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Microbially – related redox changes in a subtropical lake 2. Simulation of metalimnetic conditions in a chemostat

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Abstract. The individual influence of sulfate reducing and brown phototrophic sulfur bacteria from Lake Kinneret on the metalimnetic redox conditions was investigated by simulating the hydrochemical and microbiological conditions in a specially designed chemostat. The results show a strong correlation between measured redox values and the prevailing hydrogen sulfide concentration leading to a linear relationship. Changes in this relationship allowed differentiation between the on-going microbial processes, sulfate reduction and sulfide oxidation. The comparison of *in vitro* and *in situ* redox values shows that the results of the simulation experiments agree with the data previously measured in the metalimnion of Lake Kinneret.

Introduction

The annual redox cycle of Lake Kinneret was investigated recently by means of a multi probe for the *in situ* measurement of highly resolving vertical profiles of redox, pH, and pH_2S values, temperature and dissolved oxygen (DO) (Eckert & Trüper 1993). The measured platinum electrode potentials were expressed as pe values following the sensor specific redox concept (Frevert 1979, 1984), with:

$$pe \equiv -\log \left(\sum_i \{e^-\}_i \right)_{platinum} . \quad (1)$$

According to this concept an equilibrium exists between the platinum sensor and the investigated aquatic system leading to an electrode reading representative for the sum of all electron exchange reactions. Because of large differences within the kinetics of the electron exchange reactions

(Bockris & Reddy 1970) the net- or mixed potential measured is dominated by the redox compound with the highest tendency to exchange electrons (i.e. electron exchange density). To identify the origin of the electrode reading two main questions have to be addressed:

1. What are the decisive electroactive compounds within the test solution?
2. To what extent are microbes involved in the electron exchange reactions, since most of the *in situ* redox processes are mediated by bacterial activity (Frevert 1984; Kjaergaard 1977; Wimpenny 1969, 1984; Wimpenny & Necklen 1971; Eckert 1987; Eckert & Trüper 1993)

When relating the pe values obtained for Lake Kinneret to the *in situ* hydrochemical conditions (Eckert & Trüper 1993), a linear function evolved between meta- and hypolimnetic pe and pH_2S values ($pH_2S = -\log [H_2S]$) during the evolution of the chemocline. During the metalimnetic bloom period of phototrophic sulfur bacteria a variation within the pe – pH_2S function emerged as depicted by a steeper slope. It was the central task of this study to investigate the extent to which this change in the redox conditions in the bloom layer is related to the presence of hydrogen sulfide oxidizing bacteria by monitoring the redox changes in a chemostat system that simulated metalimnetic conditions with the *in situ* prevailing bacteria.

To better understand the specific influence of two physiologically different bacterial groups on the redox conditions prevailing in the metalimnion of Lake Kinneret we conducted growth experiments with pure and co-cultures of sulfate reducing bacteria (SRB) and phototrophic sulfur bacteria (PSB). The corresponding experiments required a highly sensitive detection method for hydrogen sulfide a point which is emphasized chiefly in the theoretical part of this study.

Theoretical

The pe concept, as applied to the metalimnion of Lake Kinneret, requires the identification of the decisive electroactive compounds in this sulfide enriched system. In sulfide containing sediments (Berner 1963), platinum electrode potentials corresponded quantitatively to the concentration of sulfide present and approximate the thermodynamical relationship (Stumm & Morgan 1981):

$$pe \simeq p\varepsilon = 2.4 - pH + 0.5 \cdot pH_2S \qquad (2)$$

($p\varepsilon$ = redox intensity).

In view of the different protonation states (H_2S, HS^-, S^{2-}), Boulegue (1978) showed that in dilute H_2S-H_2O systems ($I \rightarrow 0$; I = ionic strength) a platinum electrode selectively exchanges electrons with the H_2S species. Thus, besides the regular pe/pH dependency (e.g. the standard hydrogen electrode, Frevert 1979), a change in pH can cause an additional change in pe due to the H_2S/HS^{-1} ratio. When applying equation (2) one has to consider two cases:

1. For $[H_2S] \approx [S_{tot}]$ or $pH < pK_1$ (pK_1 = first protolysis constant of the H_2S-HS^--S^{2-} system) the pe value is directly related to the pH with:

$$\Delta pe = -\Delta pH. \tag{3}$$

2. For $[H_2S] < [S_{tot}]$ or $pH > pK_1$ the changing H_2S concentration has to be taken into account while using equation (2). The pH dependent variation of the pH_2S can be calculated as:

$$10^{-pH_2S} = [H_2S] = \frac{[S_{tot}]}{K_1/[H^+] + 1}. \tag{4}$$

The direct and indirect pH effect is summarized for different total sulfide concentrations in Fig. 1.

The pe-pH dependency is important for the calculation of pe_7 values (i.e. the correction of measured pe values to pH 7.0). Excluding the hydrogen ion effect on the platinum sensor allows the direct comparison of pe values measured under different redox conditions. As long as equation (3) is valid, the correction can be done according to equation (5) as derived from the standard hydrogen electrode (Frevert 1979):

$$pe_7 = pH - 7 + pe. \tag{5}$$

As can be seen in Fig. 1, equation (5) can not be used in aquatic systems enriched with hydrogen sulfide when the pH exceeds pK_1. According to the pe redox concept, the extent to which measured data correspond to the pe-pH-pH_2S relationship drawn by Fig. 1 (i.e. the relationship between pe values and the theoretical $p\varepsilon$) must be investigated for each aquatic system.

