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The effect of temperature on rate of bacterial oxidation of Fe(II)

D. Tekin[1]*, S. Yörük[1] ,Y.K. Bayhan[2]

[1] *Faculty of Engineering, Department of Environmental Engineering, Atatürk University, 25240 Erzurum, Turkey*
[2] *Faculty of Engineering, Department of Chemical Engineering, Atatürk University, 25240 Erzurum, Turkey*

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In this study, the effect of temperature on the rate of bacterial oxidation of Fe (II) at different microbial concentrations has been investigated. Reactions were carried out by using r4A1FC2B3 strain of *Acidithiobacillus ferrooxidans* at 15, 25 and 35 ºC and maximum bacterial activity, thus maximum oxidation rate, was reached at 35 ºC. At this temperature, the lag phase, the adaptation period of the bacteria, was very short and oxidation started immediately. The average bacteria count determined by microscopic method was 2.321×10^6 cells/mL.

Key words: Fe (II), bacterial oxidation, *Acidithiobacillus ferrooxidans*, effect of temperature

INTRODUCTION

The bioleaching involves the extraction of metallic compounds from ores and concentrates by using the catalyst effects of microorganisms under normal pressure and between 5-90 ºC. It is a simple, efficient and environmentally friendly method to process ores and has been successfully applied in the industrial scale for the recovery of copper, gold and uranium over 25 years. The process utilizes water, air, and microorganisms which all can easily be found from the environment [1,2].

Belonging to the chemotrophic organisms, Acidithiobacillus ferrooxidans are the most used bacteria in the bioleaching process. The organism is rod shaped, non-spored, a gram-negative, self spontaneous, single pole whipped. It uses carbondioxide as the carbon source and ammonium as nitrogen source [3-5]. Espejo et al.[6] studied the oxidation of Fe(II) and elemental sulfur by both, adsorbed and non-adsorbed *A. ferrooxidans* on solid surface. Nestor et al.[7] investigated the leaching mechanisms of refractory gold minerals by *A. ferrooxidans.* Mason and Rice [8], following an adaptation process, studied the leaching of iron and nickel from Ni-Fe(II)-FeS and Cu-Ni-Fe concentrates by using *A. Ferrooxidans.*

Sand et.al.[9] studied the bacterial leaching of metal sulfides by different bacteria and observed diverse effects of different bacteria on leaching and the generation of sulfuric acid and Fe(III) ions by bacteria such as *Acidithiobacillus ferrooxidans, Leptospirillum ferrooxidans* and *Sulfolobus/ Acidionus.* Ojumu et al. [10] presented a review on published studies and related rate equations for microbial ferrous-iron oxidation. They reported a broad range of kinetics models and large discrepancies on kinetics constants. They also indicated the lack of data on the effects of some factors such as pH and temperature on biooxidation.

As the case for chemical reactions, biological reactions are also temperature dependent. However, unlike chemical reactions, the rate of biological reactions starts decreasing over a certain temperature and it is important to determine optimum reaction temperature where the rate is maximum. Bosecker [11] found out that, with the Acidithiobacillus ferrooxidans the rate of oxidation of Fe(II) was lower temperatures. In lower temperatures, there is a reduction in the extraction of metals. However, at higher temperatures thermophilic bacteria can be used for leaching. The bacterial oxidation of ferrous iron is based on the reaction:

$$4FeSO_4 + 2H_2SO_4 + O_2 \xleftrightarrow{Bacteria} 2Fe_2(SO_4)_3 + 2H_2O \quad (1)$$

EXPERIMENTAL

Bacteria and Medium

The bacteria *Acidithiobacillus ferrooxidans* which are non-spore cells growing under aerobic conditions were obtained from acid mine drainages. The bacteria, also used by Kocadağıstan [12], were the type *r4A1FC2B3* modified by the use of 9K33 growth medium of 9K by Silverman and Lundgren [13]. The medium was sterilized by autoclaving,

* To whom all correspondence should be sent:
E-mail: deryatekin@atauni.edu.tr

where the basal salt and iron solution being autoclaved separately and combined when cool. In order to reduce the precipitation of ferric iron, 10 *N* H_2SO_4 was added to the iron solution. Cultures of *r4A1FC2B3* were incubated in 500-mL Erlenmeyer flasks each containing 200 mL of 9K medium and 10% (v/v) inoculum at a constant temperature of 30°C on a rotary shaker at 200 rpm.

