DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
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BIOLOGICAL LEACHING OF SHALES: BLACK SHALE AND OIL SHALE

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TARTU 1998

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PRESS

Dissertation is accepted for the commencement of the Doctor of Philosophy (in Genetics) on June 22th, 1998 by the Council of the Institute of Molecular and Cell Biology, University of Tartu

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Commencement: October 23th, 1998

The publication of this dissertation is granted by the University of Tartu

Tartu Ülikooli Kirjastuse trükikoda
Tiigi 78, EE2400 Tartu
Tellimus nr. 235

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The presented thesis is based on the following original papers which will be referred to by their Roman numerals:

I. **Tasa, A.**, Garcia, O. Jr., Bigham, J. M., Vuorinen, A. and Tuovinen, O. H. (1995) Acid and biological leaching of a black shale from Toolse, Estonia. In: Biohydrometallurgical Processing, Vol. I (Vargas, T., Jerez, C. A., Wiertz, J. V., Toledo, H. eds.), University of Chile, Santiago, Chile, 229–238.

II. **Tasa, A.** and Lindström, E. B. (1996) Biological desulphurization of Estonian oil shale. Oil Shale 13: 133–143.

III. **Tasa, A.**, Vuorinen, A., Garcia, O. Jr. and Tuovinen, O. H. (1997) Biologically enhanced dissolution of a pyrite-rich black shale concentrate. J. Environ. Sci. Health A32 (9&10): 2683–2695.

IV. **Tasa, A.** and Tuovinen, O. H: Biological leaching of shales — a review. Oil Shale 15: in press.

LIST OF ABBREVIATIONS

AAS	atomic absorption spectroscopy
AGP	acid generation potential
ANP	acid neutralisation potential
E_a	activation energy
L.O.I.	loss of ignitation
NAG	net acid generation
Q_{10}	temperature quotient
S-compounds	sulphurous compounds
STR	stirred tank reactor
XRD	X-ray diffraction
9K	growth medium for *Thiobacillus ferrooxidans* and other chemolithotrophic acidophiles

1. INTRODUCTION

Micro-organisms' possibility to extract metals from the ores, a process known as bioleaching, has gained increasing attention in recent years. Beginning from 40-ties, when Colmer and Hinkle (1947) isolated and described bacteria with the capacity of acceleration the oxidation of ferrous iron, both the fundamental and applied aspects of mineral biodegradation phenomena have been studied. The recovery of metals (copper, uranium, etc.) by biologically assisted leaching methods (dump and heap mining techniques) are currently practised in a number of countries. Reactor- and high temperature leaching have been applied for the release of high-value components from the ores (gold, silver). It is estimated that 10 to 50 million USD worth of gold was recovered by biooxidation in 1988 and that this value would increase to 2 to 3 billion USD by 1998 (Blake *et al.*, 1994). Micro-organisms play beneficial role in the mineral processing of metals, but on the other hand, the same bacteria may cause environmental problems. Acid rock drainage and leaching of heavy metals from waste rock heaps have to be mentioned. Most of the bioleaching studies have been carried out with sulphide ores or high-sulphur coal samples. There are only few reports on the bacterial leaching of shale materials available.

Oil shale is the most important mineral resource in Estonia. The other shale, black shale, has been mentioned as potential mineral resource in Estonia (Maremäe, 1988). The extensive deposits of oil shale around the world are considered important fuel reserves for the future (Russel, 1990). The shale studies have strong output to practice. Using the results of the studies new alternative and environmentally sound methods might be tested for industry as well as environmental protection.

The aim of the present work was to assess the bacterial leaching of Estonian shale samples and to broaden the understanding of basic processes of shale bioleaching. This interdisciplinary study might be of interest for microbiologists, geologists, environmentalists and technologists. In the first part of the present paper, the present state of studies of leaching of Estonian shales and the subject of bioleaching will be reviewed by literature. In the second part of the paper, experimental results of the studies of Estonian shales bioleaching are presented.

2. REVIEW OF LITERATURE

2.1. Estonian shales

Shales are fine-grained sedimentary rocks that are formed by the consolidation of beds of mud, clay or silt. These beds were deposited slowly over geological time. Shales consist mainly of clay minerals such as illite and montmorillonite, mixed with fine particles of quartz and mica. Two type of shales have been represented in Estonia, black shale and oil shale, both of them belong to the group of oil shale.

2.1.1. Black shale

Black shale (also called as dictyonema argillite, graptolite argillite, Dictyonema shale) layers with the thickness up to 7 m are distributed in Northern-Estonia. The black shale includes organic material (kerogen), illite, quartz, K-feldspar, pyrite and some other minerals in small quantities (muscovite, biotite, etc.) (Kleesment, Kurvits, 1987). Estonian black shale contains up to 20% of organic matter and 4 to 6% of pyrite. Most of the pyrite presents in the shale with a fine-dispersed form (Kallaste and Pukkonen, 1992). Common feature for Estonian black shales as all others Baltoscandian black shales of lower paleozoic age is relatively high concentration of metals (Andersson, *et al.*, 1985). For example Estonian black shale consists molybdenum up to 1990 ppm, uranium up to 1038 ppm and vanadium up to 1910 ppm (Pukkonen, 1989, Pukkonen and Rammo, 1992). According to Palvadre and Kleemeier (1982) about 30% of uranium in the black shale is bind with organic matter, about 30% with clay minerals, about 30% with phosphates and about 6% with pyrite.

Black shale has been titled as a potential mineral resource in Estonia. According to Maremäe (1988) the possible ways of utilisation of black shale might be following: (i) the raw material for building materials; (ii) the component for cement production in the mixture with limestone; (iii) fertiliser and plant growth stimulant and (iiii) raw material for extraction of metals. The last way has been considered the most advantageous. After the World War II Soviet government, looking for raw material for nuclear industry, selected the black shale as one of the potential source of uranium. Investigations were started at the Institute of Mineral Resources, at the Institute of Chemical Technology (both in Moscow) and at the Institute of Chemistry, Estonian Academy of Sciences (in Tallinn). Three different leaching technologies were tested: autoclavical, biochemical and chemical (Althausen, 1992). Autoclavical and biochemical leaching studies were carried out by Russian scientists. These results were treated as strategic studies and up to now the data of the studies are not

known to the public. The only published material about the work is the presentation at the International Conference on Use of Micro-organisms in Hydrometallurgy in 1980 (Iskra *et al.*, 1980). The presentation gives no details of the studies, it mentions only that the shales are good material for leaching. The results of black shale chemical leaching studies have been published (Palvadre and Kleemeier, 1982; Maremae and Kirret, 1989, 1990; Palvadre *et al.*, 1990; Maremae *et al.*, 1991; Palvadre and Ahelik, 1992; etc.). The influence of the treatment conditions and experimental parameters as well as substrate material (ash, mineral —, pyrite — and organic rich ore) on the leaching of metals from the ore were studied. Higher amount of leached metals was gained by percolative leaching of sulphated black shale ash. As a result of chemical leaching 73% of molybdenum, 90% of vanadium, 85% of uranium, 66% of titanium, 74% of iron, 48% of aluminium and 26% of potassium was extracted (Maremae *et al.*, 1991).

The main environmental problem concerning black shale in Estonia is due to its geological position. Phosphoritic layers often underlie the shale deposits and during the open cast mining of phosphorite the black shale are disposed in mined-out parts as a waste material. These waste materials have a history of self-combustion and have been a continuous source of dissolved metals in leach waters. Phosphorite has been mined in Estonia since 1923, firstly underground and since middle 1950-ties by opencast way. The area of 1300 hectares was detoriated, 24.1 million m^3 of black shale as a waste was buried in mined-out parts. Naumov (1991) reported that from 1977 to 1986 19 t of Cu, 90.4 kt of Mn and 162 kt of [SO_4^-] were leached to the Gulf of Finland from the black shale dumps.

2.1.2. Oil shale

Oil shale is called "oil shale", because it contains organical matter, that can be processed to yield a petroleum-like oil. Like petroleum, shale oil can be refined into gasoline, fuel oil and many other products. Comparing with the black shale the content of organical matter in oil shale is higher than in black shale (up to 45%). Organical substance of the rock, called kerogen, is in most of oil shales interspersed among fine-grained minerals. In addition to organical matter oil shale contains quartz, carbonates, silicates, pyrite, gypsum and some other minerals in small quantities. Oil shale may contain metals (U, Mo, V, Re, Ge, Be), but the concentration of them is much lower than in black shale.

Commercial extraction of Estonian oil shale began in 1916. The use of oil shale for energy began in 1924, when Tallinn Thermal Power Plant was rearranged to burn oil shale. In 1992 the output of electrical power generated by thermal power plants was about 16.5 to 17 Twh and for that reason has consumed 22.2 Mt of oil shale (Ots, 1992).

Main environmental problem connected with the oil shale in the Baltic Sea region is caused by the release of the SO_2 during the combustion of the material in thermal power plants. Estonian oil shales contain about 1.5% sulphur on an average. Each year 150 000 t of SO_2 will evaporate to atmosphere (Kaljuvee and Kuusik, 1993).

There are two different methods to prevent air pollution with SO_2 from fossil fuels. First way is to clean the released flue gases and the other is to pretreat the oil shale ore before combustion. For flue gas cleaning there are also two methods: using air filters in the chimneys of thermal power plants or to use chemical reagents for desulphurization. Limestone, calcium oxide, calcium hydroxide, dolomite, sodium carbonate, silicalite and other alkali compounds as dry sorbents as well as different ashes (furnace ash, cyclone ash) have been tested for desulphurisation of flue gases (Kaljuvee and Kuusik, 1993). Pretreatment methods for desulphurization are divided into chemical- and biological methods. Biological pre-treatment method will be described in detail in following paragraphes. For chemical desulphurization H-donor potential of oil shale organic matter and pyrogasification have been studied. These studies are in the stage of development now and there are no pilot-scale test results available. (Yefimov *et al.*, 1995).

2.2. Biological leaching of ores

Biological leaching is defined as the chemical attack of an ore in the presence of bacteria, directly or indirectly participating in the process (Ballester *et al.*, 1992). In the last twenty years many investigators all over the world have studied the process and mechanism of biological leaching of low grade sulphide minerals as well as the factors influenced on and bacteria involved.

The microbial oxidation of iron and sulphur compounds are two key processes in mineral biotechnology. Micro-organisms capable of degrading inorganic sulphur- and ferrous-compounds include mesophiles (optimal growth temperature at 20–40°C), moderate thermophiles (35–55°C), and thermophiles (45–80°C). Some samples of micro-organisms that have potential capacity in the iron- or/and sulphurous compounds oxidation are presented in Table 1. Most of these micro-organisms are autotrophic acidophiles, i.e., they use CO_2 as a carbon source and prefer acidic environment (pH 1 to 3). The biological oxidation of pyrite and ferrous iron is confined to aerobic environments where oxygen is the electron acceptor. The oxidation of sulphurous compounds also occurs under anaerobic conditions and it is coupled with ferric iron as the electron acceptor in acidophiles, and with nitrate in neutrophilic, denitrifying thiobacilli (e.g., *Thiobacillus denitrificans*).

Thiobacillus ferrooxidans is a Gram-negative mesophilic bacterium, which is regarded as the principal organism involved in the leaching of metal sulphide ores (Hutchins *et al.*, 1986). Its physiological and biochemical properties (especially related to commercial exploitation) have been quite well studied. *Thiobacillus ferrooxidans* derives energy for growth by the oxidation of reduced compounds of iron and sulphur including many different sulphide minerals.

For bioleaching two principal mechanisms, direct and indirect, have been proposed.

Table 1. Examples of micro-organisms that have potential capacity in the iron- or/and sulphurous-compounds oxidation.

Broad Temperature Range	Micro-organisms	Characteristic Inorganic Substrate Spectrum
Mesophiles (up to 45°C)	*Thiobacillus ferrooxidans*	Fe^{2+} and S-compounds, sulphide minerals
	Thiobacillus thiooxidans	S-compounds, sulphide minerals
	Leptospirillum ferrooxidans	Fe^{2+}, pyrite
Moderate thermophiles (35 to 55°C)	*Thiobacillus caldus*	S-compounds, sulphide minerals
	Leptospirillum thermoferrooxidans	Fe^{2+}, pyrite
	Sulfobacillus thermosulfidooxidans	Fe^{2+}, sulphide minerals
	Sulfobacillus acidophilus	Fe^{2+}, sulphide minerals
	Acidimicrobium ferrooxidans	Fe^{2+}, pyrite
Thermophiles (50–80°C)	*Acidianus brierleyi*	Fe^{2+}, S-compounds, sulphide minerals
	Sulfolobus acidocaldarius	Fe^{2+}, S-compounds, sulphide minerals
	Sulfolobus metallicus	S^{o}, sulphide minerals
	Sulfurococcus yellowstonii	Fe^{2+}, sulphide minerals, S^{o}
	Metalloshaera sedula	Fe^{2+}, sulphide minerals, S^{o}
	Metallosharea prunae	Sulphide minerals, S^{o}, H_2

In the case of direct bacterial leaching mechanism the bacteria are in direct contact with the substrate and the oxidation of the material will be as following:

$$4\ Fe^{2+} + O_2 + 4\ H^+ \rightarrow 4\ Fe^{3+} + 2\ H_2O$$

$$S^0 + 1.5\ O_2 + H_2O \rightarrow H_2SO_4$$

$$2\ FeS_2 + 7.5\ O_2 + H_2O \rightarrow 2\ Fe^{3+} + 4\ SO_4^{2-} + 2\ H^+$$

In the case of indirect mechanism the micro-organisms catalyse the oxidation of ferrous iron to ferric iron. The ferric iron is a chemical oxidising agent

of pyrite and thereby enhances the bacterially mediated oxidation. The abiotic redox reaction reduces ferric iron to ferrous, which is then re-oxidised by *Thiobacillus ferrooxidans*.

$$FeS_2 + 2Fe^{3+} \rightarrow 3Fe^{2+} + 2\ S^0$$

Other acidophilic micro-organisms with somewhat similar metabolic activities include *Thiobacillus thiooxidans* and *Leptospirillum ferrooxidans*. *Thiobacillus thiooxidans* oxidises inorganic S-compounds but not iron, and is active at pH values as low as 0.5. *Leptospirillum ferrooxidans*, originally described by Markosyan (1972), oxidises only inorganic ferrous compounds. All three acidophiles are common among micro-organisms in active and abandoned mine sites. Factors contributing to the relative predominance of *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, and *Leptospirillum ferrooxidans* in mine sites and acidic runoffs are poorly understood. Natural environments are usually characterised by diverse iron- and sulphur oxidising micro-organisms, and such microbial consortia have many interactions and commonly involve various heterotrophic organisms and facultative anaerobes (Johnson, *et al.*, 1992). In laboratory conditions mixed cultures can rapidly oxidise FeS_2 but it is difficult to maintain consortia that have stable proportions of *Thiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* and other bacteria in laboratory experiments (Tuovinen *et al.*, 1991).

Mesophiles thrive at temperature ranges of up to about 40 to 42°C but are inactivated at excessively high temperatures. However, reaction kinetics and growth rates can be greatly improved by increasing the prevailing temperatures to the thermophilic range that supports growth between 40 to 80°C.

Moderately thermophilic acidophiles have been described as comparable to the mesophilic *Thiobacillus* and *Leptospirillum* in terms of the range of inorganic sulphur- and iron-compounds that they can oxidise for energy. *Thiobacillus caldus* (Hallberg and Lindström, 1994) is a sulphur-oxidiser and grows best at temperatures from 45 to 50°C. It seems to be present in mixed cultures to afford sulphur oxidation capacity that is completely absent in *Leptospirillum ferrooxidans*-like iron-oxidising bacteria. Some *Leptospirillum* isolates (e.g., *Leptospirillum thermoferrooxidans*) have been noted as being moderately thermophilic and capable of growing in the temperatures 40 to 50°C range (Golovacheva *et al.*, 1992). *Sulfobacillus thermosulfidooxidans* and *Sulfobacillus acidophilus* as well as many other gram-positive, unnamed isolates have been characterised capable of oxidising several sulphide minerals (Norris, 1997). Other moderate thermophiles include iron-oxidising isolates of *Acidimicrobium ferrooxidans* derived from *Sulfobacillus* cultures (Clark and Norris, 1996). In general, there is considerable diversity in substrate oxidation by different isolates of moderately thermophilic acidophiles. These organisms have been isolated from stable mixed cultures, suggesting cultural interactions at least at a nutritional level.

Thermophilic Archaea of the genera *Sulfolobus* and *Acidianus* can oxidise inorganic sulphurous-compounds associated with coal and oil shales. Larsson *et al.* (1990, 1994) tested several species of Archaea and obtained the most promising results of sulphur removal from coal with *Acidianus brierleyi*. The organism also enhanced the solubilisation of organic sulphurous compounds although the biological mechanism remains unknown (Larsson *et al.*, 1990). It was also reported that *Acidianus brierleyi* was not adversely influenced by organic compounds leached from coal, whereas *Sulfolobus acidocaldarius* and *Sulfolobus solfataricus* were inhibited by coal leachates. Such findings are likely to be coal-specific and may not be reproducible with samples from other sources.

Several concurrent reactions take place during the mineral leaching. In addition to the processes caused by bacteria the minerals themselves have the influence on the leaching processes. The presence of different mineral phases causes the formation of galvanic couples in which the solid with the higher and more negative rest potential is leached preferentially. The process described is called electrochemical reaction and it is based on the electrode potential of the participating mineral and the redox potential of the leach solution.

The kinetics and efficiency of biological leaching is a complex function of many interactive factors. These include the metabolic capabilities of microorganisms involved, temperature, acidity, pO_2, pCO_2, nutrients (e.g., P, N, microelements), toxic compounds, mass transfer rates, mineralogy and chemical composition of ore, and specific surface area of solids. Many of these factors have been discussed in reviews that focus on the bacterial leaching of metals from sulphide ores (Tuovinen *et al.*, 1991; Tuovinen, Fry, 1993; Morin, 1995; Schnell, 1997). Testing of experimental variables is extremely crucial in developing information that can eventually be used for optimisation and kinetic modelling.

Most of the iron and sulphur oxidising bacteria have the optimum pH between 1.5 and 3. Both, the biological and chemical leaching, rates are influenced on pH. The thiobacilli can tolerate pH values to 6, but in practice at pH values above 3 the chemical oxidation of ferrous iron exceed many times the biological and the bacteria can not successfully compete for the substrate (Kuenen *et al.*, 1992).

As with all biological reactions, the rate of iron and sulphur oxidation is highly dependent on the temperature. Decrease in the temperature below the optimum is accompanied by a decline in the rate of substrate oxidation and an increase in the generation time. Different strains of the same species of bacteria may have slightly different temperature optima. With *Thiobacillus ferrooxidans*, optimum temperatures reported in the literature range mostly from 25 to 35°C. The maximum permissive temperatures for mesophilic thiobacilli are around 40–44°C (Niemelä *et al.*, 1994) but considerable strain-dependent variation is likely to exist.

Ahonen and Tuovinen (1990) reported that the temperature quotient Q_{10} for iron and sulphur oxidation was about 2. The Q_{10} is defined as the increase in the rate or rate constant of the reaction for each 10°C in the temperature. The activation energies (E_a) were 80 kJ mol^{-1} and 65 kJ mol^{-1}, respectively. The effect of temperature in biological leaching systems is complex because of the formation of reaction zones that before diffusion barriers and decrease the temperature-dependence of the reaction. Moreover, elevated temperatures favour the rate of chemical reactions but decrease the solubility of O_2 and CO_2.

