ELSEVIER

Thermochimica Acta 309 (1998) 79–85

thermochimica acta

Determination of reaction energy values for biological pyrite oxidation by calorimetry[1]

Thore Rohwerder*, Axel Schippers, Wolfgang Sand

Universität Hamburg, Institut für Allgemeine Botanik, Abteilung Mikrobiologie, Ohnhorststr. 18, D-22609 Hamburg, Germany

Received 3 June 1997; received in revised form 4 August 1997; accepted 28 August 1997

Abstract

The reaction energy values for pyrite oxidation by chemolithotrophic leaching bacteria (pure cultures of *Thiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, and mixed cultures of *L. ferrooxidans* and *T. thiooxidans*) were calorimetrically measured in batch experiments. The obtained data were compared with the nonconservative values for the corresponding abiotic processes that were calculated from standard enthalpies and chemical analyses of the leaching products. Due to the different abilities of the investigated species to oxidize the sulphur moiety of pyrite, the measured reaction energy values ranged from −1100 to −1600 kJ/mol with an accuracy of 4–16%. In samples with *T. ferrooxidans* and with the mixed cultures, no significant difference between the calorimetric and theoretical reaction energy values occurred. In contrast, pure cultures of *L. ferrooxidans* exhibited measured values which were up to 200 kJ/mol lower than the theoretical ones. It is highly unlikely that this difference may be explained by a higher energy conservation efficiency of *L. ferrooxidans* compared to the one of *T. ferrooxidans* or *T. thiooxidans*. Besides use in efficiency studies, the collected data can be used to determine the activity of leaching bacteria in natural biotopes by calorimetric measurements. © 1998 Elsevier Science B.V.

Keywords: Bioleaching; Calorimetry; Chemolithotrophy; Energy efficiency

1. Introduction

Leaching bacteria are acidophilic chemolithotrophic organisms that live in extreme biotopes such as deposits of sulphide minerals at pH values around 2–3 [1]. These bacteria are able to use reduced inorganic sulphur and metal compounds as the sole energy source for autotrophic growth. The most important mesophilic leaching bacteria are *Thiobacillus ferrooxidans*, able to oxidize both iron(II) ions and reduced sulphur compounds, *Leptospirillum ferrooxidans*, only able to use iron(II) ions, and *T. thiooxidans*, oxidizing only reduced sulphur compounds. In leaching biotopes, the metal sulphide pyrite (FeS_2) is an abundant substrate that can be oxidized completely to sulfate and iron(III) ions:

$$FeS_2 + 3.75O_2 + 0.5H_2O \rightarrow 2SO_4^{2-} + Fe^{3+} + H^+ \quad \Delta_f H^0 = -1546\,\text{kJ/mol} \qquad (1)$$

This biological process is applied by the mining industry for metal recovery. On the other hand, bio-

*Corresponding author. Tel.: +49 40 822 82 427; fax: +49 40 822 82 423

[1]Presented at the Tenth Conference of the International Society for Biological Calorimetry, Ascona, Switzerland, 27–30 April, 1997.

PII S0040-6031(97)00352-3

leaching can cause a detrimental impact to the environment if the acidic heavy metal containing drainages of leaching sites enters rivers or groundwater [2,3].

