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Microbiological Oxidation of the Waste Ferrous Sulphate

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Abstract

Ferrous sulphate, a waste product in titanium white production at the Chemical Plant "Police" S.A., was subjected to microbiological oxidation with *Thiobacillus ferrooxidans* bacteria. The kinetic parameters of the culture growth were calculated on the basis of the Monod equation. The process was found to be best described by the model of the autocatalytic 1st order reaction with respect to the product and 1st order reaction with respect to the substrate. The effect of temperature and pH on the course of the process was studied. The kinetics of oxidation of ferrous ions coming from the waste ferrous sulphate was studied in the process with laboratory bacteria strain as well as the strain adapted to the waste product and compared with that of pure substrate oxidation. Analysis of the precipitates formed during microbiological oxidation of the waste ferrous sulphate proved that $(NH_4)Fe3(SO_4)2(OH)_6$ with the admixture of $NaFe_3(SO_4)_2(OH)_6$ and $KFe_3(SO_4)_2(OH)_6$ were formed.

Keywords: *Thiobacillus ferrooxidans*, ferrous sulphate, utilization, biooxidation kinetics of iron (II) ions

Introduction

Economic and scientific attraction in *Thiobacillus ferrooxidans* has been steadily growing since its original isolation in 1947. Economic interest is caused mainly by the fact that *Thiobacillus ferrooxidans* can be used in hydrometallurgy and various desulfurization processes, being quite an undemanding organism, requiring only a few mineral nutrients which usually can be obtained from the surrounding rock.

Generally, *Thiobacillus ferrooxidans* has been regarded as a mesophilic organism with the optimum temperature between 30° and 40°C [1], depending strongly on the strain and pH [2]. The highest and the lowest temperatures in which these bacteria were observed to grow are: 42°, 43°C [3] and 2°C, respectively. Temperature is a very important parameter for bacteria growth and the ferrous ions oxidation process, as it affects inhibition caused by heavy metals constants. It has been established that the higher the temperature the greater the tolerance towards heavy metals [5]. However, the mechanism of this interaction remains unsolved. Moreover, temperature affects the amount of the precipitates formed - their formation is much reduced below 13°C [4] and below 4°C no precipitates formation was observed [2].

Sediments produced by *Thiobacillus ferrooxidans* are extremely insoluble jarosites of the composition of $MFe(SO_4)2(OH)_6$, where M is one of the following cations: H_3O^+, Na^+, K^+ and NH_4^+. Additionally, in certain conditions, amorphous ferric hydroxysulfates and oxohydroxides are formed. It has been shown that the amorphous precipitates are formed especially when there is no excess of sulphates in the medium or one of the appropriate monovalent cations is not present [6].

Thiobacillus ferrooxidans is extremely acidophilic and grows between pH 1.0 and 6.5 [7]. However, the optimum pH values vary from 1.0 to 3.6 [8, 9] with different strains and depending upon the available substrate. The widest range of pH supporting bacteria growth was noted when they were grown on sulphur. Moreover, pH was found to affect the form of the sediments - the lower its value the less amount of jarosites formed.

As far as the kinetics of the process is concerned, there is the lack of consensus in literature. Different approaches applied by different researchers were a consequence of a variety of the bacteria strains characterized by different cell surface properties. Some authors [10, 11] postulate the mechanism of competitive inhibition of ferric ions, suggesting that cells cover the substrate surface with a coat of Fe (III) complexes which reduces the ferrous oxidizing activity. Lizama et al. [12] additionally postulated the inhibition effect of increasing concentration of cells. The idea

was that the growing number of cells competes with Fe (II) ions for the binding sites of the cells. The recognition of the mechanism of Fe (II) oxidation could be profitably used for optimizing of the leaching operations.

Materials and Methods

Microorganisms

Thiobacillus ferrooxidans bacteria were isolated from waters of the "Siersza" colliery. Standard culture was grown on Silverman medium 9K in Erlenmayer flasks of 350 ml in volume containing 100 ml of the medium, at 37°C and pH 2.20. The flasks were kept in thermostated shakers Elpan 357. The bacteria were inoculated every 48 hours. The inoculum introduced to the reactors made 10% (vol./vol.) medium, and the initial number of the bacteria was of the order of 10^7 cells/ml, which is the value typical of *Thiobacillus ferrooxidans* [10]. Greater efficiency can be obtained only by electrochemical reduction of soluble ferric ions in the medium. Blake et al. reported obtaining by this method a concentration of about 10^{10} cells/ml [13].

The bacteria were adapted to the waste ferrous sulphate by subsequent passages of the culture into the medium containing this compound. After two months of these passages the rate of ferrous ions oxidation remained constant. The number of the bacteria was determined by direct counting in a haematological camera with Thoma system incisions, as the mean of three independent measurements.

