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The Kinetics of Microbiological Oxidation of Pyrite

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Abstract

The kinetics of pyrite oxidation by *Thiobacillus ferrooxidans* bacteria were studied in different conditions of reaction (different size of pyrite sample relative to the leaching medium, medium pH and temperature). A model of the kinetics of the process has been proposed according to which it can be described by an autocatalytic reaction of order II, i.e. I order with respect to pyrite iron and I order with respect to the forming Fe(III) ions. On the basis of this model the rate constants and induction times of the reactions taking place in different conditions have been determined. The results obtained within the model enable us to show the optimum conditions of microbiological oxidation of pyrite.

Keywords: *Thiobacillus ferrooxidans*, kinetics of pyrite oxidation.

Introduction

The main idea behind the biological desulfurication of coal is oxidation of the pyrite it contains, which leads to the formation of soluble sulphur compounds. It has been established that bacteria from *Thiobaccillus ferroxidans,* besides *Thiobacillus thiooxidans* and *Thiobacillus acidophillus,* are most effective in this process [1]. These chemolitotrophic bacteria, commonly found in the waters accompanying coal beds, obtain the energy they need to maintain their life functions from oxidation of Fe (II) and reduced inorganic sulphur compounds. Their additional advantages are the exceptional adaptativity and tolerance of even extreme external conditions.These values make them interesting from the point of view of searching for new industrial methods of fuel desulfurication. Although the processes of biological leaching have been known for a long time [2], the most important direction of biotechnological studies is isolation of mutants showing increased activity.

The mechanism of biological oxidation of sulphide minerals depends on the activity of enzymatic systems related to the energetic processes occurring in a bacterial cell. Metabolic transformations of sulphides in *Thiobacillus ferrooxidans* can be described by the following simplified diagram [3, 4, 5]:

$$S^{2-} \xrightarrow[\text{oxidase}]{\text{sulphide}} S^0 \xrightarrow[\text{oxidase}]{\text{sulphur}} SO_3^{2-} \xrightarrow[\text{oxidase}]{\text{sulphite}} SO_4^{2-}$$

(1) (2) (3)

$$(1)\ S^{2-} + 1/2O_2 + 2H^+ \xrightarrow[\text{oxidase}]{\text{sulphide}} S^0 + H_2O$$

$$(2)\ S^0 + O_2 + H_2O \xrightarrow[\text{oxidase}]{\text{sulphur}} SO_3^{2-} + 2H^+$$

$$(3)\ SO_3^{2-} + 2Fe^{3+}\ (\text{cytochrome c}) + H_2O \xrightarrow[\text{oxidase}]{\text{sulphite}} SO_4^{2-} + 2Fe^{2+}\ (\text{cytochrome c}) + 2H^+$$

It seems particularly interesting that *Thiobaccillus ferrooxidans* can synthesize two different enzymes responsible for transformation of the same substrate depending on actual conditions.

Although studies on the *Thiobacillus* genus have been conducted for many years, the path of metabolic transformations of sulfur in these organisms is still the subject of controversy. Many authors assume that bacteria play only an intermediate role in leaching of sulfur minerals [6, 7]. According to this theory, enzymatic oxidation of the reduced sulfur compounds takes place to a rather insignificant degree. It is Fe(III) ion forming as a result of intracellular oxidation of Fe (II) ion , which acts as an efficient oxidant. However, according to some recent reports [8, 9], the presence of active enzymes involved in sulfur metabolism significantly increases the efficiency of sulfide mineral oxidation. This effect is particularly enhanced were the defects of pyrite crystalline lattice appear due to a deficit of cations.The elemental sulfur occurring at such spots is dissolved in lipides and phospholipides contained in a bacterial cell. According to the postulated mechanism of sulfur transport into the cell, it is passed from one lipide molecule to another because of the existing sulfur concentration gradient between them [10, 11]. At the next stage sulfur is oxidized to sulfate by the enzymatic system of the cell . Then, the forming biogenic sulfuric acid initiates chemical and electrochemical processes on the surface of pyrite. The Fe(II) ions released from the mineral are oxidized by the *Thiobacillus ferrooxidans* cells to Fe(III) ions, which play the role of sulfite ion oxidants. A consequence of the release of iron ions into the solution is the appearance of new defected sites characterized by increased concentration of sulfur, which are the subsequent sites of microorganism attacks.

