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A kinetic study of the depyritization of oil shale HCl-kerogen concentrate by *Thiobacillus ferrooxidans* at different temperatures*

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Abstract: The results of kinetic studies of bacterial depyritization of HCl-kerogen concentrate of Aleksinac (Serbia) oil shale by the chemolithoautotrophic thionic bacteria *Thiobacillus ferrooxidans* under discontinuous laboratory conditions at various temperatures (0, 20, 28 and 37 ºC) at a pH of *ca.* 1.5 are presented in this paper. Low pH prevents the occurrence of the precipitation of iron(III)-ion hydrolysis products on the substrate particles and thereby reduces the process efficiency. Bacterial depyritization is developed as per kinetics of the first order. The activation energy which points to a successive mechanism of pyrite biooxidation, was computed from the Arrhenius plot. The biochemical kinetics indicators point to a high affinity of the bacteria toward pyrite but small values of V_{max}, which are probably the result of decelerated metabolic processes due to the low pH value of the environment resp. the large difference of the pH between the external medium and the cell interior.

Keywords: oil shale, *Thiobacillus ferrooxidans*, depyritization, kinetics.

INTRODUCTION

Oil shales (compact sedimentary rocks of homogeneous fine-grained composition) are potentially an important source of hydrocarbons, due to which they are the subject of geochemical investigations and are of economic interest. The majority of the components of oil shales (about 80 %) are inorganic *viz*. carbonates, alumino-silicates and pyrite. Kerogen, which is insoluble with a heterogeneous macromolecular cross-linked structure, is the dominant organic substance (approx. 95 % out of the total organic matter) while the in organic solvents soluble bitumen is present in amounts of several percents.[1,2] Funda-

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mental organic-geochemical studies of kerogen require the preparation of concentrates of relatively pure and unaltered kerogen. The removal of carbonates and alumino-silicates is realized by the action of mineral acids (first dilute hydrochloric acid and then a mixture of concentrated hydrochloric and hydrofluoric acids), while the removal of pyrite, which is closely associated with kerogen, causes through the chemical procedures (oxidation and reduction) a change in its chemical structure.[3–5] Bacterial depyritization of crude oil shale and its concentrates by the chemolithoautotrophic thionic bacteria *Thiobacillus ferrooxidans* (*Th. f.*) is exceptionally efficient (approx. 97 % within four weeks) after which rich concentrates of kerogen with unchanged organic substance are obtained.[6–8]

HCl-concentrate (crude oil shale from which the carbonates have been removed) and HCl-kerogen concentrate (HCl-concentrate from which the bitumen has been extracted) are shown to be the best substrates for bacterial depyritization, while the HCl-kerogen concentrate has certain advantages.[9] Also, at lower initial pH value, in relation to the optimal pH of 2.5, there is less precipitation of the hydrolysis products of iron(III)-ion (the product of bacterial oxidation-bacterial leaching of pyrite) on the substrate particles, leading to a reduction of the efficiency of pyrite removal.[10] For these reasons, this research work is dedicated to the kinetic study of bacterial depyritization of HCl-kerogen concentrate at pH approx. 1.5 at different temperatures.

EXPERIMENTAL

The examined sample which originated from the oil shale deposit near Aleksinac (Serbia), represented a composite obtained by combining 5 samples (476.1–491.6 m). The composite sample was crushed and then pulverized to < 63 μm.

The HCl-kerogen concentrate was obtained by extraction of the bitumen (0.95 %) from the HCl-concentrate with a benzene–methanol (1:4 V/V) mixture.

The HCl-concentrate was obtained by treatment of the powdered shale with dilute hydrochloric acid (1:4 V/V). The content of pyrite in the HCl-kerogen concentrate was 12.9 %.

A pure culture of the most active zymogenous strain of *Th. f.* (marked A_5) was isolated from an oil shale sample taken from the shale dump at the Aleksinac coal mine.[6] In preparing the bacterial biomass for the experiments,[11] a physiologically active culture of *Th. f.* was cultivated in 500 ml of 9K nutrient medium.[12]

The shaking flask technique[13] was used in the experiments carried out according to a general experimental scheme described elsewhere.[7] The ratio of solid substrate *vs.* liquid iron-free 9K medium was 1:12 (w/V), pH approx. 1.5. Each experiment lasted 192 h (8 days) at the temperatures of 0, 20, 28 and 37 ºC.