Acid leaching systems contain numerous water-soluble constituents that are potentially toxic or inhibitory to biological processes. These toxicity effects may be due to inorganic ions (metal ions) or organic compounds leached from the material. The toxicity varies with the bacterial strain, environmental conditions, the length of exposure, and the concentration of the element. By comparison with heterotrophic bacteria living in circumneutral pH values, acidophilic thiobacilli can tolerate relatively high concentrations of Cu, Co, Ni, and Zn in their growth medium. This property is one of the underlying requisites for their use in metal leaching processes. The biochemical basis for this high level of resistance is not known. Silver, mercury, and molybdenum are among the most toxic metals to iron- and sulphur-oxidisers. Specific mechanisms of toxicity and resistance are mostly unknown. Biochemical basis of resistance to toxic metals is known in the case of mercury and it involves a mercuric reductase enzyme complex that reduces Hg^{2+} to Hg^0 (Booth and Williams, 1984). Elemental mercury thus formed is removed from the cell and growth medium because of its volatility. The toxicity of metals to bacteria is subject to their bioavailability which can be altered with chelating agents; e.g., yeast extract can decrease the effective, bioavailable concentration of metals because of a complexation effect. In the presence of clay minerals (e.g., kaolinite and illite) and oxides (Al_2O_3), bacteria can be partially protected owing to cation exchange properties of clays that decrease the available concentrations of metals (Friedrich *et al.*, 1991).

2.3. Bioleaching studies of shale materials

Most of the bioleaching studies have been carried out with sulphide ores or coal. There are only few reports on the bacterial oxidation of pyrite in shale materials. Shale-containing seams have been disposed of in many mines as waste materials and, when exposed to rain water, humidity, oxygen and native bacteria over time, they become continuous sources of dissolved metals in receiving waters. Biological enhancement of metal leaching has been reported for black shales in different geographical regions (Iskra *et al.*, 1980, Konopka *et al.*, 1991, 1993).

Research about bacterial leaching of black shale in U.S.S.R. was carried out already in 60-ties and 70-ties, but there are no details of the studies since that time. The interest of soviet scientists was aimed to uranium leaching processes. The only published material about the work of Russian scientists available for general public is the presentation at the International Conference on Use of Micro-organisms in Hydrometallurgy on 1980 (Iskra *et al.*, 1980), already mentioned above.

Investigation of shale bioleaching are mostly carried out by practical reasons, first of all for the leaching of heavy metals (Konopka *et al.*, 1993). The other reasons are solubilisation of pyrite for sulphuric acid production (Mahapatra *et al.*, 1985) and preparation of kerogen concentrates (Findley, 1974; Vrvic, *et al.*, 1987).

Series of experiments about heavy metal leaching from the shales have been carried out by Polish scientists (Sztaba *et al.*, 1989; Konopka, Sztaba, 1991; Konopka *et al.*, 1993). The leaching of uranium, vanadium, molybdenum, zinc and lead was investigated in long term (7 months) experiments. Biological leaching of zinc and lead was insignificant. 95% of zinc and 55% of lead were leached chemically in the initial period of the experiments. Maximum biologically recovered amounts of uranium, molybdenum and vanadium were in these experiments as follows: 75% for uranium, 60% for molybdenum and 47% for vanadium.

Some shale materials are rich of pyrite, or the pyrite layers are between the shale layers difficult to separate. For example shale from India (Amjhore region) consists 11.43% of pyritic iron as an average (Mahapatra, 1985). Most of the shales consist the pyrite in the amount, which guarantees the life of acidophilic bacteria. In shales, where the pyrite concentration is quite high, the pyrite itself might be interesting economically for sulphuric acid production. In most ores the pyrite can be looked as a substrate supporting bacterial growth and bioleaching activities. In fossil fuels the sulphur (inorganic sulphur is present mostly as pyrite) is the source for environmental pollution and the subject for removal.

Vrvic *et al.* (1987) reported that 95% of the sulphur was removed from the oil shale (from Aleksinac, former Yugoslavia) in a week by exposing the finely ground oil shale to the leaching action of *Thiobacillus ferrooxidans*. Mahapatra *et al.* (1985) achieved 96% removal of pyrite from a black shale sample (from Amjhore, India) after three weeks of leaching in *Thiobacillus ferrooxidans* cultures.

While pyrite inclusions in shales are biologically degradable, the depyritisation process greatly depends on the substrate type. Alkaline minerals (calcite, dolomite) cause acid-consumption that needs to be neutralised with pre-treatment with sulphuric acid (Vrvic, 1987, 1988). In past efforts to neutralise acid-consumption due to dolomite-rich zones in shales, H_2SO_4 has been produced via biological oxidation of supplementary elemental sulphur (Findley *et al.* 1974).

3. MATERIALS AND METHODS

3.1. Shale samples

The black shale samples used in this work were from Toolse and Maardu phosphorite deposit in Estonia. The material included samples of the native (unprocessed) shale, flotation concentrates with high organic C (kerogen) content, flotation concentrates with high pyrite content, and ash residues after combustion of the shale at 800°C. The samples were finely ground (68.9% ¨ <50μm; 14.9% 50μm < ¨ > 100 μm; 9.4% 100 μm < ¨ > 200 μm; 6.8% ¨ > 200 μm) in ball mill. Mineralogical characteristics and the partial elemental analysis of the shale samples are presented in Table 2 and Table 3. All samples contained elevated levels of U, Mo and V.

The oil shale samples used in this work were from Estonian oil shale mining area (Ida-Virumaa, Estonia). The samples with 7.5 to 14.77 mg/g of iron (pyrite) were used in the experiments. The oil shale was finely ground in a ball mill to particle size <75 μm. Mineralogical characteristics and the partial elemental analysis of the ores are presented in Table 2 and Table 3. The acid consumption of the oil shale was about 400 g of concentrated H_2SO_4 per kg.

3.2. Bacteria and culture media

Acidophilic thiobacilli, *Thiobacillus ferrooxidans* (strains TF-135 [Tuovinen *et al.*, 1994]; TF-LR [Garcia, 1991]); *Thiobacillus thiooxidans* (strain Tt-FG01 [Garcia, 1991]), and thermophilic archaea, *Sulfolobus acidocaldarius* (strain BC [Norris and Parrot, 1986]) were used in this study. The thiobaccilli cultures were grown aerobically at 22±2°C and *Sulfolobus acidocaldarius* cultures at 65°C in shake flasks (200 rev/min) in mineral salts medium 9K (Silverman and Lundgren, 1959), which contained (per litre) 0.5 g $(NH_4)_2SO_4$, 0.5 g K_2HPO_4, and 0.5 g $MgSO_4*7H_2O$. Media for *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* were supplemented with 33.3 g $FeSO_4*7H_2O$/litre or 5% (wt/vol) of pyrite and 10 g S^0/litre, respectively. Media for *Sulfolobus acidocaldarius* were supplemented with pyrite (5%, wt/vol). The media were adjusted to pH 1.8 with 5 M H_2SO_4 before inoculation with bacteria.

Table 2. Mineralogical characteristics of Estonian shale samples used in leaching experiments.

Sample	Qualitative Mineralogical Composition
Natural (unprocessed) black shale from Toolse phosphorite deposit	Quartz, K-feldspar (orthoclase), pyrite, illite, kaolinite
Kerogen-rich flotation concentrate of Toolse black shale	Quartz, K-feldspar (orthoclase), pyrite, illite, kaolinite
Pyrite-rich flotation concentrate of Toolse black shale	Quartz, K-feldspar (orthoclase), pyrite, illite, kaolinite, marcasite
Ash residue of Toolse black shale	Quartz, K-feldspar (orthoclase), hematite, illite, gypsum
Natural (unprocessed) black shale from Maardu phosphorite deposit	Quartz, K-feldspar (orthoclase), pyrite, illite,
Oil shale from Ida-Virumaa	Quartz, dolomite, calcite, pyrite

Table 3. Partial elemental analysis of shale samples used in leaching experiments (concentration % wt/wt).

Component	Black shale (Toolse)	Black shale (Maardu)	Oil shale
SiO_2	55.30	52.90	1.60
Al_2O_3	8.18	13.40	3.05
Fe_2O_3	7.85	5.15	4.15
MgO	1.08	1.00	3.23
CaO	2.95	0.22	30.00
Na_2O	0.82	0.80	0.04
K_2O	4.92	8.30	1.00
MnO	0.04	0.02	NA
Ti_2O	0.51	0.83	0.18
S	2.21	1.47	4.75
U	0.0095	0.0026	NA
Mo	0.0245	0.0013	NA
V	0.0740	0.0240	NA
Loss of Ignitation	18.00	17.90	56.00

NA, not analysed

3.3. Leaching experiments

Shake flask experiments were carried out as follows: 100 ml of cultures, amended with 5% (wt/vol) to 30% (wt/vol) shale material, were grown in 250-ml shake flasks with agitation (200 rpm). Initial acid consumption was neutralised to pH 2 with H_2SO_4 by preincubating the suspension for 2 to 4 days before inoculation. Additional substrates (6 g Fe^{2+} [as $FeSO_4*7H_2O$] or 10 g S_o [as flowers of

sulphur] per litre) were used in some experiments to enhance the bacterial activity. Samples (3 ml or 10 ml) were periodically withdrawn for pH, redox potential and iron concentration measurements. The samples were filtered (0.45 μm pore size) for removing the solids before chemical analysis of dissolved metals. The solids were recovered from the filters and washed with 0.01 M H_2SO_4 for mineralogical analyses.

Stirred tank reactor (STR) (1.5 l working volume) (Lindström and Gunneriusson, 1990) experiments were conducted at constant aeration rate (300 ml air/l*min) with CO_2 enriched (2% vol/vol) air and stirring (300 rpm). In STR experiments 1.5 l of mineral salts solutions (modified 9K, modified 9K two-fold diluted; modified 9K ten-fold diluted) were amended with 10% (wt/vol) of shale material. After the initial pH adjustment the solutions were inoculated with 150 ml of bacterial culture. Samples of solution (10 ml) were removed at indicated intervals for measuring the pH, Eh, total leached iron, iron in solution and the iron content in the residue.

3.4. Analytical methodology

The samples were prepared (i) for measurements of total leached iron — 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 — after centrifugation 0.2 ml supernatant was diluted with 1.8 ml 5M HCl, (iii) for measurements of iron content in oil shale residues — 8 ml of the sample was centrifuged and the pellet was dried for two days at 105°C. Subsequently the pellet was weight, treated with 5M HCl (at 65°C for 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% (vol/vol) of HCl and used for total iron measurement by atomic absorption spectroscopy (AAS).

The concentration of iron in leach solution samples was determined titrimetrically with either 0.5 mM $KMnO_4$ (Fe^{2+} in black shale experiments), 1 mM $K_2Cr_2O_7$ (Fe^{2+} and total soluble iron in black shale experiments) or cerric sulphate using 1.1-phenantroline as indicator (in oil shale experiments) (Kolthoff and Sandell, 1963). The concentrations of dissolved Al, Mo, V, U, and Si, and the elemental composition of the shale samples were measured by inductively coupled plasma spectroscopy (ICP). A model Jobin Yvon 70+ ICP was used with plasma-torch, cooling, and carrier-gas flow rates of 12.3, 0.2 and 0.4 litres/min, respectively. The elements were analysed sequentially with a monochromator. Aliquots of leach solutions (2.4 ml) in polyethylene tubes were mixed with 1.2 ml matrix solution (0.07 M Li-metaborate, 0.0136 M Li-

sulphate, 0.9 M HCl) to normalise the salt concentration, followed by 8.4 ml of double-distilled water.

For elemental analysis, solid samples (100 g) were mixed in Pt-crucible with 0.7 g of $LiBO_2$. Anhydrous Li_2SO_4 (0.3 g) was added to accelerate the subsequent HCl-dissolution step. The samples were covered and fused for 5 min on a Bunsen burner. After cooling, the samples were dissolved in 15 ml of 6 M HCl at 90°C. The cooled samples were made up to 200 ml in double-distilled water containing 0.25 ml of H_2O_2. For S and trace element analysis, samples (500 mg) were sintered with 3500 mg of Na_2O_2 in a covered Pt-dish for 45 min at 460°C. After cooling, 50 ml of double-distilled water and 17 ml of 6 M HCl were added, and the samples were made up to 100 ml in double-distilled water. Standard reference rock samples were used for calibration of sample pretreatment and ICP methodology for elemental analysis.

The loss of ignitation was determined as a weight loss of the sample materials after ignitation at ca 1000°C as follows. Clean Pt-crucibles (10 ml) were heated on a Bunsen burner and cooled for 15 min over silica gel in a desiccator (one per crucible). The crucibles were weighed rapidly and a preweighed amount (*ca* 60 mg) of a ground shale sample was added. The sample was moistened with acetone and the aggregates were disintegrated with ultrasonication (48 kHz, Brasonic 12). The acetone was evaporated using a mini-blower, and the samples were ignitated for 5 min on a Bunsen burner (*ca* 1000°C) and cooled in the desiccator before the weight loss determined.

For x-ray diffraction (XRD) analysis solid residues were ground gently in a agate mortar and analysed as topfill mounts using CuKα radiation and a wide-range goniometer equipped with a diffracted-beam monochromator and a Θ compensating slit. Steps scans were conducted from 5 to 68° 2Θ increments using a 4-sec step time.

4. BIOLOGICAL LEACHING OF ESTONIAN SHALES

The aim of the study was to assess the bacterial leaching of Estonian shale samples and at the same time to broaden the understanding of basic processes of shale bioleaching. No previous information was available on the reactivity of Estonian shales when in contact with iron- and sulphur-oxidising bacteria. Moderately thermophilic thiobacilli and thermophilic archaea were used in this work. The leaching process was monitored by measuring the changes in the mineralogical composition of solid residues and by the release of major inorganic constituents in leach solutions.

4.1. Comparison of bacterial strains for the following experiments

First task of the study was to select the most appropriate strains of the bacteria for the shale leaching experiments.

The results of previous studies clearly suggest that the strains of isolated acidophilic bacteria are different from each other (Colwell and Wichlacz, 1988; Leduc *et al.*, 1993). Bhattacharyya *et al.* (1992) proposed that *Thiobacillus ferrooxidans* isolates may be characterised on the basis of utilisation of various oxidisable sulphur compounds and resistance to organic compounds or heavy metal ions.

Bacterial adaptation has a great influence on the bioleaching process. Generally, the adaptation of bacteria on a mineral increases its bioleaching rate two to four times and eliminates the lag phase at the beginning of process (Attia and Zeky, 1990). As a result of natural selection the bacterial strains, growing in the heavy metal rich environments, have acquired tolerance to them (Tuovinen and Kelly, 1974; Leduc *et al.*, 1977). The selection of resistant strains for bioleaching will increase the efficiency of the process.

Most of the published investigations with *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* have been carried out by the strains isolated from the mining- or other acidophilic environments. Assuming that local strains of the bacteria are best adapted to the substrate (shale, in our studies), *Thiobacillus ferrooxidans* strains from heavy metal rich black shale environments (from Maardu, Aa, Suhkrumäe, all in Estonia) were isolated and their characteristics with the strains from Ohio, USA (strain TF-135) and from Sao Paolo, Brasil (strain TF-LR) were compared. Enrichment-dilution and single colony isolation techniques were used to isolate *Thiobacillus thiooxidans* strains from the black shale material (from Maardu, Aa and Suhkrumäe, in Estonia). The results of these isolation experiments confirmed the missing of *Thiobacillus thiooxidans*

strains on the substrates tested. The limited spread of *Thiobacillus thiooxidans* compared with *Thiobacillus ferrooxidans* has been reported earlier (Garcia Jr, 1991).

For *Thiobacillus ferrooxidans* isolation 2.5 g of shale material was inoculated in liquid media, 9K-Fe^{2+}, and incubated at 25°C in rotary shaker. After bacterial growth, indicated by ferrous iron oxidation (9K medium changed colour from green to red-brown) and by a decrease in pH, the cultures were reinoculated (5% w/v) into the respective fresh media. Five transfers in liquid medium were carried out as well as the purification from accompanied heterotrophes with 2.5 g/l $CuSO_4$ before the final purification, which was made by dilution plating and single-colony isolation on 9K-Fe^{2+}-agarose medium. Single colonies were picked out and inoculated as described above into the respective liquid medium. The purity of the isolated strains were checked using fish-extract agar-, potato agar-, Ashby- and Manning medium (Biogeotechnology of metals, 1988).

The growth of *Thiobacillus ferrooxidans* strains were measured as already mentioned above by ferrous iron oxidation (indicated by the change of colour of the liquid medium) and by a decrease in pH. Red-brown colour of the substrate medium was achieved by three days in the experiments with TF-135 and TF-LR, and by seven to twenty days in the experiments with the strains isolated from Estonia. The growth of most of the strains isolated from Estonia decreased after each reinoculation. Probably there were some microelements in the substrate (black shale) necessary for the bacterial growth. Each dilution probably diminished the concentration of the microelements, causing the decrease of growth rate. Two strains isolated from Estonia achieving the red-brown colour of substrate medium by seven days were chosen for the following metal resistance experiments. These strains were called as TF-KJ1 and TF-KJ2.

The resistance of *Thiobacillus ferrooxidans* strains to different metal salts were tested in microplate experiments, where 0.3 ml of 9K-Fe^{2+} medium was inoculated with 15 µl of bacterial culture. The growth of the bacteria was tested by ferrous iron oxidation (achievement of red-brown colour). The results were checked with the interval of seven days (7, 14 and 21 of day of the experiment). The results of the metal resistance studies with the *Thiobacillus ferrooxidans* strains TF-135, TF-LR, TF-KJ1 and TF-KJ2 are presented in Table 4. Potassium nitrate and potassium chloride as the reference salts for measuring the influence of nitrate and chloride on the bacteria were tested for screening.

Shortly summarising the results:

It was clearly demonstrated that the effect of toxicity depends on metal salt used. The resistance to the metals decreased in the row $SO_4^{2-} > Cl^- > NO_3^-$. The most toxic metal for all of the tested strains was mercury.

The strain TF-KJ2 was supersensitive to mercury. These characteristics of the strain might be interesting in the point of view of the mercury resistance studies. The assumption that the bacteria grown in uranium rich environment

will tolerate uranium in higher concentrations that the others was approved. Estonian strains TF-KJ1 and TF-KJ2 tolerated ten-fold higher concentrations of uranium than the strains TF-135 and TF-LR. There were no other big differences in resistance to the other metals between the tested strains.

Table 4. Maximum concentrations of some heavy metal salts where the growth of *Thiobacillus ferrooxidans* strains TF-135, TF-LR, TF-KJ1 and TF-KJ2 occurred.