As follows from the latest reports, the effectiveness of biological oxidation of pyrite depends on a strain of *Thiobacillus ferrooxidans*, on the size of mineral particles, temperature, pH and composition of the medium , aeration, presence of surfactants and access of light. This paper reports results of the study on the effect of some essential parameters on the kinetics of pyrite oxidation by the isolated strain of *Thiobaciullus ferrooxidans*.

Materials and Methods

The strain of *Thiobacillus ferrooxidans* used in the studies was isolated from the Siersza colliery and passed subsequently onto Silverman 9K medium. The experiments on the sample containing pyrite were carried out in 50 ml volume reactors containing 20 ml of iron-free medium (Silverman medium containing no iron (II) sulfate) and the inoculum ensuring cell concentration of 10^{18} cells in a litre (inoculum taken from the growth medium after 22 hours of incubation). The number of the bacteria cells was determined by the McCrady method [12] in the three test-tube system. The initial pH of the media was changed in the range from 1.0 to 3.5. The reactors were placed into thermostated shakers (Shaker type 357, Elpan) at temperatures 20, 25, 30, 35 and 40°C and a shaking rate 150 c.p.m. (amplitude 9).

Pyrite was refined to a particle size not exceeding 0.06 mm and tested for the contents of iron by the fenantroline method [13] (Beckman DU 640). The results were checked by atomic absorption (SAA Unicam SP 90A). The sulfur cotent in pyrite, after its dissolution and oxidation of sulfur to sulfates, was determined by the weight method [14]. The pyrite contained 48wt% of iron and 49 wt% of sulfur. The pyrite used in the kinetic studies was sterilized for three hours at 100°C and added to the medium at 0.25 to 2.5 wt% per volume.

The kinetic model was chosen from the possible descriptions of the process by different kinetic equations (Enzfiter 1.05, Elsevier Biosoft). The model which ensured the smallest values of statistical errors was selected. In choosing the model we used results of measurements concerning changes in the concentration of iron (II) and iron (III) ions (the fenantroline method), and sulfates (weight method) in the leaching medium. A reference standard was the sample of pyrite incubated in the same conditions as the tested samples but with no inoculums added. In this way we measured only the effects related to biological oxidation of pyrite, and the contribution of chemical processes was eliminated.

Results and Disscussion

The Kinetic Model

From among the approximations considered the best description of the process was achieved by the autocatalytic equation of the 1st order with respect to the concentration of the inorganic substrate, pyrite iron, and the 1st order with respect to the concentration of iron (III) ion forming in the process. This equation is:

$$s = \frac{S_o(S_o + X_o)e^{-k(Xo+So)(t-to)}}{X_o + S_o e^{-k(Xo+So)(t-to)}}$$

where:

s - concentration of pyrite iron [g/dm^3]
S_0 - initial concentration of pyrite iron [g/dm^3]
X_0 - initial concentration of Fe (III) ions [g/dm^3]
t_0 - induction time of the process [h]
k - rate constant [dm^{3}g^{-1} h^{-1}]

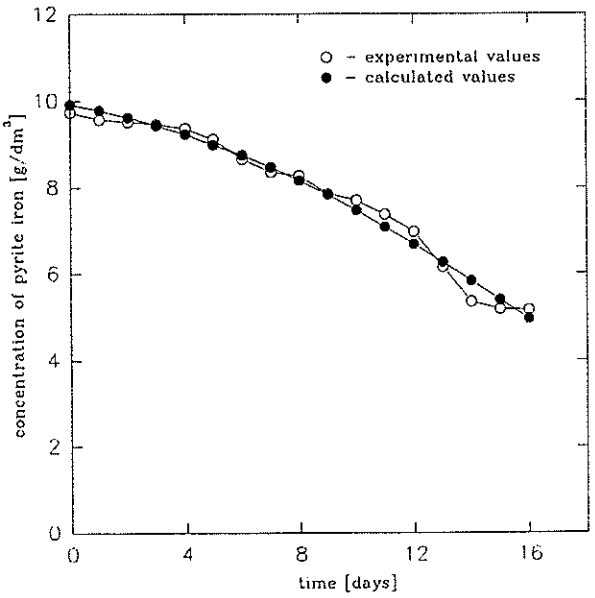


Fig. 1. Correlation of experimental values with calculated ones based on autocatalitic (35°C, pH 2.2, 0.25 wt%/vol.)

The agreement of the model predictions with experimental data is illustrated in Fig. 1.