Preparation of Cell Suspension

Cultures of *r4A1FC2B3* grown under the conditions already described were used to prepare the cell suspension. Bacteria were harvested toward the end of their exponential phase of growth (36–48 h after inoculation). In order to remove the insoluble ferric iron compounds, the cultures were filtered. Cells were washed twice with 10 mL of a solution of sulfuric acid (pH 2.0) to remove the remaining ferric iron. The cell pellet was finally suspended in 6 mL of a sulfuric acid solution of pH 2.0. In order to make cell suspensions of different concentrations, different volumes of *r4A1FC2B3* culture were used. Before using a cell suspension in initial-rate experiments, its bacterial concentration was determined.

Bacterial oxidation experiments

Bacterial oxidation experiments of Fe(II) were carried out in a shaker of the type ROSI 1000 Thermolyne Orbital Shaking Incubator. During the experiments, several samples at certain intervals were taken for Fe(II) and Fe(III) analyses. For Fe(II) analyses, Shimadzu UV160A spectrophotometer was used. Fe(III) analyses were completed by titration using EDTA solution with sulfosalisilic acid indicator.

RESULTS AND DISCUSSION

The growth of bacteria was followed by microscopic method proposed by Gürgün and Halkman [14]. The average count of bacteria was found to be 2,321x 10^6.

Since in the isolation experiments of the *r4A1FC2B3* bacteria strain, the bacteria was adapted to 15, 25 and 35 Celsius degrees, the effect of temperature on the bacterial oxidation of Fe(II) is investigated with experiments at these temperatures. All experiments were made using the pH value of 2 and repeated for each bacterial concentration. In each experiment Fe(II) and Fe(III) analysis were made at different times and the results obtained are shown at Figure 1-4. As seen from the Figures, maximum oxidation was obtained at 35°C. This was in agreement with others, that the maximum temperature for bacteria to grow is pH dependent, and found to be 45°C over the pH range of 2.5 to 3.5 and 35°C at a pH of 1.5 [15,16], where maximum temperature for bacteria to grow is pH dependent and a lower optimum temperature with decreasing pH was expressed [16].

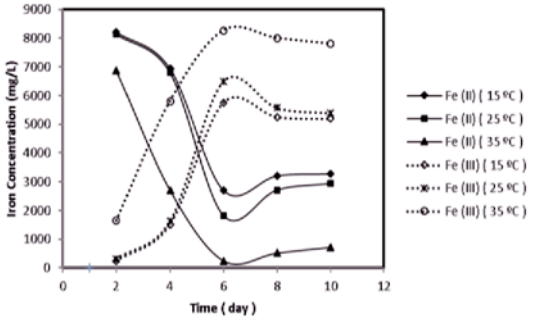

Fig. 1. Concentrations of Fe(II) and Fe(III) vs. time, for different temperatures (For initial bacteria conc. 0,5x10^5 cells/ml)

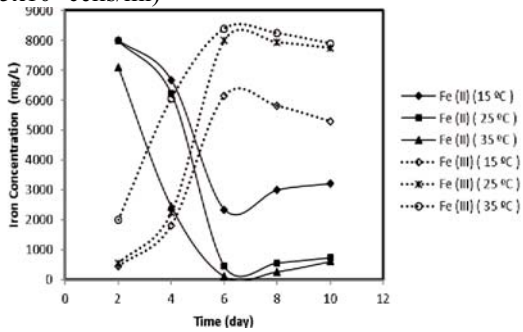

Fig. 2. Concentrations of Fe(II) and Fe(III) vs. time, for different temperatures (For initial bacteria conc. 1x10^5 cells/ml)