The Waste Ferrous Sulphate

$FeSO_4$ x $7H_2O$ is the waste product in the production of titanium white, obtained from the Chemical Plant "Police" S.A.. The detailed composition of the waste is as follows: 78% $FeSO_4$ x $7H_2O$, 2% H_2SO_4, 0.6% Mg, 0.4% TiO_2, 0.05% SiO_2, 0.004% Zn, 0.003% Pb and 0.001% As.

Analytical Methods

The kinetics of microbiological oxidation of ferrous ions coming from the waste $FeSO_4$ x $7H_2O$ with the laboratory strain of *Th. ferrooxidans* bacteria (series B) and the same strain adapted to the waste (series C) was studied, and compared with that of oxidation of pure $FeSO_4$ x $7H_2O$ (Fluka, pure for analysis, series A).

The experiments were conducted in 50 ml reactors containing 30 ml of the medium. At certain time intervals the samples were taken out from the thermostated shaker, supplemented with distilled water to the initial volume and their pH and red-ox potential were measured. The precipitates were dissolved in hot 3M HCl.

The consumption of the substrate was detected spectrophotometrically (Beckman DU 640) by the measurement of ferrous ion concentrations employing the o-phenentroline method. The concentration of iron (III) ions was measured by the rhodanate method [14].

The pH values were determined on a pH meter N5170E with a complex calomel electrode, whereas the red-ox potential was measured on a platinum electrode with a silver-chlorine electrode used as reference.

The IR spectra of the precipitates were taken on a Brucker Vector 22 spectrometer using the KBr tablets.

The X-ray diffraction studies on the crystals of the precipitates formed as a result of the oxidation reaction were performed by the powder method on a powder diffractometer, in the angular range 2-30 θ. The photographs were taken at the scanning electron microscope Philips SEN 515, under a magnification of 7500x.

Kinetics Calculations

The kinetic parameters of the bacteria growth were found from the Monod equation:

$$\mu = \mu_{max} \frac{[Fe^{2+}]}{K_s + [Fe^{2+}]}$$

where:
μ_{max} - the maximum growth rate of the bacteria,
μ - specific growth rate,
Ks - the Monod constant.

The process of microbiological oxidation of iron (II) ions was best described within the model of autocatalitic reaction of the 1st order with respect to the product (Fe^{3+}) and the 1st order with respect to the substrate (Fe^{2+}):

$$\frac{d[S]}{dt} = k[S][X]$$

thus

$$S = \frac{S_o(S_o + X_o)e^{-k(X_o + S_o)(t - t_o)}}{X_0 + S_0\, e^{-k(X_0 + S_0)(t - t_o)}}$$

where:
$[S]_o$ - the initial concentration of iron (II) ions,
$[X]_o$ - the initial concentration of iron (III) ions,
[S] - the concentration of iron (II) ions at the time t.

From this equation the following kinetic parameters were determined: rate constant (k) and induction period (t_o). The curve following from the theoretical model was fit to the experimental data by non-linear regression by the least square method.

Results and Discussion

Bacterial Growth

The dependence of the growth rate of the bacteria (μ_{max}) on the concentration of ferrous ions is illustrated in Fig. 1. The kinetic parameters calculated from the Monod equation are given in Table 1.

As follows from these results, the maximum growth rate of the bacteria (μ_{max}) is almost the same and equal to 0.135-0.141 h^{-1} for all three series (A, B and C). This value is one of the lowest obtained for *Th. ferrooxidans.* Most often found values are from the range 0.05 to 1.3 h-1 [15,

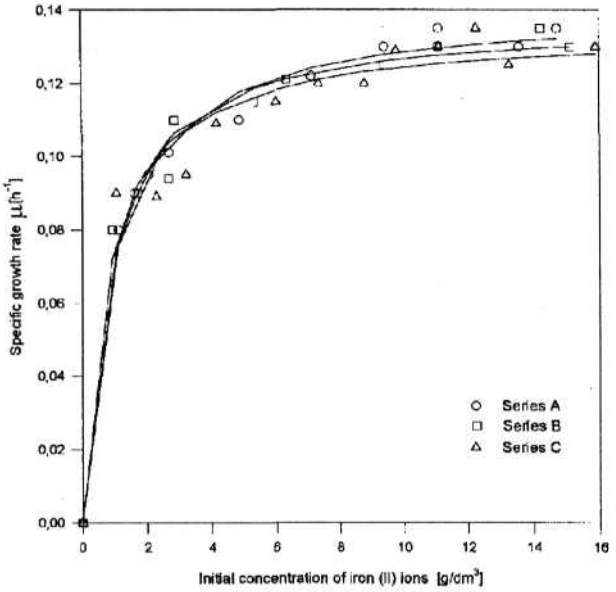

Fig. 1. The rate of *Thiobacillus ferrooxidans* bacteria growth versus the initial concentration of the substrate (37˚C; pH = 2.2; dots - experimental data; solid lines - obtained from the Monod equation).