For all studied parameters we calculated the rate constants of the process and induction times. The statistical errors charging the rate constants are from two to eight percent. Significantly larger is the error in calculation of the induction times,which can reach from 15 to 69%. This fact should be attributed to a small number of points of measurement obtainable within the time comparable with to induction time. Measurements were performed once every 24 hours because of small changes of the substrate concentration in this time interval; in shorter time intervals the differences in concentrations were close to errors of the measurement methods.

The Effect of Temperature

The effect of temperature on pyrite oxidation is illustrated by the diagram in Fig.2. Maximum efficiency of the process , 61%, was obtained for temperatures from 30 to 35°C. When the temperature fell below 25°C pyrite oxidation efficiency decreased to 33%. When the temperature increased to 40°C, the efficiency of the process decreased to 16.5%. At 45°C no growth of *Thiobacillus ferrooxidans* was observed. The rate constants and induction times measured for different temperatures are collected in Table 1. As follows from these data the process of pyrite oxidation is much more sensitive to higher temperatures than the optimum one. An increase of temperature by 5°C gives almost three times reduction of the rate constant, whereas its reduction by 15°C leads to a rate constant reduction of only 46%.

The Effect of pH

The effect of the pH value of the medium on the process is illustrated in Fig.3. Efficiency was highest if the initial pH of the medium was from 2.0 to 2.2 (61%).

A small decrease of pH, to a value below 1.8, caused a significant decrease in the efficency of the process (28%). When pH was below 1, no growth of bacteria was observed. An increase of pH by only 0.3 relative to the optimum value, that is to 2.5, caused a decrease in the efficiency of the process to 46.5%. At pH above 3 the decrease of efficiency was considerable (19.5%). Table 2 presents parameters characteristic of the process conducted for different pH values.

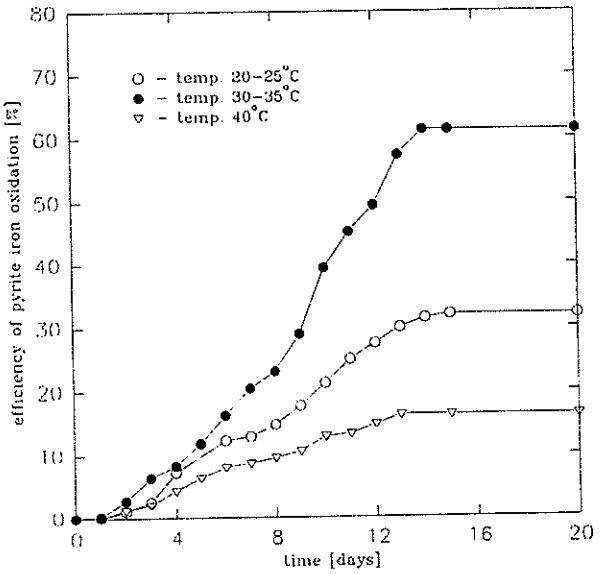


Fig. 2. The effect of temperature on the course of piryte oxidation, pH 2.2, 1 wt%/vol. sample size.

Table 1. The effect of temperature on the parameters of pyrite oxidation, pH 2.2, size 1 wt%/vol.

Temperature [°C]	20-25	30-35	40	45
Induction time [h]	23 ± 6	41 ± 6	13 ± 9	bacteria growth undetected
Rate constant [$dm^3 g^{-1} h^{-1}$] 10^{-3}	0.81 ± 0.02	1.42 ± 0.04	0.52 ± 0.03	
Max efficiency [%]	33	61	16.5	
Length of process [days]	15	14	13	

The rate constant of the process at the pH of the leaching solution between 2.0 and 2.2 is almost 50% greater than the corresponding value obtained at pH below 1.8 and twice higher than for pH above 3.0. These data prove that the process is highly sensitive to changes of pH of the leaching solution.