The following methods and instruments were used.

A Radiometer Copenhagen, type pHM 26 pH-meter with a combined electrode GK 2401 B of the same manufacturer.

Number of microorganisms was determined by the McCredys method of the most probable number (MPN).[14]

pH Measurements Residual pyrite was calculated from the quantity of iron determined in the 800 ºC ash by atomic absorption spectrophotometry (Varian 475 atomic absorption spectrophotometer). The soluble and precipitated iron compounds were previously removed by treatment with HCl (1:4 V/V).

Data processing. The results for the kinetic computations were processed by the Microcal Origin 5.0 program, and the kinetics of the biochemical processes were computed by the iterative method as per Roberts and Elmore[14] and Wentworth[15,16] program for Michaelis–Menten kinetics, which was adapted for a PC.

RESULTS AND DISCUSSION

The changes of pH and the number of microorganisms in the inoculated specimen during the experiments are shown in Table I.

TABLE I. Changes of pH and the number of *Th. f.* in inoculated samples as a function of time

Temperature	Time / h									
ºC	0	48	96	144	192	0	48	96	144	192
	pH					Number of cells *Th.f.* [$\times 10^8$ ml^{-1}]				
0	1.5	1.6	1.5	1.3	1.4	2.9	1.1	1.1	1.1	1.5
20	1.7	1.5	1.4	1.4	1.4	2.9	4.5	4.5	4.5	4.5
28	1.4	1.3	1.4	1.3	1.3	2.9	45	45	45	45
37	1.5	1.6	1.4	1.4	1.4	2.9	0.004	0	0	0

The pH value in all specimens during the bacterial process remains practically unchanged resp. it ranges within the limits of the pH equilibrium irrespective of the temperature, which is also an indication of a reduced degree of iron(III)-ion hydrolysis and precipitation of basic-iron(III)-sulphates on the substrate particles.[7,10,17] The number of *Th. f.* cells is slightly reduced at the lowest temperature after 48 h after which it remains unchanged until the end of the test, while at 20 ºC the number is somewhat increased after the same initial time period and again remained unchanged until the end. At the optimal temperature for the majority of *Th. f.* strains which is 28 ºC, after two days the number of bacteria cells had increased by about 16 times and again remained unchanged. The temperature of 37 ºC is lethal for this strain so that already after 4 days there are no living cells left (half reduction time is approx. 24 h). The dynamics of the bacterial population subject to temperature is an indicator of the efficiency of the bacterial depyritization process.

The best indicator of successful pyrite removal is the cumulative leaching in the inoculated and control (abiotic) test computed on the basis of the pyrite content in the HCl-concentrate at the beginning of experiments ("zero-time") and the amount of residual pyrite upon expiry of the overall process time resp. depyritization efficiency (*Ef*), which represents the ratio of the cumulative leaching of pyrite in the inoculated and control test. These parameters are shown in Table II.

Bacterial leaching as well as pyrite leaching in the control test increase as expected with increasing temperature (except at 37 ºC). However, in all non-inoculated tests, the leachings are considerably higher in relation to the abiotic processes at the optimal pH of about 2.5 for bioleaching,[7] which is the result of more intensive chemical processes at lower pH values[10] due to which the efficiency is lower. Since the temperature of 37 ºC is lethal for the bacteria, the results obtained in the inoculated test are mosty the result of chemical oxidation, which is also confirmed by the result for the control test. Therefore, these results cannot be used for kinetic computations relating to the bacterial process.

The function $\ln c_0/c_r = f(t)$, where c_0 and c_r are the pyrite concentrations at the beginning and residual pyrite concentration at times t, is followed for the relevant temperatures,

which means that the kinetics of the bacterial depyritization is of first order.[18] Under the assumption that the system is unstructured and non-segregated,[19] the rate constants (k) are defined. From their values, the van't Hoff's temperature coefficient[20] of pyrite bioleaching rate (Q_{10}) can be computed. The computed values are given in Table III.

