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Kinetic Model of CuS Oxidation by *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* Bacteria

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Abstract

Kinetics of the process of microbiological oxidation of synthetic CuS performed with the involvement of pure and mixed cultures of *T. ferrooxidans* and *T. lluooxidans* bacteria were studied. The CuS oxidation with *T. ferrooxidans* bacteria was found to be best described by the model of inhibition of the first order with respect to the substrate and the product-inhibitor, while the process with *T. thiooxidans* and mixed cultures of these bacteria is best described by the first order reaction with respect to the substrate.

It was also shown that the adaptation of the bacteria to the CuS tested significantly shortens the induction period and increases reaction rates. The optimum conditions of CuS oxidation were established. The yield of the reaction was established as ca. 30% and an increase in the reaction rate was observed when using mixed cultures of *T. feirooxidans* i *T. thiooxidans* bacteria adapted to CuS at 35°C, in the medium containing 1 % (wt./vol.) CuS at the initial pH of the medium of 1.82.

Keywords: *Thiobacillus ferrooxidans, Thiobacillus thiooxidans,* mineral leaching, kinetics

Introduction

Microbiological processes of CuS oxidation are most often used in hydrometallurgy for recovery of metals from ores [1], and in technologies for utilization of industrial waste [2, 3] and recovery of metals from hardly soluble sulphides [4, 5]. The effectiveness of these processes depends on the forms of sulphides and on the parameters of particular technologies of metal recovery [6, 7].

Recently, the possibility of using *Thiobacillus* bacteria for utilization of postflotation wastes and copper concentrate processing wastes has aroused much interest [8, 9]. The effort is directed to recovery of metals from low grade raw products in environmentally friendly conditions.

The study reported in this paper was aimed at determining of the optimum kinetic parameters of synthetic CuS oxidation carried out with the involvement of *Thiobacillus* bacteria. The study on synthetic CuS is expected to throw some light on the interactions between the microorganisms and minerals leached in natural conditions.

Materials and Methods

The Mineral

Synthetic CuS purchased from Aldrich (over 99% pure) contained 66% Cu and 33% sulphur. The fraction of refinement of the order of 100 mesh was used in the study.

Bacteria

The bacteria species *Thiobacillus ferrooxidans* was isolated from mine waters from the Siersza colliery. Standard culture was grown on Silvermana 9K medium (37°C, pH=2.2). The species *Thiobacillus thiooxidans* was obtained from the Faculty of Chemical and Process Engineering at the Technical University in Warsaw and grown on a standard Starkey medium (30°C, pH = 4.0).

The bacteria were adapted to the support on the medium containing 1% (wt./vol.) CuS. The adaptation was considered successful when the rate of Cu leaching in subsequent passages ceased to change.

Kinetic Studies

Kinetic studies were carried out in thermostated shakers made by Elpan (100 rpm) for the samples placed in 350 cm^3 Erlemayer flasks. The samples contained 100 ml of the medium and the CuS tested and then they were inoculated with 10% inoculum of pure cultures of *T. ferrooxidans* or *T. thiooxidans.* The processes with a mixture of the bacteria species were run simultaneously. The oxidation of CuS with the use of *T. ferrooxidans* and mixed cultures of the bacteria were carried out in the ironless Silverman medium, while the process with *T. thiooxidans* was run in the Starkey medium.

The influence of the initial pH of the medium, temperature, and the percent content of CuS on the kinetics of oxidation was analyzed. The rate of the process was determined by periodical measurements of the concentration of copper ions in the solution, pH and redox potential of the system. Moreover, the influence of the adaptation of the bacteria to the mineral on the kinetic of CuS oxidation was checked.

The kinetic parameters of the process run with the *Thiobacillus ferrooxidans* bacteria were determined assuming the inhibition reaction model of the first order with respect to the substrate and the product - inhibitor, from the following equation:

$$-\frac{dx}{dt} = k_1 x - k_2(x_o - x)$$

$$x = \frac{x_o}{k_1 + k_2[k_2 + k_1 e^{(k_1 + k_2)(t - t_o)}]}$$

where:

x_o and x - are the initial and the current concentration of the substrate,

k_1 - is the rate constant of oxidation,

k_2 - the rate constant of the inhibition reaction,

t_0 - induction period,

t - time.