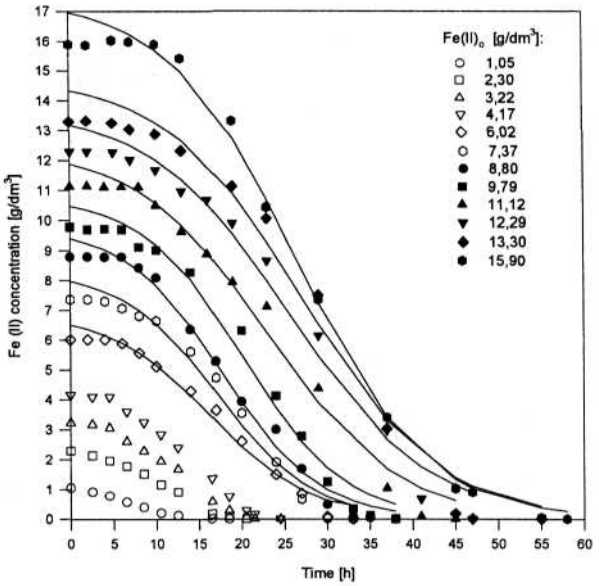

Fig. 2. The kinetic curves of the microbiological oxidation of the iron (II) ions from waste $FeSO_4$ x $7H_2O$ used at different initial concentrations; *(Thiobacillus ferrooxidans;* 37°C; pH = 2.2; dots - experimental data; solid lines - model curves).

Table 1. Kinetic parameters of growth of *Thiobacillus ferrooxidans* (37˚C, pH = 2.2)

$FeSO_4 \times 7H_2O$	μ_{max} [h^{-1}]	K_s [g/dm^3]
Series A	0.141 ± 0.003	0.93 ± 0.14
Series B	0.137 ± 0.003	0.83 ± 0.11
Series C	0.135 ± 0.004	0.85 ± 0.18

16]. The generation time of the bacteria strain used in our experiments was from 7.2 to 7.4 h what represents slightly smaller values than the ones obtained by other authors [17]. The bacteria adaptation to the substrate does not affect these values.

The Monod constant (Ks) takes values from 0.83 to 0.93 g/dm^3 and is charged with a relatively great error (15-21%). For this reason interpretation of the differences between the A, B and C series seems a bit risky. The values of Ks calculated in this work fall within the range of values given in literature (from 0.0 to 1.0 g/dm^3) [15].

Kinetics of Fe(II) Ions Oxidation

Fig. 2 presents the kinetic curves of oxidation of ferrous ions coming from the waste ferrous sulphate, taken at different initial concentrations, conducted with the bacteria adapted to the waste substrate (series B, dots). The solid lines in the figure represent the curves calculated on the basis of the theoretical model. The shapes of the curves are almost identical and comparable with the results obtained by Harvey et al. [10], who studied the process at the initial concentrations from 1 to 4 g Fe (II) /dm^3 and assumed the product inhibition mechanism. Lizam et al. [12] assumed the model of competitive inhibition by the product (ferric ions) and the bacteria cells. The use of so different kinetic models for a descriptions of microbiological oxidation of ferrous ions is justified by the different properties of cell surfaces of the laboratory strains used by different authors. These properties are responsible for different mechanisms of adhesion and coprecipitation of the cells and jarosites [11, 18].

The rate constants and induction periods of the microbiological oxidation of ferrous ions were determined for the Fe (II) concentration from 1-15 g Fe (II)/dm^3 for series A, B and C. The mean values of the reaction rate constants for the initial concentrations from 6 to 15 g Fe (II)/dm^3 are the same and independent of the series (Table 2). For the initial concentrations from 1 to 6 g Fe (II)/dm^3 the rate constants decreased with the increase of this concentration which suggested that the mechanism of the process might have changed.

Table 2. Rate constants (k) and induction periods (t,,) of process of oxidation of iron (II) sulphate, for the initial concentrations from 6 to 15 g Fe (II) / dm^3; *(Thiobacillus ferrooxidans,* 37˚C, pH = 2.2).

Parameter	Series A	Series B	Series C
t_o [h]	6.6 ± 1.6	10.1 ± 2.9	7.3 ± 1.7
k [$dm^3/g \cdot h$]	0.013 ± 0.002	0.013 ± 0.003	0.014 ± 0.004

The induction period of the process performed with the non-adapted bacteria and waste substrate (series B) increased by over 50% relative to the standard culture (series A). The adaptation of the bacteria to the waste ferrous sulphate prior to oxidation resulted in the induction period value very closed to that obtained in oxidation of the pure substrate (Table 2).

