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Electrochemical identification of microbially mediated hydrogen sulfide oxidation

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Abstract. The process of H_2S oxidation by the phototrophic bacteria *Thiocapsa roseopersicina* and *Chlorobium phaeobacteroides*, respectively, was monitored using a Pt-glass-Ag^0, Ag_2S electrode combination without liquid junction. Due to the resulting pe(pH) and pH_2S plottings three steps can be distinguished: oxidation of H_2S to an S (0) state, oxidation of S (0) to SO_4^{2-}, and oxidation of the remaining H_2S directly to SO_4^{2-}. Differences between the investigated bacteria exist with respect to their individual oxidation strategies. *Thiocapsa* apparently stops oxidizing H_2S at pH_2S 7.5 (e.g. $10^{-7.5}$M H_2S) and shifts to the utilization of the intracellularly stored S (0). In contrast *Chlorobium* utilizes its extracellularly stored sulfur parallel to the extracellular H_2S fraction. The corresponding Pt-sensor responses (pe_7 values) were found to be similar to the corresponding partial redox equilibria ($p\varepsilon_7$ values) of H_2S oxidation stoichiometries as proposed by Van Niel (1931) and Trüper (1964). It is concluded that the recording of pe enables investigators to understand (and control) in situ redox processes, independent of their thermodynamic equilibration, only bound to changes of electroactivity vs. sensor.

Introduction

The interrelationship between microbial activity and the prevailing hydrochemical conditions is of decisive significance in the state of aquatic ecosystems (Stumm and Stumm-Zollingher, 1971). In particular, the time-variant chemical composition of the metalimnetic layer of a thermally stratified lake has to be seen as a result of the succession of microbial communities mediating distinct redox reactions (Eckert and Frevert, 1984). It is apparent that the interface between biological activity and the corresponding chemical reactions is of extreme interest.

Exempli gratia, bio-oxidation of hydrogen sulfide could be shown for several groups of mainly autotrophic bacteria (Schlegel, 1981), especially the phototrophic bacteria that use reduced sulfur compounds as electron donor for photosynthetic growth under appropriate light conditions. The oxidation of H_2S to SO_4^{2-} by *Chromatiaceae* and *Chlorobiaceae* was reported as a two step reaction (Van Niel, 1931; Trüper, 1964):

$$2H_2S + CO_2 \xrightarrow{h\cdot\nu} 2S + H_2O + (CH_2O) \quad (1)$$

$$2S + 3CO_2 + 5H_2O \xrightarrow{h\cdot\nu} 2H_2SO_4 + 3(CH_2O) \quad (2)$$

Intermediately formed sulfur is stored intracellularly by *Chromatiaceae* species and extracellularly by *Chlorobiaceae*. A strictly thermodynamical description of the relevant redox processes, as for instance exemplified by Stumm & Morgan (1981), is narrowed by incalculable reaction kinetics and an overlapping of sequential redox processes. Thus, an application of the thermodynamic master variable pε, which is defined as:

$$\begin{aligned} p\varepsilon &= p\varepsilon^0 + \frac{1}{n} \log \frac{(ox)}{(red)}, \qquad \text{with} \\ p\varepsilon^0 &= \frac{Eh^0}{2.303 \cdot RTF^{-1}} \end{aligned} \tag{3}$$

becomes unfavourable by its restriction to equilibrium conditions.

The informatory value of measured redox data from multicomponent systems as obtained with Pt-metal or glassy carbon sensors was discussed repeatedly.

Proceeding from the assumption that the intracellular microbial metabolism reflects the extracellular conditions, several authors proposed an operational application of redox measurements in microbial cultures (Jacob, 1974; Wimpenny, 1976; Kjaergaard, 1977). Analogous to the pε concept, Frevert (1979) introduced the pe redox concept:

$$pe = - \log a_{e^-}, \tag{4}$$

where a_{e^-}, defined as electroactivity, represents the sum of all electrochemically effective components within the respective solution. In continuation Frevert (1984) established a sensor specific redox concept due to a stationary redox state in the test solution:

$$pe = - \log a_{e^-} \text{ (Pt-sensor)} \tag{5}$$

"Unlike pε, the pe values are independent of the internal redox equilibrium in the test solution but depend on the equilibrium between the test solution and an appropriate sensor".