The process run with a pure culture of *Thiobacillus thiooxidans* and a mixture of these species with *Thiobacillus ferrooxidans* was best described by the model of the first order reaction:

$$x = x_0 e^{(-k(t - t_0))}$$

where:

k - is the rate constant of oxidation,

t_0 - induction period,

t - time.

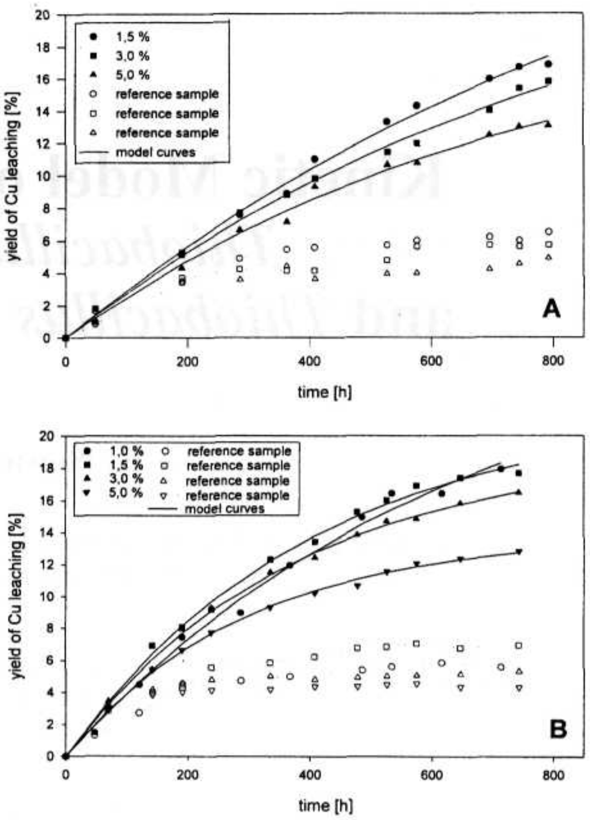

Fig. 1. The effect of CuS content on the kinetics of oxidation run with the non-adapted (A) and adapted (B) *T. ferrooxidans* bacteria; - model curves, •experimental points, oreference samples; (30°C, Silverman medium 9K, pH = 2.3).

Analytical Methods

The concentration of copper (II) ions was measured spectrophotometrically by the cuprison method [10]; the pH value was measured by a complex calomel electrode OSH 1000, and the redox potential by a complex platinum electrode EPtAgP-329W.

Results and Discussion

As follows from the results of the study, the kinetics of CuS oxidation run with the involvement of the *Thiobacillus ferrooxidans* bacteria is best described by the model of the inhibition reaction. When the *Thiobacillus thiooxidans* bacteria or a mixture of these two species are used the process is best described by the first order reaction with respect to the substrate. For these models the best correlations between the experimental and theoretical data were achieved.

Analyzing the influence of the percent concentration of CuS in the medium on the kinetic of its oxidation with the use of non-adapted *Thiobacillus ferrooxidans* bacteria, it was established that with increasing concentration of CuS from 1.0 to 5.0%, the rate constant of oxidation decreased by one order of magnitude from 0.31 to

0.03 [h^{-1}] x 10^{-3}, whereas the rate constant of the inhibition reaction increased from 0.62 to 1.30 [h^{-1}] x 10^{-3}. (Fig. 1, Table 1.)

Table 1. The effect of CuS content on the kinetics of oxidation run with the non-adapted (A) and adapted (B) *T. feirooxidans* bacteria (pH = 2.3, 30°C).

