation of Fe^{2+} in presence of Li^+ and Co^{2+}.

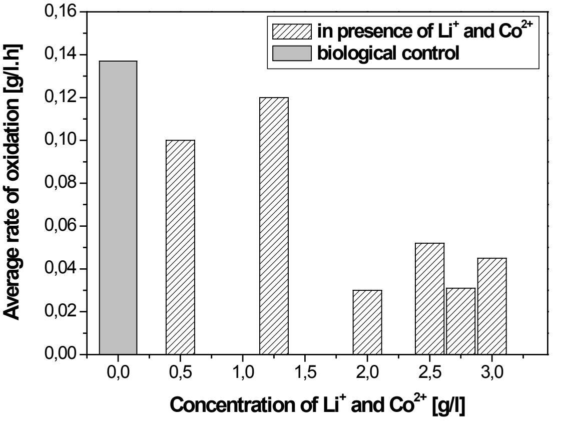

Figure 5. Average rate of oxidation in presence of Li^+ and Co^{2+}.

The presence of mixture of Li^+ and Co^{2+} in nutrient media led to significant delay of the process. The average rates of oxidation at 2.0 – 3.0 g/l ions have been 3 or 4 times lower compared with biological control. Complete inhibition occurred at concentration of both ions of 3.25 g/l and dynamics was similar to that of the chemical control. To determine the type of inhibition 1 ml of inhibited culture has been inoculated into medium 9K and has been cultivated in the same conditions. After 120 hours of cultivation, no oxidation of Fe^{2+} has been observed. It could accept that the inhibition is irreversible.

Determination of average rates of ferrous ion oxidation of different concentration of Li^+ and Co^{2+} at $\eta = 60\%$ (fig. 5) showed that after 1.25 g/l of each ion, the rate has been sharply decreased.

The results show that cobalt ions are more toxic for oxidation activity of *Acidithiobacillus ferrooxidans* JCM 3863. The joint effect of the mixture of Li^+ and Co^{2+} is stronger than single action of Li^+ but lower than single action of Co^{2+}. Obviously, lithium ions provide protection against cobalt ions and *Acidithiobacillus ferrooxidans* JCM 3863 retains their oxidation activity to 3.0 g/l. The presence of oxidation activity in the range of 0.5 – 3.0 g/l could be used for development of biotechnology for recycling lithium-ions batteries.

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