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BIOLOGICAL DESULPHURIZATION OF ESTONIAN OIL SHALE

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Pretreatment of Estonian oil shale by thermophilic Sulfolobus acidocaldarius and mesophilic Thiobacillus ferrooxidans in order to remove inorganic sulphur compounds (pyrite) has been studied. Shake flask experiments showed bacterial growth on the internal pyrite of the shale and no substrate toxicity. The bioreactor experiments demonstrated that the oxidation of pyrite by S. acidocaldarius is especially fast (96 % in four days). However, to evaluate the economic feasibility of the process, the high acid consumption of this oil shale has to be taken into consideration.

Introduction

Combustion of oil shale at thermal power plants gives rise to air pollutants such as fly ash, sulphur dioxide, nitrogen oxides, and carbon dioxide. Estonian oil shales used in Estonian and Baltic thermal power plants contain about 1.5 % (w/w) sulphur on an average, resulting in a major SO_2 pollution problem in the Baltic Sea region. About 20 million tonnes of oil shale are burnt every year at these plants, which produce 150 000 t of SO_2 per year [1].

There are three principle methods to prevent air pollution with SO_2 from fossil fuel: using air filters for cleaning flue gases, pretreatment with chemicals and pretreatment with sulphur oxidizing bacteria. According to Rossi [2] the microbial elimination of sulphur from fossil fuels has many preferences as compared with existing chemical and physical cleaning methods. Biological desulphurization can e. g. eliminate pyrite without destruction of the matrix and the biological process also decreases the amount of ash and is less expensive. The desulphurization of fossil fuels is based on the bacterial oxidation of reduced sulphur compounds present in the material.

Two groups of microorganisms have been used to remove inorganic sulphur from fossil fuels. One of these groups is mesophilic, acidophilic thiobacilli, which oxidize sulphur compounds at temperatures 25 to 40 °C. *Thiobacillus ferrooxidans* has been used in most of these studies of desulphurization [3]. *Thiobacillus ferrooxidans* is capable of oxidizing ferrous iron, reduced S compounds as well as sulphidic minerals such as pyrite.

The other group consists of thermophilic archaea, such as *Sulfolobus acidocaldarius*, *Sulfolobus thermosulfidooxidans* and *Acidianus brierleyi*, which oxidize reduced iron- and sulphur compounds at 50-80 °C.

These acidophilic microorganisms are also relevant in mineral beneficiation processes which employ biologically produced lixiviants for sulphide mineral solubilization [4]. Dump and heap leaching processes and biological pretreatment of refractory iron sulphides for gold recovery belong to such biohydrometallurgical applications of acidophilic microorganisms. It is well recognized in these applications that elevated temperatures and thermophilic microorganisms can greatly improve the kinetics of the bioleaching reactions [5].

Torma and Murr [6] reported that acidophilic thiobacilli (*Thiobacillus ferrooxidans*) can remove 90 % of the pyritic sulphur from the coal in ten days. By using thermophiles, more than 90 % of pyritic sulphur has been removed from coal materials [7]. Several authors have investigated coal desulphurization, whereas few investigations have dealt with removal of sulphur from oil shale. Vrvic et al. [8] reported that 95 % of the sulphur may be removed from the oil shale in one week by the mesophilic thiobacilli. At present there are no published data about the desulphurization of oil shale by thermophilic microorganisms.

Biological desulphurization of fossil fuels is influenced by several factors. One of them is the chemical content of metals, which can be inhibitory to the microorganisms. Also, ores from different places contain different amounts of pyrite, which therefore cause large variations in reaction rates [9].

The main objective of this work was to investigate the feasibility of Estonian oil shale for bacterial desulphurization, and to compare mesophilic and thermophilic bacteria in this process.

