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# INFLUENCE OF $Fe^{2+}$ AND $Fe^{3+}$ ON THE GROWTH OF *LEPTOSPIRILLUM FERRIPHILUM CC* AND OXIDATION OF $Fe^{2+}$

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In the paper kinetics of ferrous iron oxidation by isolated *Leptospirillum ferriphilum* CC was studied in conical flasks on rotary shaker. The effect of initial ferrous iron concentration on bacterial growth and substrate oxidation was studied in the concentration range of 50-400 mM $FeSO_4$ $x7H_2O$. The highest specific growth (0.41-0.48 $h^{-1}$) and $Fe^{2+}$ oxidation rates (6.0-6.2 mM/l h) were detected at ferrous ion concentrations of 100-200 mM. At higher concentrations, the growth of bacteria and $Fe^{2+}$ oxidation suppression was observed, reaching maximum values at 400 mM $Fe^{2+}$. The maximum specific growth rate ($\mu_{max}$) of bacteria and half saturation constant (Ks) determined using Monod equation were 6.2mM /h, and 0.83$h^{-1}$, respectively. It was shown that $Fe^{2+}$ oxidation competitively inhibited by $Fe^{3+}$ and the inhibition constant (Ki) was 61.95 mM. The comparison of kinetic parameters obtained for *L. ferriphilum* with other bacteria indicates the high potential of *L.ferriphilum* CC in leaching processes of ores and concentrates for biogenic regeneration of concentrated ferric iron.

*Leptospirillum ferriphilum – iron oxidation kinetics – specific growth rate – saturation constant*

Հոդվածում ուսումնասիրվել է երկարժեք երկաթի օքսիդացման կինետիկան մեկուսացված *Leptospirillum ferriphilum* CC-ի մոտ 250 մլ էրլենմեյերի կոլբաներում թափահարման պայմաններում: Երկարժեք երկաթի սկզբնական կոնցենտրացիաների ազդեցությունը բակտերիայի աճի և սուբստրատի օքսիդացման վրա ուսումնասիրվել է $FeSO_4x7H_2O$ 50-400 մՄ կոնցենտրացիաների միջակայքում: Բակտերիայի աճի առավելագույն տեսակարար արագությունը (0.41-0.48 $ժ^{-1}$) և սուբստրատի օքսիդացման առավելագույն արագությունը (6.0-6.2 մՄ/լ ժամ) դիտվել է $FeSO_4$ $x7H_2O$-ի 100-200 մՄ կոնցենտրացիայի պայմաններում: Ավելի բարձր կոնցենտրացիաների դեպքում դիտվել է բակտերիայի աճի և երկաթի օքսիդացման ճնշում, որն առավելագույն մեծության է հասել 400 մՄ-ի դեպքում: Մոնոյի հավասարման կիրառմամբ որոշված բակտերիայի աճի առավելագույն տեսակարար արագության ($\mu_{max}$) և հագեցման հաստատունի ($\mu_{max}$) արժեքները կազմել են 6.2 մՄ/ժ, և 0.83 $ժ^{-1}$ համապատասխանաբար: Ցույց է տրվել, որ եռարժեք երկաթի իոնները մրցակցորեն ճնշում են երկաթի օքսիդացումը, ճնշման հաստատունը (Ki) կազմել է 61.95 մՄ: *L. ferriphilum*-ի և այլ բակտերիաների կինետիկական պարամետրերի համեմատությունը վկայում է առաջինի մեծ ներուժի մասին հանքաքարերի և խտանյութերի տարրալուծման գործընթացում՝ եռարժեք երկաթի կենսածին վերականգնման համար:

*Leptospirillum ferriphilum – երկաթի օքսիդացման կինետիկա – աճի տեսակարար արագություն – հագեցման հաստատուն*

В статье изучали кинетику окислениян железа у выделенного нами *Leptospirillum ferriphilum* CC в 250 мл конических колбах, в условиях встряхивания. Влияние начальных концентраций двухвалентного железа на рост

бактерии и окисление субстрата изучали при содержании в среде 50-400 мМ $FeSO_4$ x7$H_2O$. Наибольшее значение удельной скорости роста (0.41-0.48 ч-1) и скорости окисления железа (6.0-6.2 мМ $ч^{-1}$) наблюдалось при концентрации 100-200 мМ железа. При более высоких концентрациях железа наблюдалось подавление роста бактерии и окисления $Fe^{2+}$, которое достигало максимума при 400 мМ. Максимальное значение удельной скорости роста ($\mu_{max}$) и константа насыщения (Ks), определенные из уравнения Моно, составляли 6.2мМ $ч^{-1}$ и 0.83 $ч^{-1}$ соответственно. Ионы $Fe^{3+}$ конкурентно ингибировали окисление $Fe^{2+}$, константа ингибирования (Ki) составляла 61.95 мМ. Сравнение кинети- ческих параметров *L. ferriphilum* и других бактерий показало высокий потенциал *L. ferriphilum* для регенерации концентрированных растворов $Fe^{3+}$ в процессах выщелачивания руд и концентратов.