	Maximum metal concentration tolerated (mM)			
Metal salt	TF-135	TF-LR	TF-KJ1	TF-KJ2
$CuSO_4$	250	250	500	250
$Cu(NO_3)_2$	10	5	10	5
$NiSO_4$	250	250	250	100
$NiCl_2$	50	50	50	25
$Cd(NO_3)_2$	10	1	10	10
$CdCl_2$	50	50	25	25
$ZnCl_2$	50	50	50	50
$ZnSO_4$	750	750	750	500
$AlCl_3$	25	25	25	50
$UO_2(NO_3)_2$	0.1	0.1	1	1
$VOSO_4$	100	100	100	50
$CsCl_2$	50	100	25	25
$HgCl_2$	1μM	1μM	0.1μM	0
$Co(NO_3)_2$	10	10	5	10
$CoCl_2$	50	50	25	50
$CoSO_4$	750	500	250	250
Na_2SeO_4	10	1	1	1
KNO_3	10	10	10	10
KCl	250	250	150	50

As a result of the comparison the characteristics of all tested *Thiobacillus ferrooxidans* cultures, the strains TF-135 and TF-LR as the most appropriate ones were chosen for the following experiments. *Thiobacillus thiooxidans* strain Tt-FG01, isolated from Sao Paolo region, Brasilia, kindly offered by Dr. O. Garcia Jr. was used in shale leaching studies.

The results of the isolation and comparison studies were presented in the Annual Meeting of Estonian Society for Microbiology in 1996.

4.2. Leaching studies of black shale

For the characterisation of the ore for bacterial growth suitability each mineral component has to be assessed. To simplify the calculations only sulphide minerals as main acid generating sources and carbonate minerals as primary acid consuming materials have to be taken into account. Brierly and Brierly (1996) have described in detail the method for ore biological acid production potential. They use the following terms and formulas: net acid generation (NAG), acid generation potential (AGP) and acid neutralisation potential (ANP);

$$NAG = AGP - ANP$$

where NAG, AGP and ANP are expressed in tons of $CaCO_3$ per ktons;

AGP = (% sulphide-sulphur) × 10 × 100.09/32.06
where, 100.09 and 32.06 are the molecular weights of $CaCO_3$ and sulphur (g/mol) accordingly;

ANP = (% carbonate-carbon) × 10 × 100.09/12.01
where, 100.09 and 12.01 are the molecular weights of $CaCO_3$ and carbon (g/mol) accordingly.

Estonian shales dry mass consists of organic-, sandy-clay- and carbonate ingredients. The latter consists mostly (90%) of calcite (Ots, 1992). High concentration of acid consuming components and the fast dissolution of alkaline material are not supporting the growth of acidophilic bacteria. To overcome the problem the preliminary treatment of ore has been carried out in practical experiments (Vrvic, 1987). During the preliminary treatment the acid consumption of the shale was satisfied with concentrated H_2SO_4 till the pH of the medium were stabilised at 2.

Acid consumption of the black shale samples varied between 10 and 53 g H_2SO_4/kg. Acid consumption was practically satisfied with three days of preleaching. Little acid consumption occurred when the time course was extended to 6 days. The samples with the highest acid consumption were the native (unprocessed) black shales and the ash residues. These two materials contained the highest levels of CaO. The pyrite-rich sample was under particular interest in this study because pyrite is a substrate for oxidative dissolution by thiobacilli.

The experiments were performed at five to thirty percent of pulp densities of ore, with and without various amendments (S^0, $FeSO_4$, or S^0 and $FeSO_4$) and with inoculations of iron- and sulphur-oxidising thiobacilli (*Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*) in shake flasks at room temperature (22°C) (article III, fig. 2 and fig. 3).

The oxidation of native shale and flotation products were monitored through changes in pH and redox potential (article I, fig. 2 and fig. 3). The leaching of molybdenum, vanadium, aluminium and silica from the materials were examined (article I, fig. 4). The growth of the bacteria and enhancement of the

leached metals were present in all of the experiments, except the ash residue of black shale without supplemental iron. The last might be explained with the high concentration of acid consuming components in the ash residue. As expected, the most suitable substrate for *Thiobacillus ferrooxidans* growth was pyrite-rich fraction.

Cultures supplemented with elemental sulphur showed strong acid production, with final pH values of 0.9 in *Thiobacillus ferrooxidans* cultures and 0.4–0.5 in the presence of *Thiobacillus thioooxidans*. Without supplemental substrate, the final pH values were 1.2–1.4 in all cultures. The redox potentials increased up to 540–560 mV in *Thiobacillus ferrooxidans* cultures, but remained at 400 to 430 mV when only *Thiobacillus thioooxidans* was used as an inoculum.

Ferrous iron was oxidised by *Thiobacillus ferrooxidans* culture with and without elemental sulphur amendment. About 40% of the added ferrous iron was oxidised in *Thiobacillus thioooxidans* cultures. This was attributed to abiotic oxidation over the seven week time course because *Thiobacillus thioooxidans* is not capable of iron oxidation. In mixed culture, iron oxidation declined when elemental sulphur was also present, concurrently with decrease in pH to 0.5. The final ferrous iron concentration increased, presumably because of pyrite dissolution and the presence of elemental sulphur as a reductant for ferric iron.

Thiobacillus thioooxidans did not dissolve iron from the black shale where as *Thiobacillus ferrooxidans* and the mixed culture did. These data suggested that iron dissolution was mediated by *Thiobacillus ferrooxidans*. Total dissolved iron concentrations were 3 to 50 times higher in the cultures inoculated with *Thiobacillus ferrooxidans* when compared to *Thiobacillus thioooxidans* and sterile controls.

The initial leaching experiment with the black shale sample suggested that the material was toxic to the inoculum at >5% pulp density. In an attempt to evaluate the potential toxicity associated with the black shale sample, the leach solutions that were replaced in 5, 15, and 30% pulp densities were amended with ferrous iron and re-inoculated with *Thiobacillus ferrooxidans*. Ferrous iron was completely oxidised within four days in samples removed from 5% pulp densities while no oxidation took place in the 15 and 30% pulp density samples. Thus, these data suggested that at least some of the toxicity was associated with a soluble component leached from the parent material.

The black shale sample was acid-producing at 5% but not at 20% pulp density. Acid production was evidence of pyrite and marcasite oxidation which were the only sulphide phases detected by XRD in the sample. Acid production was non-existent at 30% but variable at 10 to 15% pulp density. The respective Eh values increased in the acid-producing suspensions, in keeping with the oxidation of the Fe-sulphide phases. Ferrous iron oxidation with time was evident at 5% pulp density whereas it was negligible at 20% pulp density. Iron-

oxidation also took place at 10 and 15% pulp densities. Total iron values suggested iron precipitation during the time course in all pulp densities.

The dissolution of both major and minor elements was monitored in the experiments, namely U, Mo, V, Al and Si were measured (article I, fig. 4 and fig. 5; article III, fig. 3). The concentration of uranium in the leach solutions remained below the level of detection (<5mg U/l) in all experiments. In *Thiobacillus ferrooxidans* inoculates, the concentrations of iron and molybdenum increased with time. Approximately 45% of the molybdenum in the pyrite-rich shale was released to solution during the time course. Changes in the concentrations of dissolved aluminium and silica were mostly comparable in the inoculated and abiotic samples, and probably were mediated by precipitation reactions during the time course.

In *Thiobacillus thiooxidans* cultures the concentration of dissolved molybdenum increased most dramatically in the S^{o}+$FeSO_4$ amended experiments. The concentration of silica was also somewhat elevated in the S^{o} and S^{o}+$FeSO_4$ amended experiments. The dissolution of vanadium was similar in the inoculated and abiotic samples.

The leaching trends of metals under investigation suggest, that silicate dissolution was deemed as a chemical, proton-facilitated attack whereas molybdenum solubilisation was based on a combined microbial and chemical reaction. These differences suggest that molybdenum was at least partially associated with sulphide phase, which was susceptible to the bacterial oxidation and dissolution.

4.3. Leaching studies of oil shale

Testing the feasibility of Estonian oil shale for bacterial leaching shake flask (article II, fig. 1 and fig. 2) and stirred tank reactor experiments (article II, fig. 3 to fig. 5) were carried out. The experiments were performed at 1% to 15% of pulp densities with and without an additional energy source (120 mM Fe as $FeSO_4$) using 9K as a basic medium. *Thiobacillus ferrooxidans* strain TF-LR and *Sulfolobus acidocaldarius* strain BC were used for inoculation.

Shake flask experiments were carried out to test: (i) substrate toxicity and (ii) growth of bacteria on the internal pyrite of the shale. Shale material consists of many metals and some organic compounds, which may solubilise and inhibit the growth of bacteria (Mahapatra *et al.*, 1985). The previous experiments with Estonian black shale indicated that in the course of chemical leaching (adjustment of pH) some elements were released from the substrate inhibiting the growth of *Thiobacillus ferrooxidans*.

During the course of chemical leaching (period of stabilisation pH in the shake flasks, 3 days) already 58% of the iron was remained. At the end of the

bioleaching experiment about 95% of total iron was released by the *Sulfolobus acidocaldarius* cultures and about 90% by the *Thiobacillus ferrooxidans* cultures. The cultures on different substrate pulp density showed the same amount of leached iron, indicating that no inhibiting substances were released (article II, Table 2).

These shake flask experiments have demonstrated that *Thiobacillus ferrooxidans* and *Sulfolobus acidocaldarius* can use oil shale as substrate for their growth. The amount of internal pyrite leached was similar in samples with and without additional energy source, suggesting the active use of the oil shale internal pyrite. There was no inhibitory effect on the growth of *Thiobacillus ferrooxidans* or *Sulfolobus acidocaldarius*, even at the higher concentrations of substrate.

Bioreactor experiments were carried out to test oil shale pyrite oxidation under more controlled conditions. As the result of bioreactor experiments the *Thiobacillus ferrooxidans* cultures leached 53% to 67% of pyrite from the shale during 11 days. In the bioreactor experiments only 5% to 15% of the initial iron was leached due to 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 (Larsson *et al.*, 1990).

Thermophilic *Sulfolobus acidocaldarius* leached about 94% of pyrite from oil shale. The leaching was in principal finished after 4 days. In the course of chemical leaching during the pH adjustment 30% of the total iron was solubilised. The higher amount 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.

4.4. Mineral alterations during the biological leaching of shales

Illite, kaolinite, feldspar and quartz are common silicate minerals in shales. Quartz is relatively inert in oxidative sulphide leaching processes, but clay minerals and feldspars may be altered by acid dissolution (Curutchet *et al.*, 1990) releasing Na^+ and K^+-ions for the precipitation formation. The precipitation of iron hydroxides, jarosite or the other by products of the process inhibit the bacterial oxidation decreasing the diffusion processes, covering the mineral surface.

$$Fe_2(SO_4)_3 + 6\ H_2O \rightarrow 2Fe(OH)_3 + 3\ H_2SO_4$$
$$Fe_2(SO_4)_3 + 2\ H_2O \rightarrow 2Fe(OH)SO_4 + H_2SO_4$$
$$3\ Fe_2(SO_4)_3 + 12\ H_2O \rightarrow 2H[Fe_3(SO_4)_2(OH)_6] + 5\ H_2SO_4$$

$$3\ Fe^{2+} + HSO_4^- + K+ 13\ H_2O + SO_4^{2-} \rightarrow K[Fe_3(SO_4)_2(OH)_6] + 7\ H_3O + 3\ e^-$$

During the experiments the bacterial dissolution and structural alterations of minerals, first of all clay minerals (illite, kaolinite) were examined. The studies were carried out with black shale samples as well as with special clay samples. Illite-rich clay from Vladivostok area, Russia, and illite-, chlorite- and kaolinite-rich glacial clay from Vittige, Sweden, were used as the reference clay samples. In addition to inoculated sample experiments, parallel experiments with sterile samples for testing the influence of ferric- and ferrous ions, sulphur and low pH on the leaching were carried out. pH and redox potential of the leaching solutions were monitored. Alterations in mineralogy of the samples were measured by XRD. The results of the studies will be presented in the paper that is being prepared at the moment.

XRD analysis of black shale samples revealed jarosite lines in solid residues from the *Thiobacillus ferrooxidans* inoculated cultures. Jarosite lines were absent in solid residues from *Thiobacillus thiooxidans* cultures and only trace amounts of jarosite were detected in the abiotic controls. Both pyrite and marcasite lines decreased in all inoculated experiments. Sulphur was virtually depleted during the time course in the *Thiobacillus thiooxidans* cultures which were initially amended with S^o. Silicate minerals (illite, feldspar, kaolinite) were partially dissolved, and this was most pronounced in the *Thiobacillus thiooxidans* cultures as well as sterile controls with low pH, suggesting dissolution due to proton attack. The formation of new minerals during the process was not detected.

5. CONCLUSIONS AND FURTHER DEVELOPMENT

As the result of the studies the basic data about biological leaching of Estonian shales were obtained. By the results the following conclusions were made:

1. Iron- and sulphur oxidising bacteria can grow at oil shale and black shale material, using internal pyrite and/or marcasite as an energy source.

2. Alkaline material (calcite mostly) inside shale material should be neutralised before the active biological process starts.

3. Metals from the shales are biologically leachable by bacteria. Molybdenum solubilisation was based on a combined biological and chemical reaction, probably involving direct leaching mechanism. Vanadium, aluminium and silica leaching were chemical, proton-facilitated attacks.

4. During the leaching processes some elements (metals, organic compounds) may accumulate in concentrations inhibiting the growth of the bacteria. At least part of the toxicity effect is connected with leaching solution.

5. Clay minerals (illite, kaolinite) in the shales are easily degradable by bacteria. The degradation of the classminerals is caused by the proton attack, generated during bacterial metabolism.

6. During the leaching of shales basic iron sulphate, jarosite, was formed and precipitated.

Shale bioleaching is the topic with practical application in Estonia. Understanding of basic processes of shale bioleaching might clear up environmental (sulphur oxides pollution, black shale heaping during phosphorite mining) and industrial (heavy metal leaching, sulphur removal from shales) problems.

There have been very dramatical articles in Estonian press concerning the heavy metal leaching from black shale. Selfstarting of natural nuclear reactor has been predicted next to the Estonian capital Tallinn, in Maardu (Veski, 1990). Using the results of the present study the scientific model for prediction of leaching processes can be constructed. For the realistic model construction additional studies about microbial consortia and the interactions between bacteria in shale environments have to be carried out.

Black shale has been titled as potential mineral resource in Estonia. The most advantageous way of using the material has been considered as a raw material for extraction of metals. The results of the present study may be used in economical feasibility studies of metal extraction processes. Chemical and biological leaching methods may be compared and evaluated.

The advantages of the biological method of sulphur removal from fossil fuels over the other methods are first of all the cheapness, sustainability to environment and the method cause negligible alterations in the fuel structure and characteristics (Rossi, 1990). According to the present study sulphur from

Estonian oil shale is easily removable by bacteria. Further economical calculations have to be carried out for the evaluation of the economical feasibility of the method.

In addition to practical applications the following fundamental topics might be solved by bioleaching of shale materials:

* Chemical depyritisation of oil shales often leads to substantial alterations in the organic fraction (kerogen) in shales. Biological removal of pyrite from oil shale is the least non-destructive method. Biological removal of nonorganic part of shales may be perspective for purification of nondestructed kerogen and enhance the quality of kerogen structural studies.

* Mineral composition of the shales is challenging in the point of view of the mineral interaction studies (quartz as inert mineral, clay minerals as active minerals, organic particles, pyrite). Most of the shales consist toxic metals in elevated concentrations. Usually elevated concentrations of two or more metals are present at the same time and the co-effects of the metals can be investigated. The consistence of shales might be worth to serve as a model for research of leaching processes and interactions between minerals and metals.

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SAVIKILTADE BIOLEOSTAMINE: PÕLEVKIVI JA DIKTÜONEEMAKILT

Kokkuvõte

Bioleostamine on protsess, mille käigus mikroorganismid kas otseselt või kaudselt lagundavad maake ja mineraale. Mikroorganismide kaasabil toodetakse vaestest maakidest metalle (vask, tsink, kuld, hõbe, uraan jm.), puhastatakse fossiilseid kütuseid väävliühenditest, toodetakse väävelhapet jne. Enamus bioleostamisalastest uurimustest on tehtud sulfiidsete mineraalide või suure sulfiididesisaldusega maakidega, savikiltadega on tehtud vaid üksikuid katseid.

Käesoleva töö eesmärgiks oli uurida Eestis leiduvate savikiltade (diktüoneemakilda ja põlevkivi) bioleostumist ning seekaudu avardada teadmisi savikiltade leostumisprotsessidest.

Kõigepealt valiti materjali leostamiseks sobivad mikroorganismide tüved. Bioleostamist võivad läbi viia mitmed mikroorganismid, näited bioleostamisvõimelistest mikroorganismidest on toodud tabelis 1. Levinumad ja ka siin töös kasutatud liigid on atsidofiilsed thiobatsillid *Thiobacillus ferrooxidans* ning *Thiobacillus thiooxidans* ja arhebakter *Sulfolobus acidocaldarius*. Põhja-Eesti savikiltadelt eraldati bakteritüved, mida võrreldi USA-st, Ohiost ja Brasiiliast, Sao Paolost eraldatud tüvedega. Võrreldi bakteritüvede kasvukiirust ning resistentsust mitmesuguste metallide sooladele. Katsete tulemusena valiti edaspidisteks tööks *Thiobacillus ferrooxidans*'i tüved TF-135, Ohiost ja TF-LR, Brasiiliast. *Thiobacillus thiooxidans*'i Eesti kiltadelt isoleerida ei õnnestunud. Katsetes kasutati Brasiiliast isoleeritud tüve Tt-FG01.

Diktüoneemakilda leostumiskatsetes testiti loodusliku materjali, selle mitmesuguste flotatsiooniproduktide ning põletusjääkide leostumist. Uuriti materjali kontsentratsiooni, toitainete ning protsessis osalevate bakterite mõju püriidi ja markasiidi ning maagis sisalduvate metallide leostumisele. Katsete tulemusena selgitati, et atsidofiilsed thiobatsillid on võimelised kasvusubstraadina kasutama diktüoneemakildas sisalduvat püriiti ning markasiiti. Leostumisprotsessi käigus vabanesid diktüoneemakildast bakterite tegevuse tulemusena metallid. Tehti kindlaks, et silikaatsete elementide leostamine põhines keemilisel, prootonite mõjul toimuval protsessil, molübdeeni leostamisel osalesid lisaks keemilistele teguritele otseselt bakterid. Kõrgematel diktüoneemakilda kontsentratsioonidel bakterite tegevus inhibeerus. Selgitati välja, et vähemasti osaliselt oli inhibitsioon põhjustatud leostuvate komponentide poolt.

Röntgendifraktomeeterkatsetes tõestati jarosiidi moodustumine bioleostumisprotsessides *Thiobacillus ferrooxidans*'i juuresolekul. Tehti kindlaks, et silikaatsete mineraalide (illiit, kaoliniit) leostumine savikiltadest on tingitud

prootonreaktsioonist. Uuritud savikiltade leostumisprotsessides teisi mineraale peale jarosiidi ei moodustunud.

Põlevkivi leostamiskatsed viidi läbi mesofiilsete thiobatsillidega (*Thiobacillus ferrooxidans*, tüvi TF-LR) ning termofiilsete arhebakteritega (*Sulfolobus acidocaldarius* tüvi BC) loksutil ning reaktoris. Testiti materjali hulga, toitainete, mitmesuguste bakterite- ning katsetingimuste mõju bioleostamisprotsessidele. Põlevkivi leostamisel ei toimunud bakterite inhibitsiooni ka kõrgetel materjalide kontsentratsioonidel. Selgitati välja, et uuritud bakterid võivad kasvada põlevkivis sisalduval püriidil. Reaktorkatsetes, mis on lähedasemad looduses toimuvatele ja tööstuses kasutatavatele protsessidele, leostas *Thiobacillus ferrooxidans* 11 päevaga kuni 67% ja *Sulfolobus acidocaldarius* nelja päevaga 94% põlevkivis sisalduvast püriidist.