Material and Methods

Microorganisms

The experiments were carried out with cultures of *Thiobacillus ferrooxidans* strain TF-LR [10] and *Sulfolobus acidocaldarius* strain BC [11]. The bacterial cultures were grown aerobically in shake flasks on modified 9K medium (without iron as energy source) [12], using pyrite (5 % w/v) as an energy source. *Thiobacillus ferrooxidans* cultures were grown at 22 °C (room temperature), and *Sulfolobus acidocaldarius* cultures at 65 °C. The media were adjusted to pH 2 with 5M H_2SO_4 before inoculation with bacteria.

Oil Shale

The oil shale samples used in this work were from Estonian oil shale mining area (Ida-Virumaa, Estonia). Two samples with different iron (pyrite) content were used in the experiments. In shake flask experiments the sample with the lower, and in the bioreactor experiments - with the higher pyrite concentration was used. Oil shale was finely ground in a ball mill to particle size <75μm. The chemical composition and the acid consumption of the material are presented in Table 1.

Table 1. **Chemical Composition and Acid Consumption Data for Oil Shales Used**

	Oil shale	
	Sample 1	Sample 2
SiO_2, %	n.d.	1.60
Fe_2O_3, %	n.d.	4.15
MgO, %	n.d.	3.23
CaO, %	n.d.	30.00
Fe, mg/g	7.5	14.77
Na_2O, %	n.d.	0.04
K_2O, %	n.d.	1.00
P_2O_5, %	n.d.	0.07
L.O.I. , %	n.d.	56.00
Ti_2O, %	n.d.	0.18
Al_2O_3, %	n.d.	3.05
S, %	n.d.	4.75
Consumed H_2SO_4, g/kg (3 days)	428	400

Experiment Design

Shake flask experiments were carried out as follows: 100 ml of cultures, amended with (i) 5 % (w/v), (ii) 10 % (w/v) or (iii) 15 % (w/v) shale material, were grown in 250-ml shake flasks with agitation (200 rpm). Initially pH was adjusted to pH 2 with H_2SO_4 during three days before inoculation. In some cases preincubation solutions were removed after sedimentation and replaced with fresh modified 9K solution (called pre-treatment). This pretreatment was carried out in order to remove any solubilized compound, released due the pH adjustment. An additional energy source (120 mM Fe as $FeSO_4$) was used to enhance the bacterial growth. Samples of 3 ml were periodically withdrawn for pH, redox potential and iron concentration measurements.

Experiments were also carried out in stirred tank reactors (STR) (1.5 l working volume) [13] at constant aeration rate (300 ml air/l · min) and stirring (300 rpm). The STR experiments were conducted with CO_2 enriched (2 % v/v) air. 1.5 l of mineral salts solutions (modified 9K; modified 9K two-fold diluted; modified 9K ten-fold diluted) were amended with 10 % (w/v) of shale material. After the initial pH

adjustment the solutions were inoculated with 150 ml of an active bacterial culture ($5.2 \cdot 10^8$ cell/ml).

Samples of 10 ml were removed at indicated intervals for measuring the pH , redox potential and iron concentrations. The samples were prepared (i) for measurements of total leached iron, Fe (tot), - 0.2 ml sample was digested with 1.8 ml of 5M HCl at 65 °C for 2 hours to dissolve any precipitates formed during leaching; (ii) for measurements of iron in solution, Fe (sup), - after centrifugation 0.2 ml supernatant was diluted with 1.8 ml 5M HCl; (iii) for measurements of iron content in oil shale residues, Fe (res), - 8 ml of the sample was centrifuged and the pellet was dried for two days at 105 °C. Subsequently the pellet was weighed, treated with 5M HCl (at 65°C and 2h) to dissolve all precipitates, washed twice with 1M HCl and once with distilled water. The remaining residue was digested in 10 ml of HNO_3-HCl mixture (1 : 3) and boiled until dryness. The process was repeated. Then the dry sample was dissolved in 1 % (v/v) of HCl and used for total iron measurement by atomic absorption spectroscopy (AAS).

The concentration of ferrous iron in the leach solutions was measured titrimetrically with cerric sulphate using the 1.10-phenantroline as indicator [14].