Consequently, pe defines electrochemically stationary redox conditions in the test solution. This consideration enables a systematic identification of certain predominant redox systems. As far as the electrochemically (pe) defined redox conditions can be seen as close to redox equlibria, pe can usefully be compared to pε. Note however, that the pε will strictly depend on the assumed stoichiometry of the considered redox reactions. Here pε's for sulfur half reactions are to be calculated according to eq.'s (1) and (2) (in p-notation):

$$p\varepsilon = 2.89 - pH + 0.5\,pH_2S - 0.5\,pS^0 \tag{6}$$

$$p\varepsilon = 6.03 - 1.33\,pH + 0.166\,pS^0 - 0.166\,pSO_4^{2-}. \tag{7}$$

For the discussion of different redox equilibria it is more convenient to relate pε values to a certain pH value, e.g. pH 7, according to the equation:

$$p\varepsilon_7 = p\varepsilon + (pH - 7) \text{ (Frevert, 1979)} \quad (8)$$

The concommitance of so obtained $p\varepsilon_7$ values and measured pe_7 values indicates to what extent the electrochemically defined redox conditions correspond to the thermodynamic equilibria of the assumed redox reactions and their stoichiometric coefficients.

Due to the fact that the investigated bacteria are oxidizing H_2S according to a known stoichiometry, a direct application of eq.'s (6) and (7) could become possible if $p\varepsilon_7 \simeq pe_7$ and if the relevant oxidation reactions can be distinguished electrochemically.

A prerequisite for the corresponding investigations is a precise and selective determination technique concerning the crucial hydrochemical variables. Using direct potentiometric detection methods which meet best the mentioned requirements, the present work gives an example of an electrochemical identification of a microbially mediated redox reaction within the redox system S(+VI)/S(−II).

Experimental

The corresponding batch culture experiments were carried out with two species of phototrophic bacteria: *Thiocapsa roseopersiciana* Fam. *Chromatiaceae* and *Chlorobium phaeobacteroides* Fam. *Chlorobiaceae*, both isolated from Lake Kinneret (the biblical Sea of Gallilee). Cultures were grown in Pfennig's medium (Pfennig, 1965) slightly modified in the main components according to Bergstein et al., (1979): (gL^{-1}), KH_2PO_4 0.33, NH_4Cl 0.33, $MgCl_2 \cdot 6H_2O$ 0.33, KCl 0.33, $NaHCO_3$ 1.5, NH_4-acetate 0.2, $CaCl_2 \cdot 2H_2O$ 0.33 and (μgL^{-1}), vitamin B_{12} 10.0. Physiologically optimum conditions were adjusted with respect to medium pH, temperature and light intensity as follows: pH 7.5, 30 °C, 50 μEin $m^{-2}s^{-1}$ for *Thiocapsa* and pH 6.5, 30 °C, 20 μEin $m^{-2}s^{-1}$ for *Chlorobium*. When reaching stationary state, bacteria were harvested and transferred into a 300 ml airtight rection vessel containing fresh de-aerated medium, previously conditioned to the medium pH and the initial total sulfide concentration (S_{tot}). For *Thiocapsa* the S_{tot} concentration was adjusted to 4.4 mM and for *Chlorobium* to 3.0 mM, respectively. Permanent stirring of the bacteria solution sufficed to prevent a stratification within the reaction vessel. The oxidation experiments were carried out three times for each bacteria strain.

The realization of the electrochemical identification of the subsequent oxidation processes required keeping certain chemical and physical parameters (pH, temperature, light intensity) constant and a continuous recording of pe- and pH_2S values. For the pH_2S detection the glass/silver

silver-sulfide electrode without liquid junction (Ag-275-85-6329, INGOLD, Frankfurt/M) was used, introduced by Frevert & Galster (1978) and Frevert (1980). This electrode, described as:

$$Ag^0, AgI/0.2\,M\ HI/glass/soln./Ag_2S, Ag^0$$

is ideally suited for the designed experiments. Responsive to H_3O^+ and S^{2-}, the resulting mV-readings correspond exactly to the H_2S activity, whereas organic and inorganic sulfides, sulfate, thiosulfate and other intermediate products are not "seen". (Polysulfide interactions with the Ag/Ag_2S electrode, as they were reported by Boulègue and Michard (1979) for pH > 9, interfere according to findings of Frevert (1983) at total sulfide concentrations $> 10^{-1}$ M). Therefore the oxidative H_2S depletion by the bacteria can be monitored with highest accuracy only limited by the reading precision of the measuring device. A lower detection limit of total sulfide activities corresponding to pH_2S 18.2 could be verified in a sulfide buffered system (Peiffer and Frevert, submitted).