Since the studies of Baas-Becking and Parks [4], the efficiencies of chemolithotrophic energy conservation are a matter of controversial discussion. Chemolithotrophic bacteria were regarded as inefficient because efficiencies that were calculated from growth yields and quantifications of CO_2 fixation were low compared with the corresponding data for chemoorganotrophic organisms. Kelly [5] pointed out that aforesaid calculations do not consider that these bacteria could not gain directly reducing equivalents, i.e. mainly NAD(P)H, from their inorganic substrates, but have to spend part of their proton motive force energy on the production of reducing power for CO_2 fixation. If this energy consumption is taken into account, an efficiency of 50% can be calculated. Nevertheless, more experimental data are needed to elucidate the true energy-conserving potential of chemolithotrophs. In addition to growth yields, data for efficiency calculations could also be obtained by calorimetric measurements of the conservative reaction energy, i.e. the energy change that occurs during the oxidation of a substrate by growing cells. Until now, however, calorimetric studies on the energy conservation of chemolithotrophic growth on pyrite or other substrates of leaching bacteria do not exist or the collected data were insufficient for efficiency calculations [6]. Generally, the investigations include only characterizations of strains and metabolic pathways [7–11]. Schröter [6] presented calorimetrically determined reaction enthalpies of the biological iron(II)-ion oxidation, but the corresponding nonconservative enthalpies could not be calculated with sufficient accuracy due to the poorly characterized reaction products. The only other calorimetric studies on the energy efficiency of chemolithotrophic metabolism were conducted with nitrifying bacteria [12,13].

In this communication, we present an attempt to determine the reaction energy of pyrite oxidation by pure and mixed cultures of leaching bacteria. The calorimetrically determined values for reaction energies were compared with the theoretical ones that would occur in the corresponding abiotic, i.e. nonconservative, oxidation process.

2. Experimental

2.1. Organisms and growth conditions

T. ferrooxidans (strain R1), *L. ferrooxidans* (ATCC 49879), and *T. thiooxidans* (strain R20) [14], were grown in a medium containing iron(II) sulphate [15] or elemental sulphur [14], respectively, at 28°C. Cells were harvested by centrifugation. Cell numbers were determined by counting with a Helber chamber.

2.2. Leaching experiments

Pyrite used for the leaching experiments (flotation grade, Scanore, Germany) was sieved and washed as described previously [16]. For all experiments, a grain size of 50 to 100 μm was used. For leaching experiments, sterilized 300 mL conical flasks, each containing 10 g of pyrite and 150 mL of medium [15] without iron(II) sulphate (pH 1.9) were inoculated with pure cultures of *T. ferrooxidans* or *L. ferrooxidans* or with mixed cultures of *L. ferrooxidans* and *T. thiooxidans* (10^9 cells/g of each strain). The flasks were shaken (140 rpm) and incubated at 28°C.

2.3. Calorimetric measurements

For the determination of the reaction energy of biological pyrite oxidation, the heat evolution and the iron ion mobilization of pyrite from shake cultures were measured by batch experiments. A thermal activity monitor (Thermometric AB, Sweden) was used for isothermal heat conduction measurements at 30°C. Heat evolution of pyrite samples was measured using sealed glass ampoules with a volume of 3 mL. Cultures of different age were filtered (pore size <0.1 μm). The residue obtained, pyrite with cells, was filled in glass ampoules (1–2 g wet weight per ampoule, water content ca. 10%), and calorimetrically measured. Because of the restricted oxygen concentration in the sealed ampoules, the measuring time of the experiments was limited. In previous experiments it was found that, once the heat output had entered the plateau phase, the calorimetric signal was nearly constant until a total heat evolution of 7–8 J was reached. During this phase only a slow decrease of power of up to 20% was observed. This phase was followed by a rapid decrease to nearly zero within 1 h,

because of oxygen depletion in the sealed ampoules. Therefore, the experiments were stopped after a heat evolution of 5–6 J, corresponding to an incubation time of 3–40 h, depending on the microbial activity. With the pyrite of one culture, 6 to 8 ampoules were filled of which 3 to 4 parallels were used for calorimetric measurements. The remaining ampoules were incubated at 30°C and leaching activity was stopped by freezing when the heat output of the samples had reached the plateau phase. At the end of the experiment, the samples were removed from the calorimeter and were also frozen. Thus, the amount of solubilized iron ions (using the values at the beginning of the plateau phase and at the end of the experiment) and the corresponding heat output could be determined.

