4 Sensors for *In situ* Analysis of Sulfide in Aquatic Systems

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1 INTRODUCTION

Sulfur cycling is of major importance for the biogeochemistry of ecosystems, and here microbial and chemically catalyzed sulfide conversions play a key role [1]. In the environment, sulfide is not only a strong poison to all aerobic organisms through its high affinity to metal containing enzymes [2,3] but is also an important product of anaerobic microbial activity. Under oxic conditions microorganisms are preferentially utilizing oxygen as an electron acceptor, whereas under anoxic conditions other substances such as metal ions (Fe(III), Mn(IV)), nitrate, sulfate or even carbon dioxide are used. In the marine environment sulfate is usually the most available electron acceptor owing to its high
concentration in seawater (ca 25 mmol L⁻¹) and more than 50% of the carbon mineralization in marine sediments can go via sulfate respiration [4]. This leads to a high H₂S production by microbial sulfate reduction below the oxic/anoxic interface in sediments [5,6], biofilms [7], and in stratified water masses [8,9], where oxygen concentration is low and sulfate availability is high. The sulfide produced reacts with heavy metal ions in the environment and forms precipitates such as FeS, and FeS₂, which in the latter case is preserved in the geological record. A significant amount of sulfide is oxidized via various chemical and microbial pathways involving O₂, NO₃⁻, Fe(III) and Mn(IV) [5]. Sulfide oxidation thus binds the sulfur cycle together with the cycling of other key elements in nature [1,5].

In certain areas of industry and waste water treatment, sulfide poses problems. In sewage treatment plants, excessive sulfide production can lead to inhibition of efficient nutrient removal and clogging of activated sludge basins. In sewers, sulfide-containing waste water can lead to unpleasant odors and, when oxidized to sulfate, severe corrosion of sewer pipes [10]. Sulfur removal from solids, liquids and gases is of major industrial interest, e.g. in the paper industry [11,12] and the mining and fossil fuel industry [13,14]. Also, corrosion processes due to biofilm formation on immersed surfaces are in part induced by the presence of sulfide-producing bacteria [15,16]. A better understanding of sulfide conversion processes is thus of general biogeochemical interest and has important socioeconomic implications. This calls for suitable analytical techniques for detecting sulfide in the environment.

In this chapter, we review the current methodology for measuring sulfide in aquatic systems. While we give an overview of currently available analytical techniques, we will focus on direct measurements of sulfide with sensors, that is, devices that can be used directly in natural waters without previous conditioning steps, and that respond specifically and reversibly to sulfide. Special attention is given to sulfide micro-sensors that allow measurements at high spatial (< 0.1 mm) and temporal (t₉₀ = 90% of response time < 1–10 s) resolution, with minimal consumption of the analyte, and, therefore, without significant effects on the sulfide equilibria and gradients present in the aquatic environment. More general reviews on microsensors and their application in environmental analysis appear elsewhere [17–24] and in other chapters of this book.

2 SULFIDE IN AQUATIC SYSTEMS

Here, we only briefly summarize some important characteristics of sulfide in natural waters. More detailed accounts can be found e.g. in the work of Millero and co-workers [25–27]. In aqueous solution hydrogen sulfide is found to be a weak acid and, neglecting metal complexes and solid phases, the total sulfide concentration, [S(–II)]ₜ, consists of H₂S (dissolved hydrogen sulfide), HS⁻

![](image)

Figure 1. Relative concentrations of the dissociation products of H₂S at different pH (T = 298 K, f = 1 mol L⁻¹) calculated from equations (3)–(5). For the calculations we used pK₁ = 6.921 (298 K) as calculated from equation (6) [28] and pK₂ = 14 [29](hydrogen sulfide ion), and S²⁻ (sulfide ion). The relation of the actual concentrations of these species is determined by the dissociation constants K₁ and K₂ (equations (1) and (2); Figure 1), and [S(–II)]ₜ can be calculated from the measured species concentrations, pH, ionic strength and temperature using equations (3)–(5):

\[
\begin{align*}
H₂S &\rightleftharpoons H₂O^+ + HS^- \quad K_1 = \frac{[H₂O^+][HS^-]}{[H₂S]} \\
HS^- &\rightleftharpoons H₂O^+ + S²⁻ \quad K_2 = \frac{[H₂O^+][S²⁻]}{[HS^-]}
\end{align*}
\]