CuS content [%]	k_1 [h^{-1}] × 10^{-3}	k_2 [h^{-1}] × 10^{-3}	Yield of CuS leaching [%]	
			Sample	Reference
A				
1.5	0.31 ± 0.01	0.62 ± 0.15	16.8	6.5
3.0	0.074 ± 0.003	1.07 ± 0.16	15.8	5.7
5.0	0.025 ± 0.001	1.30 ± 0.20	13.1	4.9
B				
1.0(pH=2.02)	0.43 ± 0.03	1.15 ± 0.24	17.9	5.6
1.5	0.54 ± 0.02	1.95 ± 0.15	17.7	6.9
3.0	0.52 ± 0.01	2.19 ± 0.12	16.5	5.3
5.0	0.47 ± 0.01	2.92 ± 0.11	12.8	4.3

The use of the bacteria which had been adapted to CuS caused a considerable increase of the rate constant of oxidation, to the mean of 0.51 [h^{-1}] x 10^{-3}. These results and in particular the fact that the CuS oxidation rate does not change with increasing CuS concentration, confirm that the model proposed well describes the process. The inhibition reaction rate constant is higher than in the corresponding systems with non-adapted bacteria and increases by about 30%, from about 1.95 to 2.92 [h^{-1}] x 10^{-3} with the percent concentration of CuS from 1.0 to 5.0%.

It is interesting to note that the oxidation of CuS by both adapted and non-adapted bacteria of the two species separately runs with the zero induction period.

The CuS oxidation with the use of a mixture of the two non-adapted bacteria species runs at the rate constant equal 0.35 [h^{-1}] x 10^{-3}. With increasing concentration of CuS in the medium the induction period t_0 increases from 164 to 184 [h]. It has been shown that the yield of CuS oxidation does not depend on its concentration and is equal to about 19.6%. Moreover, a decrease in the yield of the chemical oxidation of CuS with its increasing concentration was observed (see the data for the reference sample).

The initial adaptation of the bacteria to the CuS results in a small increase in the rate constant of the process to 0.37 [h^{-1}] x 10^{-3}, and a significant shortening of the induction period to about 24 [h]. The yield of the CuS oxidation increased by about 5%. The results are given in Fig. 2 and Table 2.

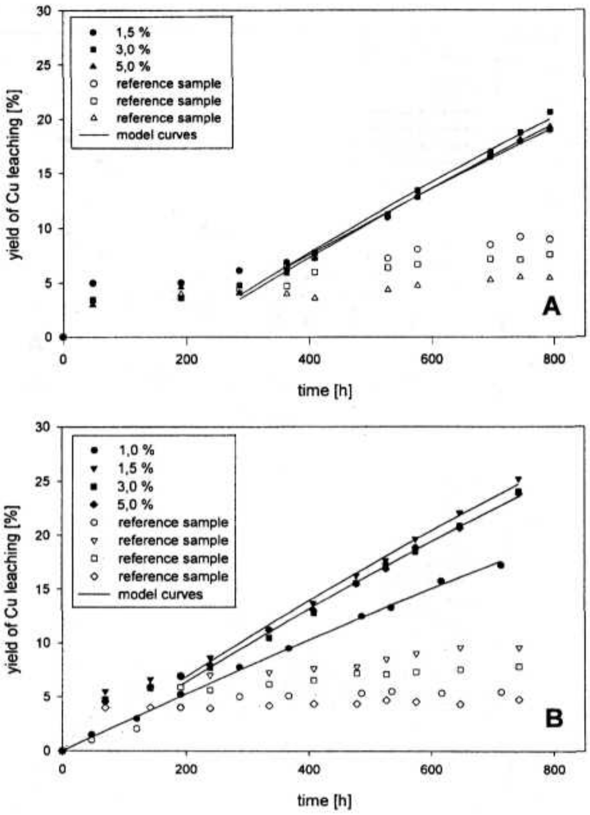

Fig. 2. The effect of CuS content on the kinetics of oxidation run with the non-adapted (A) and adapted (B) mixed cultures 71 *feirooxidans* and *T. thiooxidans* bacteria; — model curves, • experimental points, o reference samples; (30°C, Silverman medium 9K, pH = 1.5).