The calculation of pH_2S values from commonly used sulfide electrodes is very unreliable due to the uncertainty of the second acidity constant (pK_2) where literature values range from 12 to 21 (Dyrssen & Kremling 1990). Because the precision of pH_2S values was essential for this study

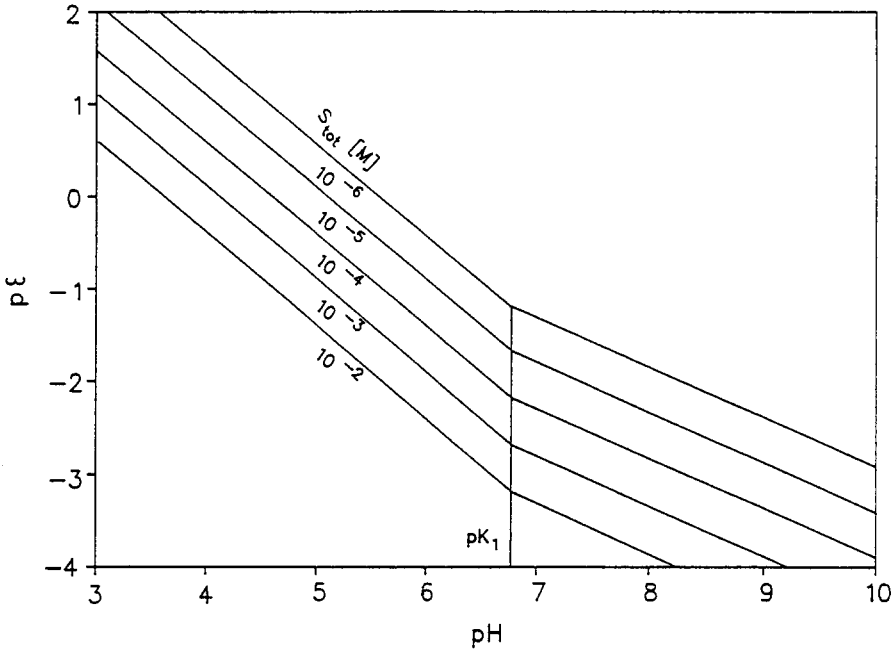

Fig. 1. pε-pH relationship in a H_2O-H_2S system for different total reduced sulfur concentrations.

we avoided the problem of choosing the proper pK_2 value by using the liquid junction free glass/Ag_2S electrode introduced by Frevert & Galster (1978). This electrode combination is described by:

Ag^0, AgI / 0.2 M HI / pH-glass / solution / Ag_2S, Ag^0.

The electromotive force (emf) reading of this cell is:

$$\text{emf} = \Delta\phi_{\text{glass}} - \Delta\phi_{\text{Ag}} \tag{6}$$

with $\Delta\phi_{\text{glass,Ag}}$ = potential difference of the glass and silver halfcell, respectively.

For K_{sp,Ag_2S} (solubility product) = $[S^{2-}] \cdot [Ag^+]^2$ the halfcell potentials are:

$$\Delta\phi_{\text{Ag}} = \phi^0_{\text{Ag}} - \frac{2.3\ RT}{2\ F}\ pK_{sp} + \frac{2.3\ RT}{2\ F}\ pS^{2-} \tag{7}$$

and:

$$\Delta\phi_{glass} = \phi^0_{glass} - \frac{2.3\ RT}{F}\ pH \tag{8}$$

with ϕ^0 = standard potential, $pK_{sp} = -\log K_{sp}$, R = gas constant, T = temperature [K], F = Farraday constant.

Replacing the halfcell potentials in equation (6) results into:

$$emf = \Delta\phi^0_{glass} - \Delta\phi^0_{Ag} - \frac{2.3\ RT}{F}\ pH +$$

$$+\frac{2.3\ RT}{2\ F}\ pK_{sp} - \frac{2.3\ RT}{2\ F}\ pS^{2-}. \tag{9}$$

According to the law of mass action pS^{2-} in equation (9) can be substituted by:

$$pS^{2-} = pK_1 + pK_2 + pH_2S - 2 \cdot pH. \tag{10}$$

Because

$$\Delta\phi^0_{glass} - \Delta\phi^0_{Ag} + \frac{2.3\ RT}{2\ F}\ pK_{sp} - \frac{2.3\ RT}{2\ F}\ (pK_1 + pK_2) = const, \tag{11}$$

equation (9) simplifies to

$$emf = const - \frac{2.3\ RT}{F}\ pH - \frac{2.3\ RT}{2\ F}\ pH_2S + \frac{2.3\ RT}{F}\ pH \tag{12}$$

and

$$emf = const - \frac{2.3\ RT}{2\ F}\ pH_2S. \tag{13}$$

As can be seen from equation (13) the emf reading of the electrode combination corresponds solely to the H_2S fraction. A major advantage of this electrode type is the possibility to calculate total sulfide concentrations by means of the well-defined first protolysis constant (Broderius & Smith 1977). The sensitivity of the pH_2S electrode is a function of the solubility product of Ag_2S leading to a lower detection limit of $10^{-18.2}$ mol $H_2S \cdot l^{-1}$ (Peiffer & Frevert 1987).

Materials and methods

The experiments carried out with this study in order to simulate the metalimnetic redox conditions of Lake Kinneret can be divided into two categories:

1. simulation of the hydrochemical conditions in the absence of biota
2. simulation of the microbiological processes under given hydrochemical conditions.

Both types of experiments were carried out in a specially designed chemostat (Fig. 2). The central part of the device is a 1.5 l double-jacket reaction vessel (Schott) covered by a custom made teflon-coated stainless steel lid (workshop, Univ. Groningen) equipped with standard fittings (Schott) for electrodes (GL 25) and supply tubing (GL 14 with butyl rubber septa). pe and pH values were assessed by measuring the halfcells of a combined platinum/glass electrode, respectively, against a double junction Ag^0, AgCl reference electrode. pH_2S values were measured with the glass/Ag_2S electrode. All electrode material was obtained from Ingold.