\[
\begin{align*}
[H₂S] = \frac{[S(–II)]ₜ}{1 + \frac{[S(–II)]ₜ}{K_1 K_2}} \quad (3)
\end{align*}
\]

\[
\begin{align*}
[HS^-] = \frac{[S(–II)]ₜ}{1 + \frac{[H₂O^+]}{K_1} + \frac{[H₂O^+]^2}{K_2}} \quad (4)
\end{align*}
\]

\[
\begin{align*}
[S²⁻] = \frac{[S(–II)]ₜ}{1 + \frac{[H₂O^+]}{K_2} + \frac{[H₂O^+]^2}{K_1 K_2}} \quad (5)
\end{align*}
\]

The determination of the exact value of the dissociation constants has been a subject of scientific debate. For the first constant, Broderius and Smith[30]
performed a direct photometric determination of H$_2$S in the gas phase and found the empirical formula, at infinite dilution:

$$pK_1 = 3.122 + \frac{1132}{T} \quad \text{for} \quad 283\,^\circ\text{K} \leq T \leq 298\,^\circ\text{K}$$

(6)

giving a value of $pK_1 = 6.921$ at $298\,^\circ\text{K}$ which is in good agreement with the constant found by Barbero et al. [31] using the empirical expression:

$$pK_1 = 19.840 + \frac{930.8}{T} - 2.800 \ln T$$

(7a)

Other empirical relations for the first dissociation constant as a function of temperature and salinity in natural waters are given by Millero et al. [26]:

$$pK_1 = -98.080 + \frac{5765.4}{T} + 15.0455 \ln T \quad \text{at infinite dilution}$$

(7b)

$$pK_1^* = pK_1 - 0.1498\sqrt{S} + 0.0119S \quad \text{at salinity, S, (in ppt)}$$

The main problem in the determination of the second dissociation constant is to determine very precisely one of the components HS$^-$ or S$^{2-}$ [32]. Licht and co-workers [32–34] tried to measure sulfide ion using different methods and found a very low value for $K_2$ of around $10^{-17}$. This would mean for most natural conditions (pH 7–9 and 0–10 mmol L$^{-1}$ total sulfide) that the sulfide ion could not be detected by any currently available analytical method except ISE in S(–II) buffered samples.

Licht and co-workers [32–34] used highly concentrated sulfide solutions of 3–6 mol L$^{-1}$. Physico-chemical parameters in aqueous solutions can, however, only be readily calculated from experimental data when the ionic strength of the solution is located inside the Debye–Hückel region, which describes properties of aqueous solutions up to a maximum ionic strength of about 1 mol L$^{-1}$ (provided empirical corrections are included). Ionic strength outside this region, resulting for instance from sulfide concentrations of 3 mol L$^{-1}$, leads to a destruction of the water structure and hence to undefined conditions. In addition, there is no way to extrapolate values of Licht and co-workers to infinitely diluted solutions or even to conditions where the ionic strength is inside the Debye–Hückel region.

A more reliable method to get information about the acidity of HS$^-$ was reported by Widmer and Schwarzenbach [29]. They investigated the complex formation of [HgS$_2$]$^{2-}$ at a mercury electrode and found at an ionic strength of 1 mol L$^{-1}$ that

$$pK_1(20\,^\circ\text{C}) = 6.88 \pm 0.02 \quad pK_2(20\,^\circ\text{C}) = 14.15 \pm 0.05$$

(8)

The existence of dissociation constants $pK_0$ and $pK_{00}$ corresponding to the equilibria:

$$H_2S^+ = H^+ + H_2S \quad K_0 = \frac{[H^+][H_2S]}{[H_2S^+]}$$

(9)

$$H_2S^{2+} = H^+ + H_2S^+ \quad K_{00} = \frac{[H^+][H_2S^+]}{[H_2S^{2+}]}$$

(10)

as proposed by Su et al. [35] is not very likely since a decrease of H$_2$S concentration at lower pH is generally not observed. In addition, the authors used a sulfide ion selective electrode (sulfide ISE) at pH<5, which is not adequate as these electrodes are responding specifically to the sulfide ion. Even with the amplified technique of Su et al., there is no way to overcome the limitations of the detection principle. Their potential readings must therefore be caused by other phenomena such as chemical destruction of the membrane and formation of potentials at the silver electrode due to the high solubility of Ag$_2$S in acidic media (see Section 4.2).