Table 2. The effect of CuS content on the kinetics of oxidation run with the non-adapted (A) and adapted (B) mixed cultures *T.ferrooxidans* and *T.tiooxidans* (pH = 1.5, 30°C).

CuS content [%]	k_1 [h^{-1}] × 10^{-3}	k_2 [h^{-1}] × 10^{-3}	Yield of CuS leaching [%]	
			Sample	Reference
A				
1.5	0.34 ± 0.01	164 ± 12	19.0	9.0
3.0	0.36 ± 0.01	176 ± 14	20.6	7.6
5.0	0.35 ± 0.01	184 ± 10	19.3	5.5
B				
1.00(pH=1.91)	0.27 ± 0.01	0	17.2	5.4
1.5	0.37 ± 0.01	23 ± 13	25.2	9.6
3.0	0.37 ± 0.01	26 ± 15	24.0	7.8
5.0	0.37 ± 0.01	22 ± 9	23.8	4.8

The influence of the percent concentration of CuS on the kinetics of its oxidation with the use of non-adapted *Thiobacillus thiooxidans* is best seen by the change in the induction period which increases with increasing concen-

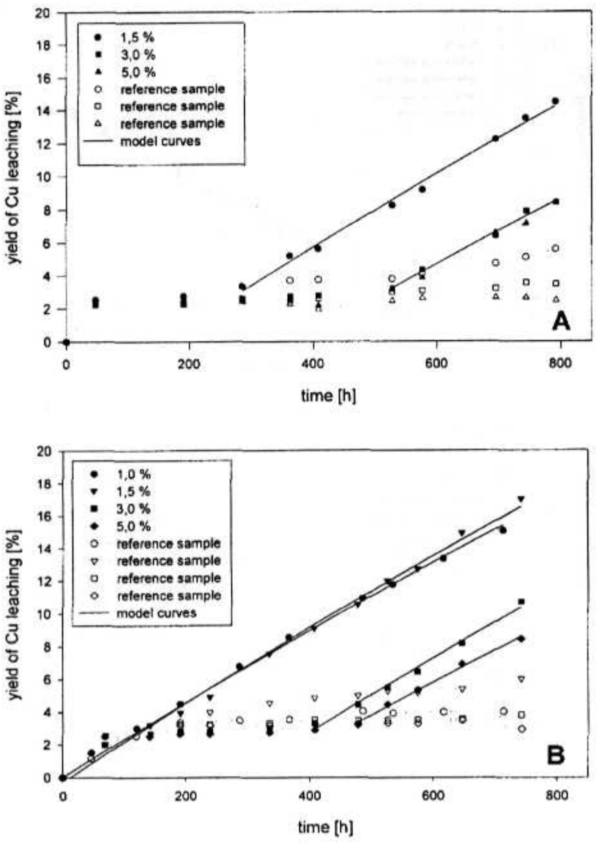

Fig. 3. The effect of CuS content on the kinetics of oxidation run with the non-adapted (A) and adapted (B) *T. tiooxidans* bacteria; — model curves, • experimental points, o reference samples; (30°C, Silverman medium 9K, pH = 1.5).

tration of CuS. The rate constant of oxidation in this case decreases. Adaptation of the bacteria to CuS, in the variants with its concentrations of 1.5 and 3.0%, leads to an increase in the rate constant of the process, shortening of the induction period and an increase in yield of the process by about 2%. No change in the rate constant was observed for the case with 5.0% concentration of CuS, only the induction period was shortened by 67 [h], at the yield of the process of about 8.4%. The results are shown in Fig. 3 and Table 3.

Table 3. The effect of CuS content on the kinetics of oxidation run with the non-adapted (A) and adapted (B) *T. thiooxidans* (pH = 1.5, 30°C).

