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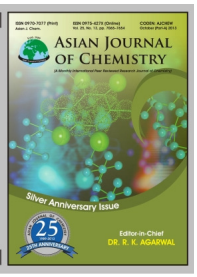

Kinetics of Bacterial Reduction of Hematite by *Acidithiobacillus ferrooxidans*-r4A1FC2B3

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The kinetics of bacterial reduction of hematite has been studied by using r4A1FC2B3 strain of *Acidithiobacillus ferrooxidans*. Hematite is dissolved in acidic medium and with the Fe(II) ions it is oxidized to Fe(III). Then Fe(III) ions are reduced to Fe(II) ions again with the bacteria. For the experiments carried out to study the kinetics of bacterial reduction of hematite, the concentration values of Fe(III) samples taken during exponential phase, which took place after the 14th day were used. It was seen that bacterial reduction of hematite fits to the Monod kinetics.

Key Words: Bacterial reduction, Hematite, *Thiobacillius ferrooxidans*, Monod kinetics.

INTRODUCTION

Bioleaching of sulphide minerals can be implemented either by the direct or by the indirect mechanism. The direct dissolution of minerals is caused by the attack on sulphide by the enzymatic system of the microorganisms situated at the mineral surface. In the indirect mechanism the primary attack on the sulphide mineral is assumed to be a ferric iron chemical leaching with the role of microorganisms, whether at the mineral surface or not, being to oxidize ferrous iron to ferric iron, maintain a high redox potential and also to oxidize the sulphur produced to sulphate. *Thiobacillus ferrooxidans, Thiobacillus thiooxidans* and *Leptospirillum ferrooxidans* are conventionally implemented in bioleaching processes at temperatures ranging from 30-45 ºC and as such the mesophilic biooxidation of ferrous iron has been studied extensively[1].

Bacterial Fe(III) oxide reduction strongly influences the geochemistry of anaerobic soil and sedimentary environments as well as the persistence and mobility of various types of organic and inorganic contaminants in such environments[2,3]. Recent studies indicate that the surface chemical properties of both the iron(III) oxide and the dissimilatory Fe(III)-reducing bacteria (FeRB) control the initial rate and long-term extent of microbial reduction of synthetic iron(III) oxides in defined growth medium[4,5].

The bacterium *Acidithiobacillus ferrooxidans* is a chemolithotrophic aerobic bacterium capable of oxidizing both ferrous iron to ferric iron and sulfide to sulfate, utilizing the energy derived from the oxidation to support carbon dioxide fixation and cell growth[6]. *Acidithiobacillus ferrooxidans (A. ferrooxidans)* is one of the few microorganisms known to gain energy by the oxidation of ferrous iron in acidic environments. The microorganism can make an important contribution to the biogeochemical cycling of metals in the environment and has the potential to help the recovery of metal contaminated sites by its ability to oxidize and reduce metals. Fe(III) ions and sulfuric acid are major by-products of the processes and these chemicals can mobilize metals in the environment including toxic metals such as arsenic[7]. It can also reduce Fe(III) ions and elemental sulfur, thus promoting the recycling of iron and sulfur compounds under anaerobic conditions[8-11]. Since the microorganism can use CO_2 as carbon source and fix nitrogen, it is thought to be a primary producer of carbon and nitrogen in acidic, nutrient-poor environments[12]. Owing to the potential applications of ferrous iron biooxidation, numerous works were carried out with *A. ferrooxidans*.

The Monod rate expression is often used to describe microbial growth and single substrate degradation kinetics. Monod kinetics have been widely applied in biogeochemical reactive transport models[13-17] and the kinetics of bacterial degradation of organic compounds and transfer of electrons to acceptors generally follows the Monod rate expression. Several rate equations published in literature related to the bactaerial oxidation of ferrous iron, where generally Monod kinetics was involved were given by Molchanov *et al.*[18]. They found large discrapencies in kinetics constants, up to 3 orders of magnitude, between published dat sets. Therefore, the aim of this study was to provide additional data on the microbial oxidation of Fe(II).