Aqueous sulfide solutions are easily oxidized by oxygen, peroxides (e.g. H$_2$O$_2$), halogens, nitric acid, lead dioxide and other oxidants [26,36,37]. Thereby, the final products (see Table 1) as well as the reaction rates are highly determined by the pH. At pH<6 the reaction rate is very slow, shows maxima at pH 8 and 11 and decreases at pH>11 [38]. On the overall pH range, heavy metal ions (mainly Fe$^{2+}$, Fe$^{3+}$ and Ni$^{2+}$) are increasing the reaction rate [41]. In the case of iron, this is due to the local formation of H$_2$O$_2$:

$$\text{Fe}^{2+} + \text{O}_2 \rightarrow \text{Fe}^{3+} + \text{HO}_2$$

(11)

$$2\text{HO}_2 \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$$

(12)

The sensitivity of sulfide solutions to oxidation plays an important role in the accuracy of analytical determination. Some established analytical procedures require sampling, and sample stabilization, e.g. by the addition of zinc acetate to form zinc sulfide, before the actual analysis is performed. Zinc sulfide is stable for several weeks against oxidation by oxygen [42]. The most important problems involved with sampling are the losses of the analyte by evaporation, adsorption and oxidation prior to the stabilization step. This requires careful handling of samples and specialized procedures for the calibration of all analytical methods, for the determination of sulfide. This is just as important when in situ-methods are used and calibrated.

**Table 1. Primary oxidation products of sulfide species at different pH**

<table>
<thead>
<tr>
<th>pH range</th>
<th>Main sulfide species</th>
<th>Primary oxidation products</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>acidic</td>
<td>H$_2$S</td>
<td>S$^0$, SO$_4^{2-}$</td>
<td>[38]</td>
</tr>
<tr>
<td>neutral</td>
<td>HS$^-$</td>
<td>SO$_2^{2-}$, S$_2$O$_3^{2-}$, SO$_3^{2-}$, SO$_4^{2-}$, S$^{2-}$</td>
<td>[38,39]</td>
</tr>
<tr>
<td>alkaline</td>
<td>HS$^-$ and S$^{2-}$</td>
<td>SO$_2^{2-}$, SO$_3^{2-}$, S$^0$</td>
<td>[40]</td>
</tr>
</tbody>
</table>
Calibration of analytical methods for sulfide determination in aqueous samples should involve the following steps:

- A relatively concentrated sulfide solution of about 0.1 molL\(^{-1}\) [S\((-II)\)] is prepared by weighing a certain amount of Na\(_2\)S (7-9)H\(_2\)O and adding degerated, deionized water. The determination of the exact sulfide content of this stock solution can be done by iodometric titration [43]. Such concentrated sulfide solutions are stable for months when protected against the impact of oxygen, light and heavy metals, e.g. by storing the solution under argon in brown gas-tight glass flasks. It is not optimal to use flasks with rubber washers or other parts made of silicone, rubber, PVC, Teflon etc. for such long time storage, as these materials are permeable for oxygen and can contain unknown amounts of heavy metals. Furthermore, some rubber materials tend to adsorb sulfide.

- The stock solution is used to make a diluted working solution. The sulfide content can be determined by the methylene blue method [43,44] (see Section 3.1). This diluted working solution is then used to calibrate the analytical method by exactly the same procedure, which is applied on the samples, i.e. stabilization with zinc acetate, dilution steps etc. The working solution cannot be stored and is stable only for some hours.

Another more accurate and easy way to perform calibration of analytical methods for sulfide determination was proposed by Jeroschewski and Schmuhl [45,46]. The method is based on the use of a novel sulfide generator (Figure 2), which performs an electrochemical reduction of HgS in a flow-through apparatus, whereby H\(_2\)S is produced in a degerated, acidic carrier solution. The exact H\(_2\)S concentration [\(\mu\)mol L\(^{-1}\)] is determined by the flow velocity and the applied current according to the Faraday law:

\[
\text{c}_{\text{H}_2\text{S}} = \frac{6 \times 10^4 I}{F n v}
\]

where \(I\) is the applied generator current in \(\mu\)A, \(F\) is the Faraday constant in c.mol\(^{-1}\), \(n\) is the number of exchanged electrons (2), and \(v\) is the flow velocity [mL min\(^{-1}\)] of the carrier solution through the generator cell.