pe Values were obtained indirectly by calculation from measured pe (pH) values (conceptually known as rH values) due to the relationship:

$$pe(pH) = 2pe + 2pH \tag{9}$$

"rH" was not used as this notion is conceptually defined as $rH = 2p\varepsilon + 2\,pH$. pe_7 values were calculated by introducing the equation:

$$pe_7 = pe + (pH - 7) \tag{10}$$

into eq. (9) leading to the following relationship:

$$pe = [pe(pH)/2] - 7 \tag{11}$$

The pe(pH) detection was done by combining a Pt-metal electrode (Pt-275-85, INGOLD, Frankfurt/M) to the glass halfcell of the pH_2S sensor which under constant pH conditions is suitable as reference electrode for the pe sensor due to the response to H_3O^+. A PtS coating of the metal surface of the Pt-electrode as predicted by Whitfield (1974) was not observed. The used Pt sensor which was rinsed and calibrated carefully for each experiment remained bright throughout all experiments which corresponds to findings of Boulègue and Michard (1979).

Figure 1 contains a circuit diagram of experiments mounted. The glass halfcell is connected directly to the high impedance input of an mV-meter (pH Digi 88, WTW, Germany), whereas the low impedance signals of the Pt- and Ag^0, Ag_2S halfcells were every half hour switched by a relay time switch. The resulting mV-readings were plotted on a recorder.

The further comparison of the pe_7 and $p\varepsilon_7$ values required the determination of the oxidation products S^0 and SO_4^{2-} from Eqs. (6) and (7), respectively. But due to the lack of similar sensitive in situ detection

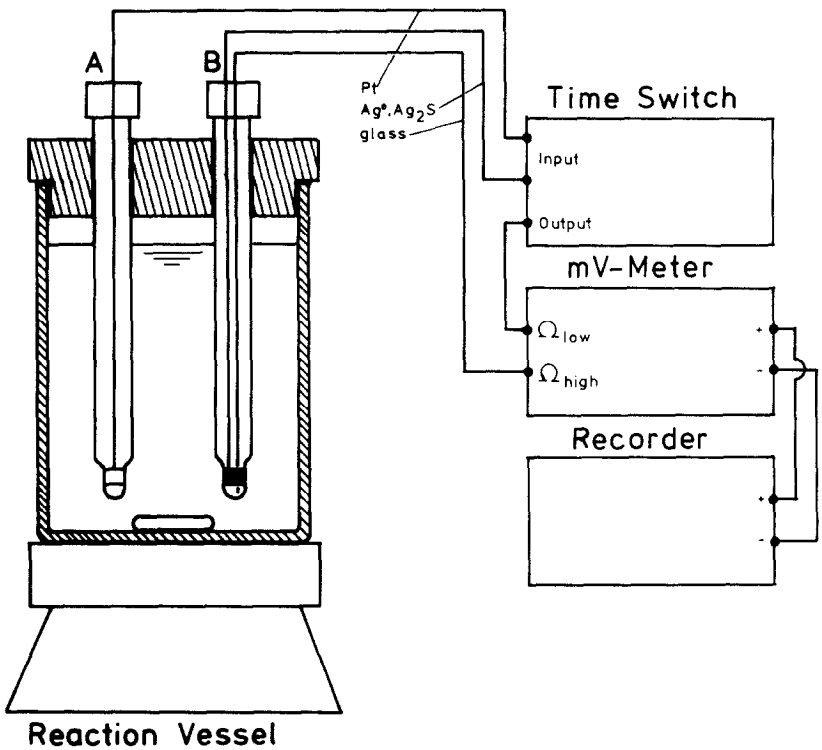

Figure 1. Experimental mounting: (A) Pt-metal sensor, (B) pH_2S electrode.

methods for these molecular species, only eq. (6) could be used after approximation of the S^0 concentration as:

$$(S^0) = (S_{tot}) - \frac{(H_2S)}{\alpha_0}, \qquad \text{with:}$$

$$(H_2S) = 10^{-pH_2S}M$$

$$\alpha_0 = \text{protolysis constant}$$

$$= \frac{1}{1 + K_1/(H_3O^+) + K_1K_2(H_3O^+)^2}$$

$$K_{1,2} = \text{equilibrium constants} \qquad (12)$$

α_0 values were taken from Frevert (1983), whose calculations are based on the equilibrium data of Broderius and Smith (1977). Eq. (12) introduced into Eq. (6) rendered the calculation of $p\varepsilon$ values of the first oxidation step (see Eq. (1):

$$p\varepsilon = 2.89 - pH + 0.5\,pH_2S - 0.5\,p[(S_{tot}) - (H_2S)/\alpha_0] \qquad (13)$$