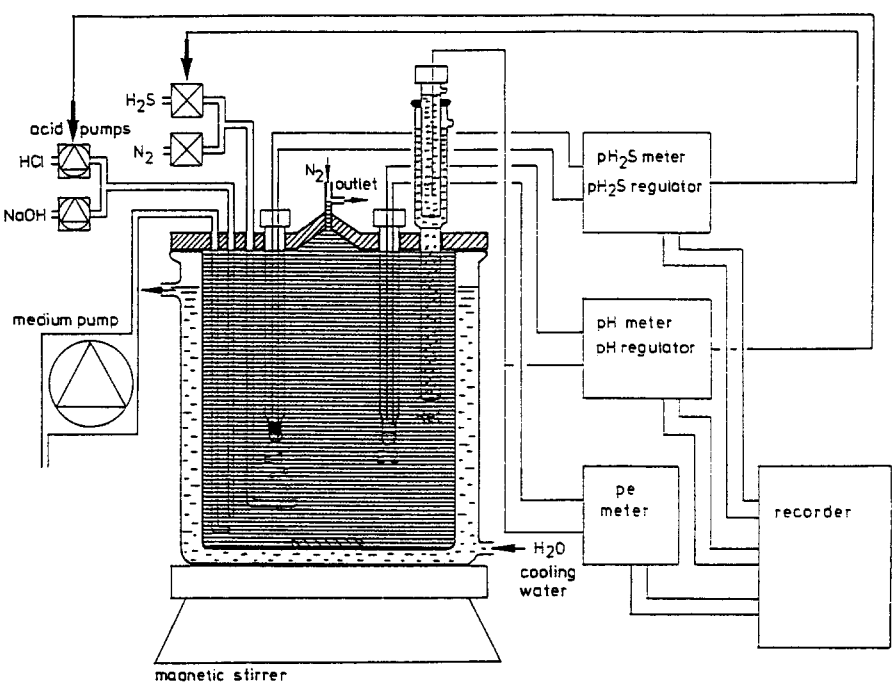

Fig. 2. Layout of the chemostat. E1: pH_2S electrode, E2: glass/Pt electrode, Ref: double junction reference cell.

pe and pH electrode cells were calibrated simultaneously in two pH buffer solutions (pH 7 & 5) saturated with quinhydrone ($pe_{20\,°C,\,pH\,7.00}$ = 5.09, $pe_{20\,°C,\,pH\,5.00}$ = 7.09). The pH_2S electrode was calibrated by the silver iodine method described by Peiffer & Frevert (1987). The mV signals of the pe cell were read on a digital mV-meter (pH 96, WTW). The pH and pH_2S signals were fed after pre-amplification (IWA 11, Dr. Kuntze) into two combined measuring regulation units (PR 12, Dr. Kuntze). A three channel recorder (SE 130, BBC/Metrawatt) allowed a continuous monitoring of pe, pH and pH_2S values. The regulators facilitated a proportional two-way regulation of pH and pH_2S values by alternative switching of acid-base pumps (Mini-Micro 2/6, Ismatek) or solenoid valves (Honeywell) for the regulation of gases (CO_2, H_2S, N_2), where CO_2 could be used alternatively for the pH regulation.

The basic medium for all titration and simulation experiments was filter-sterilized deaerated epilimnion water. A temperature bath (UC/F10, Julabo) and continuous stirring maintained constant physical conditions within the test solution. For the chemostat experiments, a peristaltic pump (Miniplus 2, Gilson) provided a continuous medium supply. To stimulate bacterial growth, a nutrient solution ($[g \cdot l^{-1}]$ 0.3 KH_2PO_4, 1.0 NH_4-acetate, 0.1 vitamin B_{12} (Pfennig 1965)) was added in parallel at 0.2 × medium flow rate. Backdiffusion of air through the outflow was avoided by two traps and a permanent overpressure of 0.1 atm oxygen-free N_2. The same overpressure maintained anaerobic conditions within the medium and acid and base flasks.

The H_2S gas needed for some of the experiments was produced by means of a Kipp system (Fig. 3). O_2-free CO_2 gas, — cleaned by passing through a heated column filled with copper powder (c) — was bubbled through a 'Normag' gas-washing bottle (j) filled with a Na_2S solution, thus, controlling the release of small amounts of hydrogen sulfide. Backdiffusion of H_2S into the copper column was prevented by a Zn-acetate trap (h). The desired flowrate was adjusted by means of a fine regulation valve (k). For feedback-controlled gas addition, the gas input could be regulated by a solenoid valve (l).

Simulation of the metalimnetic hydrochemistry

In order to investigate the pe-pH-pH_2S relationship in the metalimnion of Lake Kinneret the chemostat was filled with lake water of a preset sodium sulfide concentration (10^{-5}—10^{-3} M). Different pH values were adjusted automatically via feedback regulators described above while monitoring pe and pH_2S values. Similarly the platinum electrode response to the sulfide concentration at a given pH was tested whereby H_2S was produced