The sulfide generator thus performs a coulometrically controlled formation of hydrogen sulfide. The method can only be used in a flow-through configuration, but it is in principle possible to prepare stabilized sulfide solutions with an exactly known content by adding the sulfide-containing carrier solution to a zinc acetate solution. While the sulfide generator is ideal for calibration of e.g. amperometric H\(_2\)S sensors, it cannot be used for calibration of sulfide ion-selective electrodes as small amounts of mercury are released, which will interact with the Ag/Ag\(_2\)S membrane of such electrodes (see section 4).

**Figure 2.** Scheme of a coulometric H\(_2\)S generator. CL, carrier solution of \(5 \times 10^{-3}\) mol \(\text{L}^{-1}\) H\(_2\)SO\(_4\); CE, counter electrode; the generator electrode contains HgS; DC, direct current

### 3 MEASURING TECHNIQUES FOR SULFIDE

A large variety of techniques are available for measuring either total sulfide, sulfide ion or dissolved hydrogen sulfide. The techniques for the determination of [S\((-II)\)], usually are \textit{ex situ} methods, i.e. sampling is required. Here the most important techniques are using the methylene blue reaction, other spectrophotometric methods, or chromatographic methods (see Table 2 and 3).

#### 3.1 SPECTROPHOTOMETRIC METHODS

Spectrophotometric methods usually involve conditioning and treatment of the analyte sample with chemical reactions prior to the spectroscopic analysis. There exists a large variety of such analytical methods (Table 2). Only a few authors have proposed direct spectrophotometric determination of H\(_2\)S, mostly in the gas phase [76-83]. Although the analytical procedure in this case is rather simple, the direct measurement of H\(_2\)S demands a significant amount of (expensive) technical equipment, it suffers from serious interferences, especially from SO\(_2\), and it requires great care to avoid analyte losses through evaporation, adsorption and rapid oxidation. The method is, therefore, seldom used for environmental analysis of sulfide. This is also true for kinetic methods, i.e. sulfide-catalyzied or -inhibited reactions [84-87,103,104] because under most conditions the parameter time cannot be controlled accurately. Furthermore, in natural waters interference with the kinetic reactions, e.g. by metal ions, is a problem.
<table>
<thead>
<tr>
<th>Reference</th>
<th>LOD (mol/L)</th>
<th>Significance</th>
<th>Interference</th>
<th>Dynamic Range</th>
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<tbody>
<tr>
<td>[78]</td>
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<td>[86]</td>
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</table>

**Table 2:** Section photometric methods for 4-hydroxylation determination in various systems (1L = fluorosence, PL = fluorescence, PPA = photometry, PP = fluorescence, PFP = fluorescence, PFA = fluorescence).
Another modification of the original methylene blue method was introduced by Cline [56], and is widely applied in limnological and marine research. Instead of the standardized method, which uses separate solutions of \(N,N\)-dimethyl-1,4-phenylenediamine and \(\text{FeCl}_3\), Cline proposed a mixed solution of the two reagents to overcome problems arising with salinity, temperature and pH. We recently compared this approach with the standard method and were not able to realize any advantage (Steuckart et al., unpublished data). Furthermore, the method of Cline shows an important disadvantage if the sample is pH buffered (which is the case in many natural waters). Reliable quantitative results can only be obtained when the pH of the reaction mixture (reagents + sample) is kept very low, as the absorption spectrum of methylene blue depends on its state of protonation (Figure 3A). Also, the sensitivity characteristics of the method vary when mixed reagent solutions are used. Under the same conditions, i.e. same sulfide and reagent concentration and same protonation state of

![Figure 3](image-url)
the methylene blue, the mixing of the reagents some hours prior to the analytical procedure leads to a significant drop in sensitivity, which increases with the age of the mixed solution (Figure 3B). The experiments were not performed at the upper border of the dynamic range, i.e., the concentrations of the reagents were always high enough to convert all sulfide to methylene blue, hence resulting in the same theoretical methylene blue concentration. Therefore, the drop in sensitivity can only be explained by a deterioration of the reagent mixture and/or the formation of interfering substances over time. This is consistent with a reaction mechanism proposed by Kubán et al. [105], where the first step of the methylene blue reaction is the oxidation of the diaminoaniline by Fe(III) leading to a slowly established equilibrium between a cation radical and a quinone diimine, where the latter is not involved in the further reaction with \( \text{H}_2\text{S} \). In conclusion, we recommend the use of separate reaction solutions when using the methylene blue method.