EXPERIMENTAL

Acidithiobacillus ferrooxidans used in the experiments are chemolithoautotrophic type of bacteria utilizing CO_2 in the atmosphere as carbon source and usually recovered from acid mine drainages. They are rod-shaped gram negative bacterias in non-spore form growing in aerobic environment. The bacteria, also used by Kocadaðýstan[19], were the type r4A1FC2B3 modified by the use of 9K33 growth medium of 9K by Silverman and Lundgren[20]. The optimum pH is between 2.3 for the R4A1FC2B3 strain.

The hematite ore used in the study was provided from Attepe, Turkey iron mine and after it was grounded sieve analysis was made. The chemical analysis of the hematite ore is as follows : Fe 57.04 %, SiO_2 5.58 %, CaO 0.8 %, Al_2O_3 0.85 %, MgO 0.43 % and H_2O 10.85 %. A schematic illustration of the experimental set-up is shown in Fig. 1. Bacterial reduction experiments of hematite was done by Velp Scientifica, Leaching test/Jar Test JLT6 brand Jar Test instrument in a 1000 mL reactors. During the experiments, severel samples at certain intervals were taken for Fe(II) and Fe(III) analyses. For Fe(II) analyses, Shimadzu UV160A spectrophotometer was used. However, Fe(III) analyses were completed by titration using EDTA solution with sulfosalicylic acid indicator.

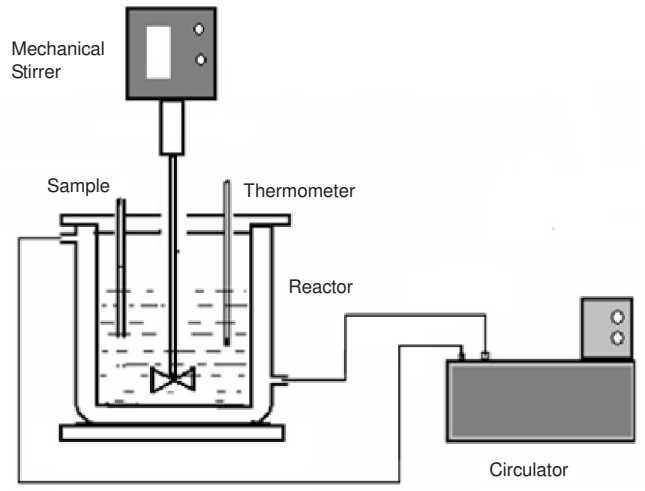

Fig. 1. Experimental apparatus

RESULTS AND DISCUSSION

The bacterial reduction kinetics of hematite were carried out at 25 ºC, 145 mm constant particle sizes and at a pH value of 2, respectively. The results obtained from the experiments using this parameters are shown in Figs. 2 and 3. The bioleaching of the hematite is reduced to Fe(II) due to the indirect bacterial leaching mechanisms. The bacteria in the medium was fed by the medium ingredients like (Fe^{2+}, Mg^{2+}, K^+, SO_4^{2-}, Cl^- *etc*.). As can be seen from the reactions (1) and (2) as a result of the Fe(II) ions oxidized to Fe(III) by the bacteria, in the medium the Fe(III) ions were procreated and then Fe(III) is reduced to Fe(II) by the means of bacteria[21].

$$Fe_2O_3 + 3H_2SO_4 \xrightarrow{\text{Chemical, Bacteria}} Fe_2(SO_4)_3 + 3H_2O \quad (1)$$

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

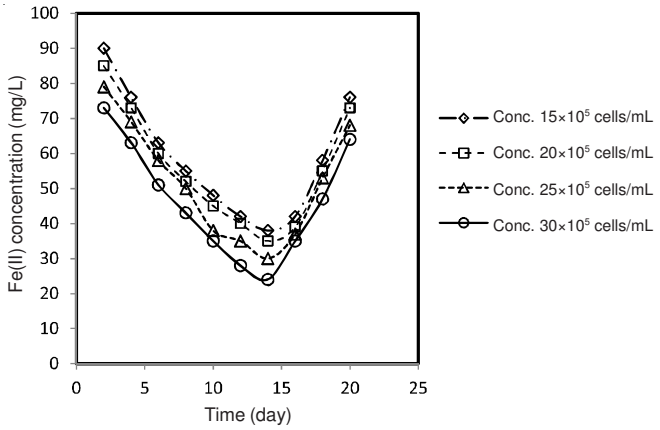

Fig. 2. Concentrations of Fe(II) *vs*. time, for different bacteria concentration