TABLE II. Cumulatively removed pyrite and depyritization efficiency (*Ef*).

Temperature	Cumulatively leached pyrite / %		*Ef*
ºC	Inoculated test	Control test	
0	30.1	19.8	1.5
20	45.9	30.7	1.5
28	80.8	33.6	2.4
37	49.1	38.8	1.3

The highest rate constant of the microbiological process is at the temperature which is optimal for the used strain *Th. f.*, and the average temperature coefficient of the depyritization rate is 3.5, identical to the computed value over the whole temperature interval.

The activation energy (E_a) of the pyrite bacterial oxidation was computed by linear regression of the $-\ln k = f(1/T)$ – Arrhenius plot.[18] The obtained value is 86 kJ/mol of pyrite (correlation coefficient: $r = 1.000$) which is similar to the E_a of 83 kJ/mol (g-ion Fe^{2+}),[21] for iron(II)-ion oxidation at pH 1.5, which indicates that the biooxidation of pyrite can be represented by the overall reaction.

$$2FeS_2 + 7.5\ O_2 + H_2O \rightarrow Fe_2(SO_4)_3 + H_2SO_4$$

which proceeds *via* the following sequence of reactions:

$$2FeS_2 + 4\ O_2 \rightarrow 2FeSO_4 + 2S$$
$$2S + 3\ O_2 + 2\ H_2O \rightarrow 2\ H_2SO_4$$
$$2FeSO_4 + 0.5\ O_2 + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + H_2O$$

TABLE III. Kinetic indicators of bacterial depyritization

Temperature / ºC	$k \times 10^{-3}/h^{-1}$	Temperature interval/ºC	Q_{10}
0	0.23	0–20	3.7
20	3.1	20–28	3.2
28	7.9	0–28	3.5

The interaction of *Th. f.* cells with pyrite from oil shale concentrate, which means also HCl-kerogen concentrate, can also be dealt with as an enzyme reaction in which the biochemical activity is proportional to the overall enzyme activity of the bacterial cells which use pyrite as an energetic substrate and as a source of electrons. This means that Michaelis–Menten biochemical kinetics can be applied to this process.[22] Processing of results as per the stated program for the linearized form of the Michaelis–Menten equation (Lineweaver–Burk equation) gave the results presented in Table IV.

TABLE IV. Indicators of biochemical kinetics for bacteria depyritization

Temperature / ºC	V_{max} ×10^{-3}/mol l^{-1} h^{-1}	K_m ×10^{-1}/mol^{-1}
0	0.093	0.010
20	0.193	7.66
28	8.05	5.36

The biochemical parameters of depyritization at 20 and 28 ºC indicate that increased acidity chemically activates the pyrite surface released from bitumen as a hydrophobic "protection". Also, due to a smaller degree of iron(III)-ion hydrolysis, the active centers on the external surface of the *Th. f.* cells are "free" for contact with the substrate,[23,24] *i.e.*, the affinity of the cells for pyrite is increased which can be seen from the value for K_m. Consequently, a greater number of cell-substrate interactions arise at lower pH values which agrees with previous results.[9] K_m at 28 ºC is smaller than the same parameter at 20 ºC, which is the result of the increased degree of hydrolysis at the higher temperature. However, the values for V_{max} are considerably lower compared with the same parameter at the optimal pH[9] which is, after the initial efficient contact of the bacterial cells with the pyrite, probably the result of a deceleration of further transport of electrons and the process of oxidative phosphorylation due to the great pH difference between the external environment and the cell interior. This means that in the adaption process of this *Th. f.* strain to a pH of about 1.5, the induction of the biosynthesis of an acidoresistent cytochrome, which would ensure the bypassing of the existing pH barrier, is not possible.[25,26] For this reason, the yield of ATP resynthesis is small resulting in a small biomass growth and thereby also smaller depyritization effects. This is also confirmed by the results presented in Tables I and II. The results at 0 ºC indicate the negative influence of low temperatures and low pH on the biochemical activity of *Th. f.*