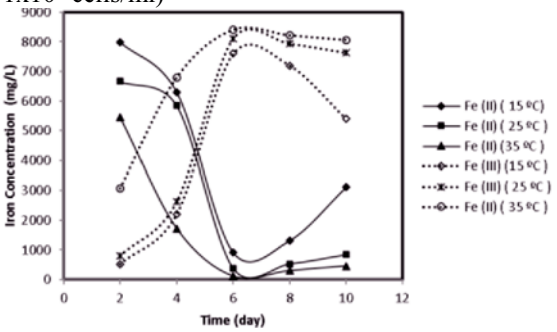

Fig. 3. Concentrations of Fe(II) and Fe(III) vs. time, for different temperatures (For initial bacteria conc. 1,5x10^5 cells/ml)

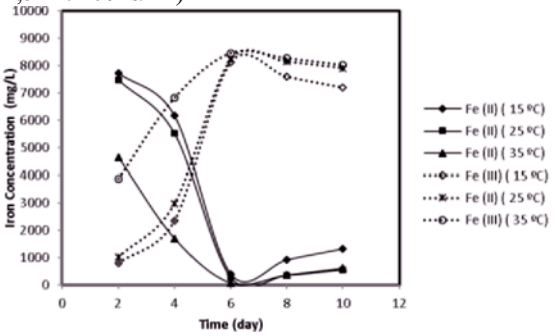

Fig. 4. Concentrations of Fe(II) and Fe(III) vs. time, for different temperatures (For initial bacteria conc. 2x10^5 cells/ml)

CONCLUSIONS

From the Figures 1-4 obtained for the bacterial oxidations of Fe(II) at 15 ^{O}C, 25 ^{O}C and 35 ^{O}C, the following conclusions may be drawn.

Highest conversion of Fe(II) was reached at the end of the 6th day. For the experiments carried out at 15 ^{O}C by adding 0.5 mL bacteria initially, concentration of Fe(II) was 8,210 mg/L at the end of the 2nd day which dropped to 2,706 mg/L at the end of 6th day. However, the corresponding concentration values of Fe(II) at 25^{O}C were 8,150 mg/L and 1,820 mg/L, whereas for 35^{O}C, Fe(II) concentrations were 6875 mg/L and 516 mg/L at the end of 2nd and 6th day, respectively.

When 1 ml of bacteria is added initially, these values of Fe(II) dropped from 8,009 mg/L to 2,350 mg/L for 15^{O}C , from 7,993 mg/L to 446 mg/L for 25^{O}C and from 7,100 mg/L to 116 mg/L for 35^{O}C. For an initial bacteria concentration of 1.5 ml, the Fe(II) concentrations at the end of 2nd and 6th day were 7,991 mg/L and 912 mg/L for 15^{O}C, 7,660 mg/L and 374 mg/L for 25^{O}C, 5,449 mg/L and 106 mg/L for 35^{O}C. If the initial concentration of bacteria increases to 2 mL, corresponding Fe(II) concentrations of 7,726 mg/L and 392 mg/L for 15^{O}C, 7,465 mg/L and 265 mg/L for 25^{O}C and 4,649 mg/L and 66 mg/L for 35^{O}C were obtained.

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ВЛИЯНИЕ НА ТЕМПЕАРТУРАТА ВЪРХУ СКОРОСТТА НА БАКТЕРИАЛНО ОКИСЛЕНИЕ НА Fe(II)

Д. Текин*, С. Йорюк[1] ,Й.К. Байхан

[1] *Департамент по екологично инженерство, Факултет по инженерство, Университет Ататюрк, Ерзорум, Турция*

[2] *Департамент по химиично инженерство, Факултет по инженерство, Университет Ататюрк, Ерзорум, Турция*

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Ш настощщата работа се изследва скоростта на бактериално окисление на Fe (II) при различни концентрации на микроорганизми. Реакциите са извършени с щам r4A1FC2B3 на *Acidithiobacillus ferrooxidans* при 15, 25 и 35ºC. Максимална микробна активност и съответно скорост на окисление са постигнати при 35 ºC. При тази температура лаг-фазата (периодът за адаптиране) на бактериите е много кратък и окислението започва незабавно. Среднищт брой на бактериите е 2.321x10^{6} клетки/мл.