The formation of ethylene blue instead of methylene blue, by using \( \text{p} \)-diethylaminoaniline as the reactive substrate, has been successfully established for the determination of sulfide in Kraft liquors [64–66]. This method is well suited for sulfide determination even in complex wastewater from paper mills (white, green and black liquors consisting of highly concentrated mixtures of hydroxide and sulfide).

An alternative to the classical methylene blue method for environmental sulfide analysis was proposed by Koh et al. [68–71] and is based on several variations of the oxidative reaction of sulfide with cyanide to thiocyanate:

\[
\text{S}^2^- + \text{I}_2 + \text{CN}^- \rightarrow \text{SCN}^- + 2\text{I}^- \tag{14}
\]

Though the methodological parameters (e.g., linear dynamic range, LOD, precision) are similar to those of the methylene blue method, the method is significantly more time consuming and complicated. We conclude that the classical methylene blue method is still the spectrophotometric method of choice for sulfide determination in natural waters.

3.2 CHROMATOGRAPHIC METHODS

Chromatographic methods find many applications in environmental chemistry. They combine powerful separation methods and sensitive detection techniques. The main disadvantage of chromatography is the need for sampling prior to analysis, and \textit{in situ} measurements using chromatographic methods are very complicated to realize. In addition, sample treatment (pre-column or post-column) as well as the separation procedure itself change the chemical and/or biological state of the sample. Therefore, information about the actual situation with respect to sulfide speciation, pH, redox equilibria etc. cannot easily be obtained. In spite of this, gas chromatography (GC), ion chromatography (IC), and high performance liquid chromatography (HPLC) are used for the separation and determination of sulfide in waters (Table 3). Gas chromatography requires the removal of the analyte (\( \text{H}_2\text{S} \)) from the aqueous matrix prior to the analysis followed by enrichment procedures, such as cryofocusing of the headspace [89,90]. Tang and Heaton [91] have shown that it is possible to inject aqueous samples into the gas chromatograph. The detectors normally used for the gas chromatographic determination of \( \text{H}_2\text{S} \) are the photo-ionization detector (PID), the flame photometric detector (FPD), and the sulfur chemiluminescence detector (SCD), which is extremely selective towards sulfur compounds.

Ion chromatographic methods can be performed directly with filtered aqueous samples but problems such as (i) enrichment due to the reaction of sulfide with heavy metal contaminations of the reagents, or (ii) a strong adsorption of sulfide onto the analytical column can be observed [106]. Because of the low dissociation of \( \text{H}_2\text{S} \), the widespread conductivity detectors cannot be used for sulfide determination. In most cases, ion chromatographic methods are combined with amperometric (Ag versus SCE) [92–95] or photometric (e.g. post-column oxidation with iodine) [97,98] detection principles. More sensitive HPLC methods involve a pre-column derivatization of sulfide mostly to methylene blue and these techniques have detection limits in the pmol L\(^{-1}\) range [100–102].

3.3 ELECTROCHEMICAL METHODS

Electrochemistry offers a variety of measuring principles for determining specific sulfur species directly in natural waters (see Table 4). Currently, three types of electrochemical techniques are mostly used for \textit{in situ} environmetal analysis of sulfide speciation: (i) the potentiometric sulfide ion-selective electrode, (ii) the amperometric \( \text{H}_2\text{S} \) sensor, and (iii) methods based on voltammetry with either amalgamated gold or Hg-coated iridium microelectrodes. These methods will be discussed in more detail in the following section.