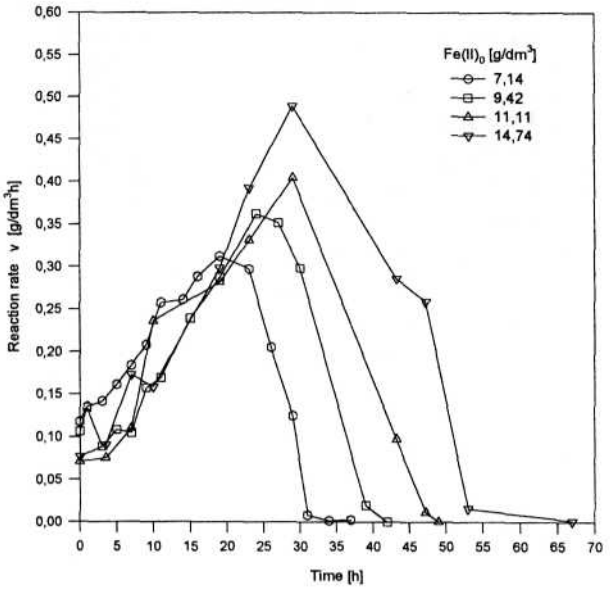

Fig. 3. The kinetic curves illustrating changes in the reaction rate depending on the initial concentration of the substrate (series A) *(Th. ferrooxidans;* 37°C; pH = 2.2).

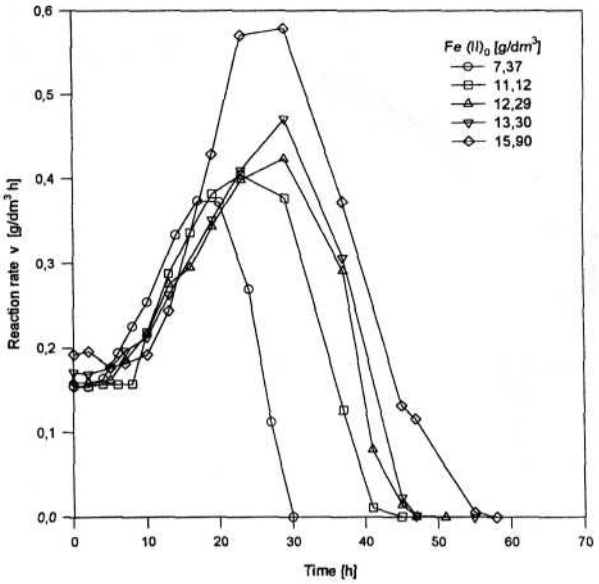

Fig. 5. The kinetic curves illustrating changes in the reaction rate depending on the initial concentration of the substrate (series C). *(Th. ferrooxidans;* 37°C; pH = 2.2).

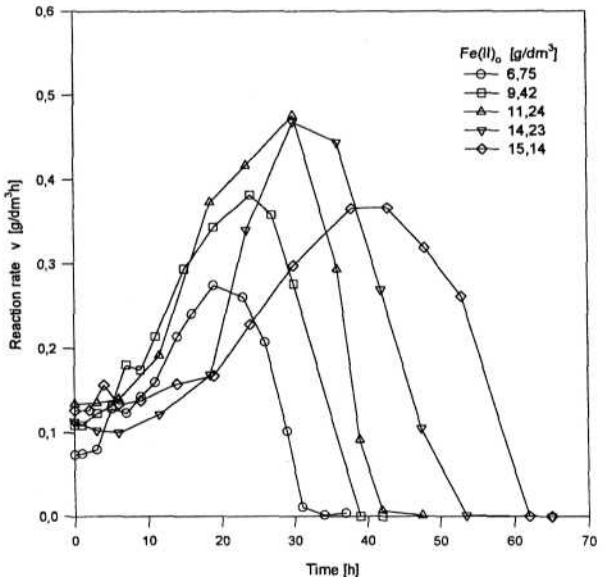

Fig. 4. The kinetic curves illustrating changes in the reaction rate depending on the initial concentration of the substrate (series B). *(Th. ferrooxidans;* 37°C; pH = 2.2).

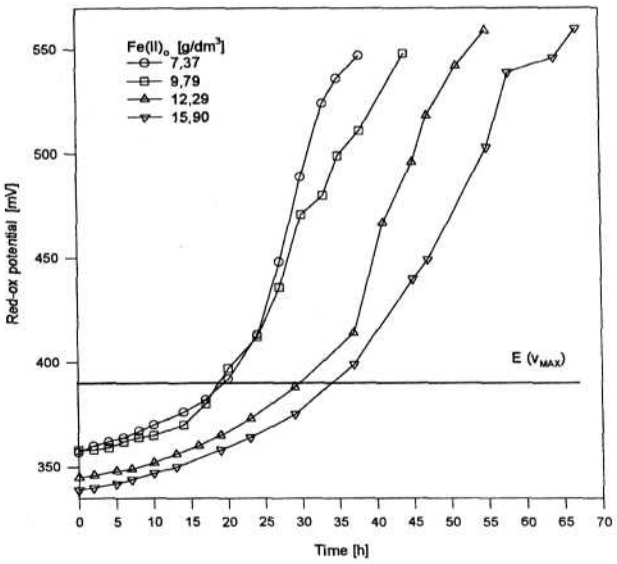

Fig. 6. Changes in the red-ox potential during microbiological oxidation of iron (II) ions; *(Th. ferrooxidans;* Series C; E(v_{max}) - potential for the point of maximum reaction rate; 37°C; pH = 2.2).