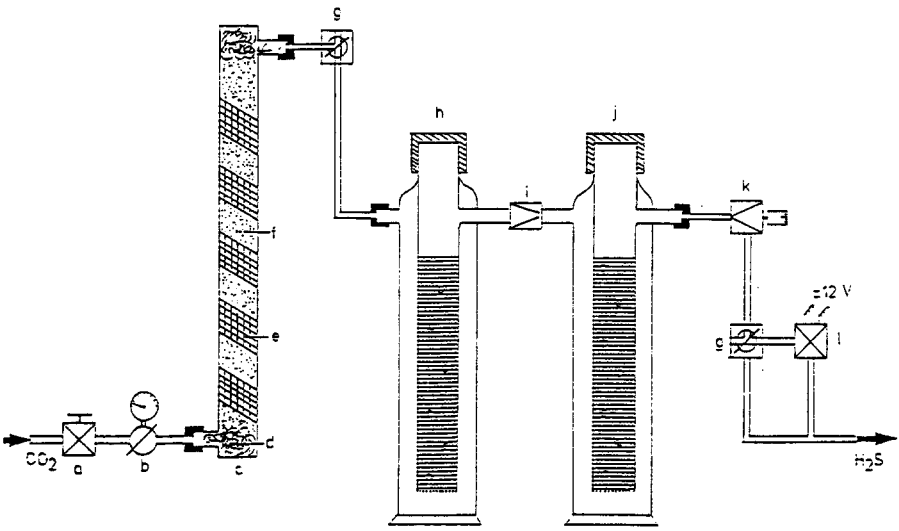

Fig. 3. 'Kipp' system for the generation of $H_2S_{(g)}$ a: main valve, b: pressure gauge, c: purifying column, d: glass wool, e: heating tape (450 °C), f: cooper powder (40 mesh), g: three way stopcock, h: Zn-acetate trap, i: check valve, j: Na_2S solution, k: fine regulation valve, l: solenoid valve.

using the 'Kipp' system. Besides sulfide, the possibility of redox changes following the addition of SO_3^-, SO_4^-, NO_3^-, $S_2O_3^-$, and NH_4^+ ions was investigated. These ions were injected as dissolved salts through the septum of one of the chemostat inlets.

Simulation of the metalimnetic microbiology

Chemostat experiments were carried out with pure- and co-cultures of a SRB (*Desulfovibrio sp.*) and a brown PSB (*Chlorobium phaeobacteroides*) isolated from Lake Kinneret. Before starting an experiment all wetted parts of the chemostat were autoclaved including the double junction of the reference electrode previously filled with electrolyte (3 M KCl, 1% agar). The electrodes were UV sterilized. The dilution rate was set at $0.005 \cdot h^{-1}$, unless otherwise stated. The pH value was feedback-regulated to 7.0. Three sets of experiments were conducted:

1. Redox changes due to sulfate reduction were measured in a chemostat inoculated with a pure culture of SRB. $1\ g \cdot l^{-1}$ sodium lactate was added to the nutrient solution to stimulate the bacterial growth (Postgate 1984).
2. The continuous culture experiment with PSB required the installation

of a light source (100 μEinstein $\cdot$ $m^{-2} \cdot sec^{-1}$). $H_2S_{(g)}$, produced by the 'Kipp' system, was the only sulfide source. In order to find the optimal $H_2S_{(g)}$ flow rate for the given dilution rate, the input was regulated using the pH_2S electrode signal as a feedback parameter. Optimal flow rate was determined at that rate which yielded constant pH_2S.

3. The co-culture experiment was started like experiment (1) until the amount of H_2S released reached 1 mM. At that stage an exponentially growing PSB culture was inoculated and the light turned on. The only sulfide source for the PSB was due to the activity of the SRB. The same experiment was repeated by inoculating the system with a subsample taken from a sediment core with a second subsample added after illumination.

Results

Hydrochemical experiments

In Fig. 4 the pe values measured during the acid-base-titration of metalimnon waters containing a total sulfide concentration of 10^{-4} M are related to the theoretical curve calculated from equation (2). As in the $p\varepsilon$-pH relationship the pe curve deviated from the linear function at pH $>$ pK_1. However, the final pe data for this pH range changed less rapidly with changing pH. Hence, use of equation (2) for calculating pe_7 would lead to an increasing error with increasing pH the pH dependency of the measured pe values in sulfide rich Kinneret water was related for the pH range: $pK_1 < pH < 9$ to an empirical equation:

$$pe = 300 \cdot e^{(-0.89 \cdot pH)} - 2.7 \tag{14}$$

in which pe as a function of pH was measured for $[S_{tot}] = 10^{-3}$, 10^{-4}, and 10^{-5} M. From equation (14) the following correction term for the calculation of pe_7 values was derived:

$$pe_7 = pe + 0.59 - 300 \cdot e^{(-0.89 \cdot pH)}. \tag{15}$$

This equation was used by Eckert and Trüper (1993) for correcting meta- and hypolimnetic pe values.

The platinum electrode responded instantaneously to the addition of H_2S (Fig. 5). The result was the pe-pH_2S relationship (pH 7.00):