4 SENSORS FOR MEASURING SULFIDE IN AQUATIC SYSTEMS

The quantitative determination of chemical variables in the environment requires analytical methods that are minimally invasive in terms of disturbance of local chemical or redox equilibria and in terms of mechanical disturbance. Electrochemical sensors are suitable analytical tools for this purpose because no sampling and/or sample treatment is required. Such sensors can be constructed with geometric parameters of the sensor tip in the micrometer range, which minimizes mechanical disturbance and, in the case of electrochemical methods, offers many other advantages compared with macroelectrodes. For instance, amperometric and voltammetric methods are characterized by a consumption
**Table 4.** Electrochemical methods for sulfide determination in aqueous solutions (RDE, rotating disc electrode; SMDE, static mercury drop electrode; ISE, ion-selective electrode; LOD, limit of detection)

<table>
<thead>
<tr>
<th>Method</th>
<th>Procedure</th>
<th>Dynamic range (μmol L⁻¹)</th>
<th>LOD (μmol L⁻¹)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polarography</td>
<td>cathodic stripping polargraphy</td>
<td>no data</td>
<td>0.09</td>
<td>[107]</td>
</tr>
<tr>
<td>Polarography</td>
<td>differential pulse polargraphy</td>
<td>&lt; 1500</td>
<td>0.1</td>
<td>[108-110]</td>
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<tr>
<td>Voltaametry (Ag-RDE)</td>
<td>cathodic stripping voltagmetry</td>
<td>0.01-10</td>
<td>0.01</td>
<td>[111,112]</td>
</tr>
<tr>
<td>Voltaametry (SMDE)</td>
<td>alternating current voltagmetry</td>
<td>0.01-900</td>
<td>no data</td>
<td>[113]</td>
</tr>
<tr>
<td>Potentiometry S²⁻-ISE(Ag/AgCl)</td>
<td>&lt; 10⁶</td>
<td>&lt; 10⁻¹⁷</td>
<td>[114-117]</td>
<td></td>
</tr>
<tr>
<td>Potentiometry S²⁻-ISE(Ag/AgCl/PH)</td>
<td>&lt; 10⁶</td>
<td>&lt; 10⁻¹⁷</td>
<td>[118-126]</td>
<td></td>
</tr>
<tr>
<td>Potentiometry S²⁻-ISE(Ag/AgCl with/without gas dialysis)</td>
<td>15-1500</td>
<td>S(−II)ₙ 1-6</td>
<td>[127-130]</td>
<td></td>
</tr>
<tr>
<td>Potentiometry S²⁻-ISE (air-gap variation of Ag/AgCl)</td>
<td>&gt; 10; [S(−II)ₙ], [S(−II)ₙ]</td>
<td>0.01; [S(−II)ₙ]</td>
<td>[131]</td>
<td></td>
</tr>
<tr>
<td>Potentiometry CN⁻-ISE (Ag/AgCN with acidic evaporation)</td>
<td>&gt; 12; [S(−II)ₙ], [S(−II)ₙ]</td>
<td>0.3; [S(−II)ₙ]</td>
<td>[132]</td>
<td></td>
</tr>
<tr>
<td>Potentiometry S²⁻/Cl⁻-ISE (Ag/Agx) (S,Cl) with FIA</td>
<td>no data</td>
<td>&lt; 10; [S(−II)ₙ]</td>
<td>[133]</td>
<td></td>
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<tr>
<td>Potentiometry potentiometric titration with Pb(II) with Pb-ISE</td>
<td>no data</td>
<td>no data</td>
<td>[134]</td>
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<tr>
<td>Potentiometry S²⁻-ISE (Co(II)-phthalocyanines and -porphyrines as electrocatalyst)</td>
<td>depends on pH</td>
<td>depends on pH</td>
<td>[135-137]</td>
<td></td>
</tr>
<tr>
<td>Potentiometry HS⁻-ISE (carrier system)</td>
<td>0.2-20; [S(−II)ₙ], 0.06; [S(−II)ₙ]</td>
<td>&lt; 1000; HS⁻ no data</td>
<td>[138]</td>
<td></td>
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<tr>
<td>Potentiometry HS⁻-ISE (carbon paste electrode)</td>
<td>&lt; 1000; HS⁻ no data</td>
<td>[139]</td>
<td></td>
<td></td>
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<td>Amperometry porous Au electrode with pneumonia-amperometry</td>
<td>3-3300</td>
<td>no data</td>
<td>[140]</td>
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<td>Amperometry H₂S specific biosensor with immobilized Thiothrix flocculenta</td>
<td>20-400</td>
<td>no data</td>
<td>[141]</td>
<td></td>
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<tr>
<td>Amperometry H₂S specific sensor with redox mediator</td>
<td>3-90</td>
<td>~ 1</td>
<td>[142-146]</td>
<td></td>
</tr>
<tr>
<td>Amperometry H₂S specific sensor with redox mediator (FIA)</td>
<td>1-750</td>
<td>~ 1</td>
<td>[147,148]</td>
<td></td>
</tr>
<tr>
<td>Amperometry H₂S microsensor</td>
<td>1-750</td>
<td>&lt; 1</td>
<td>[6,149,150]</td>
<td></td>
</tr>
</tbody>
</table>

