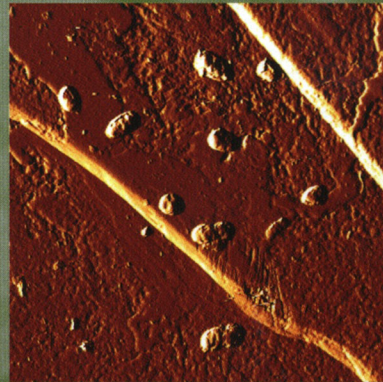
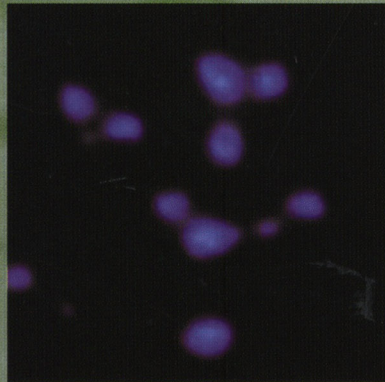


# Biohydrometallurgy: From the single cell to the environment



FM- and AFM-Images of Cells on a Pyrite Surface (Mangold & Sand, Biofilm Centre, University Duisburg-Essen, Germany)



Lichtenberg open pit in 1991 and "New Ronneburg Landscape" with reclaimed Lichtenberg open pit mine (Wismut GmbH, Germany)

Edited by

**Axel Schippers, Wolfgang Sand,  
Franz Glombitza, Sabine Willscher**



Proceedings of the  
17th International Biohydrometallurgy Symposium

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The International Biohydrometallurgy Symposium 2007 is the 17th event in this series. The first symposium took place in Wolfenbüttel, Germany, in the year 1977. In recent years attention has focused increasingly on the role that microorganisms play in the treatment of minerals, metals, coal, oil, waste materials and also in related environmental issues. Today, besides new technologies for the production of raw materials and valuable substances, the main tasks are focused on the remediation of former mining sites and environmental protection connected with the different kinds of mining and the mining industries. Increasing contributions to the field from disciplines like genetics, biochemistry, electrochemistry, microbiology, hydrometallurgy, chemistry, geology and process engineering have affected the rapid development as well as pressure from legislation of the European Union and of national governments. The International Biohydrometallurgy Symposium IBS2007 is held at the well-equipped DECHEMA premises in Frankfurt am Main, Germany. The Symposium provides a forum to present the latest scientific and technological advances in this area, and an opportunity for biotechnologists, practitioners and environmentalists to meet and discuss challenging future trends in the field.



ISBN 0-87849-452-9, ISBN-13 978-0-87849-452-1  
Advanced Materials Research Vols. 20-21  
Electronically available at <http://www.scientific.net>

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**(IBS 2007)**

2-5 September 2007  
DECHEMA Gesellschaft für Chemische Technik  
und Biotechnologie e.V  
Frankfurt am Main, Germany

*Edited by*

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Volumes 20-21 of  
*Advanced Materials Research*  
ISSN 1022-6680

Full text available online at <http://www.scientific.net>

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Cover page pictures provide by  
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## Removal of organically bound sulfur from oil shale by iron(III)-ion generated-regenerated from pyrite by the action of *Acidithiobacillus ferrooxidans*

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**Keywords:** oil shale, *Acidithiobacillus ferrooxidans*, iron(III)-ion, dibenzothiophene (DBT), desulfurization

**Abstract.** Oil shales are one of the alternative sources of hydrocarbon fuels (“synthetic petroleum”), characterized by the increased sulfur and nitrogen content which represent even greater ecological problem in use, compared to classical fuels.

*Acidithiobacillus ferrooxidans* is capable of oxidizing pyrite to iron (III)-ion, providing a strong oxidation agent at low pH. We have used this oxidizing agent for oxidation of sulfur present in DBT as a substrate model to demonstrate its potential to oxidize organically bound sulfur in oil shales. An HCl-concentrate was used as the hydrocarbon matrix. *Acidithiobacillus ferrooxidans* is already recognized to oxidize the pyritic sulfur component, thereby potentially providing a complete sulfur removal system.

By applying GC-MS we established that DBT transformation occurred by oxidation or elimination of sulfur. The products obtained are more soluble in water than parent compounds and this reduces concentration of organic sulfur.

### Introduction

Oil shales as a raw material for obtaining “synthetic petroleum” are of economic interest and therefore are the subject of interest and research. The biggest share of oil shales (cca. 80%) are inorganic components, such as carbonates, aluminosilicates and pyrite. Kerogen, which is insoluble and of diverse, heterogeneous, cross-linked macromolecular structure, is a dominant organic substance (over 90%), while bitumen, soluble in the organic solvents is present as only a few percent. Sulfur as the main unwanted element in oil shales is predominantly present in pyrite and is also organically bonded. The share of sulphate and elementary sulfur is minor (irrelevant). Nitrogen in shales is far less abundant than sulfur and the ingredient is of organic substance. In the process of obtaining the “synthetic petroleum” and its further processing and use, both sulfur and nitrogen give environmental unfriendly SO<sub>x</sub> and NO<sub>x</sub> [1].