Figs. 3, 4 and 5 show the kinetic curves of the rate of the process as a function of time for different initial concentrations of ferrous ions, for series A, B and C. In all cases the rate of the process at first increased to the maximum value (which was higher, the greater the initial concentration of the substrate) and then it decreased.

The potential at the point at which the rate of the process reached the maximum value (Ev_{max}) was the same for all series (A, B, C) and equal to 390 mV, irrespective of the initial concentration of the substrate, so also the initial potential of the sample ($E_{f=0}$). The point of the maximum process rate represents the moment when the concentrations of ferrous and ferric ions were the same. Therefore, the changes of potential during the process (Fig. 6) are of the key importance for its evaluation [10].

Temperature Studies

The process was conducted at a range from 22 to 45°C. For each temperature the kinetic parameters were calculated and collected in Table 3. The respective kinetic curves are shown in Figs. 7 and 8.

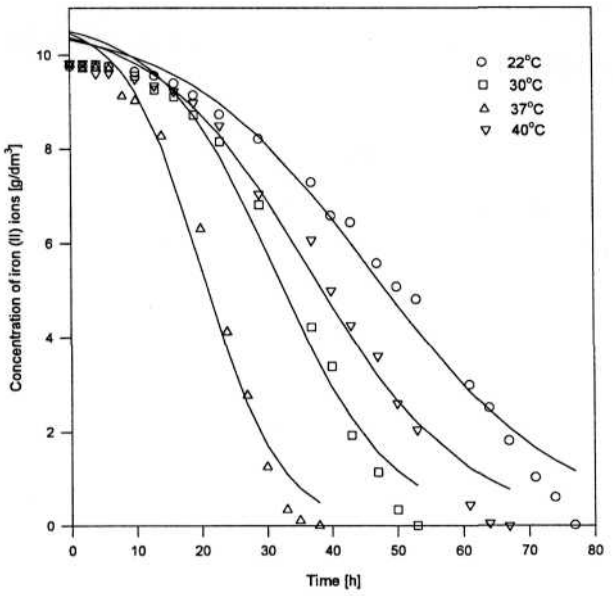

Fig. 7. The effect of temperature on the oxidation of iron (II) ions coming from the waste $FeSo_4$ x $7H_2O$; *(Th. ferrooxidans;* pH = 2.2; dots - experimental data; solid lines - model curves).

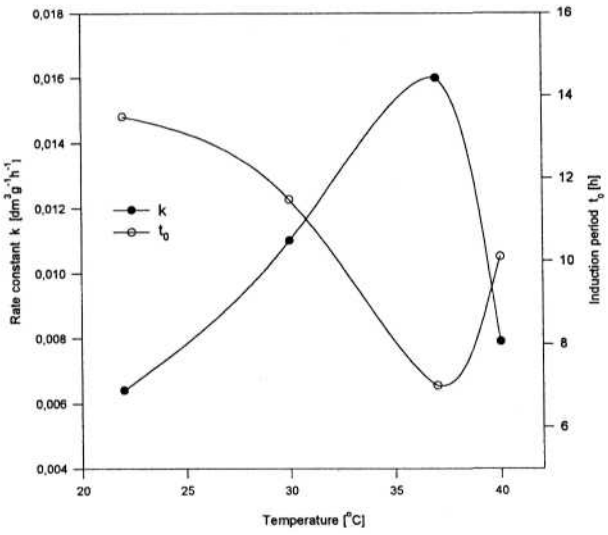

Fig. 8. The effect of temperature on the rate constant (k) and induction period (to) of the microbiological oxidation of the waste $FeSO_4$ x $7H_2O$; *(Thiobacillus ferrooxidans).*

Table 3. The effect of temperature on the kinetics of oxidation of the waste $FeSO_4$ x $7H_2O$; *(Thiobacillus ferrooxidans,* pH = 2.2).