Most of the sulphur in oil shale is present as pyrite. Because of difficulties to measure the amount of leached sulphur (the sulphur content in 9K solution is too high comparing with sulphur content of the ore) we measured the iron (pyrite) instead of it.

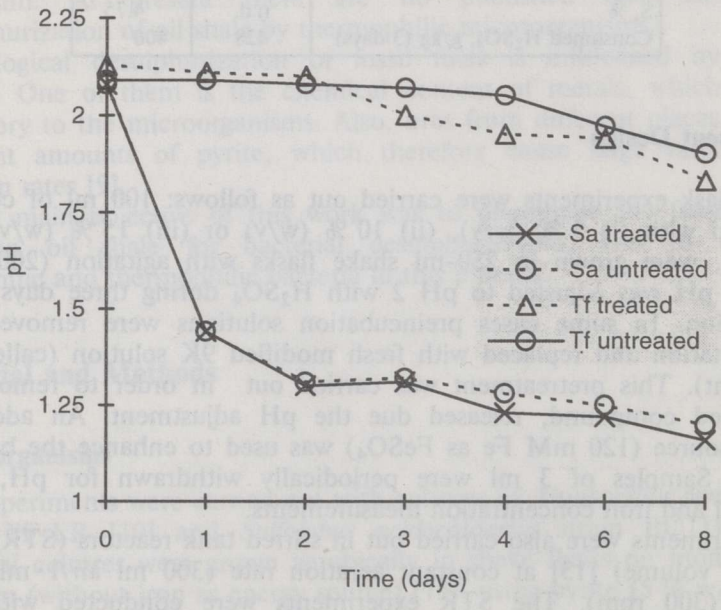

Fig. 1. Shake flask experiments with *Sulfolobus acidocaldarius* (Sa) and *Thiobacillus ferrooxidants* (Tf) showing changes in pretreated and unpretreated samples, both with additional energy source (as $FeSO_4$)

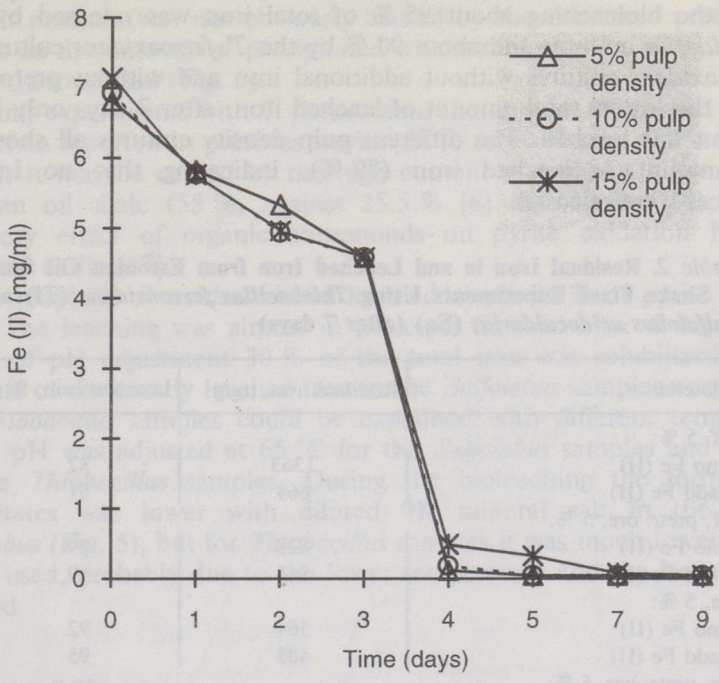

Fig. 2. Shake flask experiments with *Sulfolobus acidocaldarius* showing ferrous iron oxidation for different pulp densities of oil shale (added 120 mM Fe as $FeSO_4$)

Results and Discussion

Shake Flask Experiments

Shake flask experiments were carried out to test: (i) substrate toxicity, and (ii) growth of bacteria on the internal pyrite of shale.