2.4. Chemical analyses

Iron ions were photometrically determined [17]. Sulphur compounds were determined by high pressure liquid chromatography (elemental sulphur, polythionates), atomic absorption spectrophotometry, and ion chromatography (sulfate), as previously described [16]. Sulphate and iron ions from pyrite samples were extracted with 1 N HCl.

2.5. Calculation of theoretical reaction energy values

Reaction energy values were calculated from standard enthalpies of formation ($\Delta_f H^0$) [18]. The obtained standard reaction enthalpy values ($\Delta_r H^0$) were corrected due to the nonstandard experimental conditions. Calorimetric measurements were performed at constant volume; therefore, the enthalpy was transformed into the internal energy change ($\Delta_r U$) according to Eq. (2), where $\Delta\nu$ is the oxygen consumption and R and T, respectively, the gas constant and the thermodynamic temperature

$$\Delta_r U = \Delta_r H - \Delta\nu RT \quad (2)$$

Furthermore, the experimental ion concentrations of 20–200 mM of total ions deviated significantly from the standard condition of infinite solution. Therefore, the heat of solution and, consequently, the heat of formation of the relevant ions had to be corrected. With data from [18], for nonstandard $\Delta_f H$ at ca. 20 to 200 mM, the $\Delta_r H$ of reaction (1) was reduced by ca. 3%. Therefore, all calculated reaction energies were corrected by 3% as a sufficient approximation to the real values. Contrary to the deviations mentioned above, the temperature difference of experimental 30°C in comparison to the standard temperature of 25°C has only a negligible effect on the reaction energy.

3. Results

3.1. Leaching experiments

For the calorimetric experiments, the pyrite and cells of whole shake cultures of different ages were used in order to compare the reaction energy values of cells of different physiological state. Leaching graphs of pure cultures of *T. ferrooxidans* and *L. ferrooxidans* are shown in Fig. 1(a), Fig. 2(a), respectively. The iron ion mobilization by *T. ferrooxidans* in batch cultures was characterized by a short lag phase of 2–3 days, then an increasing leaching rate with a maximum at 15 days and a subsequent decline of activity was noted. The inhibition of the oxidation rate was also indicated by the increase of iron(II) ions from 25 days onwards and was probably caused by the accumulation of leaching endproducts (protons and iron(III) ions [14,19]). The inoculum of 10^8 cells/ml attached almost completely to the pyrite surface during the lag phase. Afterwards, an exponential increase of planktonic cells occurred from days 2 to 7. Pure cultures of *L. ferrooxidans* showed a similar lag phase, followed by an exponential increase of iron ions. An inhibition of leaching activity did not occur, even at high iron(III)-ion concentrations and low pH [14,19]. The increase of single planktonic cells was slow. Cells of *L. ferrooxidans* were mainly organized in aggregates as was demonstrated previously [20] and, therefore, difficult to quantify. Cultures investigated in parallel had similar leaching graphs as indicated by the final iron ion concentrations of all cultures as compared with the described ones (Fig. 1(a), Fig. 2(a)). Mixed cultures of *L. ferrooxidans* and *T. thiooxidans* showed the same leaching results as pure cultures of *L. ferrooxidans*. Sterile controls demonstrated a very low activity and a linear increase of iron ions (data not shown).