Temperature [°C]	Rate constant k [$dm^3g^{-1}h^{-1}$]	Induction period t_0 [h]	Reaction time [h]
22	0.0064 ± 0.0003	3.5 ± 1.9	77
30	0.011 ± 0.001	11.5 ± 1.7	53
37	0.016 ± 0.001	7.0 ± 1.3	38
40	0.0079 ± 0.0005	10.1 ± 1.8	67
45	–		

The process was found to be the most efficient at 37°C, at which the rate constant is 0.016 $dm^3g^{-1}h^{-1}$, the induction period is 7 hours and the complete oxidation of ferrous ions occurs after 38h. A temperature decrease to 30°C caused a slight reduction in the reaction rate constant (to 0.011 $dm^3g^{-1}h^{-1}$) and an almost two fold increase in the induction period of the reaction (to 11.5 hours). At this temperature complete oxidation occurs after 53 hours. A further decrease in temperature, to 22°C, resulted in a nearly three-fold decrease in the reaction rate constant and an almost two-fold increase in the induction period, when referred to the corresponding values at 37°C. An increase of temperature to 40°C gives a two-fold decrease in the reaction rate constant and elongation of the induction period by 3 hours with respect to the corresponding parameters at 37°C. Only when temperature was increased to 45°C were no oxidation of ferrous ions and no growth of the bacteria observed.

These observations prove that the process is very sensitive to temperature changes and its response is more drastic to higher temperatures than the optimum one. The temperature increase by 3°C results in a substantial decrease in the reaction rate constant and the increase in the induction period. These results are consistent with those reported by Hubert et al. [19]. It has to be stressed that the bacteria strain was not adapted to temperatures other than 37°C. Such an adaptation, suggested by Ahonen et al. [2], would extend the range of the optimum temperatures towards lower ones. Also, a decrease in pH value causes a decrease in the optimum temperature of the process [2], which is important with regard to the fact that at lower temperatures the problem causing jarosites formation at much lower amounts [13].

The activation energy (E_a) of the process was calculated from the Arrhenius equation and its mean value is 46.3 ± 2.7 kJ/mol, which is about 50% lower than the value reported by Ahonen et al. (83 kJ/mol) [2], or by Ferroni et al. (95 kJ/mol) [3]. Our value was close to that obtained by Lundgren (40 kJ/mol) [20] or Guay et al. (50 kJ/mol) [21], calculated for cell suspensions or cell membrane preparations. The value of the activation energy indicates that the process is limited by biochemical and not diffusion factors, as if the latter was true, its value would be much lower.

The temperature coefficient Q_8 calculated from our results was 1.97, which is close to the value obtained by Sakaguchi et al. (1.9 - 2.4) [22]. A lower value (1.06 - 1.22) was given by Cwalina [17], who studied the process of leaching of minerals.

pH Studies

Studies were performed with the initial pH varied in the range 1.00-2.80, in the 9K medium and at 37°C. The results are illustrated in Fig. 9 and collected in Table 4. The greatest rate constant of the process (0.016) was noted at pH 2.20. For pH changes in the range from 1.50 to 2.00, the rate constant was only 15-20% lower than the maximum value, so pH changes in this range had an insignificant effect on the rate constant. The values of the induction period in this pH range were very similar, except for the specific situation at pH 1.50 when the induction period increased to 10 hours and the whole process duration increased by 12 hours with respect to that at pH 2.20.

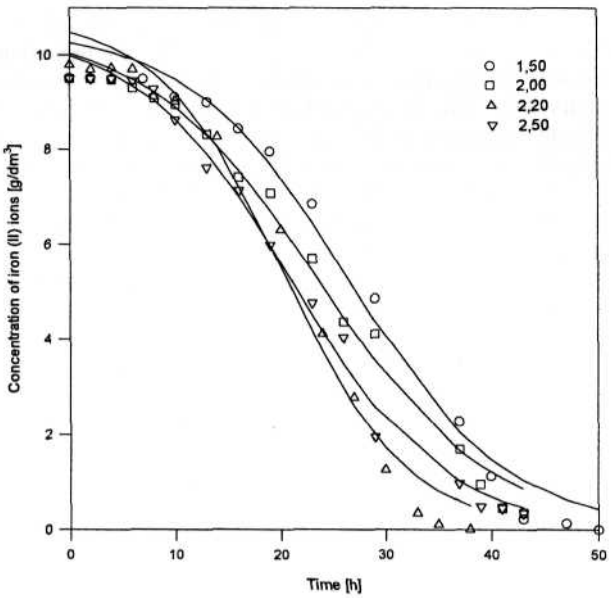

Fig. 9. The effect of the initial pH of the medium on the microbiological oxidation of iron (II) ions from the waste $FeSO_4$ x $7H_2O$; *(Th. ferrooxidans;* 37°C; dots - experimental data; solid lines - model curves).

Table 4. The effect of initial pH of the medium on the kinetics of oxidation of the waste FeSC>4 x $7H_2O$; *(Thiobacillus ferrooxidans,* 37°C).