Käesoleva uurimistöö tulemused on abiks praktiliste küsimuste lahendamisel. Katsete tulemusi saab kasutada keskkonnaprobleemide lahendamiseks (väävlireostus põlevkivi põletamisel, raskmetallide leostumine fosforiidi kaevandamisel kuhjatavatest aherainemägedest), samuti välja töötamaks alternatiivseid, keskkonnasõbralikke tehnoloogiaid (metallide eraldamine diktüoneemakildast).

ACKNOWLEDGEMENTS

I wish to express my gratitude to prof. Olli H. Tuovinen, Ohio State University, for the help and strong support for all of my scientific activities. I want to thank Dr. Börje Lindström, University of Umeå, Dr. Oswaldo Garcia Jr., Sao Paolo State University, Dr. Antti Vuorinen, University of Helsinki, Dr. Jerry M. Bigham, Ohio State University, for collaboration and practical help. I am grateful to Dr. R. Palvadre, Institute of Chemistry, for the shale samples used in this work, to Dr. M. Lehtinen, University of Helsinki, for providing additional mineralogical information and to Dr. Å. Sandström, University of Luleå, for helping to grind the oil shale samples. I wish to thank Ms. S. Sääf, University of Umeå, Mr. U. Soto, Ohio State University, and Mr. E. Jõgi, University of Tartu. for technical assistance.

I wish to thank Prof. Ain Heinaru, my supervisor at the University of Tartu, for his help, support and useful discussions.

The financial support for the work was received from Estonian Science Foundation, Estonia, Swedish Institute, Sweden, Viro Säätio Foundation, Finland, Rotalia Foundation, USA and Open Estonian Foundation, Estonia. I also wish to express my special thanks for "in kind" support to Ohio State University, USA, University of Umeå, Sweden, University of Tartu, Estonia and University of Helsinki, Finland.

PUBLICATIONS

I

Tasa, A., Garcia, O. Jr., Bigham, J. M., Vuorinen, A. and Tuovinen, O. H. (1995)
Acid and biological leaching of a black shale from Toolse, Estonia.
In: Biohydrometallurgical Processing, Vol. I (Vargas, T., Jerez, C. A.,
Wiertz, J. V., Toledo, H. eds.), University of Chile, Santiago, Chile, 229–238.

In: *Biohydrometallurgical Processing, Vol. I* (T. Vargas, C.A. Jerez, J.V. Wiertz, H. Toledo, eds.), p. 229-238. University of Chile, Santiago, Chile (1995).

ACID AND BIOLOGICAL LEACHING OF A BLACK SHALE FROM TOOLSE, ESTONIA

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ABSTRACT

The purpose of this study was to evaluate the oxidative dissolution of a black (dictyonema) shale and processed concentrates from a prospective mine site in Toolse, Estonia. Black shales are sedimentary rocks composed mainly of illite, quartz, feldspars, pyrite, and kerogen. Some Estonian black shale deposits also contain high levels of U, V, and Mo as well as other microelements. In Estonian phosphate mines, black shales tend to overlie phosphorite deposits. At mine sites, these shales are stored in waste heaps which become potential sources of acidic discharge with high concentrations of dissolved metals. Acid leaching under both abiotic conditions and with enhanced bacterial action (*Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*) were assessed in this study. The leaching was monitored by measurement of chemical changes in leach solutions and examination of solid leach residues by X-ray diffraction. The shale concentrate with the highest pyrite content showed the most potential for biological formation of sulfuric acid, and acidophilic thiobacilli also enhanced the dissolution of metals.

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INTRODUCTION

Dictyonema shale (also termed graptolite argillite, dictyonema argillite, black shale, alum shale) is a sedimentary rock of Lower Ordovician age that was deposited about 450-500 million years ago. The shale is named after fossil graptolite dictyonema commonly found in it. Some black shales are synonymous with low-grade oil shales, containing up to 10-20% of kerogenic material. Trace constituents (e.g., U, V, Mo) may also be present at elevated concentrations.

In Estonia, black shale deposits occur mostly in the northern and northeastern parts of the country and represent a potential source of metals, fossil fuel, and even construction material (1). Some Estonian shales were explored for U mining in the 1950-1960's, but the exploration did not develop to commercial scale (2). Phosphoritic layers often underlie the shale deposits. Phosphorite ore has been mined from the Maardu deposit near Tallinn since 1923, first by underground mining, and then by open cast-mining from the mid-1950's until 1991 when the phosphorite ore was depleted. At present, there is exploratory work on a phosphorite deposit in Toolse, near Rakvere, in northern Estonia. Exposed black shales pose several environmental problems related to the dissolution of metals in leach waters. Black shales together with limestone, another waste rock, have been disposed of in the Maardu phosphorite mine by burial in mined-out parts of the quarry. These waste materials have a history of self-combustion and have been a continuous source of dissolved metals in receiving waters. Shales with high kerogen content are presently mined in Estonia for fossil fuel combustion. These oil shales contain an average of 1.5% S, mostly as pyrite. Combustion of these shales in Estonian power plants constitutes a major airborne SO_2 pollution problem in the Baltic Sea region (3).

The black shale deposits in Estonia are mostly composed of illite, quartz, K-feldspars, and pyrite (4). Many thiobacilli can oxidize sulfide minerals as substrates, releasing metals and producing sulfuric acid from these minerals. Several non-sulfide minerals are also subject to chemical dissolution by organic and inorganic acids which may be of biological origin (5, 6). The natural weathering of these minerals is mostly mediated via proton attack, with additional leaching action by organic acids that form soluble complexes with metals. In this scheme, microorganisms need an external source of energy and carbon for growth, and the mineral dissolution does not involve an oxidative or other redox-related reaction.

Shales have been previously evaluated in bacterial leaching studies. Vrvić et al. (7) used *Thiobacillus ferrooxidans* to oxidize pyrite from a kerogen-rich shale, with 1-3% residual pyrite remaining after 4 weeks of contact in the bacterial culture. Konopka et al. (8) reported that the leaching of U, V, Mo, and Pb from a Polish black shale (0.5-2 mm particle size) was enhanced by *T. ferrooxidans* during a time course of seven months. Mishra et al. (9) and Mahapatra et al. (10) reported that *T. ferrooxidans* could remove most of the pyrite content of pyrite-rich shale samples by oxidative dissolution. The data also suggested an inhibition of the bacterial action, possibly caused by high pulp density or by toxic elements released from the shale during the leaching. Shale matrix materials may influence the bacterial oxidation of the pyritic fraction due to excessive acid demand and by physically masking the reactive surface (11).

As a prelude to estimating the potential of acid mine drainage formation in Estonian mine waste materials, the present study was undertaken to assess the bacterial leaching of Estonian black shale samples. No previous information is available on the reactivity of Estonian black shales when in contact with Fe- and S-oxidizing thiobacilli. Both *T. ferrooxidans* and *Thiobacillus thiooxidans* were used in this work. The leaching process was monitored by measuring changes in the mineralogical composition of solid residues and by the release of major inorganic constituents in leach solutions.

MATERIALS AND METHODS

Black shale samples

The shale samples used in this work were from the Toolse phosphorite deposit in Estonia, kindly supplied by Dr. R. Palvadre, Institute of Chemistry, Estonian Academy of Sciences, Tallinn. The materials were finely ground and included samples of the native black shale, a flotation concentrate with high organic C (kerogen) content, a flotation concentrate with high pyrite content, and an ash residue after combustion of the shale at 800°C. Bulk elemental composition of the samples is given in Table I. All samples contained elevated levels of U, Mo, and V. The main minerals present in the black shale sample were (in decreasing order of abundance) quartz, K-feldspar (orthoclase), pyrite, illite, and kaolinite. The kerogen-rich sample contained mainly orthoclase, quartz, pyrite, illite, and traces of kaolinite; the pyrite-rich concentrate contained quartz, pyrite, orthoclase, illite, and traces of kaolinite (Figure 1). The sample of ash residue was composed of quartz, orthoclase, hematite, illite and traces of gypsum.

Bacteria and culture media

Acidophilic thiobacilli, *T. ferrooxidans* TFI-35 (12) and *T. thiooxidans* Tt-FG01 (13), were used in this study. The cultures (5% inocula) were grown at 22±2°C in shake flasks (200 rev/min) in mineral salts medium which contained (per liter) 0.5 g $(NH_4)_2SO_4$, 0.5 g K_2HPO_4, and 0.5 g $MgSO_4 \cdot 7H_2O$. Media for *T. ferrooxidans* and *T. thiooxidans* were supplemented with 33.3 g $FeSO_4 \cdot 7H_2O$/liter and 10 g S^0/liter, respectively. The media were adjusted to pH 1.8 with H_2SO_4.

Table I - Elemental composition and acid consumption data for the shale materials

Parameter	Elemental Composition (%)			
	Native shale	Kerogen-rich concentrate	FeS_2-rich concentrate	Ash-residue
SiO_2 (%)	55.3	48.0	38.6	62.1
Al_2O_3 (%)	8.18	12.6	6.63	12.1
Fe_2O_3 (%)	7.85	5.67	20.13	9.10
MgO (%)	1.08	1.12	0.54	1.50
CaO (%)	2.95	0.41	1.35	2.88
Na_2O (%)	0.82	0.80	0.73	0.75
K_2O (%)	4.92	7.00	4.53	7.40
MnO (%)	0.04	0.02	0.02	0.04
TiO_2 (%)	0.51	0.76	0.43	0.75
L.O.I. (%)[a]	18.0	24.1	27.0	3.45
Subtotal (%)	99.6	100.5	100.0	100.0
S (%)	2.21	1.13	7.10	0.50
U (mg/kg)	95	170	155	170
Mo (mg/kg)	245	540	360	465
V (mg/kg)	740	1330	660	1120
H_2SO_4 (g/kg)				
after 3 days	51.0	11.8	18.6	48.5
after 6 days	51.9	11.8	18.6	52.9

[a] L.O.I., loss on ignition

Leaching experiments

The leaching experiments were carried out in 250-ml shake flasks in 80 ml cultures which were amended with 5% (wt/vol) shale material. Initial acid consumption was neutralized to pH 2 with H_2SO_4 by preincubating the suspensions for 2-4 days before inoculation. Additional substrates (6 g Fe^{2+} [as $FeSO_4 \cdot 7H_2O$] or 10 g S^0 [as flowers of sulfur]/liter) were used in some experiments to enhance the bacterial activity. Samples (10 ml) were periodically withdrawn for pH and redox potential measurements. The samples were filtered (0.45 µm pore size) for removing the solids before chemical analysis of dissolved metals. The solids were recovered from the filters and washed with 0.01 M H_2SO_4 for mineralogical analyses.

Analytical methodology

The concentration of iron in leach solution samples was determined titrimetrically with either 0.5 mM $KMnO_4$ (Fe^{2+}) or 1 mM $K_2Cr_2O_7$ (Fe^{2+} and total soluble Fe) as previously described (12). The concentrations of dissolved Al, Mo, V, U, and Si, and the elemental composition of the shale samples were measured by inductively coupled plasma spectroscopy (ICP). A model Jobin Yvon 70+ ICP was used with plasma-torch, cooling, and carrier-gas flow rates of 12.3, 0.2 and 0.4 liters/min, respectively. The elements were analyzed sequentially with a monochromator. Aliquots of leach solutions (2.4 ml) in polyethylene tubes were mixed with 1.2 ml matrix solution (0.07 M Li-metaborate, 0.0136 M Li-sulfate, 0.9 M HCl) to normalize the salt concentration, followed by 8.4 ml of double-distilled water.

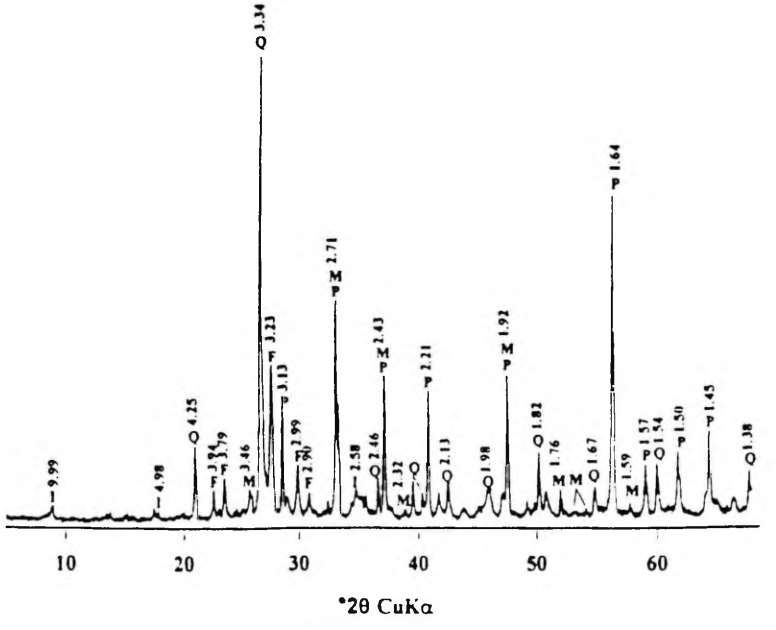

Figure 1 - X-ray diffractogram of the pyrite-rich concentrate. Numerical values, when given, are in angstroms. Letter designations: F, feldspar; I, illite; M, marcasite; P, pyrite; Q, quartz.

For elemental analysis, solid samples (100 mg) were mixed in a Pt-crucible with 0.7 g of $LiBO_2$. Anhydrous Li_2SO_4 (0.3 g) was added to accelerate the subsequent HCl-dissolution step. The samples were covered and fused for 5 min on a Bunsen burner. After cooling, the samples were dissolved in 15 ml of 6 M HCl at 90°C. The cooled samples were made up to 200 ml in double-distilled water containing 0.25 ml of H_2O_2. For S and trace element analysis, samples (500 mg) were sintered with 3500 mg of Na_2O_2 in a covered Pt-dish for 45 min at 460°C. After cooling, 50 ml of double-distilled water and 17 ml of 6 M HCl were added, and the samples were made up to 100 ml in double-distilled water. Standard reference rock samples were used for calibration of sample pretreatment and ICP methodology for elemental analysis.

The loss on ignition was determined as a weight loss of the sample materials after ignition at ca. 1000°C as follows. Clean Pt-crucibles (10 ml) were heated on a Bunsen burner and cooled for 15 min over silica gel in a desiccator (one per crucible). The crucibles were weighed rapidly and a preweighed amount (ca. 60 mg) of a ground shale sample was added. The sample was moistened with acetone and the aggregates were disintegrated with ultrasonication (48 kHz, Bransonic 12). The acetone was evaporated using a mini-blower, and the samples were ignited for 5 min on a Bunsen burner (ca. 1000°C) and cooled in the desiccator before the weight loss was determined.

For x-ray diffraction (XRD) analysis, solid residues were ground gently in an agate mortar and analyzed as topfill mounts using CuKα radiation and a wide-range goniometer equipped with a diffracted-beam monochromator and a Θ compensating slit. Step scans were conducted from 5 to 68°2Θ in 0.05°2Θ increments using a 4-sec step time.

RESULTS AND DISCUSSION

The acid consumption of the shale samples varied between 10 and 53 g H_2SO_4/kg (Table I). The acid consumption was practically satisfied within 3 days of contact time, and little additional consumption occurred when the time course was extended to 6 days. The native black shale and the ash residue samples exhibited the highest acid consumption. These two materials also contained the highest levels of CaO. The pyrite-rich concentrate had the highest S and Fe content and was of particular interest in this study for evaluation of its biological oxidation potential. The oxidation of the native shale and concentrates was monitored through changes in pH and redox potential in samples inoculated with *T. ferrooxidans* with and without additional $FeSO_4$. As expected, the decrease in pH was most noticeable in the inoculated, pyrite-rich fraction (Figure 2). The redox potential increased with time in all samples.

The pyrite-rich sample was also tested in *T. thiooxidans* cultures with and without various amendments (S^0, $FeSO_4$, or S^0 and $FeSO_4$). The culture media were replaced with fresh mineral salts solutions (with $FeSO_4$ amendment where applicable) after 3 weeks of incubation. The lowest pH values were recorded in cultures which received either S^0 or S^0 and $FeSO_4$ (Figure 3).

The dissolution of both major and minor elements was monitored in experiments with the pyrite-rich black shale sample (Figure 4). The concentration of U in the leach solutions remained below the level of detection (<5 mg U/l) in all experiments. In *T. ferrooxidans* cultures, the concentrations of Fe and Mo in solution increased with time (Figure 4). Approximately 45% of the Mo in the pyrite-rich shale was released to solution during the time course. Changes in the concentrations of dissolved Al and Si were mostly comparable in the inoculated and abiotic samples, and probably were mediated by precipitation reactions during the time course. However, trends over time were somewhat erratic.

In *T. thiooxidans* cultures the concentration of dissolved Mo increased most dramatically in the S^0 + $FeSO_4$-amended experiments (Figure 5). The concentration of Si was also somewhat elevated in the S^0- and S^0 + $FeSO_4$-amended experiments. The dissolution of V was similar in the inoculated and abiotic samples. Upon replacement of culture volumes with fresh media at 22 days,

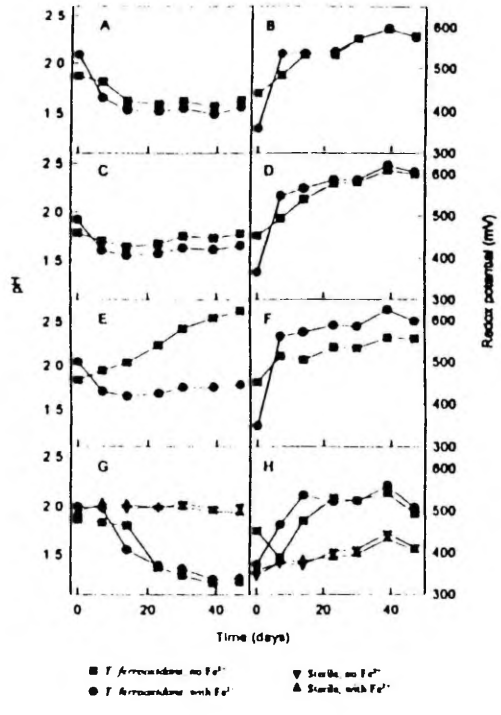

Figure 2 - Changes in pH and redox potential during the leaching of black shale samples by *T. ferrooxidans*. A and B, native black shale, C and D, kerogen-rich concentrate, E and F, ash residue, G and H, pyrite-rich concentrate

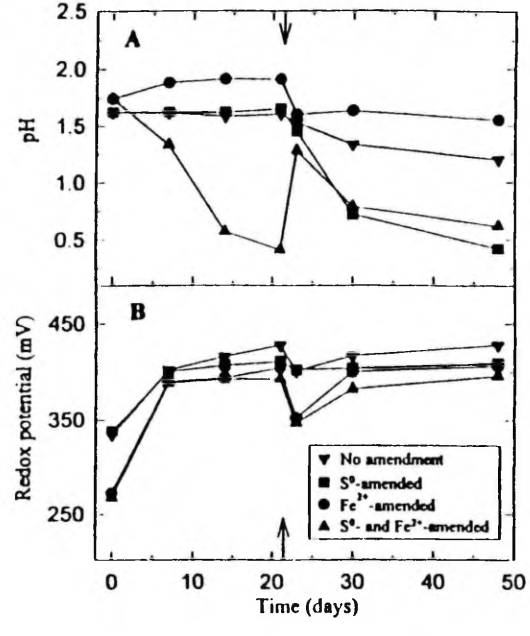

Figure 3 - Changes in pH (A) and redox potential (B) during the leaching of native black shale by *T. thiooxidans*. The mineral salts solutions were replaced on day 22 (*arrow*).