*Leptospirillum ferriphilum – кинетика окисления железа – удельная скорость роста – константа насыщения*

Bioleaching is an environmentally friendly microbiological technology that is applied worldwide for processing of mineral raw materials and recovery of copper, uranium and gold. It is considered that the main mechanism of bacterial attack on the metal sulfides is an indirect contact mechanism. According to this mechanism metal sulfide oxidation occurs by ferric ion and the role of microorganisms refers to the oxidation of ferrous ion and regeneration of ferric ion. The importance of microbiological ferrous ion oxidation in bioleaching of sulfide minerals is well known, and widely reported in the literature [3, 13]. During bioleaching processes, the sulfide minerals are chemically oxidized by ferric iron ($Fe^{3+}$) (Eq. 1). Resulted ferrous iron ($Fe^{2+}$) then is regenerated biologically by microorganisms (Eq. 2) [13, 14].

$$2MS + 4Fe^{3+} \longrightarrow 2M^{2+} + 4Fe^{2+} + 2S^0 \quad (1)$$

$$4Fe^{2+} + 4H^+ + O_2 \longrightarrow 4\,Fe^{3+} + 2H_2O \quad (2)$$

Therefore, the influence of $Fe^{3+}$ and $Fe^{2+}$ ions on the growth of bacteria and $Fe^{2+}$ oxidation activity often determines the intensity of metals leaching processes [5, 7, 9].

The most important microorganisms involved in the regeneration of $Fe^{3+}$ iron, responsible for the oxidation of exposed sulfide minerals are $Fe^{2+}$ oxidizing bacteria *Acidithiobacillus ferrooxidans, Leptospirillum ferrooxidans, L. ferriphilum, Sulfobacillus thermosulfidooxidans, Acidimicrobium ferroooxidans.* Currently, the mechanism and kinetics of $Fe^{2+}$ oxidation is well studied mainly in *At. ferrooxidans.*

The study of kinetics of $Fe^{2+}$ oxidation by *L. ferriphilum* is of great interest from the point of their wide use for leaching of metals from mineral raw materials.

In this paper the influence of $Fe^{2+}$ and $Fe^{3+}$ ions on the kinetics of $Fe^{2+}$ oxidation by isolated *L. ferriphilum* CC [15] was studied.

***Materials and methods***. *Object of investigation***.** In this study iron oxidizing bacteria *Leptospirillun ferriphilum* str. CC with optimal temperature of the growth of 37-40$^oC$, isolated in Armenia from bioleacing pulp of copper concentrate was used [15].

*Culture preparation*. *L. ferriphilum* CC was grown in Mackintosh medium, containing 20 g/l of $FeSO_4.7H_2O$ as an energy source [8]. In the logarithmic phase of the growth the bacterial cells were collected by centrifugation at 6000 g 10 min. Biomass collected was resuspended in the same medium without $Fe^{2+}$. Number of viable cells was determined by the method of tenfold dilution. The most probable number (MPN) of cells was calculated using the Mac-Credy tables (1). The maximum specific growth rate ($\mu_{max}$) of bacteria was determined using Monod equation.

***Influence of ferrous and ferric ions.*** The kinetics of ferrous ion oxidation by *L.ferriphilum* CC was studied in Mackintosh medium with ferrous ion ($Fe^{2+}$) in the concentration range of 50-400 mM. The influence of $Fe^{3+}$ ions on the growth rate and ferrous ion oxidation by *L.ferriphilum* CC was studied at the concentrations from 2 to 100 mM.

***Pyisico-chemical analysis.*** Ferrous (Fe (II)) and ferric (Fe (III)) ions were determined by the complexometric method with EDTA (1).

Saturation constant ($K_m$) was determined by the method of Lineweaver–Burk. The inhibition constant ($K_i$) was calculated from the equation $K_i = i / (K_p / K_m - 1)$ [12].

The pH was measured with a pH 121 pH meter-millivoltmeter.

Experiments were performed in triplicate. The data presented in the text are formed on the average from repeated experiments with ± 2 % variation of $Fe^{2+}$.