In this work we applied our scientific desulfurization hypothesis of DBT [2, 3] as a model substrate of the organically bound sulfur in the presence of oil shale (free from carbonate-“HCl-concentrate”) as fossil fuel hydrocarbon matrix by the action of iron(III)-ion generated by *Acidithiobacillus ferrooxidans* (*At.f.*) which is based on the following idea: “To convert DBT into the (water soluble) sulfur-free form with the oxidation by bacterially generated-regenerated iron(III) sulphate from pyrite” as shown in Fig. 1.

Thermodynamic computations of DBT desulfurization (R=S) to biphenyl (RH<sub>2</sub>) by the action of iron(III) sulphate according to the Eq. 1 indicate that free energy of this process under standard conditions (unit activity and 298 K) and at pH 2.5 has the value  $\Delta G_{2.5}^0 = -168$  kJ.

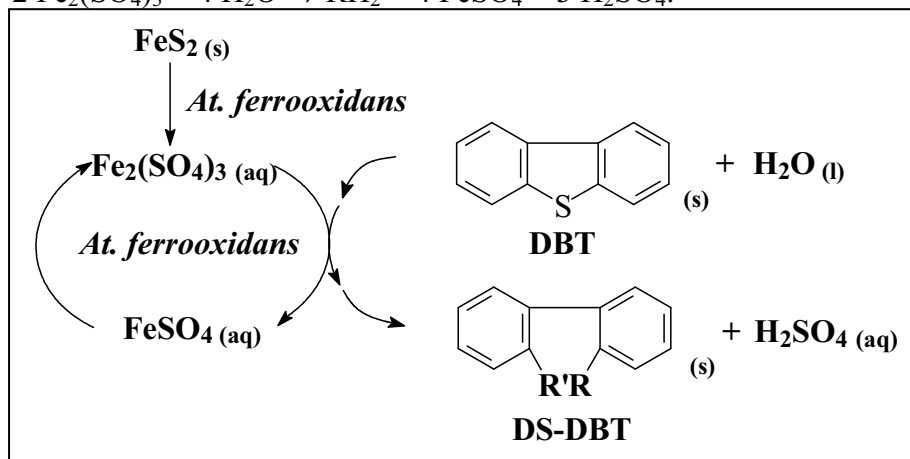


Fig. 1. Schematic presentation of hypothesis.  
DS-DBT-Desulfurized DBT.  
R=R' = -H and/or -OH.

This means that the process can be spontaneously developed, being one of the proofs that theoretically the hypothesis is correctly set.

The presence of pyrite as the source of iron(III)-ion together with DBT should favor oxidation of thiophenic sulfur.

Obtained iron(II)-ion is included again in „ferrous-ferric cycle” of *Acidithiobacillus ferrooxidans* and thereby the cycle would be closed.

## Material and Methods

The examined sample which originated from the oil shale deposit near Aleksinac (Serbia) was crushed and then pulverized to < 63µm. The HCl-concentrate was obtained by treatment of the powdered shale with dilute hydrochloric acid (1:4 V/V). The content of total sulfur was 3.36 %.

A pure culture of the most active zymogenous strain of *At.f.* was isolated from an oil shale specimen taken from the old open pit mine near Aleksinac [4]. In preparing the bacterial biomass for experiments [5], a physiologically active culture of *At.f.* was cultivated in 9K medium [6].

Pyrite concentrate was prepared from the commercial concentrate (Copper mine Bor, Serbia) by treating with sulfuric acid solution (0,5M; shaking; room temperature; 12 hours). Thereafter it was washed in demineralized water, ethyl acetate and drying at 80°C (sterilization at the same temperature). For experiments fraction < 63µm was used.

The shaking flask test technique was used in the experiments carried out according to a general experimental scheme described elsewhere [7].

The model solutions contained 9K iron-free medium with initial pH 2.5, suspended DBT (25 mg/L), pyrite concentrate as an iron(II) ion source (purity *ca.* 85 %; pulp density 2.0 %-equivalent with 9 g Fe/L), and HCl-concentrate of oil shale (100 g/L). To suspend DBT in the medium, *N,N*-dimethylformamide were used. Biotic tests were inoculated by *At. ferrooxidans* biomass. As a control, the same mixture was used, but sterilized prior to commencing incubation. Starting concentrations of microorganisms were approx. 10<sup>9</sup> cells per mL<sup>-1</sup>. Parameters were determined initially and every 7 days afterwards. Experiments lasted 28 days at 28°C temperature [3].

The following methods and instruments were used.

**Quantitative FTIR.** KBr pellet of solid substrate (1:100). 0.2 % KCNS internal standard ( $v_{max}$  2065 cm<sup>-1</sup>). Thermo Nicolet, AVATAR 370 FT-IR.