Since pyrite is a limiting substrate and the method of realization of the bacterial depyritization corresponds to a periodical culture (batch process), then, assuming that the mixing conditions enable homogeneity of the system, it is possible to apply the Monod unstructured model to the kinetics of the bacterial growth and activity. This model in terms of the mathematical formulation is identical to the Michaelis–Menten equation.[19] Hence, V_{max} is the same as the maximum specific growth rate of the biomass (μ_{max}), and K_m is the saturation constant (K_S). If the mass concentration at optimal temperature is, for example, used instead of the molar concentration of the pyrite, then the following values are obtained: μ_{max} = 1.0 g l^{-1} h^{-1} and K_S = 106.6 g l^{-1}. Such a high affinity towards the substrate is a result of the low utilization of energy (about 3 % for Fe^{2+} at pH 2.5)[17] for biosynthesis of the cellular components of the biomass.

CONCLUSION

The presented kinetic study of bacterial depyritization-bioleaching of a HCl-kerogen concentrate of Aleksinac (Serbia) oil shale by an unadapted strain *Thiobacillus ferrooxidans* A_5 at different temperatures (0, 20, 28 and 37 ºC) in the periodical culture at pH of

about 1.5 during 192 h (8 days), under laboratory conditions using the shaking flask technique indicates the following:

1. The temperature of 37 ºC is lethal for the used strain and therefore this temperature is excluded from the kinetic considerations;

2. The best effects of bacterial depyritization (cumulative leaching 80.8 %) are obtained at 28 ºC, which is the optimal one for this *Th. f.* strain;

3. Lower pH values are suitable for the chemical oxidation of pyrite-abiotic tests, and therefore the efficiencies of pyrite removal are small (the maximal is 2.4 at 28 ºC);

4. Bacterial depyritization is a first order reaction and the rate constants of the oxidation processes have been computed for the relevant temperatures;

5. From the Arrhenius plot, the activation energy (E_a = 86 kJ/mol pyrite) was calculated by linear regression and its value points to a successive mechanism pyrite bacterial oxidation;

6. The average value Q_{10} is 3.5;

7. The Michaelis–Menten biochemical kinetics resp. the Monod unstructured model point to a high affinity of *Th. f.* in relation to pyrite (high values for K_m resp. K_s), but the values of V_{max} resp. μ_{max} are small, which probably means that due to the great difference in pH between the external environment and the cell interior, transport of electrons and biosynthesis of the cell components is decelerated irrespective of a low degree of iron(III)-ion hydrolysis;

8. The biochemical processes at 0 ºC are very slow and inefficient, due both to the low temperature and the low pH values; and

9. Of importance for the potential application of depyritization is that the processes are generated within the tested temperature interval at all temperatures.

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ИЗВОД

КИНЕТИЧКО ПРОУЧАВАЊЕ ДЕПИРИТИЗАЦИЈЕ HCl-КЕРОГЕНСКОГ КОНЦЕНТРАТА БИТУМИНОЗНОГ ШКРИЉЦА ПОМОЋУ *Thiobacillus ferrooxidans* НА РАЗЛИЧИТИМ ТЕМПЕРАТУРАМА

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У раду су приказани резултати кинетичких проучавања бактеријске депиритизације HCl-керогенског концентрата алексиначког (Србија) битуминозног шкриљца помоћу хемолитоаутотрофне тионске бактерије *Thiobacillus ferrooxidans* у дисконтинуалним лабораторијским условима на различитим температурама (0, 20, 28 и 37 ºC) при pH око 1,5. Ниско pH спречава настајање производа хидролизе гвожђе(III)-јона, који се таложе на честицама суп-

страта и тиме смањују ефикасност процеса. Бактеријска депиритизација се одвија по кинетици првог реда. Из Arrhenius-овог дијаграма израчуната је активациона енергија која указује на сукцесивни механизам биооксидације пирита. Показатељи биохемијске кинетике указују на високи афинитет бактерија према пириту, али мале вредности V_{max}, што је вероватно последица успорених метаболичких процеса због ниске pH вредности средине, односно велике разлике pH између спољашње средине и унутрашњости ћелије.