***Results and Discussion.*** *The effect of substrate concentration.* Ferrous iron oxidation by *L.ferriphilum* CC was carried out on the rotary shaker. Kinetics of bacterial growth and iron oxidation is well described by Monod equation. Applying the Monod equation to the data obtained (fig. 1), ferrous iron bio-oxidation can be described by the parameters $\mu_{max}$ and $V_{max}$ as 0.488 $h^{-1}$ and 3.2mM, respectively. Studies carried out showed that the growth of *L. ferriphilum* CC and the activity of $Fe^{2+}$ oxidation depend on the concentration of the latter in the medium.

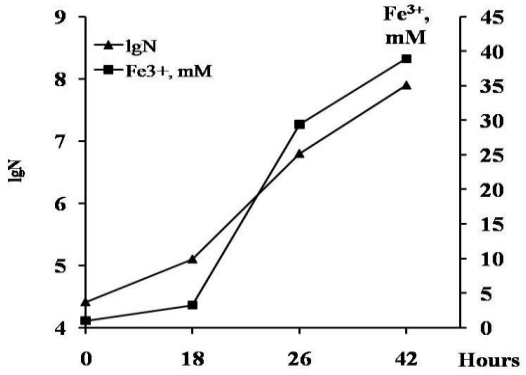


**Fig. 1.** Dynamics of growth and iron oxidation by *L.ferriphilum* CC ($Fe^{2+}$- 70mM, t-37$^0$C, 180 rpm)

Below is a quantitative characteristic of bacterial growth and ferrous iron oxidation depending on the initial concentration of $Fe^{2+}$ in the medium (tab.1).

As can be seen from tab. 1, maximal values of specific growth (0.41-0.48 $h^{-1}$) and $Fe^{2+}$ oxidation rates (6.0-6.2 mM/l h) were detected at ferrous ion concentrations of 100-200 mM. At higher concentrations, the growth of bacteria and $Fe^{2+}$ oxidation suppression was observed, reaching maximum values at 400 mM $Fe^{2+}$. At a concentration of 50 mM $Fe^{2+}$ in the medium, limitation by the substrate occurred. Thus, the optimal concentrations of $Fe^{2+}$ for the growth of *L. ferriphilum* CC are its initial concentrations of 100-200 mM.

***The effect of ferric ion concentration***. The tolerance of metal leaching bacteria to high $Fe^{3+}$ concentrations is important in indirect tank leaching applications. Ferric ion ($Fe^{3+}$), being the product of $Fe^{2+}$ oxidation, as iron oxidizing chemolithotrophic bacteria grow, it accumulates in the medium. Therefore, the effect of the initial concentrations of $Fe^{3+}$ on the oxidation of $Fe^{2+}$ can be studied only during the first hours (10-17 h) of the cultivation of bacteria, while the amount of oxidized $Fe^{2+}$ is insignificant. Results obtained are presented in tab.2.

**Table 1.**

Growth characteristics of *L.ferriphilum* str. CC at different initial concentrations of $Fe^{2+}$

| Concentration of $Fe^{2+}$, mM | Specific growth rate ($\mu_{max}$), $h^{-1}$ | $Fe^{2+}$ oxidation rate ($V_{max}$), mM/ h |
|---|---|---|
| 50mM | 0,31 | 1.7 |
| 100mM | 0,48 | 6.2 |
| 200mM | 0,41 | 6.0 |
| 300mM | 0,28 | 4.6 |
| 400mM | 0,09 | 3.7 |

**Table 2.**

Growth of *L. ferriphilum* CC and oxidation of $Fe^{2+}$ at different initial concentrations of $Fe^{3+}$

| Concentration of $Fe^{3+}$, mM | Specific rate of growth ($\mu_{max}$), $h^{-1}$ | $Fe^{2+}$ oxidation rate ($V_{max}$), mM/l h |
|---|---|---|
| 2.0 mM | 0,35 | 6.5mM |
| 20.0 mM | 0,32 | 6.1mM |
| 50.0 mM | 0,26 | 5.5mM |
| 75.0mM | 0,19 | 3.2mM |
| 100.0 mM | 0,16 | 1.9mM |

As the presented data show, in the presence of elevated concentrations of $Fe^{3+}$ ions, inhibition of the growth of *L. ferriphilum* CC and $Fe^{2+}$ oxidation was observed. Inhibition is expressed in the decrease of the specific growth rate and $Fe^{2+}$ ion oxidation rate (tab. 2, fig. 2). Furthermore, the inhibition degree increases with increasing the initial concentration of $Fe^{3+}$. The correlation between ferrous ion oxidation rate by *L. ferriphilum* CC and dynamics of changes in pH of the medium was detected (consumption of $H^+$ ions and increase of pH) (fig. 2c). At low values of $Fe^{3+}$, more active consumption of $H^+$ protons has been observed, which are necessary for the oxidation of $Fe^{2+}$ (Eq. 2).