**GC-MS.** Analysis of ethyl acetate extracts of total reaction mixture. Agilent GC System 6890N, with Injector 7673 Series Agilent, and mass selective detector 5973), equipped with 30 m type DB-



5ms column (Agilent). The carrier gas was helium, and the flow rate was 1.0 mL/min. The injection temperature was maintained at 250°C. The oven temperature was programmed to start at 50°C, and to increase to a final temperature of 250°C at the rate of 10 °C/min.

The interpretation of each spectrum was performed by comparison with the commercial NIST 02, Wiley database of spectra, using Agilent probability base matching (PBM) software for mass spectrometry search.

*At.f.* cell concentration, pH and total iron in the solution were also determined. On the basis of these, the share of bacterially dissolved pyrite were calculated. Presented were only the results which directly point out to DBT transformations and changes of hydrocarbon matrix.

## Results and Discussion

Infrared spectra were used for estimation of a change of hydrocarbon component of oil shale kerogen on the basis of characteristic bands for aliphatic structures (2920, 2850 and 1475 $\text{cm}^{-1}$ ) and carbonyl group (1720 and 1710  $\text{cm}^{-1}$ ) [8].

Band intensity ratio of aliphatic and oxygen functional groups shows occurrence of smaller oxidation changes in the kerogen structure only in the biotic model systems.

On the basis of GC-MS profiles of ethyl acetate extracts, DBT transformations were proved. Identified were dibenzothiophene S-oxide (DBT-O), dibenzothiophene S,S-dioxide (DBT-O<sub>2</sub>), 2-hydroxybiphenyl (2-HBP) in all biotic experiments (except at “zero” time) with probability greater than 90 % as the first compounds from the hit list of PBM search of Wiley database of spectra.

On the basis of the obtained results related to identified products of DBT transformation, assumed desulfurization mechanism is shown on Fig.2.

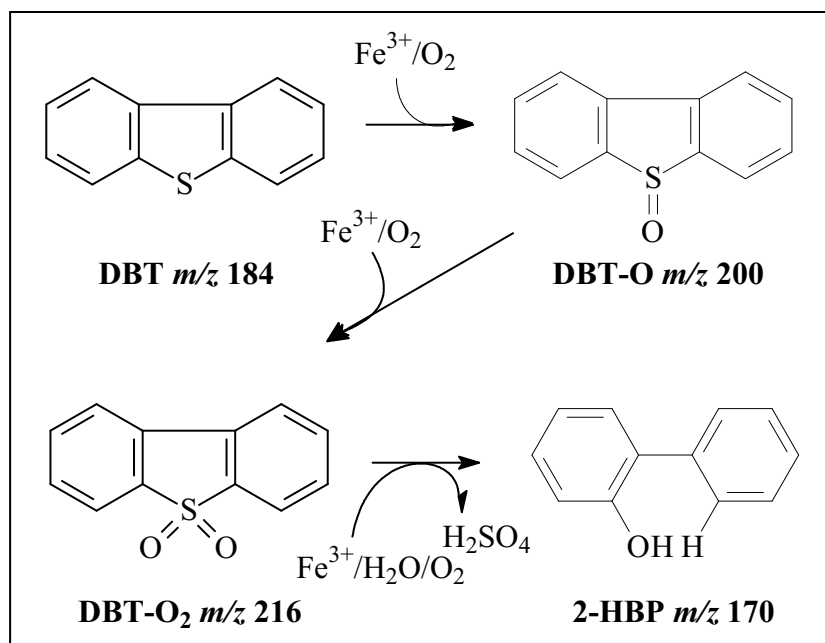


Fig. 2. Assumed mechanism of DBT desulfurization

This fact points out that DBT transformation-oxidation is fully of abiotic type by action of iron(III)-ions generated from pyrite in the biotic model system.

The reaction products obtained are more soluble in water than the basic substrate (for example, DBT-O 320 times, and DBT-O<sub>2</sub> 6 times) [9]. This gives the possibility for direct decrease of the organically bound sulfur content in fossil fuels, by dissolving more polar compounds obtained by iron(III)-ion action-oxidation.

These results confirm our basic hypothesis.

It is interesting that presence of DBT-O<sub>2</sub> was demonstrated in the sterile control test after 28 days. The assumption is that this was due to abiotic oxidation by residual iron(III)-ion present in extracellular polymeric substances (EPS) of *At.f.* [10] since abiotic tests were obtained by sterilization of inoculated experiments.

By the action of *At.f.* on oil shale, the efficient depyritization [7,8] is also carried out, which means that the process is potentially applicable also for entire desulfurization of solid fossil fuels.

## Summary

On DBT as a substrate model in the presence of oil shale (free from carbonate-“HCl-concentrate”) as hydrocarbon matrix, it was proved that there is a possibility of separation of organically bound sulfur from solid fossil fuels, by the effect of iron(III)-ion generated-regenerated by means of *At.f.* from pyrite as the oxidant source.

By means of GC-MS, the products of DBT transformation were identified. On the basis of these the DBT desulfurization mechanism was presupposed.

## Acknowledgments

This work was supported by the Ministry of Science and Environmental Protection of the Republic of Serbia. Grants No. 142018B and 7032B.

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