CuS content [%]	k_1 $[h^{-1}] \times 10^{-3}$	k_2 $[h^{-1}] \times 10^{-3}$	Yield of CuS leaching [%]	
			Sample	Reference
A				
1.5	0.24 ± 0.01	156 ± 13	14.5	5.6
3.0	0.21 ± 0.01	373 ± 16	8.4	3.5
5.0	0.21 ± 0.01	381 ± 15	8.4	2.5
B				
1.0(pH=1.97)	0.23 ± 0.01	0	15.1	4.0
1.5	0.25 ± 0.01	12 ± 9	17.0	6.0
3.0	0.24 ± 0.01	281 ± 15	10.7	3.8
5.0	0.21 ± 0.01	314 ± 12	8.4	3.0

The influence of the initial pH of the medium on the process run with the use of the bacteria species studied adapted at 30°C, in the medium containing 1.0% (wt./vol.) CuS is illustrated by the data given in Figs. 4, 5 and 6 and Tables 4, 5 and 6. The oxidation of CuS by *Thiobacillus ferrooxidans,* in the pH of the medium ranging from 1.00 to 2.02, ran at the rate constant of 0.43 $[h^{-1}] \times 10^{-3}$ and the inhibition constant from the range 1.15-1.34 $[h^{-1}] \times 10^{-3}$. The process ran at the rate and inhibition constants of 0.54 and 1.95 $[h^{-1}] \times 10^{-3}$ in the medium containing 1.5% CuS and at pH = 2.27, and the yield of the process was almost the same and equal ~ 18% at the zero induction period. With increasing initial pH of the medium the contribution of the chemical oxidation decreased, (see the results for the reference samples). These results mean that the *Thiobacillus ferrooxidans* bacteria are little sensitive to the changes in pH in the range 1-2.

The oxidation of CuS by a mixture of adapted bacteria in the medium of pH in the range 1.00-1.91 occurred according to a similar mechanism, as indicated by almost the same rate constant, zero induction period and the yield of 17%, (Figure 9 and Table 9). At pH of 1.82 the highest rate constant of $(0.37 [h^{-1}] \times 10^{-3})$ and the highest yield of 25.2% were obtained.

The species *Thiobacillus thiooxidans* proved more sensitive to pH changes. An increase in the pH value from 1.86 to 1.97 resulted in a decrease in the rate constant from 0.25 to 0.23 $[h^{-1}] \times 10^{-3}$ and the shortening of the induction period to zero.

Table 4. The influence of the initial pH of the medium on the kinetics of CuS oxidation by the adapted *T. ferrooxidans* bacteria (30"C, CuS content - 1% (wt./vol.)).

pH	k_1 $[h^{-1}] \times 10^{-3}$	k_2 $[h^{-1}] \times 10^{-3}$	Yield of CuS leaching [%]	
			Sample	Reference
1.00	0.44 ± 0.02	1.34 ± 0.21	17.8	3.5
1.5	0.43 ± 0.02	1.20 ± 0.14	18.4	7.1
2.02	0.43 ± 0.03	1.15 ± 0.24	17.9	5.6
2.27(1.5%)	0.54 ± 0.02	1.95 ± 0.15	17.7	6.9

Table 5. The influence of the initial pH of the medium on the kinetics of CuS oxidation by the adapted mixed cultures *T. ferrooxidans* and *T. tiooxidans* bacteria (30°C, CuS content - 1% (wt./vol.)).

pH	k_1 $[h^{-1}]$	t_o [h]	Yield of CuS leaching [%]	
			Sample	Reference
1.00	0.28 ± 0.01	0	17.9	6.2
1.73	0.24 ± 0.01	0	15.8	5.5
1.82(1.5%)	0.37 ± 0.01	23 ± 13	25.2	9.6
1.91	0.27 ± 0.01	0	17.2	5.4

Table 6. The influence of the initial pH of the medium on the kinetics of CuS oxidation by the adapted *T. thiooxidans* bacteria (30°C, CuS content - 1% (wt./vol.)).

pH	k_1 [h^{-1}]	t_o [h]	Yield of CuS leaching [%]	
			Sample	Reference
1.00	Chemical leaching		4.1	3.6
1.86$^{(1.5\%)}$	0.25 ± 0.01	12 ± 9	17.0	6.0
1.97	0.23 ± 0.01	0	15.1	4.0
2.99	Chemical leaching		4.3	3.8

Table 7. The influence of the temperature on the kinetics of CuS oxidation by the adapted *T. ferrooxidans* bacteria (pH = 2.27, CuS content - 1% (wt./vol.)).