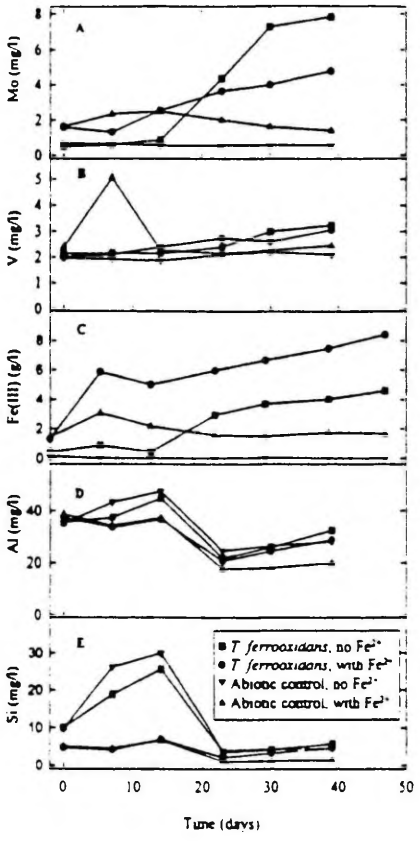

Figure 4 - Changes in the concentrations of Mo (A), V (B), Fe(III) (C), Al (D), and Si (E) during the leaching of the pyrite-rich concentrate in *T. ferrooxidans* cultures

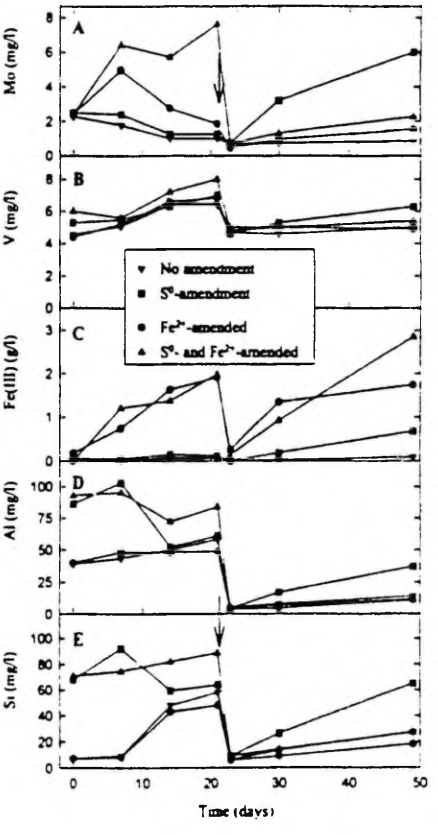

Figure 5 - Changes in the concentrations of Mo (A), V (B), Fe(III) (C), Al (D), and Si (E) during the leaching of the pyrite-rich concentrate in *T. thiooxidans* cultures. The mineral salts solutions were replaced on day 22 (*arrow*)

the concentrations of dissolved Si and other metals transiently declined because of precipitation caused by the pH change.

XRD analysis revealed jarosite lines (characteristic doublet at 3.08-3.11 Å) in solid residues from the *T. ferrooxidans* inoculated cultures (Figure 6A,B). Only trace amounts of jarosite were detected in the inoculated cultures without $FeSO_4$-amendment and in the abiotic controls. Jarosite

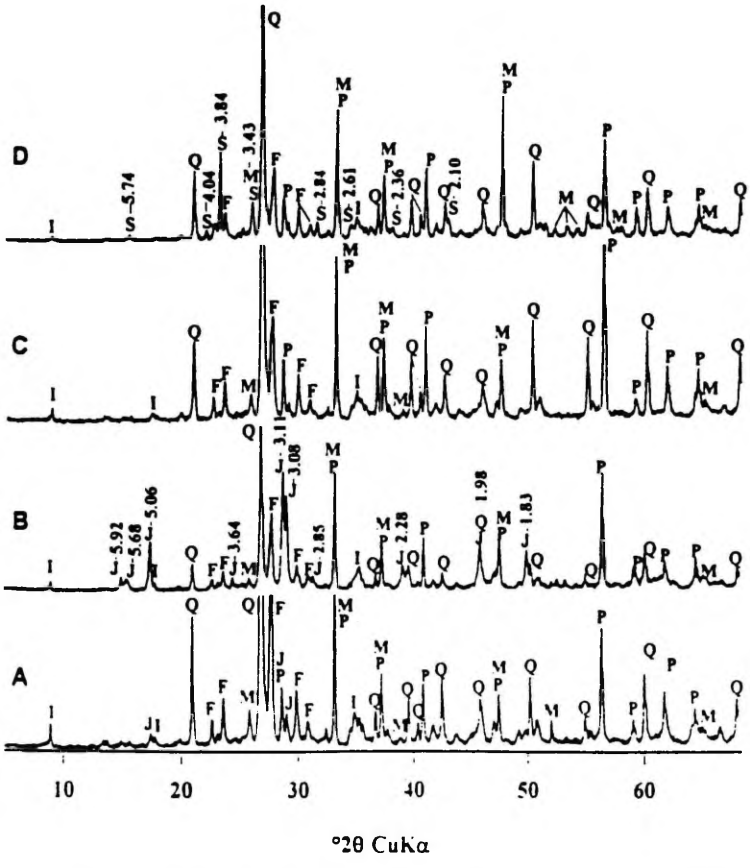

Figure 6 - X-ray diffractograms of solid residues from leaching experiments with the pyrite-rich concentrate. A. *T. ferrooxidans* without a supplemental energy source after 47 d of leaching. B. *T. ferrooxidans* with supplemental Fe^{2+} (30 mM) after 47 d of leaching; C. *T. thiooxidans* without a supplemental energy source after 49 d of leaching. D. *T. thiooxidans* with supplemental S^0 (10 g/l) after 49 d of leaching. Numerical values, when given, are in angstroms. Letter designations: F, feldspar; I, illite; J, jarosite, M, marcasite, P, pyrite, Q, quartz, S, elemental sulfur

lines were absent in solid residues from *T. thiooxidans* cultures (Figure 6C,D). Both marcasite and pyrite lines decreased in all inoculated experiments. Sulfur was virtually depleted during the time course in the *T. thiooxidans* cultures which were initially amended with S^0 (Figure 6D). Silicate minerals (illite, feldspar) were partially dissolved, and this was most pronounced in the *T. thiooxidans* cultures, suggesting dissolution due to proton attack.

In some cases, the initial lag periods preceding metal dissolution were extended for up to 2 weeks. There was no evidence for inhibitory effects when spent leach solutions were amended with Fe^{2+} and inoculated with *T. ferrooxidans*. Ferrous sulfate media supplemented with either 16 mg Mo/liter (as $Na_2MoO_4 \cdot 2H_2O$) or 28 mg V/liter (as $VOSO_4 \cdot 2H_2O$) were not inhibitory to iron oxidation by *T. ferrooxidans*. In conclusion, the results demonstrate that Fe-sulfides in black shale materials are readily susceptible to biological oxidation.

ACKNOWLEDGEMENTS

We are grateful to Dr. R. Palvadre for the shale samples used in this work and to Dr. M. Lehtinen for providing additional mineralogical information. Mr. Ubaldo Soto kindly prepared the XRD illustrations. Partial funding for the work was received from the Viro Foundation (A.T.), the Rotalia Foundation (A.T.), and the Fundação de Amparo à Pesquisa do Estado de São Paulo (O.G.).

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Tasa, A. and Lindström, E. B. (1996)
Biological desulphurization of Estonian oil shale.
Oil Shale 13: 133–143.

Oil Shale, Vol. 13, No. 2
pp. 133-143

ISSN 0208-198X

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.

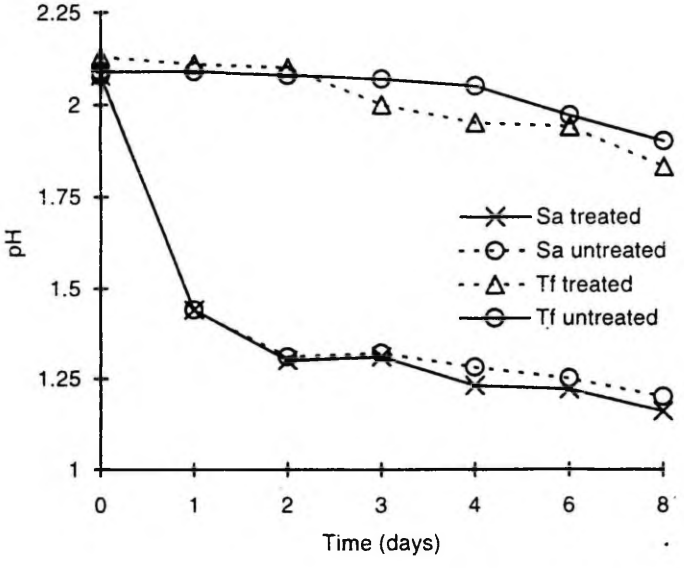

Fig. 1. Shake flask experiments with *Sulfolobus acidocaldarius* (Sa) and *Thiobacillus ferrooxidants* (Tf) showing changes in pretreated and unpretreated samples, both with additional energy source (as $FeSO_4$)

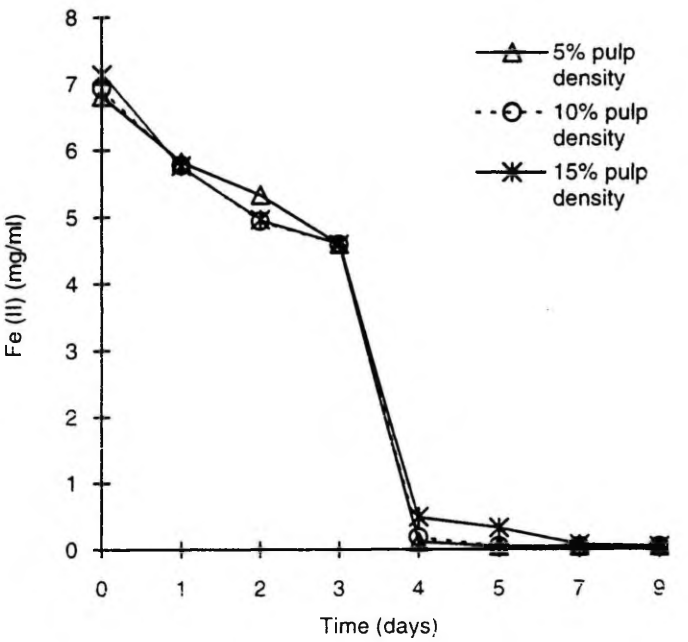

Fig. 2. Shake flask experiments with *Sulfolobus acidocaldarius* showing ferrous iron oxidation for different pulp densities of oil shale (added 120 mM Fe as $FeSO_4$)

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.

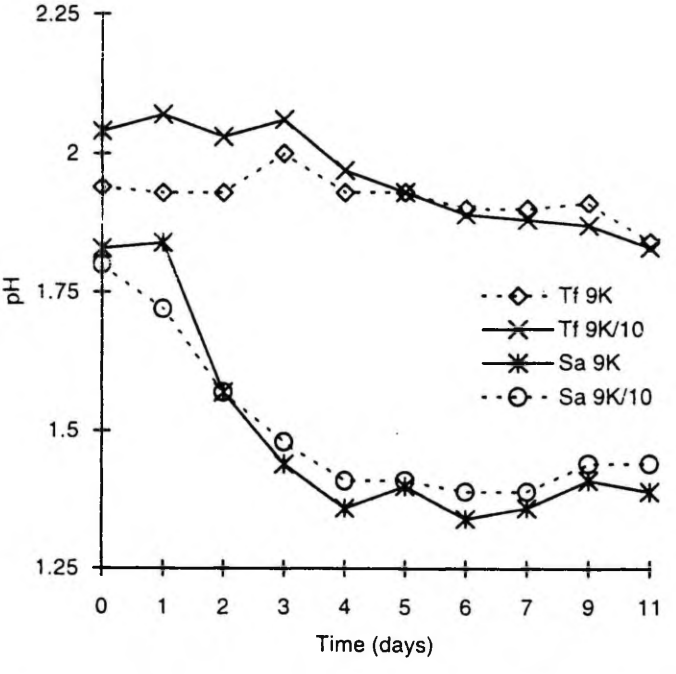

Fig. 3. pH changes in bioleaching experiments of Estonian oil shale using *Sulfolobus acidocaldarius* (Sa) and *Thiobacillus ferrooxidants* (Tf) in bioreactor experiments

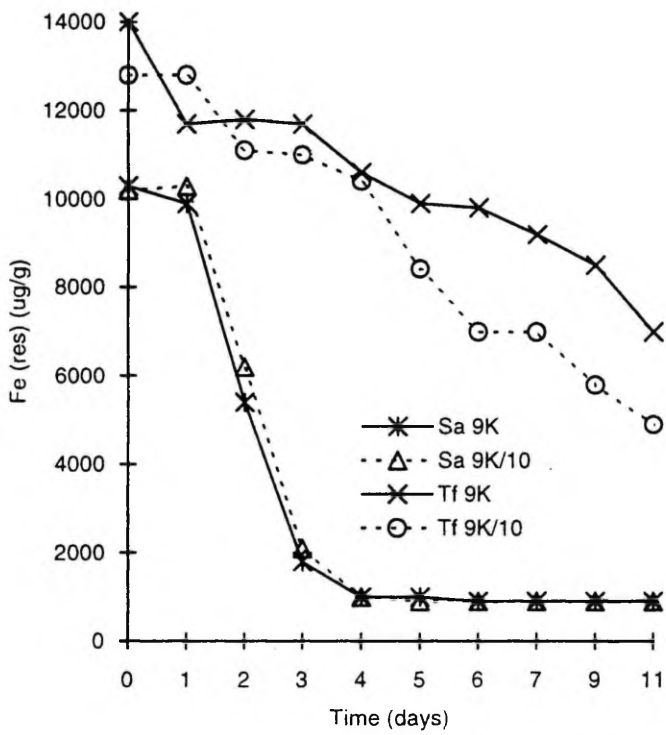

Fig. 4. Residual iron in bioleaching experiments of Estonian oil shale using *Sulfolobus acidocaldarius* (Sa) and *Thiobacillus ferrooxidants* (Tf) in bioreactor experiments

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.

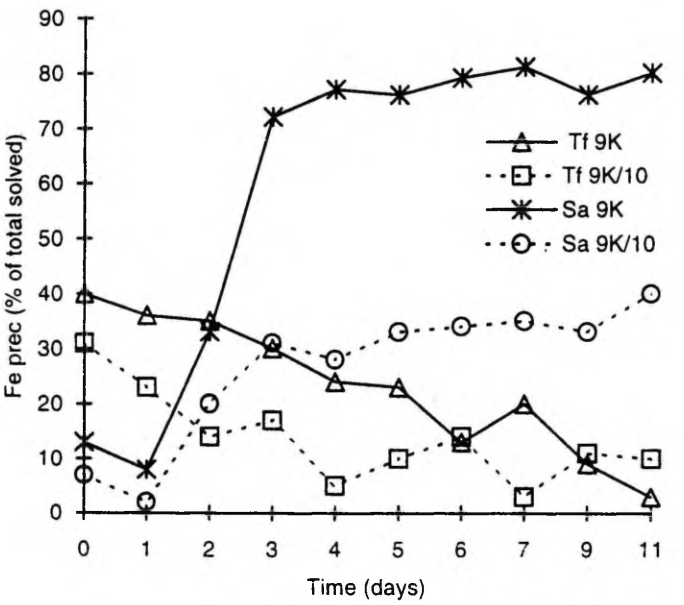

Fig. 5. Precipitation of iron in bioreactor experiments using *Thiobacillus ferrooxidants* (Tf) and *Sulfolobus acidocaldarius* (Sa) in bioreactor experiments

Acknowledgements

We thank Siv Sääf, Department of Microbiology, Umeå University for technical assistance, Dr. Åke Sandström, Luleå for helping to grind the oil shale samples, Dr. A. Vuorinen, Helsinki University for helping to analyze the oil shale samples and Dr. Olli Tuovinen, Department of Microbiology, Ohio State University for taking active interest in the project.
The financial support for the work was received from Swedish Institute (A.T.).

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Presented by J. Kann
Received March 18, 1996

III

Tasa, A., Vuorinen, A., Garcia, O. Jr. and Tuovinen, O. H. (1997)
Biologically enhanced dissolution of a pyrite-rich black shale concentrate.
J. Environ. Sci. Health A32 (9&10): 2683–2695.

J. ENVIRON. SCI. HEALTH, A32(9&10), 2683-2695 (1997)

BIOLOGICALLY ENHANCED DISSOLUTION OF A PYRITE-RICH BLACK SHALE CONCENTRATE

Key words: Bioleaching, iron oxidation, pyrite oxidation, sulfur oxidation, *Thiobacillus*

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ABSTRACT

The acid leaching of a pyrite-rich black shale concentrate (7% S) was tested in this study. The experiments were performed at 5-30% pulp densities and with inoculations of Fe- and S-oxidizing thiobacilli (*Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*). Cultures supplemented with S^0 showed strong acid production, with final pH values of 0.9 in *T. ferrooxidans* cultures and 0.4-0.5 in the presence of *T. thiooxidans*. Fe dissolution was pronounced in the *T. ferrooxidans* culture whereas *T. thiooxidans* did not dissolve Fe from the black shale. Total dissolved Fe concentrations were 3 to 50 times higher in the cultures inoculated with *T.*

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ferrooxidans when compared to *T. thiooxidans* and sterile controls. The dissolution of Mo was enhanced in the inoculated cultures as compared with the chemical controls. With V, Si, and Al this effect was not as pronounced but was still discernible in solutions acidified by bacterial oxidation of S^0. The leaching experiments suggested that the black shale was inhibitory to the inocula. The inhibition was related to the pulp density and was associated with the leach solution. The inhibition could be completely alleviated by replacing the leach solution.

INTRODUCTION

Estonian black shale deposits are typically composed of organic matter (kerogen), feldspar, quartz, clay minerals, and minor amounts of Fe-sulfides and gypsum. Some Estonian black shales contain metals such as U, V, and Mo at elevated concentrations. The black shale deposits in Estonia represent a potential mineral and fuel resource (1). Of concern are also the environmental problems due to release of metals from exposed black shales in surface runoffs and seepage in groundwater. In addition to the chemical leaching, black shales may be amenable to dissolution processes mediated by bacterial activities. The target is usually the inorganic sulfur content which is mostly pyrite (FeS_2). The bacterial oxidation of pyrite and other sulfides converts the S-entity to H_2SO_4 and soluble sulfate salts (2). Several microorganisms can be active in the oxidative removal of inorganic sulfur from minerals and fossil fuels. These include a mesophilic, acidophilic bacterium, *Thiobacillus ferrooxidans*, that oxidizes inorganic S-compounds at ≤40°C. *T. ferrooxidans* is also capable of oxidizing ferrous iron as well as sulfidic minerals such as pyrite (3). Another organism, *Thiobacillus thiooxidans*, has similar metabolic capabilities except that it does not oxidize ferrous iron. Both acidophiles are relevant in mineral biotechnology (4). This technology employs biologically produced lixiviants for solubilization of Cu-ores in dump and heap leaching processes and in the biological pre-treatment of refractory iron sulfides for gold recovery (5-7). Non-sulfide minerals can also be subject to bacterially generated acids via proton attack or complex formation (8, 9).