$$pe = -4.5 + 0.59 \cdot pH_2S. \tag{16}$$

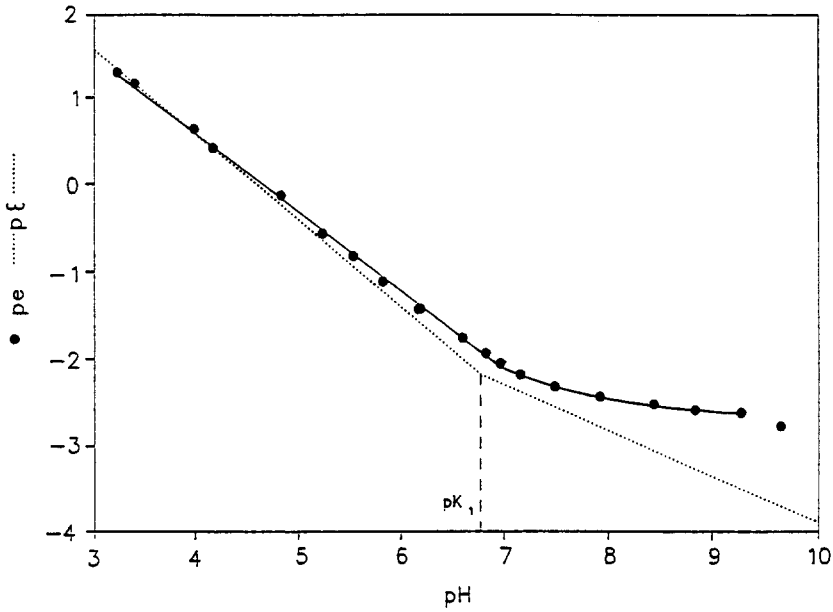

Fig. 4. pe electrode response to pH changes at a given total sulfide concentration (10^{-4} M); ●: pe values, ...: pε calculated from eq. 2; pK_1 is the first protolysis constant of the H_2S-HS^--S^{2-} system.

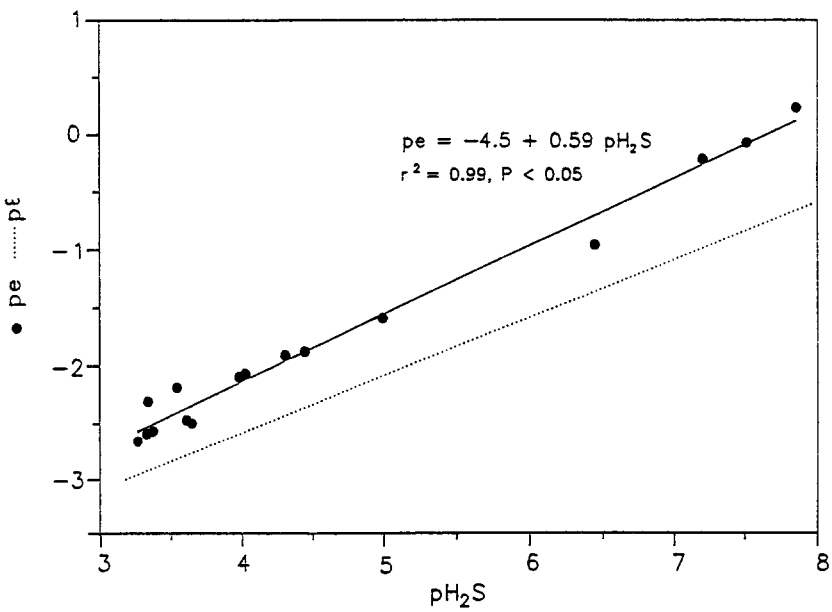

Fig. 5. pe electrode response to hydrogen sulfide at pH 7.00; ●: pe values, ...: pε calculated from eq. 2 for pH 7.

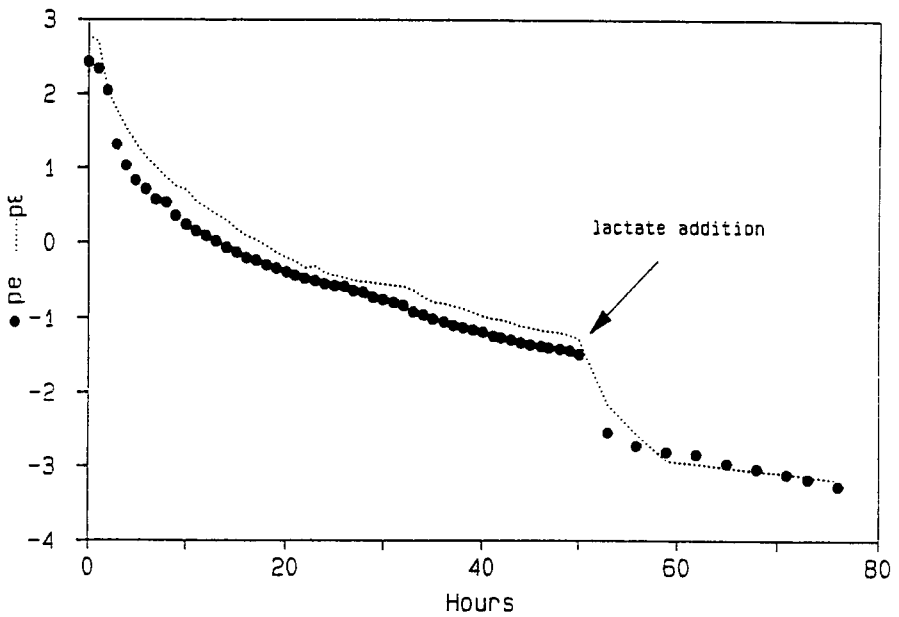

Fig. 6. pe changes during a chemostat experiment with a culture of a sulfate reducing bacterium; ●: pe values, ...: pε calculated from eq. 2 for pH 7.

The obtained regression line runs close to the thermodynamic function (equation 2) with a slightly more positive slope. None of the other system components (e.g. $SO_4^=$, NO_3^-, NO_2^-, $S_2O_3^=$, NH_4^+) that were added yielded a detectable change in the measured pe values.

Chemostat simulations

Measured pe values in the chemostat experiment with SRB closely followed the theoretical pε curve (Fig. 6). During the first 50 hours the pe decreased from 2.5 to −1.3. A one time injection of lactate (1 ml) caused a further pe drop to −2.5 due to the release of H_2S. The experiment was continued until no further pe change could be observed.