Shale materials consist of many metals and some organic compounds, which may solubilize and inhibit the growth of bacteria [15]. Our previous experiments with Estonian black shale (unpublished) indicated that in the course of chemical leaching (adjustment of pH) some elements were released from the substrate wich inhibited the growth of *Thiobacillus ferrooxidans*. The ferrous iron oxidation (not shown) and pH curves (Fig. 1) in our experiments with oil shale showed similar curve trends for the differently treated samples, indicating that no inhibiting substances were released. The pH -drop, from 2.1 to 1.3 in two days, was more drastic in the experiments with *Sulfolobus acidocaldarius* (Fig. 1.).

The growth of *Sulfolobus acidocaldarius* on 5 % (w/v), 10 % (w/v) and 15 % (w/v) pulp densities is shown in Fig. 2. No difference in ferrous iron oxidation rates by different oil shale pulp densities was seen. However, the pH decrease was highest for 5 % (w/v) of pulp density (not shown).

In Table 2 the iron remaining in the oil shale residues after seven days of bioleaching is presented. During the course of chemical leaching (period

of stabilization pH, 3 days) already 58 % of the iron was remained. At the end of the bioleaching about 95 % of total iron was released by *the S. acidocaldarius* cultures and about 90 % by the *T. ferrooxidans* cultures. The *T. ferrooxidans* cultures without additional iron and without pretreatment showed the lowest total amount of leached iron, after 7 days only 82 % of total iron was leached. The different pulp density cultures all showed the same amount of leached iron (79 %), indicating that no inhibiting substances were released.

Table 2. **Residual Iron in and Leached Iron from Estonian Oil Shale in Shake Flask Experiments Using *Thiobacillus ferrooxidans* (Tf) and *Sulfolobus acidocaldarius* (Sa) (after 7 days)**

Material	Residual iron, μg/g	Leached iron, %
Tf, 5 %:		
no Fe (II)	1363	82
add Fe (II)	669	91
Tf, pretr. ore, 5 %:		
no Fe (II)	828	89
add Fe (II)	844	89
Sa, 5 %:		
no Fe (II)	564	92
add Fe (II)	405	95
Sa, pretr. ore, 5 %:		
no Fe (II)	314	96
add Fe (II)	378	95
Sa, 5 %, add Fe (II)	1571	79
Sa, 10 %, add Fe (II)	1571	79
Sa, 15 %, add Fe (II)	1660	78
Original oil shale	7500	0
Oil shale after pH regulation	3158	58

These shake flask experiments have shown that the *T. ferrooxidans* and *S. acidocaldarius* can use oil shale as growth substrate. The amount of internal pyrite leached was similar in samples with and without additional energy source, suggesting the active use of the oil shale substrate (Table 2). There was no inhibitory effect on growth of *T. ferrooxidans* or *S. acidocaldarius*, even at the higher concentrations of substrate.

Stirred Tank Experiments

Bioreactor experiments were carried out to test oil shale pyrite oxidation under more controlled conditions. In Figure 3 to Fig. 5 the results with *T. ferrooxidans* and *S. acidocaldarius* are shown.

The *T. ferrooxidans* cultures leached 53 % to 67 % of pyrite from the shale in 11 days. In the bioreactor experiments only 5 % to 15 % of the initial iron was leached due to the chemical leaching, which is lower than in shake flask experiments. The biological leaching was highest with the more diluted mineral salt solution (9K/10), which can be explained by that the pyrite oxidation rate is sensitive to jarosite formation, which decreases

by using low salt media [16]. In our experiments the elevated leaching on low salt medium was not obviously caused by the decreased precipitation process as the amount of precipitated iron was similar in all mineral salt concentrations used (Fig. 5).

In our experiments with *T. ferrooxidans* the amount of leached iron was lower than reported in previous works (50 % to 70 % resp. 90 % to 95 %) [8, 15]. It may be caused by the high content of organics - kerogen - of Estonian oil shale (55 %, against 25.5 % [8] and 14 % [19]), because inhibitory effect of organic compounds on pyrite oxidation has been described earlier[17].