Calibration of the sensors

The pH_2S sensor was calibrated according to the method described by Peters et al., (1984). The electrode cell is screwed into a 100 ml reaction vessel fitted for anaerobic use. After adding 100 ml of a pH buffer solution (MERCK, pH 5 at 25 °C) the solution is de-aerated with purified N_2 gas. Then 1, 10, 100, 1000 μl of an iodemetrically controlled standard solution (0.1 M $Na_2S \cdot 9H_2O$ p.a.) are stepwise added corresponding to the pH_2S values 6.0, 4.96, 3.96, 2.96 ($\simeq pS_{tot}$). The mV-readings are due to the Nernst equation:

$$pH_2S = pH_2S^0 + \frac{(X - mV^0)}{S}$$

$$x = \text{reading } (-mV)$$

$$s = \text{slope } (mV/pH_2S)$$

$$mV^0 = \text{reading } (-mV \text{ at } pH_2S^0) \qquad (14)$$

The corresponding data with this study have been:

$$pH_2S = 2.96 + \frac{(X - 78)}{29} \qquad (15)$$

By analogy, the calibration curve for the pe(pH) electrode reads:

$$pe(pH) = pe(pH) - \frac{(X - mV^0)}{S}$$

$$x = \text{reading } (-mV)$$

$$s = \text{slope } (mV/\tfrac{1}{2}pH = mV/pH_2S)$$

$$mV^0 = \text{reading } (-mV \text{ at } pe(pH)^0) \qquad (16)$$

Note that the slope corresponds to one of the pH_2S sensor. The following calibration data were obtained for the cell combination:

$$pe(pH) = 23.6 - \frac{(X + 735)}{29} \qquad (17)$$

(data refer to a saturated quinhydrone solution at pH 7.0 and 25 °C).

Results

The results are pointed out by Figure 2 for *Thiocapsa* and by Figure 3 for *Chlorobium*. In each case three oxidation phases could be distinguished as indicated by the respective plottings:

I – hydrogen sulfide oxidation to S(O)

II – oxidation of the intermediate formed S(O) to SO_4^{2-}

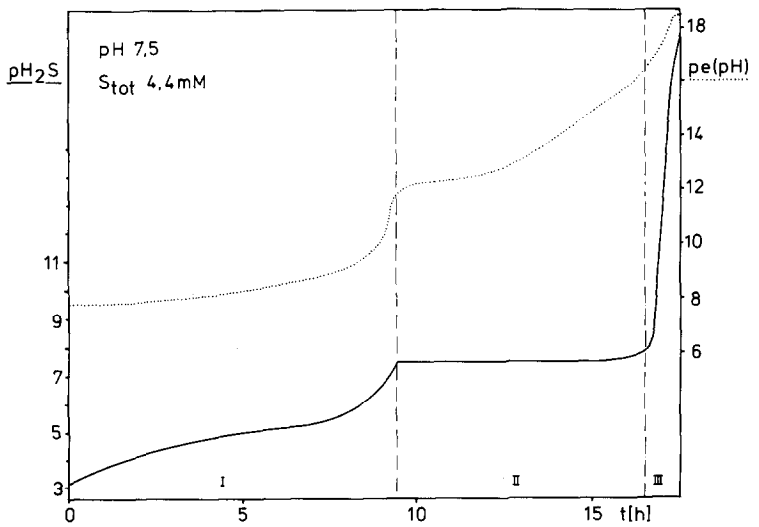

Figure 2. pe(pH) and pH_2S plottings versus time due to the H_2S oxidation by Thiocapsa: (.....) pe(pH), (———) pH_2S, (I–III) oxidation reaction.

III – oxidation of the remaining H_2S, apparently direct to SO_4^{2-}.
Differences were found with respect to the individual oxidation strategies of the investigated microorganisms.

During the first ten hours *Thiocapsa* oxidized H_2S to S(O), as stored intracellularly, until pH_2S 7.5 [$(H_2S) = 0.003\ 10^{-5}$ M] corresponding according to eq. (12), to the approximate pS^0 value 2.36 [$(S^0) \simeq 4.4$ mM].