Initial pH	Rate constant k [$dm^3g^{-1}h^{-1}$]	Induction period t_0 [h]	Reaction time [h]
1,00	reaction did not occur, even after 50 h		
1.50	0.013 ± 0.001	10 ± 1.3	50
2.00	0.012 ± 0.001	6.0 ± 0.9	43
2.20	0.016 ± 0.001	7.0 ± 1.3	38
2.50	0.013 ± 0.001	5.0 ± 0.7	43
2.80	iron precipitates		

At pH 1.00 no bacteria growth was observed, whereas at pH 2.80 the precipitates started to appear. Recognition of the process at low pH values is very important as in this range the amount of the precipitate formed is much lower, which is beneficial from a technological point of view. At pH 1.5 the amount of the precipitate is significantly reduced [23].

Precipitates

Table 5 presents the results on identification of the precipitates formed during the process of oxidation of ferrous ions, run at pH 2.20, 37°C and in the medium containing 9 g Fe(II)/dm^3.

Fig. 10 shows the IR spectra of the precipitates formed during the oxidation process in 5K, 10K and 15K media containing $FeSO_4$ of analytical grade as well as the waste $FeSO_4$. The spectra of the precipitates taken for different initial concentrations of Fe(II) revealed no differences. Additionally, the spectra obtained for the precipitates formed during the oxidation of pure substrate are identical with those obtained for the waste ferrous sulphate. The spectra of the precipitates show the lines corresponding to the vibrations of the frequency characteristic of jarosites ($MeFe_3(SO_4)2(OH)_6$), in particular v_3 (SO_4^{2-}) at 1.194 cm^{-1}, δ(OH) at 995 cm^{-1} (indistinguishable from v_1 (SO_4^{2-}), and v_4 (SO_4^2) at 626 cm^{-1} [6]. Moreover, the spectra show the stretching vibrations attributed to (OH) at 3.405 cm^{-1}, τ (OH) at 468 and 510 cm^{-1}. At 1.632 cm^{-1} there is a wide shoulder ascribed to the deformational vibrations of water, despite drying the precipitates at 110°C and washing out with acetone (Table 6). Analysis of the spectra proves that the only difference between the precipitates is that the ones formed during the oxidation of the waste $FeSO_4$ contain the ammonium cation at a higher concentration, which is manifested by a strong absorption of the deformational vibrations attributed to NH_4^+ at 1.427 cm^{-1} and the stretching vibrations (NH) at 3200 cm^{-1}. These results are consistent with chemical analysis of the precipitates.

The photographs of the precipitates formed in the standard 9K medium and 9K medium prepared with the waste iron (II) sulphate, taken on a scanning electron-microscope show the agglomerates of jarosites crystals which are much better developed for the precipitates formed as the result of oxidation of the waste $FeSO_4$ x $7H_2O$ (Photos 1 and 2).

The data obtained from X-ray powder diffraction, Fig. 11, were compared with the database of the "Powder" program and the peaks appeared at the angles 0 identified as characteristic of potassium, sodium and ammonium jarosites [24], which proves that the precipitates also contain an admixture of potassium and sodium jarosites, apart from the ammonium ones.

Table 5. Chemical composition of the precipitates formed during microbiological oxidation of the waste $FeSO_4$ x 7H.O (37°C, pH = 2.2, Silverman medium 10K)

Precipitates formed during oxidation	%Fe[a]	%SO_4[b]	Fe/SO_4[c]	%NH_4	%Na	%K	%Mg	Weight of precipitates [g/dm^3]	Colour
$FeSO_4$ × $7H_2O$ (p.a.)	36.70	39.70	0.92	2.9	0.07	1.7	0.04	0.902	yellow
$FeSO_4$ × H_2O (waste)	37.09	39.70	0.93	3.1	0.12	1.22	0.06	1.342	yellow

Mean values for jarosites [24]: [a] 33.47 ± 1.09; [b] 38.30 ± 1.10; [c] 0.87± 0.01

Table 6. Characteristics of the IR spectra of the precipitates formed during microbiological oxidation of the waste iron (II) sulphates ***(Thiobacillus** ferrooxidans*, 37°C, pH = 2.2)

Precipitate	OH stretching	NH stretching	H_2O deformational	NH_4 deformational	ν_3 (SO_4)	ν_3 (SO_4)	δ (OH)	ν_1 (SO_4)	ν_4 (SO_4)	τ (OH)	τ (OH)	ν_2 (SO_4)	τ (SO_4)
precipitates formed during oxidation of $FeSO_4 \times 7H_2O$ (p.a.)	3.405	3.200	1.632	1.427	1.194	1.078	995 indistinguishable		626	510	468		
precipitates formed during oxidation of $FeSO_4 \times 7H_2O$ (waste)	3.405	3.200	1.632	1.427	1.194	1.078	995 indistinguishable		626	510	468		
$NaFe_3(SO_4)_2(OH)_6$	3.354				1.184	1.094	1.025	1.008	629	510	478	445	346
$KFe_3(SO_4)_2(OH)_6$	3.383				1.180	1.083	1.012	1.003	628	509	474	446	336
$(NH_4)Fe_3(SO_4)_2(OH)_6$	3.408	3.200	1.630	1.423	1.193	1.076	1.000	997	626	507	469		338