Thermophilic *S. acidocaldarius* leached about 94 % of pyrite from oil shale. The leaching was almost in principal finished after 4 days. In the course of pH adjustment 30 % of the total iron was solubilized. Higher amounts of chemically leached iron in the *Sulfolobus* samples compared to the *Thiobacillus* samples could be explained with different temperatures used - pH was adjusted at 65 °C for the *Sulfolobus* samples and at 25 °C for the *Thiobacillus* samples. During the bioleaching the formation of precipitates was lower with diluted 9K mineral salt in the case of *Sulfolobus* (Fig. 5), but for *Thiobacillus* samples it was much lower for both media used, probably due to the lower temperature and the low total iron released.

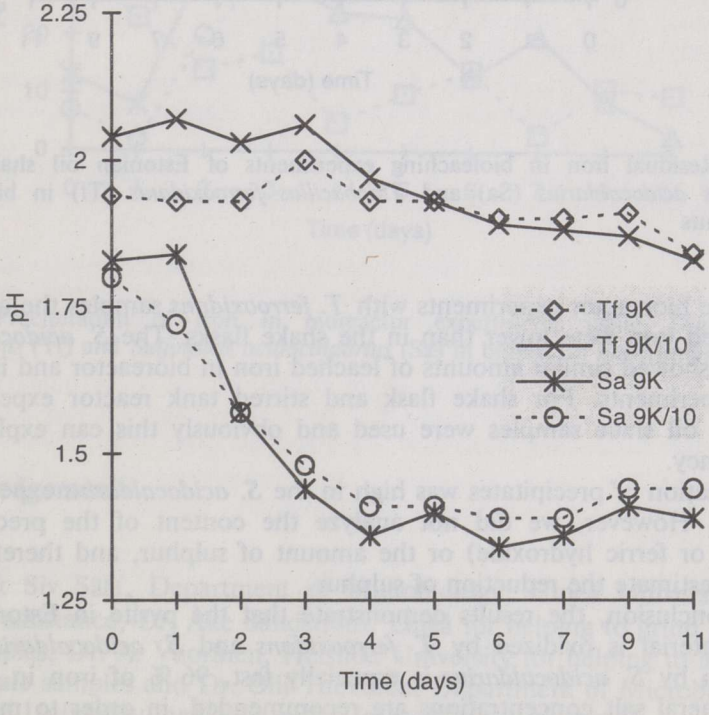

Fig. 3. pH changes in bioleaching experiments of Estonian oil shale using *Sulfolobus acidocaldarius* (Sa) and *Thiobacillus ferrooxidants* (Tf) in bioreactor experiments