Measured pe values obtained during a continuous culture experiment with PSB deviated strongly from the theoretical pε (Fig. 7). This experiment was run at three different growth rates ($0.005 \cdot h^{-1}$, $0.01 \cdot h^{-1}$, $0.02 \cdot h^{-1}$). During the first 18 hours of the experiment with low $H_2S_{(g)}$ flow rate, hydrogen sulfide was removed continuously causing an increase in the pH_2S value and hence an increase in the theoretical pε value. Measured pe values on the other hand increased according to the theoretical pε values only for the first 15 hours then remained constant until

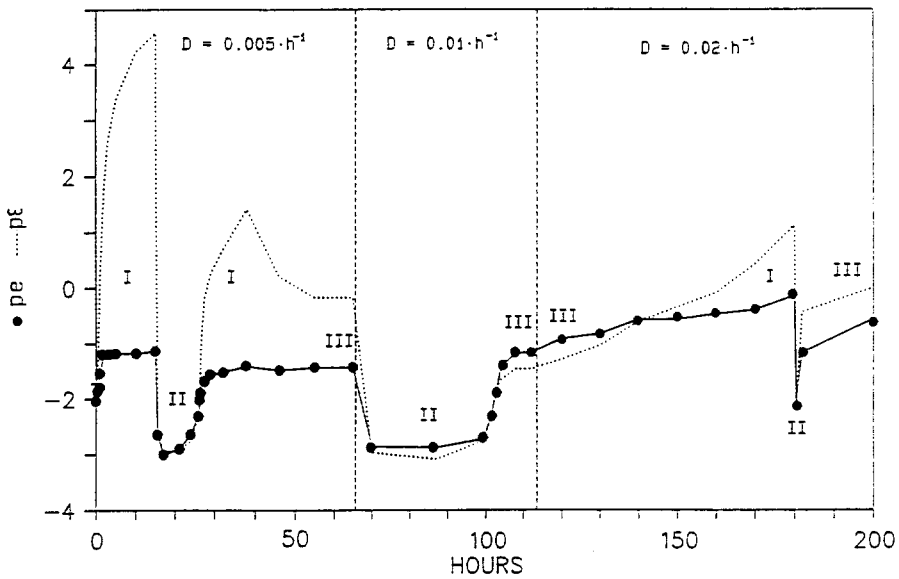

Fig. 7. pe changes during a chemostat experiment with a brown phototrophic sulfur bacterium at different dilution rates; •: pe values, ...: pε calculated from eq. 2 for pH 7; Roman numerals indicate low (I), high (II) and optimal (III) $H_2S_{(g)}$ flow rates.

H_2S addition was adjusted to a higher flow rate (condition II). During this stage, measured pe values followed the theoretical pε curve until H_2S addition was lowered again (condition I). The flow rate was re-adjusted until the parallel progress of the pe and pH_2S curves indicated the optimum rate for $H_2S_{(g)}$ addition (III).

During the first stage of the co-culture experiment in which only SRB were present pe values dropped in a similar form to the theoretical pε from −0.4 to −3.2 (Fig. 8). Upon the addition of the phototrophic bacteria culture, a rise of the electroactivity was observed with final pe values exceeding the theoretical curve. Similar results (not shown) were obtained when repeating this experiment with the sediment inoculum from a previously incubated sediment core from Lake Kinneret.

Discussion

The comparison of the acid-base titration experiment with Lake Kinneret water (Fig. 4) with similar measurements carried out by Boulegue (1977, 1978) and by Boulegue & Michard (1979) in mineral springs shows that the response of the redox sensor is dominated by H_2S. As found also by

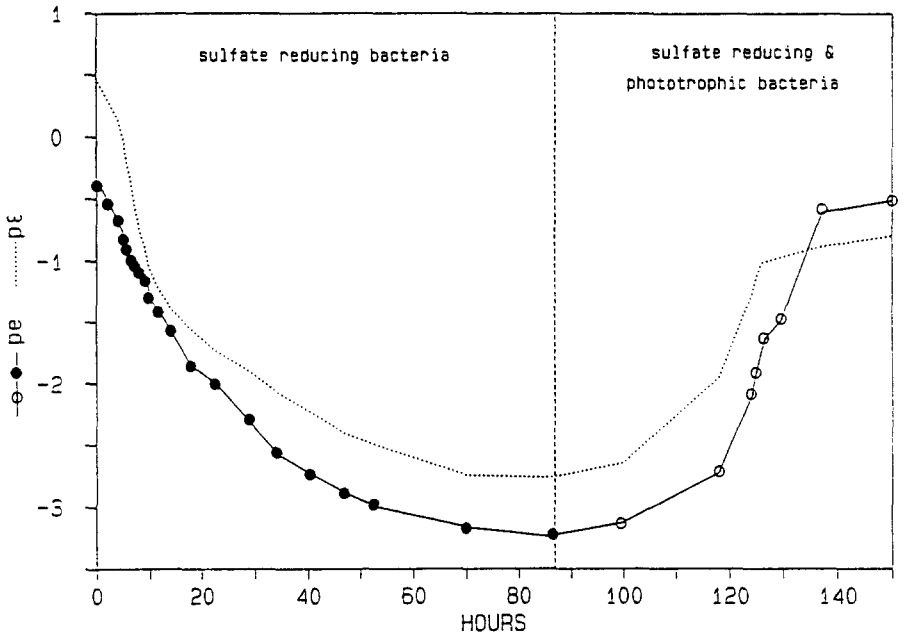

Fig. 8. pe changes during a co-culture experiment with sulfate reducing and phototrophic sulfur bacteria; ●: pe values during growth of SRB, ○: pe values during growth of SRB + PSB, ...: $p\varepsilon$ calculated from eq. 2 for pH 7.