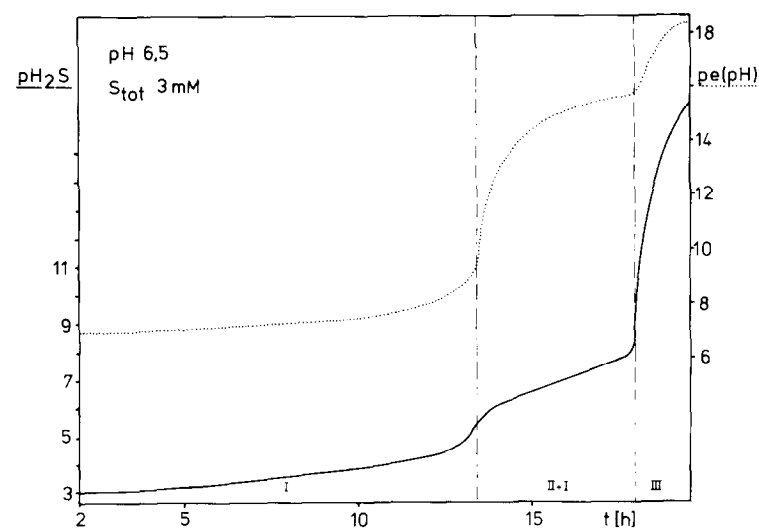

Figure 3. pe(pH) and pH_2S plottings versus time due to the H_2S oxidation by Chlorobium: (.....) pe(pH), (———) pH_2S, (I–III) oxidation reaction.

This process was accompanied by an increase in the pe(pH) value from 8 to 10 which corresponds to an increase in the pe value due to the constant pH value. The sudden step in the pe(pH) plotting to pe(pH) 12.3 indicated a shift to the oxidation of the intermediate sulfur. For all phototrophic bacteria, which are able to utilize elemental sulfur as electron donor for photosynthesis, SO_4^{2-} will be the end product of sulfur oxidation (Trüper, 1981). Note the sharp turning point in the pH_2S curve. During this state *Thiocapsa* stopped for ca. seven hours utilizing H_2S, while most likely due to the permanent release of SO_4^{2-}, the pe(pH) rose until pe(pH) 18.2 where it entered into a plateau. At this point the stored S(O) seemed to be consumed for the remaining H_2S was oxidized within minutes apparently directly to SO_4^{2-}.

The analogous plottings for *Chlorobium* differed from the former in some aspects. In course of the first oxidation step S(O) was formed and stored extracellularly until pH_2S 5 (0.01 mM), reached after 13 hours ($pS^0 \simeq 2.52$; $(S^0) \simeq 2.9$ mM). Analogous to Figure 2, the pe(pH) value rose slightly from pe(pH) 7 to 8 during this first time period. Likewise, the beginning of S(O) oxidation resulted in a strong rise of six pe(pH) units. When oxidizing S(O), *Chlorobium*, in contrast to *Thiocapsa*, continued to utilize the H_2S fraction. After the apparent depletion of the stored S(O), 18 hours after starting the experiment, the pe(pH) output remained at pe(pH) 16. Subsequently the residual H_2S ($= pH_2S$ 8) was oxidized immediately and the pe(pH) curve reached a plateau at pe(pH) 18.2.

Discussion

Considering the investigated oxidation processes, some new, and with respect to former findings, partly contradictionary aspects turned out, certainly due to the high sensitivity of the sensors used. According to Trüper & Schlegel (1964) and Van Gemerden (1967), who carried out similar oxidation experiments with *Chromatiaceae*, these bacteria start oxidizing the stored S(O) during the state of H_2S oxidation. On the other hand, we can prove for *Thiocapsa* the intermediate stop in H_2S oxidation when the bacteria shifts to the utilization of stored S(O) (Figure 2). The corresponding changes in the electron donor–electron acceptor proportion, which derive from the beginning release of SO_4^{2-}, are illustrated most clearly by the pe(pH) plotting. The conclusion is confirmed by the repeated shift of *Thiocapsa* to H_2S utilization after the apparent consumption of S(O).