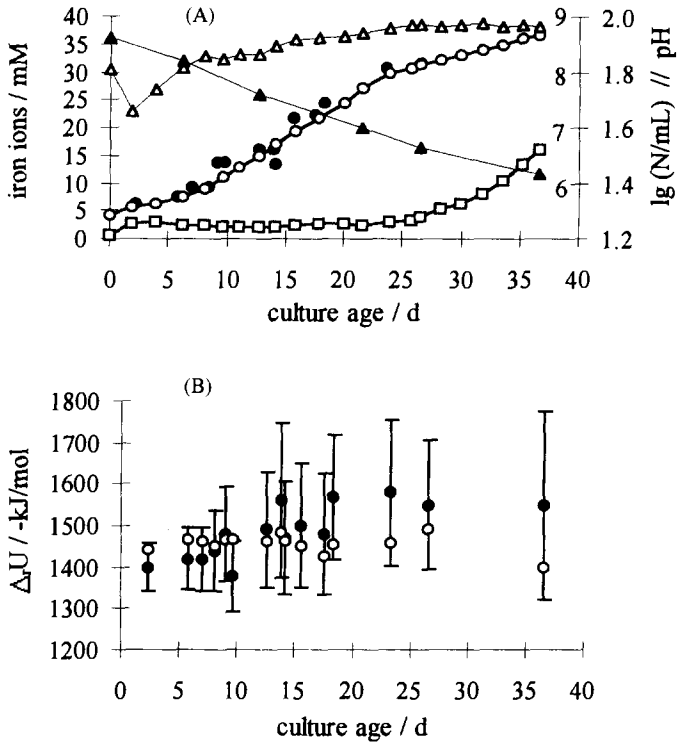

Fig. 1. Pyrite leaching by pure cultures of *Thiobacillus ferrooxidans*. (a) leaching graph for a shake culture: (▲) – pH; (△) – decadic logarithm of planktonic cell numbers (N); (○) – concentration of total iron ions; (□) – iron(II) ions; and (●) – final iron-ion concentrations of all investigated cultures. (b) Values for the reaction energy ($\Delta_r U$): (●) – calorimetrically determined values; and (○) – theoretical values.

3.2. Reaction energy values

The oxidation of pyrite by *T. ferrooxidans* was calorimetrically investigated with samples from 2–37-day old shake cultures (Fig. 1(b)). For the calculation of the theoretical $\Delta_r U$, the formation of reduced sulphur compounds could be neglected because the sulphur moiety of pyrite was oxidized nearly completely to sulphate. Elemental sulphur (<1%) and no polythionates were produced (data not shown). From 2–14 days, the theoretical $\Delta_r U$ increased slightly from −1440 to −1480 kJ/mol due to an increased amount of iron(III) ions in the total of solubilized iron ions (data not shown). On the contrary, older cultures generally showed lower oxidation activities (Fig. 1(a)) and, consequently, decreased values for the theoretical $\Delta_r U$, with a final value of ca. −1400 kJ/mol after 37 days. The corresponding calorimetric $\Delta_r U$ increased from −1400 to −1550 kJ/mol with an experimental error of 4–15%, respectively. This decrease of accuracy was due to the limited oxygen content in the calorimetric batch experiments. In all experiments, nearly the same absolute amounts of heat evolution and iron mobilization were measured, only the period for recording time differed due to the activity of a sample. As a consequence, the experimental error for the determination of iron ion mobilization increased with culture age as a result of augmenting background concentrations of iron ions in the samples. Generally, calorimetric and theoretical $\Delta_r U$ showed no significant difference. Even in samples from relatively young cultures (2–7 days), with errors of only 4–5%, both values for $\Delta_r U$ were in good agreement. In contrast to the results with *T. ferrooxidans*, the values for the reaction energy of pyrite oxidation by *L. ferrooxidans* were totally different (Fig. 2(b)). Due to the inability of this organism to oxidize reduced sulphur compounds, the sulphur moiety of pyrite was oxidized to <90% to sulphate. Instead, about 10% elemental sulphur, 1% tetrathio-