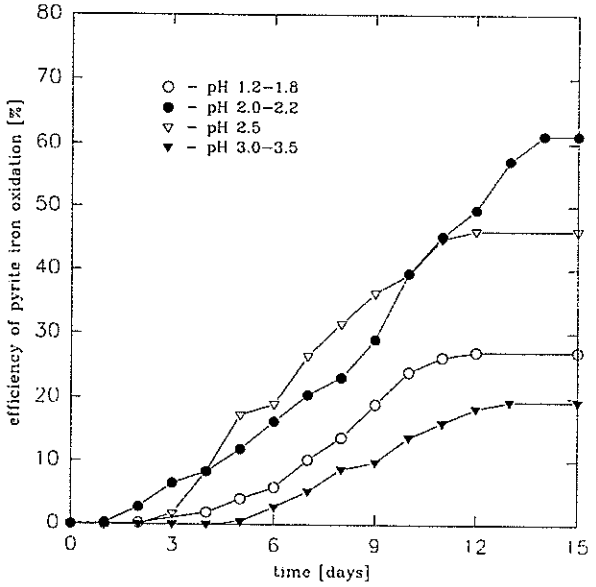


Fig. 3. The effect of pH on the course of pyrite oxidation, temp. 35°C, 1wt%/vol. sample size

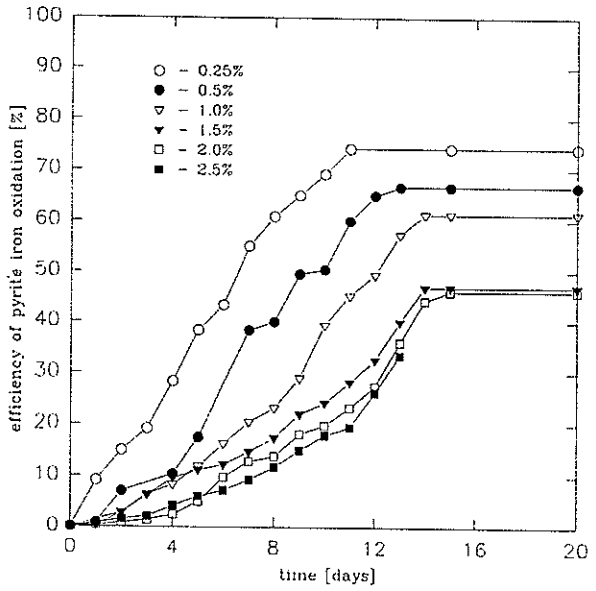


Fig. 4. The effect of the sample size (wt%/vol.) on the course of pyrite oxidation, temp. 35°C, pH 2.2.

Table 2. The effect of pH on the parameters of pyrite oxidation, 35°C, size 1 wt%/vol.

pH	1.0	1.2-1.8	2.0-2.2	2.5	3.0-3.5
Induction time [h]	bacteria growth undetected	62 ± 10	41± 6	26 ± 10	81 ± 14
Rate constant [$dm^3 g^{-1} h^{-1}$] 10^{-3}		1.00 ± 0.02	1.42 ± 0.04	1.33 ± 0.08	0.72 ± 0.06
Max efficiency [%]		28	61	46.5	19.5
Length of process [days]		11	14	12	13

Table 3. The effect of sample size on the parameters of piryte oxidation, pH 2.0, 35°C.

Sample size [wt%/vol]	0.25	0.5	1	2.0	2.5
Induction time [h]	7 ± 4	30 ± 6	41± 6	50 ± 10	50 ± 10
Rate constant [$dm^3 g^{-1} h^{-1}$] 10^{-3}	3.8±0.1	2.3±0.1	1.42±0.04	0.73±0.02	0.61±0.02
Max efficiency [%]	74	68	61	46	43
Length of process [days]	11	13	14	15	15

The Effect of the Pyrite Sample Size

The course of pyrite oxidation for its samples of different size relative to the volume of the leaching medium is illustrated by the diagram in Fig.4.

The results clearly show that the efficiency of oxidation is higher the smaller the size of the mineral sample. For a sample making 0.25 wt%/vol of the medium the efficiency is 74% and it decreases to 43% for a sample making 2.5% of the medium. The increased amount of pyrite also implies increase of the time of reaching the maximum efficiency from 11 to 15 days. The parameters of this process calculated for this series of experiments are collected in Table 3. According to these data, when the size of the sample is increased from 0.25 to 2.5% of the the medium volume, the rate constant decreases approximately 6 times and the induction time increases from 7 to 50 hours.

As follows from the results of our studies, from the point of view of the kinetics of the process, optimum parameters of pyrite oxidation with the isolated strain of *Thiobacillus ferrooxidans* are: temperature from 30 to 35°C, pH from 2.0 to 2.2, and the size of the sample making 0.25wt% of the leaching solution volume. However, depending on economic requirements, a different variant of the parameters can be applied. Therefore, it is important to know the course of the process in the whole range of parameters.

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