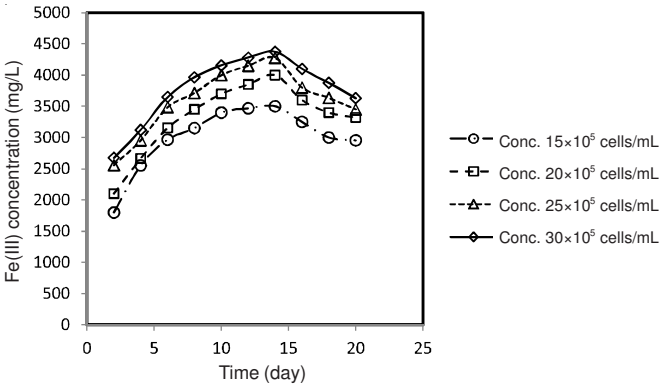

Fig. 3. Concentrations of Fe(III) *vs*. time, for different bacteria concentration

As a kinetic model of bacterial reduction of hematite, modified Monod equation which is given below was applied for this study[22].

$$\frac{dC_{Fe(III)}}{dt} = -L(t)\frac{(\mu_{max}/Y)C_x C_{Fe(III)}}{K_s + C_{Fe(III)}} \quad (3)$$

where L(t) equals to 1. Since the μ_{max}, Y and C_x values are constant, K can be written as

$$K = \left(\frac{\mu_{max}}{Y}\right)C_x \quad (4)$$

and, by re-arranging eqn. 3, gives the followings.

$$r_g = \frac{dC_{Fe(III)}}{dt} = -\frac{KC_{Fe(III)}}{K_s + C_{Fe(III)}} \quad (5)$$

In order to determine the constants K and K_s present in Monod expression, the data of exponential growth phase were used and temperature, pH and the composition of growth medium are held constant, while changing the initial concentration of bacteria (C_{xo}). For the experiments carried out to study the kinetics of bacterial reduction shown in Fig. 2, the concentration values of Fe(III) samples taken during exponential phase, which took place after the 14th day were used. Meanwhile, for an initial concentration of Fe(III) in the growth medium, the temperature and pH were held constant at the values of 25 ºC and 2, respectively. In the eqn. 5, the rates (r_g) of oxidation reaction, namely the values of derivative, were calculated by polynomial fitting[23]. These values for the each bacteria concentration are given in Table-1.

TABLE-1
REDUCTION RATES OBTANIED BY POLINOMIAL FITTING. (FOR TEMPERATURE: 25 ºC, PARTICLE SIZES: 145 mm AND pH:2)

Initial amount of bacteria (cells/mL):15×10^5			Initial amount of Bacteria (cells/mL): 20×10^5		
Time (day)	Fe^{+3} Conc. (mg/L)	$-r_g$ (mg/L.day)	Time (day)	Fe^{+3} conc. (mg/L)	$-r_g$ (mg/L.day)
0	3500	98.256	0	3884	96.504
1	3408	98.142	1	3788	96.378
2	3250	98.028	2	3600	96.252
3	3212	97.914	3	3595	96.126
4	3114	97.800	4	3500	96.000
5	3016	97.686	5	3403	95.874
6	2954	97.572	6	3320	95.748
7	2821	97.458	7	3212	95.622
8	2724	97.344	8	3180	95.496
Initial amount of Bacteria (cells/mL): 25×10^5			Initial amount of bacteria (cells/mL): 30×10^5		
0	3983	93.624	0	4077	92.284
1	3890	93.468	1	3985	92.088
2	3797	93.312	2	3892	91.892
3	3703	93.156	3	3800	91.696
4	3610	93.000	4	3709	91.500
5	3517	92.844	5	3618	91.304
6	3424	92.688	6	3527	91.108
7	3332	92.532	7	3435	90.912
8	3239	92.376	8	3344	90.716