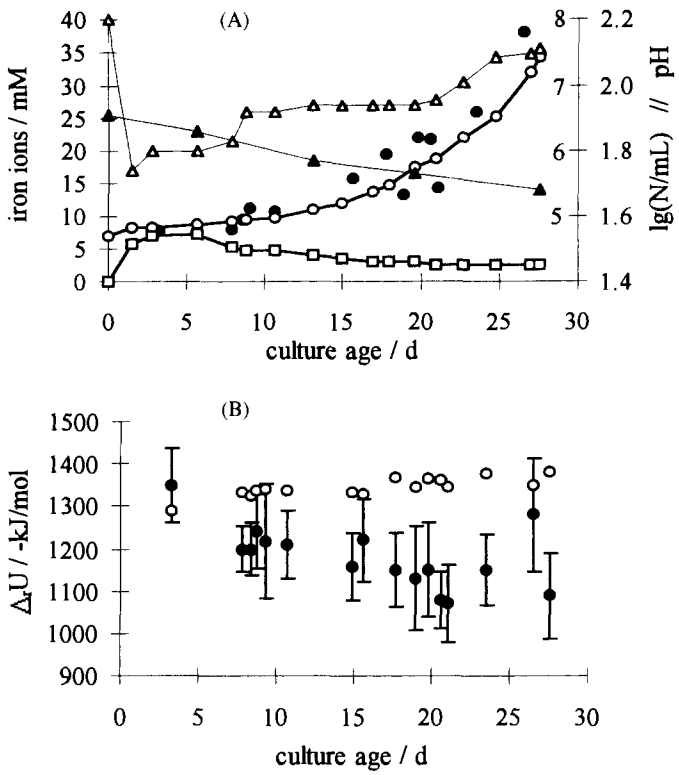

Fig. 2. Pyrite leaching by pure cultures of *Leptospirillum ferrooxidans*. (a) leaching graph for a shake culture, (▲) – pH, (△) – decadic logarithm of planktonic cell numbers (N), (○) – concentration of total iron ions (□) – iron(II) ions and (●) – final iron ion concentrations of all investigated cultures. (b) Values for the reaction energy ($\Delta_r U$): (●) – calorimetrically determined values; and (○) – theoretical values.

nate, and 2% pentathionate were formed (data not shown). The theoretical $\Delta_r U$ increased from −1290 kJ/mol at a culture age of 3 days to a final value of −1380 kJ/mol after 28 days due to an increasing iron(II) ion oxidation activity. In contrast, the calorimetric $\Delta_r U$ decreased with culture age from −1350 to ca. −1100 kJ/mol with experimental errors of 4–11%. In most samples older than 15 days, there was a significant difference between the calorimetric and theoretical value for $\Delta_r U$, that of >−100 kJ/mol, with maximal values of ca. −200 kJ/mol. Experiments with mixed cultures of *L. ferrooxidans* and *T. thiooxidans* yielded comparable values as for pure cultures of *T. ferrooxidans* (data not shown). As expected from the complementation of the ability to oxidize both iron(II) ions and reduced sulphur compounds in these cultures, the sulphur moiety of pyrite was oxidized nearly completely to sulphate, only <1% elemental sulphur was formed, and polythionates were not detectable. For the calorimetric investigations, only cultures in the exponential growth phase were used. Therefore, the oxidation activity was generally high resulting in a theoretical $\Delta_r U$ of ca. −1490 kJ/mol. The values for the calorimetric $\Delta_r U$ ranged between −1400 and −1600 kJ/mol with errors of 11–16% due to high background iron ion concentrations. As for the pure cultures of *T. ferrooxidans* no significant difference between calorimetric and theoretical values for $\Delta_r U$ was observed for this mixed culture combination. In sterile controls only very low oxidation activities were measured. About 10% elemental sulphur were formed, but no polythionates were detectable. In contrast to all bioleaching assays, under sterile conditions the iron moiety of pyrite was mobilized only in the iron(II) form (data not shown). The calorimetric $\Delta_r U$ showed values between −1300 and −1340 kJ/mol with errors between 4 and 6% (Fig. 3). Again, calorimetric values were in good