Temperature	k_1 [h^{-1}] × 10^{-3}	k_2 [h^{-1}] × 10^{-3}	Yield of CuS leaching [%]	
			Sample	Reference
20	Chemical leaching		4.2	3.5
25	0.30 ± 0.01	3.48 ± 0.37	7.5	4.5
30$^{(1.5\%)}$	0.51 ± 0.01	2.35 ± 0.25	17.7	6.9
35	0.52 ± 0.01	2.29 ± 0.23	16.2	3.3

In all systems studied, with pure or mixed cultures, an increase in the reaction rate constant and the yield of the reaction was observed with increasing temperature. For instance, when the process was run with *Thiobacillus ferrooxidans* at 20°C, only chemical leaching occurred (Fig. 7. and Table 7.). The inverse dependence was noted between the temperature of the process and the inhibition constant. An increase in the process temperature by 10°C caused the rate constant to increase by 0.22 [h^{-1}] x 10^{-3}. The temperature coefficient Q_{10} determined as 1.73 is a typical value of these bacteria for covellite leaching [1, 11].

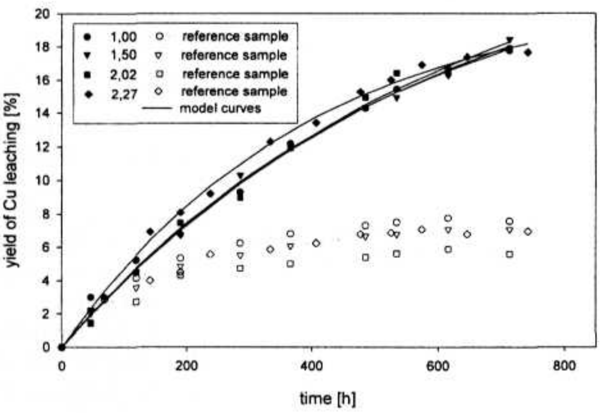

Fig. 4. The influence of the initial pH of the medium on the kinetics of CuS oxidation by the adapted *T. ferrooxidans* bacteria; — model curves, • experimental points, *o* reference samples; (30°C, Silverman medium 9K, CuS content - 1.0% (wt./vol.)).

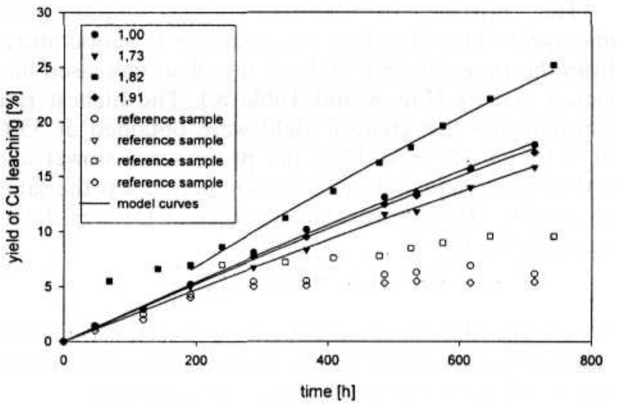

Fig. 5. The influence of the initial pH of the medium on the kinetics of CuS oxidation by the adapted mixed cultures *T. ferrooxidans* and *T. tiooxidans* bacteria; — model curves, • experimental points, o reference samples; (30°C, Silverman medium 9K, CuS content - 1.0% (wt./vol.)).