Black shales have been disposed of in some mine sites as waste material and stored in surface heaps. These waste materials can become significant sources of dissolved

metals in receiving waters. The ability to predict metal release from black shale materials is an important facet in pollution abatement. Very few investigations have been reported for the biological oxidation of inorganic S in shales, although there is abundant literature concerning the use of microorganisms for coal desulfurization. In both applications, pyrite is the main source of inorganic S. Vrvić et al. (10, 11) showed that *T. ferrooxidans* removed >95% of the pyrite content from a kerogen-rich shale within four weeks of contact time. Oxidative dissolution of pyrite from shale samples was evaluated with *T. ferrooxidans* by Mishra et al. (12) and Mahapatra et al. (13). The data suggested an inhibition of *T. ferrooxidans*, possibly due to the high pulp density or the dissolution of toxic elements from the shale during the biological treatment. The enhancement of metal leaching from shales due to the treatment with Fe- and S-oxidizing bacteria has been reported for Polish and Russian black shales (14, 15).

We have previously screened the feasibility of the biological leaching of a pyritic Estonian black shale (16). The purpose of the present work was to test the leachability of a black shale concentrate under batch experimental conditions suitable for the bacterial oxidation of the high pyrite content. As with other natural weathering processes involving pyritic surfaces (17), exposure of black shales to ambient surface conditions at mine sites constitutes a potential point-source pollution problem of metal dissolution with time. Laboratory leaching studies with black shales using oxygen, water, nutrients, and bacteria will facilitate future attempts of risk assessment of these sites.

MATERIALS AND METHODS

Black shale samples

The black shale sample used in this study was a flotation concentrate with high pyrite content from the Toolse phosphorite deposit in Estonia. The sample was finely ground (68.9% Ø <50 μm, 14.9% 50 μm < Ø < 100 μm, 9.4% 100 μm < Ø <200 μm, 6.8% Ø >200 μm) and contained quartz, pyrite, orthoclase, illite, and kaolinite as main mineral phases (16). Marcasite was also detected in the black shale concentrate. Partial elemental analysis yielded the following composition: 38.6% SiO_2, 6.63% Al_2O_3,

7.10% S, 20.13% Fe_2O_3, 0.54% MgO, 1.35% CaO, 0.73% Na_2O, 4.53% K_2O, 0.02% MnO, 0.43% TiO_2, 155 ppm U, 360 ppm Mo, and 660 ppm V. Sulfuric acid consumption was 18.6 g/kg after 3 and 6 days.

Bacteria and culture media

Two species of acidophilic thiobacilli, *Thiobacillus ferrooxidans* TFI-35 and *Thiobacillus thiooxidans* FG01 (18) were used in this study. The cultures were grown in shake flasks (200 rev/min) at 22±2°C in mineral salts medium (0.5 g $(NH_4)_2SO_4$, 0.5 g K_2HPO_4, and 0.5 g $MgSO_4 \cdot 7H_2O$ per liter). The media were adjusted to pH 1.8 with H_2SO_4. Some media were supplemented with either 33 g $FeSO_4 \cdot 7H_2O$/liter and 10 g elemental S/liter. For mixed culture experiments involving *T. ferrooxidans* and *T. thiooxidans*, inocula from the two cultures were mixed in equal volume. Initial acid consumption was neutralized to pH 2 with H_2SO_4 after 2-4 days contact time before inoculation. Either 5% (*T. ferrooxidans*, *T. thiooxidans*) or 10% v/v (mixed culture) inocula were used. Samples were withdrawn for pH and redox potential measurements and then filtered (0.45 μm) to remove the solids before chemical analysis of dissolved metals.

Analytical methodology

The concentrations of Fe^{2+} and total Fe in leach solutions were determined by titration with 1 mM $K_2Cr_2O_7$ and Fe^{3+} was calculated from the difference (18). The concentrations of dissolved Al, Fe, Mo, Si, V and U, and the elemental composition of the pyrite-rich black shale concentrate were measured by inductively coupled emission plasma spectroscopy (ICP) (16,19). A model Jobin Yvon 70 Plus ICP was used with plasma-torch, cooling and carrier-gas flow rates of 12.3, 0.2 and 0.4 liters Ar/min, respectively. The instrument was equipped with a sheath gas tube to provide an additional argon stream (0.8 liters/min) that reduced the contact of the salt-laden aerosols with the central injector. An Ar-humidifier was used to increase the moisture content of the gas flow and thereby to reduce salt deposition within the nebulizer. For ICP-analysis of dissolved elements (Al, Fe, Mo, Si, V, and U), aliquots of leach solutions (2.4 ml) in polyethylene tubes were mixed with 1.2 ml matrix solution (0.07 M $LiBO_2$, 0.0136 M Li_2SO_4, 0.9 M HCl) to normalize the salt concentration, followed by 8.4 ml of double-distilled water. For Al, Ca, Fe, Mg, and Si analysis in solids, samples (100 mg) were mixed in a Pt-crucible with 0.7 g of $LiBO_2$. Anhydrous Li_2SO_4 (0.3 g) was added to accelerate the subsequent HCl-dissolution step. The samples were covered and fused for 5 min at about 1000°C. After cooling, the samples were dissolved in 15 ml of 6 M HCl at 90°C and made up to 200 ml in double-distilled

water containing 0.25 ml of H_2O_2. For K, Mn, Mo, S, Ti, V, and U analysis in solids, samples (500 mg) were sintered with 3500 mg of Na_2O_2 in a covered Pt-dish for 45 min at 460°C. After cooling, 50 ml of double distilled water and 17 ml of 6 M HCl were added, and the samples were made up to 100 ml in double distilled water. Analytical standards were prepared using calibrated stock solutions of Al, Mo, V, Fe (all from Merck GmbH, Darmstadt, FRG), and U (Referensmaterial Ab, Ulricehamn, Sweden). For S determination, 0.5 M H_2SO_4 (reagent-grade) was used as an analytical standard. For Si and elemental analysis, Syenite rock SY-2 and Mount Royal Gabbro MRG-1 (CANMET, Ottawa, Ont., Canada), and Basalt BR, CRPG, and Basalt BE-N, GIT-IWG (Geostandards, Vandœuvre, France), were used as analytical standards for ICP.

Results and Discussion

Effect of bacteria and substrates on the leaching of the FeS_2-rich black shale concentrate

The leaching of the black shale concentrate was tested at 5% pulp density with and without Fe^{2+} and S^0 amendments. For inoculum, *T. ferrooxidans*, *T. thiooxidans*, and their mixture were used. The initial pH was 1.7-1.8 (Fig. 1). Cultures supplemented with S^0 showed strong acid production, with final pH values of 0.9 in *T. ferrooxidans* cultures and 0.4-0.5 in the presence of *T. thiooxidans* (Fig. 1). On day 21, the leach solutions were replaced with fresh mineral salts media which partially neutralized the strong acid production. Without supplemental substrate, the final pH values were 1.2-1.4 in all cultures. The redox potential values increased up to 540-560 mV in *T. ferrooxidans* cultures, but remained at 400-430 mV when only *T. thiooxidans* was used as an inoculum.

Fe^{2+} was oxidized in *T. ferrooxidans* culture with and without S^0 amendment (Fig. 2). About 40% of the added Fe^{2+} was oxidized in *T. thiooxidans* cultures. This fraction was attributed to abiotic oxidation over the seven week time course because *T. thiooxidans* is not capable of Fe oxidation. In mixed culture, Fe oxidation declined when S^0 was also present, concurrently with decrease in pH to 0.5. The final Fe^{2+} concentration increased due to pyrite dissolution and the presence of S^0 as a reductant for Fe^{3+}.

Total Fe data are shown in Fig. 2. *T. thiooxidans* did not dissolve Fe from the black shale whereas *Thiobacillus ferrooxidans* and the mixed culture did. These data

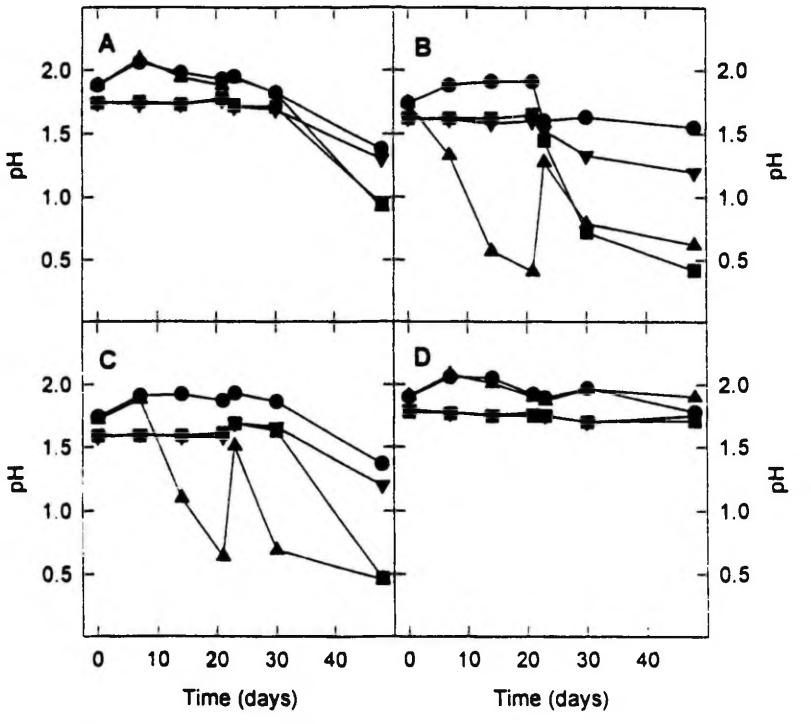

Figure 1. Changes in pH during the leaching of black shale suspensions in acid solutions. The media were inoculated with *T. ferrooxidans* (A), *T. thiooxidans* (B), or a mixture of *T. ferrooxidans* and *T. thiooxidans* (C). Symbols: ●, Fe amendment (as $FeSO_4$); ■, elemental S amendment; ▼, Fe and S amendment; ▲, no additional substrate amendment.

suggested that Fe dissolution was mediated by *T. ferrooxidans*. Total dissolved Fe concentrations were 3 to 50 times higher in the cultures inoculated with *T. ferrooxidans* when compared to *T. thiooxidans* and sterile controls.

The concentration of U in leach solutions remained below the level of detection (<2 mg U/l) in all experiments. The relative yields of dissolution of Mo were higher in the inoculated cultures as compared with the chemical controls (Table 1). With V, Si, and Al this effect was not clearly discernible, although the general trend suggested minor enhancement in solutions acidified by bacterial oxidation of S^0. Thus, silicate

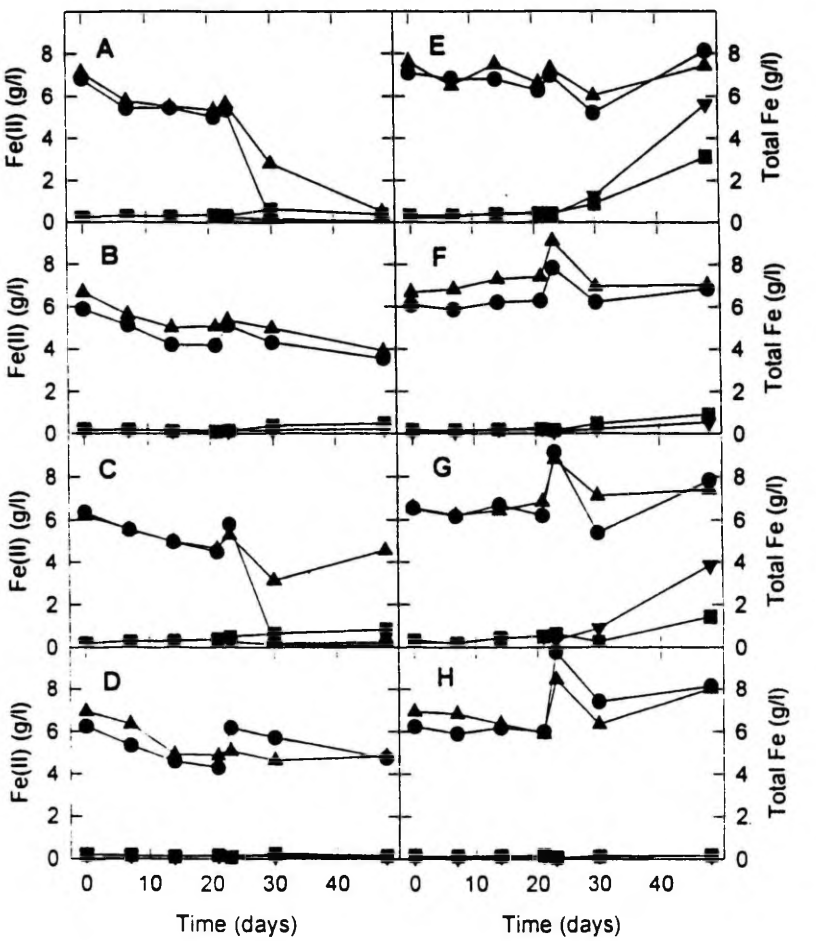

Figure 2. Changes in Fe^{2+} and total Fe concentrations during the leaching of black shale suspensions in acid solutions. The media were inoculated with *T. ferrooxidans* (A and E), *T. thiooxidans* (B and F), or a mixture of *T. ferrooxidans* and *T. thiooxidans* (C and G). Abiotic data are also shown for a sterile control (D and H). Symbols: ●, Fe amendment (added as $FeSO_4$); ■, elemental S amendment; ▼, Fe and S amendment; ▲, no additional substrate amendment.

Table 1. Dissolution of Mo, V, Si, and Al after 49 days contact time in black shale leaching experiments.

Organism	Amendment[a]	% Solubilization			
		Mo	V	Si	Al
T. ferrooxidans	None	49	37	0.8	3.8
	Fe^{2+}	37	37	0.6	3.5
	S^0	N.a.[b]	N.a.	N.a.	N.a.
	$Fe^{2+} + S^0$	N.a.	N.a.	N.a.	N.a.
T. thiooxidans	None	15	31	1.2	4.3
	Fe^{2+}	19	36	0.7	3.4
	S^0	40	40	1.5	5.6
	$Fe^{2+} + S^0$	55	41	1.3	5.4
T. ferrooxidans + *T. thiooxidans*	None	35	36	0.9	3.8
	Fe^{2+}	16	35	0.6	2.9
	S^0	34	39	1.1	4.5
	$Fe^{2+} + S^0$	55	39	1.4	5.6
Sterile control	None	10	35	1.0	4.1
	Fe^{2+}	9.0	36	0.6	3.3
	S^0	11	36	1.1	4.3
	$Fe^{2+} + S^0$	N.a.	N.a.	N.a.	N.a.

[a] Substrate amendment was 120 mM Fe^{2+} added as $FeSO_4 \cdot 7\ H_2O$, 10 g S^0/liter, or both.

[b] N.a., not analyzed.

dissolution was deemed as a chemical, proton-facilitated attack whereas Mo solubilization was based on a combined microbial and chemical reaction. These differences suggest that Mo was at least partially associated with a sulfide phase that was susceptible to the bacterial oxidation and dissolution.

Effect of pulp density on the leaching of black shale

The initial leaching experiment with the black shale sample suggested that the material was inhibitory to the inoculum at >5% pulp density. Consequently, the media that were used as leach solutions were changed and re-inoculated on day 16 of the

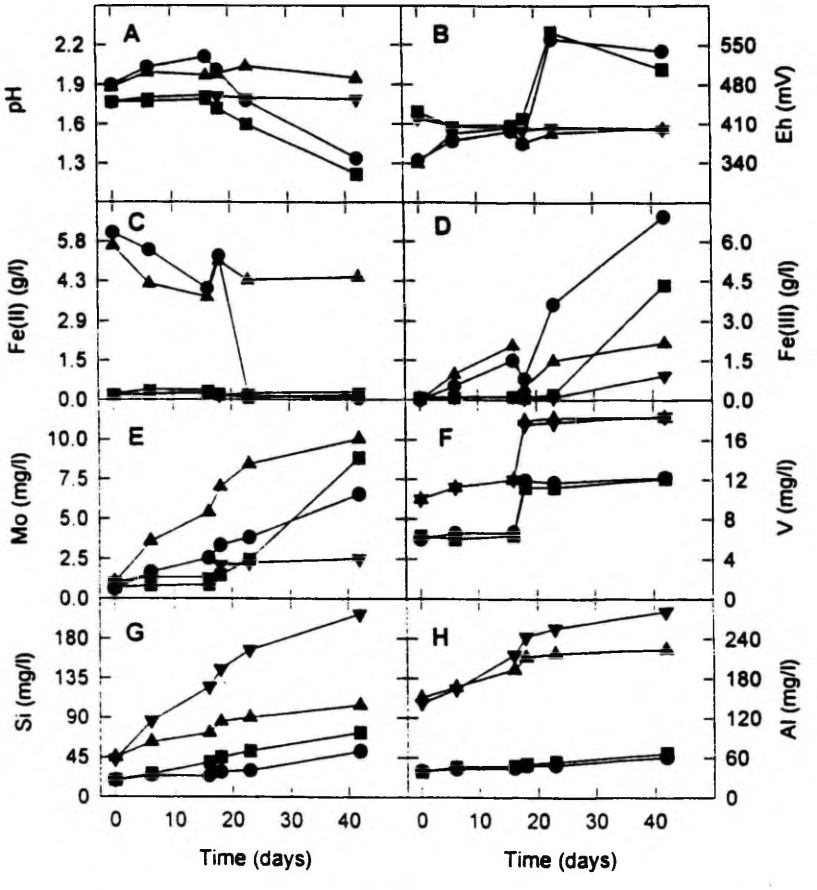

Figure 3. Leaching of black shale suspensions in acid solutions inoculated with *T. ferrooxidans*. The leaching was monitored by measurement of changes in pH (A), redox potential (B), and the concentration of dissolved Fe^{2+} (C), Fe^{3+} (D), Mo (E), V (F), Si (G), and Al (H). Symbols: ● and ■, 5% pulp (wt/vol) density with and without additional Fe; ▼ and ▲, 20% (wt/vol) pulp density with and without Fe amendment (added as $FeSO_4$).

experiment. Subsequent pH and redox potential measurements indicated that bacterial activity was resumed. As shown in Fig. 3A, the black shale sample was acid-producing at 5% but not at 20% pulp density. Acid production was evidence for the oxidation of pyrite and marcasite which were the only sulfide phases in the sample. Acid production

Table 2. Dissolution of Mo, V, Si, and Al after 42 days contact time in pulp density experiments.