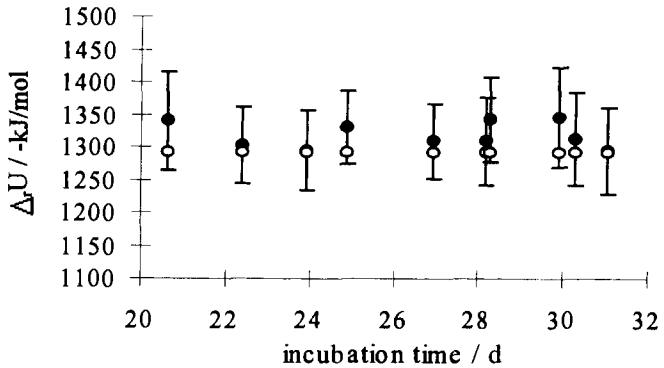

Fig. 3. Pyrite leaching under sterile conditions, reaction energy ($\Delta_r U$), (●) – calorimetrically determined values and (○) – theoretical values.

agreement with the theoretical $\Delta_r U$ value of −1290 kJ/mol.

4. Discussion

In calorimetric experiments with pyrite oxidation by defined cultures of leaching bacteria the reaction energy values for this bioleaching process were determined. In addition, the corresponding theoretical values for $\Delta_r U$ could be estimated with high accuracy by the quantification of all relevant reaction products. The good agreement between measured and theoretical $\Delta_r U$ for sterile samples demonstrates that the approximations used for the calculations of the non-standard $\Delta_r U$ from the standard enthalpies were sufficiently describing the real conditions. In pure cultures of *T. ferrooxidans*, no differences between calorimetric and theoretical values for $\Delta_r U$ were observed. Even samples with cells in the logarithmic growth phase did not show any deficits in the energy balance. From these experimental data, a conservation efficiency of only a few percent can be deduced. In contrast, for pure cultures of *L. ferrooxidans* the theoretical values for $\Delta_r U$ were significantly higher than the measured ones. It is very unlikely that these differences could be related to a totally different conservation efficiency between *T. ferrooxidans* and *L. ferrooxidans*. *T. ferrooxidans* is expected to show higher efficiency values because this bacterium can gain energy not only from iron(II) ion oxidation, but also from the oxidation of sulphur compounds that were formed during pyrite leaching [16], whereas *L. ferrooxidans* exclusively uses iron(II) ion oxidation for energy conservation. Furthermore, in the experiments with mixed cultures of *L. ferrooxidans* and *T. thiooxidans*, a higher efficiency than the one of *T. ferrooxidans* was not observed, although the only difference to the pure culture of *L. ferrooxidans* was the oxidation of the reduced sulphur compounds by *T. thiooxidans*. An explanation for the low calorimetric values for $\Delta_r U$ with pure cultures of *L. ferrooxidans* might be endothermic precipitation of iron(III) compounds, e.g. the formation of $Fe(OH)_3$ with a reaction enthalpy $\Delta_r H^0$ of +83 kJ/mol. As a consequence of the incomplete oxidation of the sulphur moiety of pyrite by *L. ferrooxidans*, the formation of protons was reduced and, thus, the formation of iron(III) precipitates was more likely to occur than in the other samples.

This first calorimetric study on the energy conservation of leaching bacteria demonstrates that it is possible, in principle, to determine enthalpy efficiencies by this technique for substrates such as pyrite. For a clarification of the observed discrepancies, measurements with an increased accuracy are needed. In addition, the biomass of planktonic and of attached cells have to be quantified for efficiency estimations. For a complete analysis of conservation efficiency, free energy efficiencies have to be calculated which cannot be measured directly by calorimetry. Therefore, in addition to the measured values for $\Delta_r U$, values for the formation entropy of cells of leaching bacteria have to be obtained.

Besides the use for thermodynamic evaluations of chemolithotrophic growth, calorimetry could be applied for monitoring the activity of leaching bacteria in mine waste heaps or other leaching sites [2,21,22],

where the leaching activity causes serious environmental problems. With the determined reaction energies for the bioleaching of pyrite, it is now possible to quantify the leaching rates of samples by calorimetric measurements. This technique can be used in rehabilitation programmes for the evaluation of measurements that are applied for the inhibition of leaching bacteria.