The experiment with *Chlorobium* (Figure 3) pointed out several differences concerning the individual oxidation behaviour, e.g. the different shapes of the pe(pH) plottings during the second oxidation period may be explained by the Pt sensor sensitivity mainly to SO_4^{2-} and to H_2S in this oxidation step. The concave progress of the pe(pH) curve on the part of

Thiocapsa may be a result of an increasing SO_4^{2-} concentration concomitant to a constant H_2S concentration, whereas the convex curve of *Chlorobium* derives from an SO_4^{2-} increase while H_2S decreases. Concerning the last oxidation step, we emphasize that after depletion of the stored S(O) the remaining H_2S concentration is oxidized in each case directly to SO_4^{2-}. This observation would confirm what was assumed by Pfenning (1975) who claimed that *Chlorobium* under low sulfide concentrations, directly oxidizes H_2S to SO_4^{2-}.

Another aspect of the present work was the investigation of the Pt sensor response to extra — and intracellular stored S(O), the oxidation product of the first oxidation step. At a first glance both pe(pH) plottings seem to resemble each other during this period. For the pe(pH) cell is responsive to the activities of H_3O^+ and e^-, respectively, the resulting mV-readings can indicate changing pH – and pe conditions as well. However, a possible common effect can be excluded for the pH values were kept constant in both experiments, and therefore the pe(pH) plottings are strictly related to the electroactivity in the solution. The corresponding pe_7 values, obtained according to Eq. (11), are summarized in Tables 1 and 2, together with the $p\varepsilon_7$ values which are calculated according to Eqs. (8) and (13). It is apparent that the pe_7 values in Table 1 are very close to the $p\varepsilon_7$ values. This can be explained by the sensors behaviour. Under the assumption that the incorporated S(O) has no electroactivity to the Pt-sensor, the pe_7 values in Table 1 can only be the result of the activity of H_2S, oxidized at the sensor's surface according to Eq. (6). On the other hand, the comparison between the pe_7 and $p\varepsilon_7$ values in Table 2 points out a clear difference. In the case of the experiment with *Chlorobium*, the oxidation product appears in the measuring solution as large, light breaking globules attached to the bacteria cells. Apparently, a possible reaction of the secreted sulfur with the remaining H_2S to polysulfides does not take place, for it would, due to the solubility of polysulfides, cause the resolution of the globules which is not observed. Thus, beside H_2S, S(O) is electroactive at the Pt sensor leading to the relatively higher pe_7 values. Considering these results, two main aspects are stressed:

Process monitoring by means of high sensitive electrochemical sensors can be useful for a better understanding and subsequent control of microbially mediated redox reactions, irrespective of thermodynamic limitations. Hereby the relative changes in the electron donor–electron acceptor proportion i.e. S(O)/S(– II) or S(+ VI)/S(O), can be pursued by electroactivity measurements, although the absolute mass exchanges per time unit can only be approximated. Furthermore, pe-monitoring of redox processes of a known stoichiometry can lead to a better understanding of the corresponding reaction kinetics, and, consequently, render the analytical application of the gained function which, according to the introduced pe-concept (Eq. (5)), is not necessarily identical with the thermodynamic

Table 1. pe_7 and $p\varepsilon_7$ values as calculated due to the H_2S oxidation of *Thiocapsa*

Time (h)	1	2	3	4	5	6	7	8	9
pe_7	−3.0	−2.9	−2.9	−2.9	−2.8	−2.7	−2.6	−2.4	−2.0
$p\varepsilon_7$	−3.46	−3.16	−3.02	−2.86	−2.78	−2.68	−2.61	−2.44	−2.14

Table 2. pe_7 and $p\varepsilon_7$ values as calculated due to the H_2S oxidation of *Chlorobium*

Time (h)	2	3	4	5	6	7	8	9	10	11	12	13
pe_7	−3.3	−3.3	−3.3	−3.2	−3.2	−3.2	−3.1	−3.1	−3.0	−2.9	−2.9	−2.7
$p\varepsilon_7$	−4.02	−3.96	−3.92	−3.90	−3.85	−3.76	−3.69	−3.61	−3.57	−3.50	−3.37	−3.23

equilibrium relation. In the case of H_2S oxidation by *Thiocapsa*, for example, the following approach is suggested:

$$pS^0 \simeq 5.78 - 2pH + pH_2S - 2pe \quad (18)$$

This equation represents the ideal case with $pe \simeq p\varepsilon$, and it is conceivable that the process-monitoring of distinct redox reactions can lead to analytically applicable empirical relations of a form similar to Eq. (18), if the concerned variables can be detected with a sufficient precision.

Acknowledgements

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