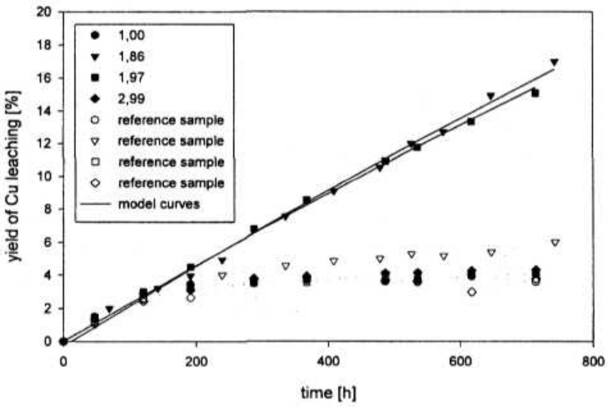

Fig. 6. The influence of the initial pH of the medium on the kinetics of CuS oxidation by the adapted *T. thiooxidans* bacteria; — model curves, • experimental points, o reference samples; (30°C, Silverman medium 9K, CuS content - 1.0% (wt./vol.)).

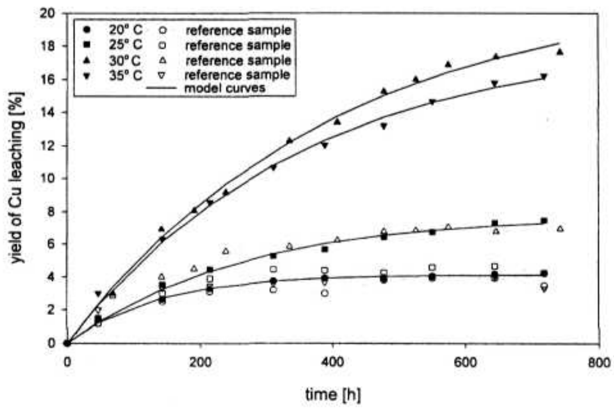

Fig. 7. The influence of the temperature on the kinetics of CuS oxidation by the adapted *T. ferrooxidans* bacteria; — model curves, • experimental points, o reference samples; (30°C, Silverman medium 9K, CuS content - 1.0% (wt./vol.)).

The mixture of *Thiobacillus ferrooxidans* and *thiooxidans* introduced into the medium at temperatures from the range 20-25°C did not reveal an increased biological activity (Fig. 8 and Table 8.). The highest rate constant and the greatest yield were obtained at 35°C (0.52 [h^{-1}] x 10^{-3}). At 25°C, the process was slower and with a lower yield, but the induction period was the same as at 35°C. The temperature coefficient Q5 was determined to be 1.41.

Table 8. The influence of the temperature on the kinetics of CuS oxidation by the adapted mixed cultures *T. ferrooxidans* and *T. thiooxidans* (pH = 1.82, CuS content - 1% (wt./vol.)).

Temperature	k_1 [h^{-1}]	t_o [h]	Yield of CuS leaching [%]	
			Sample	Reference
20	Chemical leaching		5.2	5.4
25	Chemical leaching		6.3	5.4
$30^{(1.5\%)}$	0.37 ± 0.01	23 ± 13	25.2	9.6
35	0.52 ± 0.01	24 ± 13	29.8	6.4

Contrary to *Thiobacillus ferrooxidans* and mixed species, the *T. thiooxidans* bacteria were biologically active already at 20°C. Although at this temperature the process ran at the lowest rate constant, its yield was over twice as much as that for the corresponding reference sample. For the system with the latter bacteria the rate constant of the process increased and the induction period shortened with increasing temperature. The highest rate constant and the greatest yield were obtained at 35°C (Fig. 9 and Table 9.). The temperature coefficient Q_{10} was 2.99, so an increase in the process temperature by o 10°C resulted in a threefold increase in the reaction rate.

Table 9. The influence of the temperature on the kinetics of CuS oxidation by the adapted *T. thiooxidans* bacteria (pH = 1.86, CuS content - 1% (wt./vol.)).