Acknowledgements

The work was supported, in part, by grants to W.S. from the Bundesministerium für Bildung und Forschung/via Umwettbundesamt (AZ 149054) and the Deutsche Bundesstiftung Umwelt (AZ 05333).

References

[1] H.L. Ehrlich, Geomicrobiology, Marcel Dekker, New York, 1996, p. 578.
[2] A. Schippers, R. Hallmann, S. Wentzien, W. Sand, Appl. Environ. Microbiol. 61 (1995) 2930.
[3] H.L. Lowell, Water Sci. Techn. 15 (1983) 1.
[4] L.G.M. Baas-Becking, G.S. Parks, Physiol. Rev. 7 (1927) 85.
[5] D.P. Kelly, in: T.A. Krulwich (Ed.), The Bacteria, Vol. 12, Academic Press, New York, 1990, p. 479.
[6] A.W. Schröter, Mikrokalorimetrische Untersuchungen an chemolithotrophen Eisen(II) und Thiosulfat oxidierenden Bakterien, doctoral thesis, Universität Hamburg, Hamburg, 1993.
[7] A. Goodman, B.J. Ralph, in: P.A. Trudinger, M.R. Walter, B.J. Ralph (Eds.), Biogeochemistry of Ancient and Modern Environments, Springer, Berlin, 1980, p. 477.
[8] A.W. Schröter, W. Sand, in: P.R. Norris, D.P. Kelly (Eds.), Biohydrometallurgy, Science and Technology Letters, Kew, 1988, p. 127.
[9] A.W. Schröter, W. Sand, in: J. Sally, R.C.L. McGrady, P.L. Wichlacz (Eds.), Biohydrometallurgy, CANMET, Ottawa, 1989, p. 427.
[10] A.W. Schröter, W. Sand, FEMS Microbiol. Rev. 11 (1993) 79.
[11] P. Soljanto, O.H. Tuovinen, in: P.A. Trudinger, M.R. Walter, B.J. Ralph (Eds.), Biogeochemistry of Ancient and Modern Environments, Springer, Berlin, 1980, p. 469.
[12] H. Laudelout, P.-C. Simonart, R. van Droogenbroeck, Arch. Microbiol. 63 (1968) 256.
[13] A. Dessers, C. Chiang, H. Laudelout, J. Gen. Microbiol. 64 (1970) 71.
[14] W. Sand, K. Rohde, B. Sobotke, C. Zenneck, Appl. Environ. Microbiol. 58 (1992) 85.
[15] M.E. Mackintosh, J. Gen. Microbiol. 105 (1978) 215.
[16] A. Schippers, P.-G. Jozsa, W. Sand, Appl. Environ. Microbiol. 62 (1996) 3424.
[17] N.N., Deutsche Einheitsverfahren zur Wasser-, Abwasser-und Schlammuntersuchung, Verlag Chemie, Weinheim, 1984, p. 1.
[18] D.D. Wagman, W.H. Evans, V.B. Parker, R.H. Schumm, I. Halow, S.M. Bailey, K.L. Churney, R.L. Nuthall, NBS tables of thermodynamic properties, J. Phys. Chem. Ref. Data, 11 (1982) Suppl. 2.
[19] P.R. Norris, D.W. Barr, D. Hinson, in: P.R. Norris, D.P. Kelly (Eds.), Biohydrometallurgy, Science and Technology Letters, Kew, 1988, p. 43.
[20] R. Hallmann, A. Friedrich, H.P. Koops, A. Pommering-Röser, K. Rohde, C. Zenneck, W. Sand, Geomicrobiol. J. 10 (1993) 193.
[21] W. Sand, R. Hallmann, K. Rohde, B. Sobotke, S. Wentzien, Appl. Microbiol. Biotechnol. 40 (1993) 421.
[22] S. Wentzien, R. Hallmann, W. Sand, in: W. Dubbert, J.M.L. Pila (Eds.), Fachtagung Überwachungsmethoden, Gentechniktexte 29/94, Umweltbundesamt, Berlin, 1994.