Pulp density (% w/v)	Amendment[a]	% Solubilization Mo	V	Si	Al
5	- Fe^{2+}	49	37	0.8	3.8
5	+Fe^{2+}	36	37	0.6	3.5
10	- Fe^{2+}	4.8	22	0.7	3.7
10	+ Fe^{2+}	14	21	0.3	2.8
15	- Fe^{2+}	4.2	16	0.8	3.9
15	+ Fe^{2+}	26	18	0.5	3.5
20	- Fe^{2+}	3.5	14	0.8	4.0
20	+ Fe^{2+}	14	14	0.4	3.2
30	- Fe^{2+}	2.9	11	0.5	3.5
30	+ Fe^{2+}	11	11	<0.1	2.9

[a] 120 mM Fe^{2+} was added as $FeSO_4 \cdot 7\ H_2O$.

was non-existent at 30% but variable at 10-15% pulp density. The respective redox potential values increased in the acid-producing suspensions, in keeping with the oxidation of the Fe-sulfide phases (Fig. 3B). Fe^{2+} oxidation with time was evident at 5% pulp density whereas it was negligible at 20% pulp density (Fig. 3C). Fe-oxidation also took place at 10 and 15% pulp densities. Total Fe values suggested Fe precipitation during the time course in all pulp densities, as shown in Fig. 3D for 5 and 20% pulp densities. The time courses of Mo and V are shown in Fig. 3E and F, respectively. Mo solubilization was time-dependent in most cases whereas V dissolution showed little increase with contact time. The solubilization of Si and Al also increased with time (Fig. 3G and H).

Table 2 shows the relative dissolution of Mo, V, Si and Al from the black shale sample after 42 days contact time. The relative dissolution of Mo and V was enhanced at 5% pulp density where the initial pH decreased to 1.2-1.3 after 42 days of leaching. At >10% pulp densities the solubilization of Mo was enhanced in the presence of Fe-amendment. The dissolution of the other elements did not show a consistent association with pH change or additional Fe. While the solubility of the elements in

leach solutions (minerals salts media) is unknown, the relative dissolution of Mo and V was at least one order of magnitude higher by comparison with Si and Al, suggesting that these two trace elements were not structural components in the silicate phases.

In an attempt to evaluate the potential toxicity associated with the black shale sample, the leach solutions that were replaced on day 16 in 5, 15, and 30% pulp densities were amended with Fe^{2+} and re-inoculated with *T. ferrooxidans*. Fe^{2+} was oxidized within 4 days in samples removed from 5% pulp densities while no oxidation took place in the 15 and 30% pulp density samples. These data suggested that the toxicity was associated with the fraction that was solubilized from the solid phase.

CONCLUDING REMARKS

Fe- and S-oxidizing bacteria such as *T. ferrooxidans* and *T. thiooxidans* used in this study release metals and produce H_2SO_4 because they oxidize sulfide minerals. These microorganisms are ubiquitous and native in coal and metal mine sites (20, 21). Pollution problems associated with leachates from black shales are related to acid production from exposed pyrite closures. Sulfuric acid production also enhances proton-mediated dissolution of other inorganic constituents from the shale matrix. The inhibition of bacterial activity was related to the pulp density and was associated with the leach solutions, suggesting the solubilization of toxic constituents from the black shale. The inhibition is attributed to water-soluble organic compounds or toxic metals in the leachates.

ACKNOWLEDGEMENTS

We thank Dr. R. Palvadre, Institute of Chemistry, Estonian Academy of Sciences, for the sample of black shale concentrate used in this study. Partial financial support was received from the Viro Foundation, Finland (A.T.), Rotalia Foundation, U.S.A. (A.T.), and Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil (O.G.).

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IV

Tasa, A. and Tuovinen, O. H:
Biological leaching of shales — a review.
Oil Shale 15: in press.

BIOLOGICAL LEACHING OF SHALES — A REVIEW

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INTRODUCTION

The purpose of the paper is to present an overview of microbial technology for sulfur removal from shales. Shales are fine-grained, sedimentary mixtures of clay minerals (e.g., illite, montmorillonite), quartz, and micas that have been usually formed by consolidation of mud, clay, or silt. Several types of shales exist depending on the conditions of formation and the associated materials. Some shale deposits may have economic importance because of kerogen content; environmental concerns may also be involved with their commercial mining because of water pollution by leachates containing elevated levels of metals. Table 1 lists major minerals identified in several Estonian shale samples.

Oil-containing shales constitute a significant resource of fossil fuel in many countries, including Estonia where energy production has reliance on using domestic oil shales in thermal power plants. Fossil fuel combustion (oil shale, coal) is a major source of anthropogenic SO_2 emission as well as of other air pollutants such as fly ash, CO_2, and NO_x. SO_2 emissions due to oil shale combustion can be reduced with installation and operation of air filters for cleaning flue gases, or with pre-combustion treatment with chemicals or with microorganisms. Biological removal of sulfur (Table 2) relies on the microbiological oxidation of S-compounds to water-soluble products, principally to sulfates that can be subsequently washed off before combustion. The microbial technology minimizes the loss of BTU content during sulfur removal if the organic carbon matrix is not destroyed. In principle, the biological treatment also decreases the ash content due to the leaching of metals in acid solutions and it may be economically competitive with the chemical and physical methods of S-removal. At present, the technology is in various stages of bench-scale and small pilot-plant studies. The lack of a simple, low-cost technology for removing S from oil shales has greatly suppressed the commercial exploitation of oil shale resources for energy production in many regions of the world (Yefimov *et al.*, 1995).

Microorganisms potentially useful in the removal of sulfur from oil shales are also relevant in coal desulfurization and mineral beneficiation processes which employ bacterial oxidation of sulfide minerals and biologically produced lixiviants for metal leaching (Tuovinen and Fry, 1993). For coal desulfurization, the emphasis has been on the microbiological oxidation of Fe-sulfides (mostly pyrite) that generally make up about 50% of the total S content in coal, but the technology has not been commercialized. Interested reader can find supporting and additional information regarding biological processing of coal in several review articles (Boos *et al.*, 1992; Larsson *et al.*, 1994; Murty *et al.*, 1994; Ohmura and Saiki, 1994). For metal leaching, commercial applications of Fe- and S-oxidizing microorganisms include dump and heap leaching processes for Cu-ores (Rossi, 1990; Schnell, 1997) and biological pre-treatment of refractory Fe-sulfides for

gold recovery (Lindström *et al.*, 1992; Morin, 1995; Dew *et al.*, 1997; Miller, 1997). A substantial amount of literature is available on biohydrometallurgical and coal-processing applications of microorganisms, whereas only few studies have addressed the biological removal of sulfur from shales.

SULFUR IN SHALES

Sulfur in fossil fuels exists in inorganic and organic forms. Both the inorganic and organic S-compounds occur in reduced forms and are potential sources of SO_2 emission upon combustion. In coal and shales, the bulk of the inorganic S fraction is present as Fe-disulfides (Chou, 1990), mostly as pyrite (FeS_2 cubic), although marcasite (FeS_2, orthorhombic) has also been reported. Other sulfide minerals (e.g., Fe-monosulfides, ZnS, $CuFeS_2$, PbS) (Table 3) may be present in minor quantities depending on the geochemical and mineralogical composition of geological deposit. Prolonged exposure of shales and coal seams to rainwater, humidity, and air causes pyrite oxidation to elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$), polythionates ($S_nO_6^{2-}$), and eventually to sulfates (Evangelou, 1995). Microorganisms are largely responsible for these oxidative processes. Extensive sulfate formation due to pyrite oxidation is also a source of sulfuric acid that may have an adverse environmental impact depending on the acid-neutralization capacity of alkaline minerals (carbonates) in exposed deposits and adjacent soils and sediments.

The organic sulfur fraction is complex and variable in structure. Organic sulfur compounds in fossil fuels are believed to occur as aliphatic or aromatic thiols, sulfides or disulfides, thiophenes, and their numerous derivatives (Orr and Damsté, 1990; Chou, 1990). In shales, the main matrix of organic sulfur is kerogen which is defined as sedimentary organic matter that is not soluble in common organic solvents. The S-content may display tremendous variation within short distances even in the same seam of a coal deposit. For oil shales from different geographical regions of the world, it has been reported that the total S content is in the range of 1.8% to 6% and the organic S fraction in the range of 0.1% to 5% (Yefimov *et al.*, 1995). Kerogens greatly vary in composition depending on the original nature of the organic matter and sedimentary environmental conditions. The organic matter in kerogens represents modified macromolecules from biopolymers and products from condensation, polymerization, and cross-linking reactions. Sulfur may incorporate into organic matter through these reactions or it may react with biopolymers or organic precursors. The eventual condensation of carbonaceous residue has also preserved organic sulfur content in kerogen.

BIOTRANSFORMATIONS OF ORGANIC SULFUR

Microbial metabolism of organic S-compounds in fossil fuels is poorly understood. Both aerobic and anaerobic transformations have been characterized with non-polar model compounds such as dibenzothiophene (Fedorak, 1990; Shennan, 1996), but degradative pathways for most organic S compounds in fossil fuels remain elusive. Partial oxidation of organic S-compounds has been noted for heterotrophic bacteria such as *Pseudomonas*, *Rhodococcus*, *Corynebacterium*, *Sphingomonas*, and *Brevibacterium* spp. Condensed thiophene structures such as dibenzothiophene are among the most recalcitrant

organic S compounds in fossil fuels. Dibenzothiophene metabolism may involve a carbon-destructive pathway (Kodama *et al.*, 1973) that leads to the formation of water-soluble metabolites and loss of carbon matrix. Sulfur-specific pathways also exist (Gallagher *et al.*, 1993), with the S-entity removed from dibenzothiophene directly as SO_3^{2-} and oxidized to SO_4^{2-} without a significant loss of carbon or energy from the solid matrix of fossil fuel. Partial oxidations of condensed thiophenes to the respective sulfoxides and sulfones have been reported (Kropp *et al.*, 1997). Naphthalene dioxygenase has been shown to be involved in the initial step of oxidative transformation of dibenzothiophene in a *Pseudomonas* sp. (Resnick and Gibson, 1996). Dibenzothiophene utilization as the sole sulfur source by *Rhodococcus* cultures, with monohydroxybiphenyl as the transformation product, has been demonstrated (Wang and Krawiec, 1996; Wang *et al.*, 1996). The amenability of organic S metabolism to coal and shale processing remains unclear. By comparison, microbial transformations of inorganic S-compounds have been elucidated more thoroughly, especially for the acidophilic Fe- and S-oxidizers that are common inhabitants of environments exposed through mining processes.

ACIDOPHILIC FE- AND S-OXIDIZING MICROORGANISMS

The microorganisms that have potential for desulfurization are capable of oxidizing Fe-sulfides which generally make up the most of the inorganic S fraction. Fe-sulfides can serve as substrates for both Fe- and S-oxidizing bacteria. Microorganisms capable of degrading sulfide minerals and oxidizing sulfur compounds include mesophiles (optimal growth at 20–40°C), moderate thermophiles (35–55°C), and thermophiles (45–80°C). Most of these microorganisms are autotrophic, i.e., they use CO_2 as a carbon source. The microbes most active in inorganic S oxidation are capable of oxidizing both the Fe- and S-entities of FeS_2 under acidic conditions (pH 1–3). The biological oxidation of pyrite and Fe^{2+} is confined to oxic zones and aerobic environments where O_2 is the electron acceptor. The oxidation of inorganic S-compounds also occurs under anaerobic conditions and it is coupled with Fe(III) as the electron acceptor in acidophiles. At circumneutral pH values there exist several bacteria that can couple the oxidation of S-compounds to denitrification.

Among mesophiles, *Thiobacillus ferrooxidans* derives energy for growth by the oxidation of reduced compounds of Fe and S including many different sulfide minerals.

$$4\ Fe^{2+} + O_2 + 4\ H^+ \rightarrow 4\ Fe^{3+} + 2\ H_2O$$

$$S^0 + 1.5\ O_2 + H_2O \rightarrow H_2SO_4$$

$$2\ FeS_2 + 7.5\ O_2 + H_2O \rightarrow 2\ Fe^{3+} + 4\ SO_4^{2-} + 2\ H^+$$

Ferric iron produced during oxidation is a chemical oxidizing agent of pyrite and thereby enhances the bacterially mediated oxidation. The abiotic redox reaction reduces Fe^{3+} to Fe^{2+} which is then re-oxidized by *T. ferrooxidans*.

$$FeS_2 + 14\ Fe^{3+} + 8\ H_2O \rightarrow 15\ Fe^{3+} + 2\ SO_4^{2-} + 16\ H^+$$

Other acidophilic bacteria relevant in these oxidation reactions include *Thiobacillus thiooxidans* and *Leptospirillum ferrooxidans*. *T. thiooxidans* oxidizes inorganic S-compounds (including FeS_2) but not Fe, and is active at pH values as low as 0.5. *L. ferrooxidans* oxidizes only inorganic Fe(II)-compounds. All three acidophiles are common among microorganisms in active and abandoned mine sites (Tuovinen *et al.*, 1991).

Factors contributing to the relative predominance of *T. ferrooxidans*, *T. thiooxidans*, and *L. ferrooxidans* in mine sites, acidic runoffs, and other environments are poorly understood. Natural acid environments are usually characterized by diverse Fe- and S-oxidizing microorganisms, and such microbial consortia have many interactions and commonly involve various heterotrophic organisms and facultative anaerobes (Johnson *et al.*, 1992; Johnson and Roberto, 1997). Under laboratory conditions, mixed cultures can rapidly oxidize FeS_2 but it is difficult to maintain consortia that have stable proportions of *T. ferrooxidans*, *L. ferrooxidans* and other bacteria. Mixed culture work in the area of S-removal has been largely based on empirical approaches.

Mesophilic acidophiles thrive at temperature ranges of up to about 40°C but are inactivated at temperatures that are optimal for the moderate thermophiles. However, reaction kinetics and growth rates can be greatly improved with the use of thermophilic microbes because they can be used at elevated temperatures in the range of 40 to 80°C. Examples of these temperature effects are illustrated in Figure 1 that shows a comparative time course of iron oxidation by *T. ferrooxidans* (22±2°C) and *Sulfolobus acidocaldarius* (65±2°C).

Moderately thermophilic acidophiles (Table 4) have been described that are comparable to the mesophilic *Thiobacillus* and *Leptospirillum* in terms of the range of inorganic S- and Fe-compounds that they can oxidize for energy. *Thiobacillus caldus* (Hallberg and Lindström, 1994) is a sulfur-oxidizer and grows best at 45–50°C. It seems to be present in mixed cultures to afford sulfur oxidation capacity that is completely absent *L. ferrooxidans*-like Fe-oxidizing bacteria. Some *Leptospirillum* isolates (e.g., *Leptospirillum thermoferrooxidans*) have been noted as being moderately thermophilic and capable of growing in the 40 to 50°C range (Golovacheva *et al.*, 1992). *Sulfobacillus thermosulfidooxidans* and *S. acidophilus* as well as many other gram-positive, unnamed isolates have been characterized that can oxidize several sulfide minerals (Norris, 1997). Other moderate thermophiles include Fe-oxidizing isolates of *Acidimicrobium ferrooxidans* derived from *Sulfobacillus* cultures (Clark and Norris, 1996). In general, there is considerable diversity in substrate oxidation by different isolates of moderately thermophilic acidophiles. These organisms have been isolated from stable mixed cultures, suggesting cultural interactions at least at a nutritional level.

Thermophilic acidophiles (Table 4) assigned to the genera *Sulfolobus*, *Acidianus*, *Sulfurococcus*, and *Metallosphaera* are archaea, forming a distinct lineage from the mesophilic and moderately thermophilic acidophilic bacteria. In general, these organisms oxidize inorganic S- and Fe-compounds but the spectrum of substrates and growth requirements vary with the isolates (Norris, 1997). *Sulfolobus acidocaldarius* and *S. metallicus* (formerly *S. acidocaldarius* strain BC, as well as *Acidianus brierleyi* (formerly *Sulfolobus brierleyi*), have been isolated from hot springs and coal spoils and grow at around 70°C. Several *Sulfurococcus* isolates originate from hot springs (Karavaiko *et al.*, 1995). *Metallosphaera sedula* and *M. prunae* have optimum temperatures in the range of 70 to 75°C and grow at >80°C (Huber *et al.*, 1989; Fuchs *et al.*, 1995). The thermoacidophiles have great potential in reactor leaching processes because of the favorable, rapid kinetics of bacterial growth at elevated temperatures.

Larsson *et al.* (1990, 1994) tested several thermophilic archaea for the ability to oxidize pyrite in coal. The most promising results of S-removal from coal were obtained with *Acidianus brierleyi*. There was circumstantial evidence that this organism also enhanced the removal of organic S-compounds, but the biological mechanism remains completely unknown (Larsson *et al.*, 1990). *A. brierleyi* was not adversely influenced by

organic compounds leached from coal, whereas some strains of *S. acidocaldarius* and *S. solfataricus* were sensitive to compounds leached from coal. The chemical nature of these water-soluble organic compounds was not explored further. Such findings are coal-specific and may not be reproducible with samples from other sources.

Tasa and Lindström (1996) found that about 95% of the total S-content of a finely ground oil shale sample was removed in *Sulfolobus acidocaldarius* cultures within four days and there was no evidence for inhibitory effects due to leachates. Fig. 1 shows a comparison of ferrous iron oxidation by mesophilic *Thiobacillus ferrooxidans* and thermophilic *Sulfolobus acidocaldarius* cultures. In this experiment, iron was oxidized within 24 hours in the thermophilic culture whereas it took four days for *Thiobacillus ferrooxidans*. Similarly, the leaching of pyrite from oil shales was fast with *Sulfolobus acidocaldarius* compared with the mesophilic *T. ferrooxidans*. In bioreactor experiments, *Sulfolobus acidocaldarius* removed 94% pyrite from finely ground oil shale sample within 4 days, contrasted with 67% removal of pyrite by *T. ferrooxidans* within 11 days (Tasa and Lindström, 1996).

Leaching processes involving thermophilic bacteria are considered particularly promising for the biological leaching of sulfide minerals such as chalcopyrite and pyrite that tend to require long contact times in mesophilic bacterial processes. An added advantage in thermophilic leaching processes is the heat generation from exothermic oxidation reactions. The generation of heat can be so intensive with bacterial oxidation of sulfide concentrates that a cooling system would be required for mesophilic bacteria in order to prevent prohibitively high temperatures. With thermophilic bacteria, the heat generation would help maintain the elevated temperature range.

ENVIRONMENTAL FACTORS

The kinetics and efficiency of biological removal of S from fossil fuels are a complex function of many interactive factors. These include the metabolic capabilities of microorganisms involved, temperature, acidity, pO_2, pCO_2, nutrients (e.g., P, N, microelements), toxic compounds, mass transfer rates, mineralogy, chemical composition of fossil fuel, and specific surface area of solids. Many of these factors have been discussed in reviews that focus on the bacterial leaching of metals from sulfide ores (Tuovinen *et al.*, 1991; Tuovinen, Fry, 1993; Morin, 1995; Schnell, 1997). Although the solid phase composition in sulfide ores is different from oil shales, the kinetic and thermodynamic principles governing the oxidation processes for Fe and S-compounds are applicable to both ores and shales. Testing of experimental variables is extremely crucial in developing information that can eventually be used for optimization and kinetic modeling.

Most of the bacteria able to remove sulfuric compounds from fossil fuels have the optimum pH between 1.5–3. The pH has a major influence on the biological and chemical leaching rates. Acid attack becomes increasingly more aggressive at low pH values, but the chemical stability of Fe^{2+} is increasingly improved. *T. thiooxidans* and *L. ferrooxidans* grow at pH values at least as low as pH 0.5. Acidophilic thiobacilli tolerate near circumneutral pH values but, in practice, at pH values above 3 the chemical oxidation of ferrous iron becomes increasingly faster and the product, ferric iron, precipitates and does not participate in the leaching reactions.