The study of the kinetic parameters of the oxidation of $Fe^{2+}$ has shown that the affinity of *L. ferriphilum* CC to the substrate increases in the presence of $Fe^{3+}$ ions in the medium. Thus, the value of the saturation constant ($K_m$) for $Fe^{2+}$ oxidation in *L. ferriphilum* CC was 0.83 mM $FeSO_4$ in the absence of $Fe^{3+}$, while increased to 1.5mM $FeSO_4$ at the initial concentration of 50.0 mM $Fe_2(SO_4)_3$ in the medium. It is concluded that like *S. thermosulfidooxidans* [16] and *At. ferrooxidans* [6, 7], $Fe^{3+}$ ions competitively inhibit the oxidation of $Fe^{2+}$ in *L. ferriphilum* CC. The inhibition constant (Ki) was 61.95 mM $Fe_2(SO_4)_3$.

Thus, the optimal concentrations of $Fe^{2+}$ for the growth of *L. ferriphilum* CC are in the range of 100-200mM $FeSO_4$. Higher concentrations of $Fe^{2+}$ inhibit the growth of bacteria and oxidation of $Fe^{2+}$. The saturation constant for $Fe^{2+}$ is less than that of *S.thermosulfidooxidans* (3.4-4.1mM) (16) and *At. ferrooxidans* (1.34 mM $Fe^{2+}$) [6, 10, 11]. Thus, by the affinity for $Fe^{2+}$, *L. ferriphilum* CC considerably exceed *At. ferrooxidans.*

$Fe^{3+}$ ions, like *At.ferrooxidans*, competitively inhibit the growth of *L. ferriphilum* CC and $Fe^{2+}$ oxidation [2, 4, 6, 7]. The inhibition constant of *L. ferriphilum* CC is 61.95mM. It should be noted that $Fe^{3+}$ at a concentration of 36 mM inhibits the growth of *At. ferrooxidans* causing cell lysis [4].

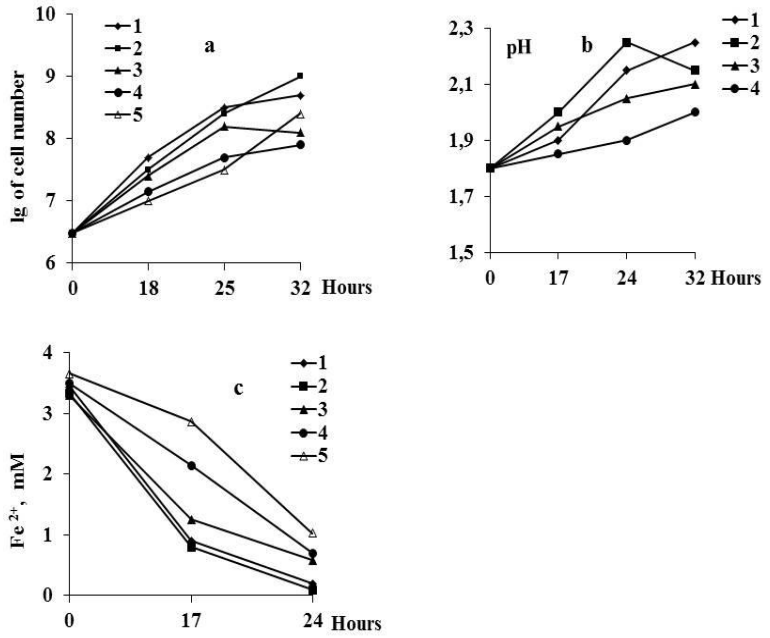


**Fig. 2.** Dynamics of growth of *L. ferriphilum* CC (b), oxidation of $Fe^{2+}$ (a) and changes in pH at $Fe^{3+}$ initial concentration (c) of: 1 - 2; 2 - 20.0; 3 - 50.0; 4 - 75.0 and 5-100.0 mM

$Fe^{2+}$ oxidation proceeds in the presence of 100 mM $Fe_2(SO_4)_3$ (40 g $Fe^{3+}$/L) allowing to use successfully *L. ferriphilum* CC in biogenic regeneration of concentrated $Fe^{3+}$ sulfate solutions for biohydrometallurgical applications.

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