Boulegue's studies, experimental pe values for pH > pK_1 in this study were higher than predicted by the theoretical $p\varepsilon$-pH relationship as defined in equation (2). Consequently, the calculation of pe_7 values following the thermodynamical relationship will induce a pH-dependent error. The chemostat experiment presented here demonstrates that a correct pe-pH relationship for a specific aquatic system can be obtained by conducting an acid-base titration experiment and by fitting the pe and pH data to an empirical curve. This strategy simplifies the comparison of redox data collected in different aquatic systems.

The investigation of the response of the platinum electrode to potentially electroactive compounds (Fig. 5) demonstrated in a highly correlated linear function between the pe and the pH_2S value. The other compounds that were added to the solution containing sulfide did not further affect the pe; an observation that agrees with the findings of Zhdanov (1975). It can be explained by a higher standard exchange current density (i_0) of H_2S in comparison to the other substances (Bockris & Reddy 1970). Literature values for i_0 of various redox systems vary from 40 $A \cdot cm^{-2}$ for the Br_2/Br^- system (Spiro 1964) to 10^{-9} $A \cdot cm^{-2}$ for the $O_2/O^=$ system

(Bockris & Huq 1956). Linear pe-pH_2S relationships are reported as well for anoxic sediments (Berner 1963; Whitfield 1969). However the question remains: How the pe value is influenced by competing microbial processes?

In the presence of SRB, pe as a function of pH_2S approached the theoretical relationship (Fig. 9). This result establishes a case of a microbially mediated redox reaction that is close to thermodynamical equilibrium.

A situation where the experimental pe values become detached from the prevailing pH_2S is given in the continuous culture experiment with PSB (Fig. 10) when the phototrophic bacteria apparently removed instantaneously the incoming $H_2S_{(g)}$ (squares). This phenomenon indicates an active buffering effect on the redox value due to microbial activity. In the case that the $H_2S_{(g)}$ flux exceeded the rate of sulfide oxidation (circles) pe values are close to the $p\varepsilon$ curve as in the experiment with SRB (Fig. 9). A scattering of pe values relative to the $p\varepsilon$ curve appears at a balanced sulfide budget (triangles).

The co-culture experiment (Fig. 8) was carried out to simulate the coexistence of the sulfate reduction and sulfide oxidation processes as they appear in the metalimnion of Lake Kinneret during the time period of the *Chlorobium* bloom. The observed increase in pe after the addition of the

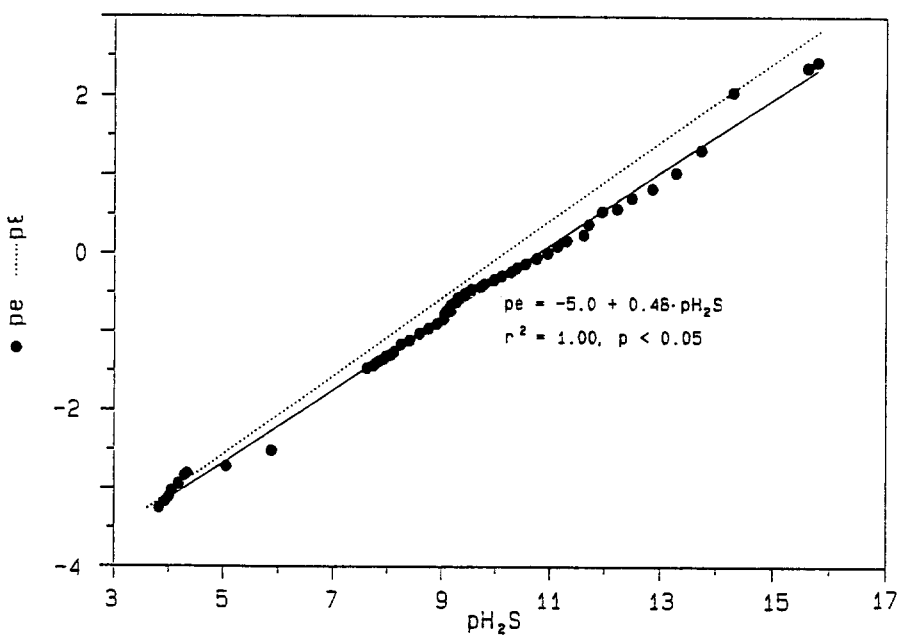

Fig. 9. pe/pH_2S plot and regression line of the data from Fig. 6; .....: $p\varepsilon$ calculated from eq. 2 for pH 7.

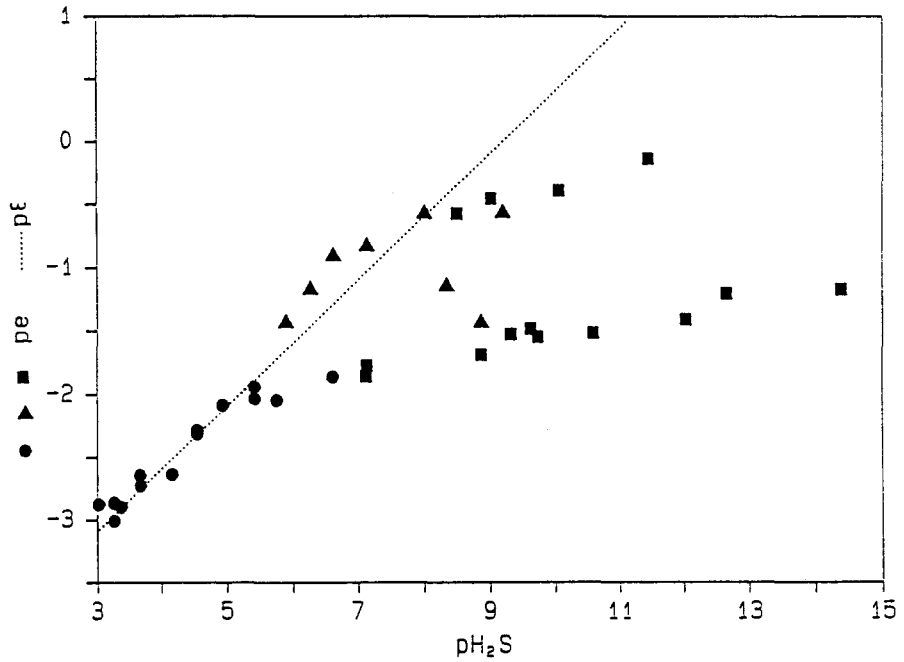

Fig. 10. pe/pH_2S plot of the data from Fig. 7; ■: pe values during condition I, ●: pe values during condition II, ▲: pe values during condition III, ...: pε calculated from eq. 2 for pH 7.