* Free concentration in buffered medium; value stated by manufacturer (see Section 4.2 for details). † blocked by I⁻, SCN⁻ and CN⁻.

of the analyte through reduction or oxidation. The analytical parameter is the corresponding current which is time dependent in the case of macroelectrodes and is described by the Cottrell equation [151]:

\[
i_t = \frac{nFA\sqrt{Dc^*}}{\sqrt{\pi t}}
\]

where, \(i_t\) is the limiting current, \(t\) is the time, \(n\) is the number of exchanged electrons, \(F\) is the Faraday constant, \(A\) is the electrode surface area, \(D\) is the analyte diffusion coefficient, and \(c^*\) is the bulk concentration of the analyte.

In the case of microelectrodes the limiting current is time independent [151]:

\[
i_t = 4\pi r_0 n F D c^*
\]

where, \(i_t\) is the limiting current, \(r_0\) is the electrode radius, and the other parameters have the same meaning as in equation (15). This important measuring characteristic makes microelectrodes very suitable for the analysis of dynamic processes and solute distribution at high spatial and temporal resolution. In addition, since the radius of the electrode, \(r\), is very small, the limiting current, \(i_t\), is also small and analysis in highly resistive solutions is possible since the \(rR\) drop is small. The formation of spherical, hemispherical and cylindrical diffusion layers enhances the mass transport of the analyte to the microelectrode leading to short response times and low dependence of the sensor signal on stirring rate of the sensor tip environment. A more detailed discussion of macro- versus microscale electrochemical sensors can be found in Chapters 8 and 9 of this book.

The different measuring characteristics of macro- and microelectrodes are illustrated in Figure 4. Cyclic voltammetry of a reversible redox system (here: hexacyanoferrate(III)/(II) in alkaline solution) at macroelectrodes shows a typical asymmetrical peak of the \(I/E\) curve due to a time-dependent diffusion layer thickness, whereas at a microelectrode spherical diffusion leads to a time-independent flux of analyte at the electrode surface, and thus a characteristic S-shape of the \(I/E\) curve.

Most sensor principles available for the determination of sulfide species in the environment are based on potentiometry (section 4.2), voltammetry (section 4.3), and amperometry (section 4.4). The available sensors respond either specifically to the sulfide ion or to H₂S, or to the whole of labile S(−II) species. Some attempts to develop HS⁻ specific sensors have been reported [138,139]. However, a reliable analytical method for HS⁻ is still to be developed. Also, reversible optical sensors for the determination of S(−II) species have not yet been developed [50-52,152].

### 4.1 OPTICAL AND BIOSENSORS FOR SULFIDE

Choi et al. [50] proposed an optical sensor based on the fluorescence quenching of fluorescein by mercury(II) acetate (FMA), which is reacting with sulfide. The measuring principle is reversible only in the presence of oxygen, which is used to regenerate the sulfide sensitivity of the sensor after analytical measurement. A similar behavior was found for the fluorescent dye thionine, which is also irreversibly quenched by sulfide in the absence of oxygen [51,52]. Evidently this imposes severe problems for the practical use of these sensor principles as S(−II) is unstable in the presence of oxygen. Therefore, the currently available
optical sensors do not allow for the continuous determination of sulfide under anaerobic conditions in natural waters. Cardoso et al. [152] presented a reversible sensor principle using FMA for the determination of atmospheric hydrogen sulfide, which cannot, however, be used in natural waters.

An interesting possibility for future sensor developments was proposed by Kurosawa et al. [141]. Their H₂S specific sensor is based on the oxidation of sulfide by Thiobacillus thioxidans (a colourless sulfur bacterium) with oxygen, which is monitored by a Clark-type oxygen sensor as an internal transducer in the biosensor. More details on various biosensors are presented in Chapter 6 in this book.