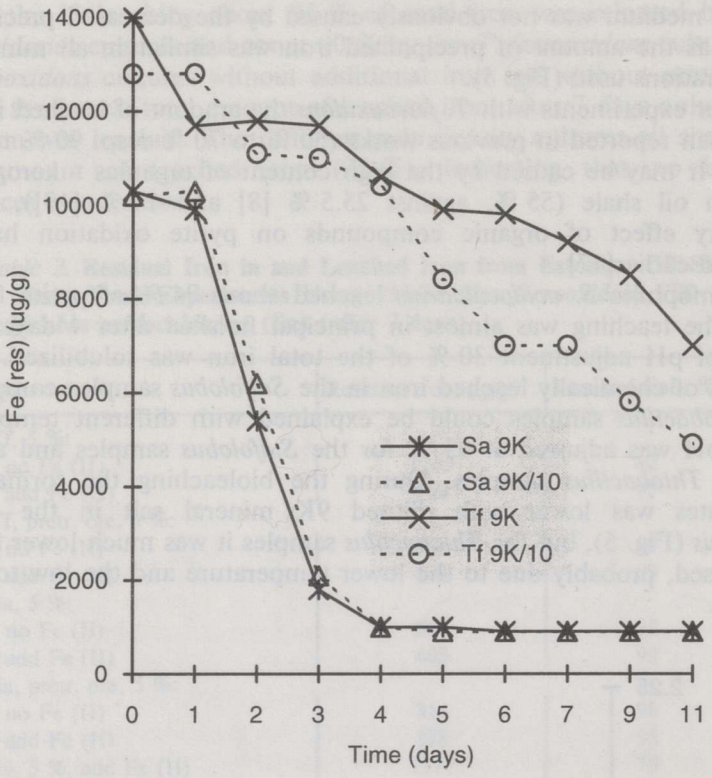

Fig. 4. Residual iron in bioleaching experiments of Estonian oil shale using *Sulfolobus acidocaldarius* (Sa) and *Thiobacillus ferrooxidants* (Tf) in bioreactor experiments

In the bioreactor experiments with *T. ferrooxidans* samples the amounts of leached iron were lower than in the shake flasks. The *S. acidocaldarius* samples showed similar amounts of leached iron in bioreactor and in shake flask experiments. For shake flask and stirred tank reactor experiments different oil shale samples were used and obviously this can explain the discrepancy.

Formation of precipitates was high in the *S. acidocaldarius* experiments (Fig. 5). However, we did not analyze the content of the precipitates (jarosite or ferric hydroxide) or the amount of sulphur, and therefore we can not estimate the reduction of sulphur.

In conclusion, the results demonstrate that the pyrite in Estonian oil shale material is oxidized by *T. ferrooxidans* and *S. acidocaldarius*. The oxidation by *S. acidocaldarius* is especially fast, 96 % of iron in 4 days. Low mineral salt concentrations are recommended, in order to minimize the formation of precipitation. To remove the remaining precipitates an extra acid washing of the residue or removing the pH adjustment solution prior to bioleaching is needed to reduce sulphur content of the starting

material. Bioleaching of oil shale by acidophilic bacteria is possible, but before evaluation the economic feasibility of the process, the reduction of the high acid consumption of oil shale is needed (for example to remove acid consuming calcite by a flotation process). Biological desulphurization of Estonian oil shale without any treatment cannot be used in practice, because of high calcite concentration. The acid consumption of the natural oil shale (about 400 g H_2SO_4/kg) is higher than economically feasible.

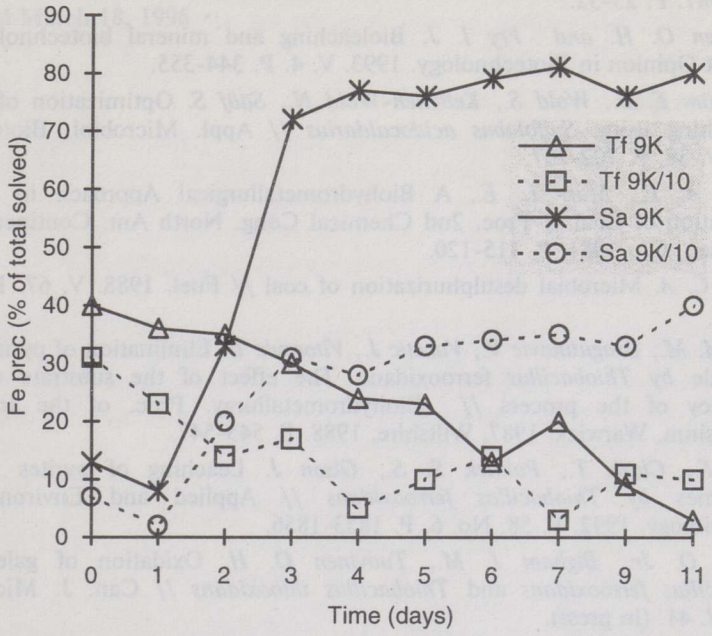

Fig. 5. Precipitation of iron in bioreactor experiments using *Thiobacillus ferrooxidants* (Tf) and *Sulfolobus acidocaldarius* (Sa) in bioreactor experiments

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