For the kinetic model, by re-arranging the eqn. 5,

$$\frac{1}{-r_g} = \frac{1}{K} + \frac{K_s}{K}\frac{1}{C_{Fe(III)}} \quad (6)$$

is obtained. The values of Monod constants (K, K_s) for each cell population were obtained from ($1/C_{Fe(III)}$) *vs.* ($1/-r_g$) drawings as presented for initial bacteria conc. 15×10^5 cells/mL in Fig. 4. The linearity of these curves, which is the result of eqn. 6, indicates that the bacterial reduction of hematite obeys the Monod kinetics. Therefore, the values of K and K_s given in Table-2 were calculated from the intercepts . On the other hand, as a result of eqn. 6, the constant K should also change linearly with the initial cell concentration. To demonstrate this, K values calculated for different cell concentrations were shown in Fig. 5, where a linear variation against cell concentration also indicates the Monod mechanisms.

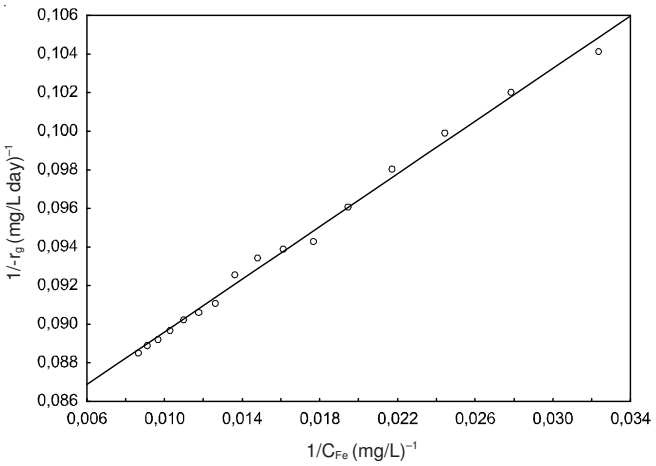

Fig. 4. Linewearer-burk plot (for initial bacteria conc. 15×10^5 cells/mL)

Conclusion

For the experiments carried out to study the kinetics of bacterial reduction of hematite, the concentration values of Fe(III) samples taken during exponential phase, which took place after the 14th day were used.

• Kinetics parameters of the monod equation were calculated from Fig. 3. The monot constant value of (μ_{max}/Y) estimated from the slope of Fig. 3 was found as 5.79. The half-saturation constant (K_s) was independent from cell concentrations and gave a constant approximate value of 251 mg/L.

• By applying these estimated values, the following equation as general rate expression for the bacterial oxidation of Fe(II) is proposed.

$$-r_g = -\frac{dC_{Fe(III)}}{dt} = \frac{5.79 C_{Fe(III)} C_x}{251 + C_{Fe(III)}} \quad (7)$$

TABLE-2
K AND K_s VALUES DETERMINED FOR DIFFERENT INITIAL BACTERIAL CONCENTRATIONS

Initial bacteria concentration (cells/mL)	K Values	K_s Values (mg/L)
15×10^5	102.0	251.0
20×10^5	114.9	250.6
25×10^5	135.1	251.0
30×10^5	181.8	250.9

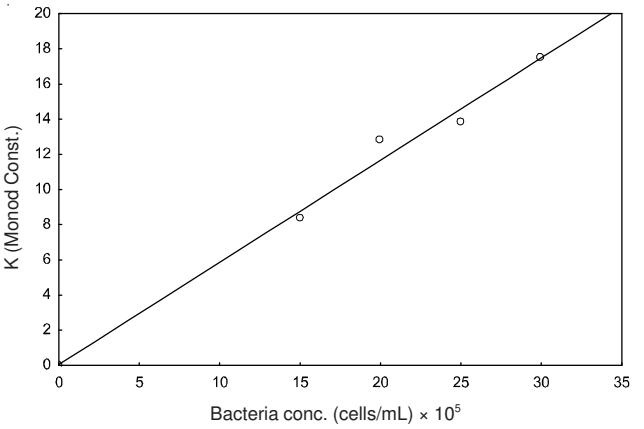

Fig. 5. Variation of monod constant (K) with initial bacterial concentration

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