Temperature	k_1 [h^{-1}]	t_o [h]	Yield of CuS leaching [%]	
			Sample	Reference
20	0.079 ± 0.001	25 ± 8	5.4	2.5
25	0.110 ± 0.001	15 ± 5	7.5	3.1
$30^{(1.5\%)}$	0.25 ± 0.01	12 ± 9	17.0	6.0
35	0.31 ± 0.01	8 ± 6	19.6	3.3

The oxidation of CuS, susceptible to the oxidation attack of Fe^{3+} and oxidation by oxygen from the air in an acidic environment, is accompanied by a significant contribution of the chemical process (reference samples). The chemical reactions lead to the formation of sulphur, which is then oxidized to sulphuric acid (VI) by the *Thiobacillus thiooxidans* bacteria [1]. The *Thiobacillus*

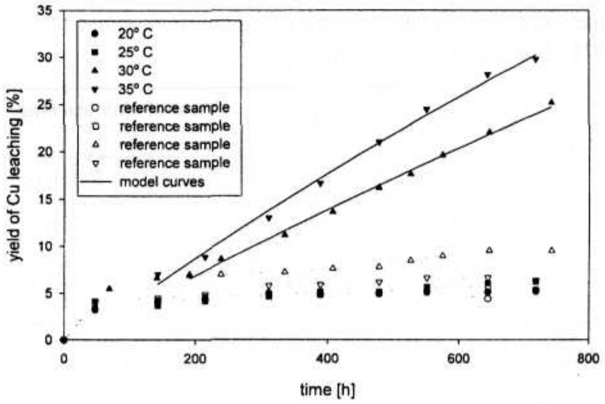

Fig. 8. The influence of the temperature on the kinetics of CuS oxidation by the adapted mixed cultures *T. ferrooxidans* and *T. thioxidans* bacteria; — model curves, • experimental points, o reference samples; (30°C, Silverman medium 9K, CuS content - 1.0% (wt./vol.)).

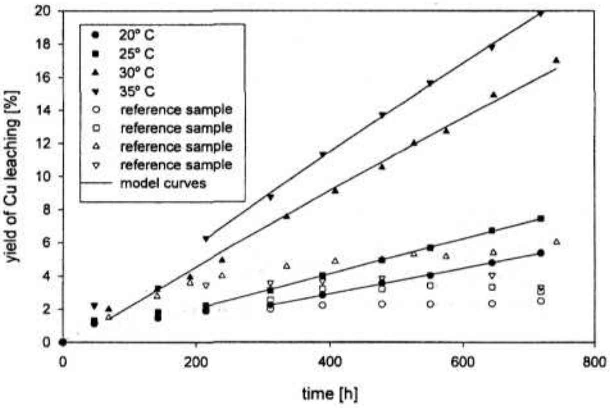

Fig. 9. The influence of the temperature on the kinetics of CuS oxidation by the adapted *T. thiooxidans* bacteria; — model curves, • experimental points, o reference samples; (pH medium 1.86, Starkey medium, CuS content - 1.0% (wt./vol.)).

ferrooxidans bacteria oxidized the iron (II) ions formed to iron (III) ions. The occurrence of these two processes taking place with the involvement of microorganisms explains the greater rate of CuS oxidation when the mixed species of the bacteria are used. In the process with *T. thiooxidans* CuS is slowly oxidized by oxygen from the air, and the sulphur formed is microbiologically oxidized to sulphuric acid, which chemically dissolves CuS. This fact explains the lower yields of CuS oxidation in the processes with the involvement of only *T. ferrooxidans.*

Depending on the species of the autotrophic bacteria used, the mechanism of CuS biooxidation may be direct - requiring direct contact with the mineral surface, or additionally an indirect mechanism may be involved, in which the products of the microorganisms metabolism are involved causing physical and chemical changes in the medium [1, 6], resulting in an increase in H_2SO_4 concentration.

Conclusions

The proposed models of the kinetics of the CuS oxidation occurring in different conditions allow a determination of the optimum parameters of leaching and can be used in industrial conditions. The results indicate that the process runs at the highest rate and with the greatest yield (= 30%), when the mixed and adapted to CuS cultures of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* are used at 35°C, in a medium containing *1%* (wt./vol.) CuS and at the initial pH of 1.82.

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