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REFERENCES

1. T. F. Yen, G. V. Chilingarian Eds., *Oil Shale*, Elsevier. Amsterdam, 1976
2. B. Durand Ed., *Kerogen, Insoluble Organic Matter from Sedimentary Rocks*, Editions Technip, Paris, 1980
3. B. Durand, G. Nicaise, in *Kerogen, Insoluble Organic Matter from Sedimentary Rocks*, B. Durand Ed., Editions Technip, Paris, 1980, p. 35
4. D. L. Lawlor, J. I. Fester, W. E. Robinson, *Fuel*, **42** (1963) 239
5. D. Vitorović, V. D. Krsmanović, P. A. Pfendt, in *Advances in Organic Geochemistry 1979*, A. G. Douglas, J. R. Maxwell Eds., Pergamon Press, Oxford, 1980, p. 587
6. M. M. Vrvić, J. Vučetić, D. Vitorović, in *Recent Progress in Biohydrometallurgy*, G. Rossi, A. E. Torma Eds., Associazione Mineraria Sarda, Iglesias 1983, p. 527
7. M. M. Mrvić, V. Djordjević, O. Savković, J. Vučetić, D. Vitorović, *Org. Geochem.* 13 (1988) 1109
8. O. Cvetković, V. Dragutinović, M. M. Vrvić, J. A. Curiale, M. Ercegovac, D. Vitorović, *Org. Geochem.* **20** (1993) 57
9. M. M. Vrvić, V. Dragutinović, J. Vučetić, D. Vitorović, in *Proceedings of the International Symposium on Biohydrometallurgy*, P. R. Norris, D. P. Kelly Eds., Science and Technology Letters, Kew Surrey 1988, p. 545
10. V. Dragutinović, M. M. Vrvić, O. Cvetković, J. Vučetić, D. Vitorović, *J. Serb. Chem. Soc.* **64** (1997) 343
11. J. Vučetić, M. M. Vrvić, B. M. Vujović, B. Grujić-Injac, in *Papers of the 3rd Yugoslav Congress of Microbiologists*, M. Likar Ed., Union of Yugoslav Microbiological Societies, Bled (Yugoslavia) 1976, p. 244 (in Serbian)
12. M. P. Silverman, D. G. Lundgren, *J. Bacteriol.* **77** (1959) 642
13. R. A. Fisher, F. Yates, *Statistical Tables for Biological, Medical and Agricultural Research*, 6th Ed., Longman, London, 1975, p. 6, 66
14. D. V. Roberts, D. T. Elmore, *Biochem. J.* **141** (1974) 545
15. W. E. Wentworth, *J. Chem. Edu.* **42** (1965) 96
16. W. E. Wentworth, *J. Chem. Edu.* **42** (1965) 162
17. M. M. Vrvić, *PhD. Thesis,* Faculty of Chemistry, University of Belgrade, Belgrade, 1991, p. 414, 440 (in Serbian)
18. V. Dondur, *Chemical Kinetics*, Faculty of Physical Chemistry, Belgrade, 1992, p. 340 (in Serbian)
19. H. W. Blanch, D. S. Clark, *Biochemical Engineering*, Marcel Dekker, New York, 1996, p. 181
20. D. A. Sukatach, A. Dziengel, *Biotechnology: A Handbook of Practical Formulae*, Longman Scientific & Technical, Harlow, 1987, p. 87
21. L. Ahonen, O. H. Tuovinen, *Appl. Environ. Microbiol.* **55** (1989) 312
22. H. Bisswanger, *Enzyme Kinetics*: *Principles and Methods*, Wiley-VCH, Weinheim, 2002, p. 55
23. D. Lundgre, T. Tano, in *Metallurgical Applications of Bacterial Leaching and Related Phenomena*, L. E. Murr, A. E. Torma, J. A. Brierley, Eds., Academic Press, New York, 1978, p. 151
24. F. K. Crundwell, in *Biohydrometallurgy: Fundamentals, Technology and Sustainable Development*, Part A, V. S. T. Ciminelli, O. Garsia Jr, Eds., Elsevier, Amsterdam, 2001, p. 149
25. W. J. Ingledew, I. C. Cox, P. I. Halling, *FEBS Lett.* **2** (1977) 193
26. W. J. Ingledew, *Biochim. Biophys. Acta* **89** (1982) 683.