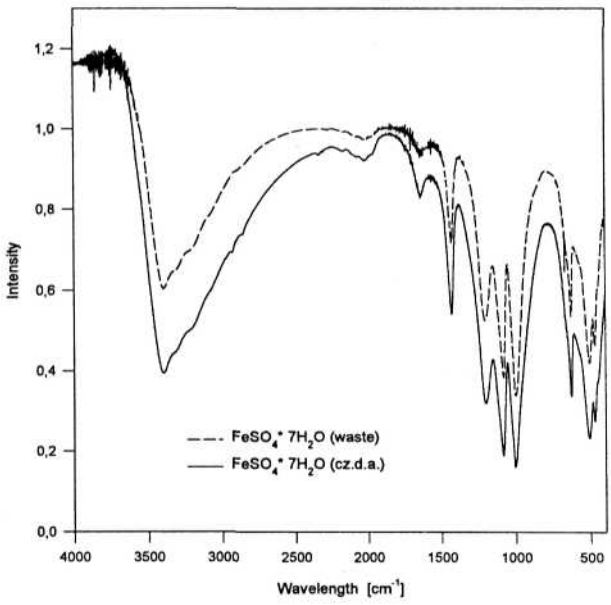

Fig. 10. IR spectra of the precipitates formed during the microbiological oxidation of $FeSO_4$ x 7 H_2O; *(Th. ferrooxidans;* 37°C; pH = 2.2).

A comparison of the kinetic parameters of microbiological oxidation of ferrous ions coming from pure substrate and waste ferrous sulphate (the waste product in production of titanium white) reveals that the laboratory strain of *Thiobacillus ferrooxidans* after adaptation is equally efficient in oxidation of the pure and waste substrate. In both cases the rate constants and the induction periods are almost the same for substrate concentrations up to 10%. The most important parameters of the oxidation reaction are pH and temperature: the process is the most efficient at pH from 1.5 to 2.5 and at temperatures from 30 to 37°C. The information about how the bacteria function in different temperatures is of a great importance as in waste dumps surface temperature can change from a few degrees below 0 to 50°C. Low pH and lack of organic substrate cause the

Precipitates formed in the process of iron (II) ion oxidation, 7500x.
Photo. 1. Standard Silverman 9K medium.
Photo. 2. 9K medium containing the waste $FeSO_4$ x $7H_2O$.

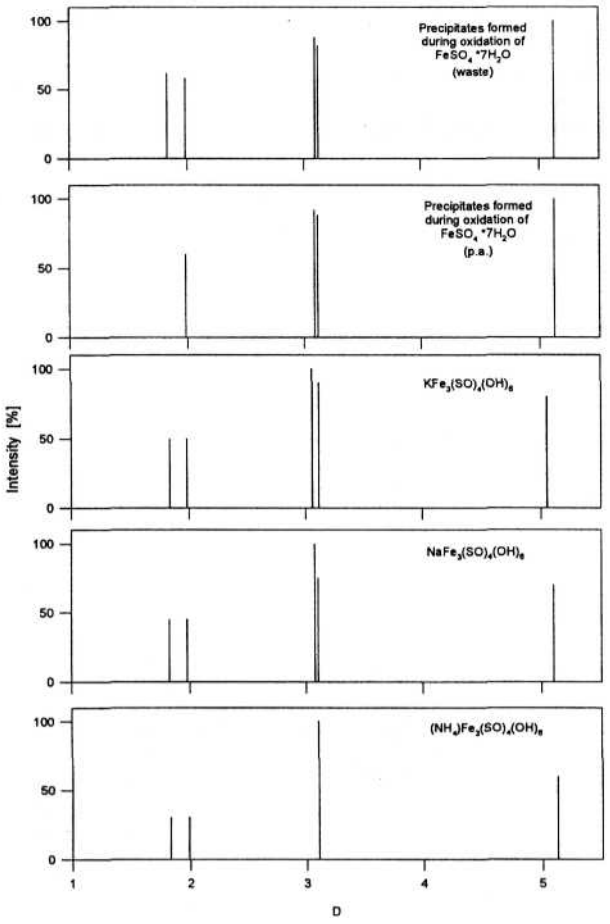

Fig. 11. Powder photographs of the precipitates formed during microbiological oxidation of the waste $FeSO_4 \times 7H_2O$.

culture to hardly get contaminated with micro-organisms what together with the mesophilic temperatures make the conditions of the process easy to achieve in industrial practice. Solution of the problem of jarosite deposition on the substrate, which blocks the sites for the bacteria, may bring additional recovery of up to 20% of ferric ions and enhance the efficiency of utilization of the waste $FeSO4x7H_2O$, providing substrates based on iron (III).

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