The suitability of sulfide-containing materials for bacterial oxidation can be assessed on the basis of acid-generating (mostly FeS_2) and acid-consuming (mostly carbonates)

minerals. Brierley and Brierley (1996) have described the biological acid production potential, based on the estimation of acid generation, acid generation potential, and acid neutralization potential.

Estonian oil shales contain organic, sandy-clay and carbonate constituents. The carbonate fraction mostly comprises calcite (Ots, 1992). Acid-consumption due to carbonate dissolution leads to increasing pH values and precipitation of $CaSO_4$, and this is counteractive to the bacterial leaching.

$$CaCO_3 + 2\ H^+ + SO_4^{2-} + H_2O \rightarrow CaSO_4{\cdot}2H_2O + CO_2$$

It is possible to deal with excessive carbonate reaction by titration with H_2SO_4 to satisfy the acid consumption in initial experiments, but the gypsum ($CaSO_4{\cdot}2H_2O$) thus formed covers mineral surfaces and acts as barrier to the diffusion of reactants and products.

The precipitation of ferric iron occurs in the form of hydroxides, jarosites, and hydroxysulfates that also decrease diffusion rates because they tend to coat mineral surfaces. Jarosite precipitation ($XFe_3(SO_4)_2(OH)_6$, where $X = Na^+$, K^+, NH_4^+, or H_3O^+) is considered to be the most important reaction controlling ferric iron solubility at pH <3 (Ahonen and Tuovinen, 1994). At higher pH values other solid phases such as schwertmannite ($Fe_8O_8(OH)_6SO_4$), ferrihydrite ($Fe_5HO_8{\cdot}4H_2O$), and goethite (α-FeOOH) are more dominant (Murad *et al.*, 1994; Bigham *et al.*, 1996).

Silicate minerals found in shales and coal are subject to weathering reactions in acid solutions, mediated by proton attack. Proton attack is enhanced at low pH values and can liberate structural monovalent cations from mica interlayer position in solution (Niemelä *et al.*, 1994a). Depending on the pH, monovalent cations from mica weathering may become incorporated into jarosites which thereby act as sinks for monovalent cations. Silicates vary in their susceptibility to weathering processes. Microbiological weathering of feldspars and micas has been reported (Robert and Berthelin, 1986; Stucki *et al.*, 1992; Barker *et al.*, 1997) but the actual rates are highly variable depending on the mineral and experimental conditions. Typical products of mica weathering in acid solutions relevant to biological desulfurization processes are expandable layer silicate phases (e.g., vermiculite) and mixed layer mica/vermiculite structures.

The prevailing temperatures at mine sites show a great deal of variation. In underground mines the temperatures vary relatively little on a seasonal basis and are usually around 10–15°C depending on geological conditions. In surface heaps, waste piles, and coal spoils, temperatures may be elevated depending on the amount of Fe-sulfides present in the pile. Elevated temperatures that are prohibitively high to mesophilic bacteria are not uncommon in coal spoils. Mesophilic Fe- and S-oxidizers are found active at near-zero temperatures, but the growth and metabolic activity are slow and the minimum temperature still supporting growth remains poorly defined.

As with all biological reactions, the rate of iron and sulfur oxidation is highly dependent on the temperature. Decrease in the temperature below the optimum is accompanied with a decline in the rate of substrate oxidation and an increase in the generation time. Different strains of the same species of bacteria may have slightly different temperature optima. With *T. ferrooxidans*, optimum temperatures reported in the literature range mostly from 25 to 35°C. The maximum permissive temperatures for mesophilic thiobacilli are around 40–44°C (Niemelä *et al.*, 1994b) but considerable strain-dependent variation is likely to exist.

Ahonen and Tuovinen (1989, 1990) reported that the temperature quotient Q_{10} for iron and sulfur oxidation was about 2. The Q_{10} is defined as the increase in the rate or

rate constant of the reaction for each 10°C in the temperature. The activation energies (E_a) for iron and sulfur oxidation by *T. ferrooxidans* were 80 kJ mol^{-1} and 65 kJ mol^{-1}, respectively. The effect of temperature in biological leaching systems is complex because of the formation of reaction zones that become diffusion barriers and decrease the temperature-dependence of the reaction. While elevated temperatures up to 70–80°C are selective for thermophilic archaea (*Sulfolobus*, *Acidianus*, *Metallosphaera*), they also increase the rates of chemical reactions and decrease the solubility of O_2 and CO_2.

Acid leaching systems contain numerous water-soluble constituents that are potentially toxic or inhibitory to biological processes. These toxicity effects may be due to inorganic ions (metal ions) or organic compounds leached from the fossil fuel matrix. The toxicity varies with the bacterial strain, environmental conditions, the length of exposure, and the concentration of the element. Figure 2 summarizes the range of metal concentrations inhibitory to acidophilic Fe- and S-oxidizing bacteria. The data in Figure 2 are all based on $FeSO_4$ oxidation data pooled from published studies, with variations in test strains, experimental conditions, and assay methodology.

By comparison with heterotrophic bacteria living in circumneutral pH values, acidophilic thiobacilli can tolerate relatively high concentrations of Cu, Co, Ni, and Zn in their growth medium. This property is one of the underlying requisites for their use in metal leaching processes. The biochemical basis of this high level of resistance is not known. Silver, mercury, and molybdenum are among the most toxic metals to Fe- and S-oxidizers. Specific mechanisms of toxicity and resistance are mostly unknown. Biochemical basis of resistance to toxic metals is known in the case of mercury and it involves a mercuric reductase enzyme complex that reduces Hg^{2+} to Hg^0 (Booth and Williams, 1984). Elemental mercury thus formed is removed from the cell and growth medium because of its volatility. The toxicity of metals to bacteria is subject to their bioavailability which can be altered with chelating agents; e.g., yeast extract can decrease the effective, bioavailable concentration of metals because of a complexation effect. In the presence of clay minerals (e.g., kaolinite and illite) and oxides (Al_2O_3), bacteria can be partially protected owing to cation exchange properties of clays that decrease the available concentrations of metals.

Some Estonian shales contain toxic elements such as uranium, molybdenum, vanadium at levels (Pukkonen, 1989) that are clearly toxic to bacteria if completely solubilized in leach solution. The geochemical reactions controlling the solubility of elements such as molybdenum and vanadium are poorly understood in biological leaching systems. Factors influencing the bioavailability of these and other elements in the presence of bacteria and mineral sulfide substrates have yet to be elucidated. Inhibitory effects attributed to the leaching of toxic elements into aqueous phase have been reported in shale leaching experiments (Mahapatra *et al.*, 1985; Tasa *et al.*, 1995) but the toxic constituents have not been identified.

BIOLOGICAL LEACHING STUDIES WITH SHALE MATERIALS

By far, most of the bioleaching studies have been carried out with sulfide ores or high-sulfur coal samples. There are only few reports on the bacterial oxidation of pyrite in shale materials. Shale-containing seams have been disposed of in many mines as waste materials and, when exposed to rain water, humidity, oxygen and native bacteria over time, they become continuous sources of dissolved metals in receiving waters. Biologi-

cal leaching of metals has been reported for black shales in different geographical regions (Iskra *et al.*, 1980, Konopka *et al.*, 1993, Tasa *et al.*, 1995). Bacterial leaching of black shales was explored in the former USSR in the 1960's and 1970's (Iskra *et al.*, 1980), but no specific details of these projects have been disclosed since that time. The underlying interest of the Soviet research program was related to uranium processing.

Shales are composed of silicate phases and organic matter, and bacterial leaching of shales is of interest in assessing the potential mobility (and recovery) of heavy metals (Konopka *et al.*, 1993, Tasa *et al.*, 1995). The solubilization of pyrite from high-sulfur shales is another reason for these studies because it is the potential source of sulfuric acid (Mahapatra *et al.*, 1985). Bacterial oxidation can also be viewed essentially as a pretreatment step in preparation of kerogen concentrates (Findley, 1974; Vrvic, *et al.*, 1987). There are also pressing issues of environmental pollution that can stem from exposed shales in waste heaps and dumps (Tasa and Lindström, 1996). As indicated in Table 5, Estonian shale samples contain toxic elements (U, Mo, V) that can potentially be solubilized when shales are exposed to acid conditions and pyrite inclusions oxidized to water-soluble products.

Bacterial leaching of U, V, Mo, Zn, and Pb from Polish shale samples was investigated in long term (7 months) experiments by Sztaba *et al.* (1989), Konopka and Sztaba (1991), and Konopka *et al.* (1993). The bacterial leaching of zinc and lead was insignificant, as most of the Zn and Pb content (95% Zn and 55% Pb) was leached chemically during the initial stage of the experiments. Maximum yields of dissolution in these experiments were 75% U, 60% Mo, and 47% V. Short-term shake flask studies with Estonian black shale samples have also suggested that Fe- and S-oxidizing bacteria enhance the dissolution of these elements (Tasa *et al.*, 1995, 1997). This enhancement may be due to co-solubilization with pyrite dissolution, or increased acid dissolution via bacterial production of sulfuric acid. Some shales are pyrite-rich or they have pyrite-containing zones interlayered with shales. Shale samples have been tested for bacterial leaching that have contained as much as 11% Fe as pyrite (Mahapatra *et al.*, 1985).

While pyrite inclusions in shales are biologically degradable, the depyritization process greatly depends on the presence of accessory minerals. Reaction of acid leach solution with alkaline minerals (calcite, dolomite) causes acid-consumption that can be satisfied with pretreatment with sulfuric acid (Vrvic, 1988; Tasa and Lindström, 1996). As the high CaO content indicates in Table 5 for the Ida-Virumaa oil shale sample, some materials contain Ca-carbonates that cause excessively high acid consumption when subjected to acid leaching conditions. Biological oxidation of supplemental S^0 has been proposed as a source of sulfuric acid to compensate for acid consumption (Findley *et al.* 1974).

Vrvic *et al.* (1987) reported that up to 95% of the sulfur content was removed from oil shale samples (from Aleksinac, former Yugoslavia) within a week by exposing the finely ground oil shale to the leaching action of *T. ferrooxidans*. In a study conducted with an Indian black shale sample, Mahapatra *et al.* (1985) achieved >95% removal of pyrite after three weeks of leaching in *T. ferrooxidans* cultures. For Estonian oil shales (from Ida-Virumaa), Tasa and Lindström (1996) reported 90 to 96% removal of pyrite (5% pulp density) within a week, based on the oxidative action of *T. ferrooxidans* or *Sulfolobus acidocaldarius* in shake flask experiments. In stirred tank bioreactors that allow for improved process control, *T. ferrooxidans* oxidized about 65% of the pyrite content in 10% pulp density to water-soluble products in 11 days, while *Sulfolobus acidocaldarius* removed 94% of the pyrite content in four days. These comparisons

clearly underscore the rate and other kinetic comparisons in favor of the thermophilic *Sulfolobus* species.

CONCLUDING REMARKS

Chemical depyritization of oil shales often leads to substantial alterations in the organic matter fraction (kerogen) and a loss of BTU content in shales. Biologically mediated, oxidative removal of pyrite from oil shale is the least non-destructive method. Biological removal can also be used for non-destructive purification of kerogen for structural studies. The complexity of shales due to inert mineral phases (quartz), clay minerals with surface active properties, organic matter fraction particles, oxidizable sulfide minerals such as pyrite, and acid-reactive carbonates poses a challenge to identify reaction mechanisms, pathways, and products. Some shales contain potentially toxic metals but their solubility, bioavailability, and geochemical speciation are unknown at this time.

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Table 1. Mineralogical characteristics of Estonian shale samples used in preliminary leaching experiments.

Sample	Qualitative Mineralogical Composition[a]
Black shale from Toolse phosphorite deposit	Quartz, K-feldspar (orthoclase), pyrite, illite, kaolinite
Kerogen-rich flotation concentrate of Toolse black shale	Quartz, K-feldspar (orthoclase), pyrite, illite, kaolinite
Pyrite-rich flotation concentrate of Toolse black shale	Quartz, K-feldspar (orthoclase), pyrite, illite, kaolinite, marcasite
Ash residue of Toolse black shale	Quartz, K-feldspar (orthoclase), hematite, illite, gypsum
Black shale from Maardu phosphorite deposit	Quartz, K-feldspar (orthoclase), pyrite, illite,
Oil shale from Ida-Virumaa	Quartz, dolomite, calcite, pyrite

[a] Major mineral phases identified by x-ray diffraction; data pooled from Tasa *et al.* (1995, 1997) and courtesy of Dr. Martti K. Lehtinen, Geological Museum, University of Helsinki, Finland

Table 2. Categorical representation of microbiological removal of sulfur from fossil fuel.

Sulfur fraction in fossil fuel	Main Groups of Compounds	Microbes
Inorganic S	Fe-sulfides	Acidophilic Fe- and S-oxidizing bacteria, oxidation to water-soluble products (Fe^{3+} and SO_4^{2-})
Organic S	Organic thiols, sulfides, and thiophenes	Heterophic bacteria, partial degradation to various non-polar and water-soluble products

Table 3. Sulfide minerals found in shales and low-grade coals, and various inorganic sulfur compounds that are intermediates or products from biochemical pathways of sulfur oxidation by thiobacilli.

S-compound	Chemical formula
Sulfide	S^{2-}, HS^-
Elemental sulfur	S^0
Thiosulfate	$S{\cdot}SO_3^{2-}$
Tetrathionate	$^-O_3S{\cdot}S{\cdot}S{\cdot}SO_3^-$
Trithionate	$^-O_3S{\cdot}S{\cdot}SO_3^-$
Sulfite	SO_3^{2-}
Sulfate	SO_4^{2-}
Pyrite	FeS_2
Marcasite	FeS_2
Pyrrhotite	$Fe_{1-x}S$
Mackinawite	FeS
Chalcopyrite	$CuFeS_2$
Sphalerite	ZnS
Galena	PbS
Arsenopyrite	FeAsS

Table 4. Examples of microorganisms that have potential capacity in the desulfurization of fossil fuel.

Broad Temperature Range	Microorganisms	Characteristic Inorganic Substrate Spectrum
Mesophiles (up to 45°C)	*Thiobacillus ferrooxidans*	Fe^{2+} and S-compounds, sulfide minerals
	Thiobacillus thiooxidans	S-compounds, sulfide minerals
	Leptospirillum ferrooxidans	Fe^{2+}, pyrite
Moderate thermophiles (35 to 55°C)	*Thiobacillus caldus*	S-compounds, sulfide minerals
	Leptospirillum thermoferrooxidans	Fe^{2+}, pyrite
	Sulfobacillus thermosulfidooxidans	Fe^{2+}, sulfide minerals
	Sulfobacillus acidophilus	Fe^{2+}, sulfide minerals
	Acidimicrobium ferrooxidans	Fe^{2+}, pyrite
Thermophiles (50–80°C)	*Acidianus brierleyi*	Fe^{2+}, S-compounds, sulfide minerals
	Sulfolobus acidocaldarius	Fe^{2+}, S-compounds, sulfide minerals
	Sulfolobus metallicus	S^{o}, sulfide minerals
	Sulfurococcus yellowstonii	Fe^{2+}, sulfide minerals, S^{o}
	Metalloshaera sedula	Fe^{2+}, sulfide minerals, S^{o}
	Metallosharea prunae	Sulfide minerals, S^{o}, H_2

Table 5. Partial elemental analysis of shale samples used in preliminary leaching experiments (concentration (% wt/wt). Data pooled from Tasa *et al.* (1995, 1997), Tasa and Lindström (1996), and courtesy of Dr. Antti Vuorinen, Department of Geology, University of Helsinki, Finland.

Component	Black shale (Toolse)	Black shale (Maardu)	Oil shale
SiO_2	55.30	52.90	1.60
Al_2O_3	8.18	13.40	3.05
Fe_2O_3	7.85	5.15	4.15
MgO	1.08	1.00	3.23
CaO	2.95	0.22	30.00
Na_2O	0.82	0.80	0.04
K_2O	4.92	8.30	1.00
MnO	0.04	0.02	NA[a]
Ti_2O	0.51	0.83	0.18
S	2.21	1.47	4.75
U	0.0095	0.0026	NA
Mo	0.0245	0.0013	NA
V	0.0740	0.0240	NA
Loss of Ignitation	18.00	17.90	56.00

[a] NA, not analyzed

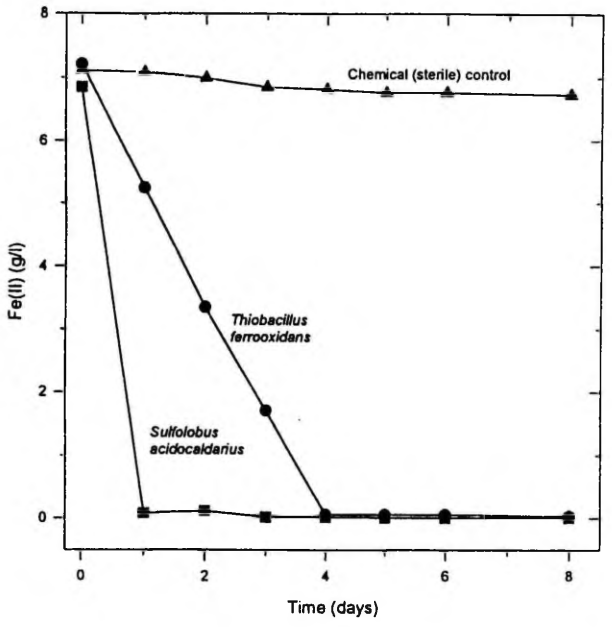

Figure 1. Ferrous iron oxidation by *T. ferrooxidans* and *Sulfolobus metallicus* (formerly *S. acidocaldarius* strain BC). The cultures received 6.5 g Fe^{2+}/l (added as $FeSO_4*7H_2O$) and were incubated at 22±2°C (*T. ferrooxidans*) and 65±2°C (*S. metallicus*). Further details can be found in Tasa and Lindström (1966).

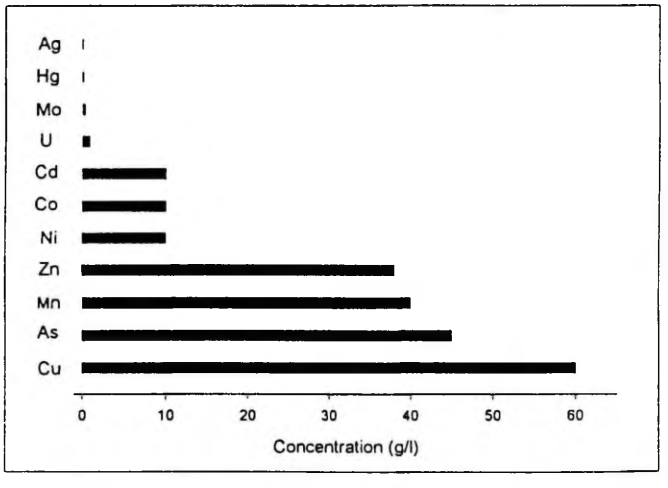

Figure 2. Spectrum of resistance of *Thiobacillus ferrooxidans* to different metals. The columns indicate the concentration ranges where activity or growth of *T. ferrooxidans* has been reported. Data have been pooled from several literature sources that have considerable differences in experimental variables and methodology of establishing the level of toxicity or resistance. Substantial variation occurs with different strains. The experimental design has not included testing for complexation, precipitation, or redox speciation. These data can be constructed as a relative ranking of metal toxicity based of magnitude in the inhibitory concentration.

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ISSN 1024–6479
ISBN 9985–56–343–3