PSB culture is reflected in the pe-pH_2S plot in Fig. 11. The initial predominating growth of SRB led to the function (pH = 7):

$$pe = -4.7 + 0.44 \cdot pH_2S, \tag{17}$$

while the coexistence of both processes corresponds to:

$$pe = -8.2 + 0.99 \cdot pH_2S. \tag{18}$$

In this continuous culture experiment the process of phototrophic sulfide oxidation apparently caused an increase of the pe value above the pε curve. The same raise was observed in the pure culture experiment with *Chlorobium* when the dilution rate was $0.01 \cdot h^{-1}$. The possibility of intermediately formed oxidation products due to microbial sulfide oxidation being responsible for the pe shift is rejected because the corresponding compounds ($SO_3^=$, $SO_4^=$, $S_2O_3^=$) do not interact with the platinum electrode when H_2S is present. A possible formation of polysulfide in the course of microbial sulfide oxidation which would cause a shift from the H_2S-S_8-

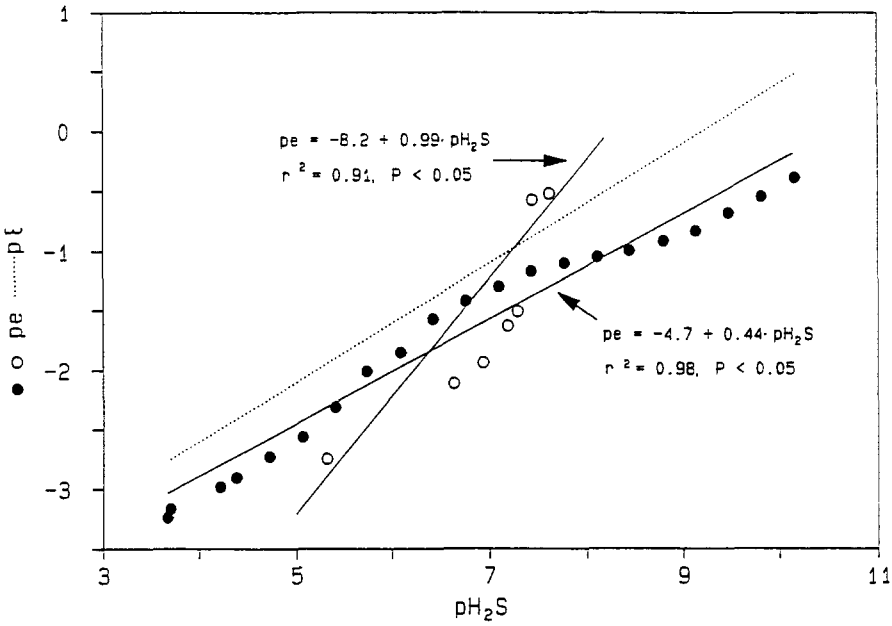

Fig. 11. pe/pH_2S plot and the regression lines of the data from the co-culture experiment in Fig. 8; ●: pe values during growth of SRB, ○: pe values during growth of SRB + PSB, ...: pε calculated from eq. 2 for pH 7.

H_2O system to the H_2S-S_n^{2-}-H_2O system can not be responsible for the observed redox shift since for a pH range below pH 8.5 both systems behave electrochemically identical (Boulegue & Michard 1979). Referring to the assumption that the prevailing redox conditions reflect the intracellular microbial metabolism (see introduction) the observed pe shift could be an indication for an direct effect of the PSB on the platinum electrode potential.

A comparison of the experimental results obtained with this study with the field data from Lake Kinneret (Eckert & Trüper 1993) shows that the pe_7-pH_2S relationship calculated from meta- and hypolimnetic data is similar to the pe-pH_2S function in Fig. 5 when hydrogen sulfide was added to metalimnion water from Lake Kinneret. Finally, the linear function (equation 18) that was calculated from the co-culture experiment resembles the one found in the metalimnion of Lake Kinneret during the first peak of the *Chlorobium* bloom in July (Eckert & Trüper 1993). It seems that this pe-pH_2S relationship is a characteristic for the coexistence of two microbially mediated processes: sulfate reduction and phototrophic sulfide oxidation.

In summary, both, the experimental as well as the *in situ* pe values from

Lake Kinneret, are linearly related to the prevailing pH_2S by a general equation of the form:

$$pe = \alpha + \beta \cdot pH_2S \tag{19}$$

with average values for $\alpha = -4.8$ and $\beta = 0.55$ for sulfate reduction and $\alpha = -7.0$ and $\beta = 1.0$ for sulfate reduction coinciding with phototrophic sulfide oxidation.

This result illustrates a situation where redox conditions are biased by the differential influence of sulfur bacteria of different physiological properties. Our research profited certainly from the high electroactivity of hydrogen sulfide and it remains questionable if the investigation of the microbially mediated redox reactions within a different redox system would confirm our hypothesis of a direct microbial influence on the redox conditions. However our research may enhance further research using redox measurements in microbially dominated systems.

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