4.2 POTENTIOMETRIC SULFIDE SENSORS

Potentiometric methods are based on the measurement of equilibrium potentials without electrochemical consumption of the analyte and are, therefore, well suited for in situ determination. The most important tools used in potentiometry are ion-selective electrodes (ISE) consisting of a solid state or (semi-)liquid membrane and a suitable transducer [153]. The sensor membrane, which has to be electrically conductive and practically insoluble in the medium to be investigated, is the sensitive part of such electrodes. Although this type of electrode is referred to as ion selective, the analytical practice shows interferences by many other species. Problems arise when species are present that may complex one of the components of the membrane or incorporate into the solid membrane (e.g., Cl⁻, Br⁻, I⁻ in the case of Ag/Ag₂S membranes) [154]. A more general discussion of potentiometric techniques and ion-selective microsensors can be found in Chapter 5 of this book.

The Ag/Ag₂S electrode, which was originally used as a reference electrode, was first introduced as a potentiometric sulfide ISE by Berner in 1963 [114]. In 1983, Revsbech et al. [155] introduced an Ag/Ag₂S microelectrode for use in environmental analysis. More robust needle-type Ag/Ag₂S sensors have also been developed [117].

Silver sulfide is a semiconductor with a high ionic conductance similar to its electrical conductance, i.e., ~ 5.4 × 10⁻⁴ S cm⁻¹, with Ag⁺ as the mobile ion in an S²⁻ network [156]. The detection principle is based on the formation of equilibrium potentials in the electrochemical chain Ag/Ag₂S/S²⁻ described by the Nernst equation:

$$E = E^0 - \frac{RT}{2F} \ln \frac{a(S^{2-})}{a(S^{2-})_{std}}$$

(17)

where a(S²⁻) is the activity of the ion S²⁻. This implies a strong influence of temperature and ionic strength mainly due to a change in the activity coefficient of the sulfide ion. With the help of equation (5) the calibration curve for sulfide ion (E versus ln(a(S²⁻))) obtained with the ISE at a given pH and ionic strength can be converted to a calibration curve for total sulfide (E versus ln[S(S⁻II)]), which has a theoretical slope of −29 mV decade⁻¹ at 298 K. As the potentiometric Ag/Ag₂S electrode responds only to the sulfide ion, and not to HS⁻ or H₂S, the E versus ln[S(S⁻II)] calibration graph exhibits a parallel shift by 29 mV pH⁻¹ at pH 7–13, while at pH < 7 the shift amounts to 59 mV pH⁻¹. The slope of the E versus ln[S²⁻] calibration curve is theoretically independent of pH. The theoretical limit of detection can be calculated from the solubility product of Ag₂S[157]:

$$[Ag^+]^2[S^{2-}] = 10^{-51}$$

(18)

giving a value of [S²⁻] = 6.3 × 10⁻¹⁸ mol L⁻¹, which corresponds to 2 pmol L⁻¹ of [S(S⁻II)]ₐ at pH 8.5. In practice, the limit of detection is far higher (about 0.1 μmol L⁻¹ of [S(S⁻II)]ₐ) owing to the formation of mixed potentials, silver complexes, elemental silver and other factors leading to the so-called super-Nernstian behavior (see below).
There are several practical problems with the use of Ag/Ag₂S electrodes in complex environmental samples. Dissolved silver ions, heavy metals incorporated (as metal sulfides) in the membrane, halides (as silver halides), and pseudo-halides (cyanide) can build new electrochemical chains of the general form Ag/(Ag,Me)ₓ₊₁(S,Hal)/(Ag⁺,Me⁺',S²⁻,Hal⁻) and hence lead to unpredictable mixed potentials and irreproducible quantitative results [154,155].

In particular, the presence of Hg(II) in solution leads to an irreversible destruction of the Ag₂S membrane by formation of HgS precipitates at the membrane surface. Because of the lower solubility product of HgS as compared with Ag₂S and the unpredictable amount of the HgS formed, uncontrolled mixed potentials occur, which can result in large analytical errors. For that reason, Dobcnik et al. [116] proposed to coat the Ag₂S membrane with mercury sulfide in order to improve the sensitivity, the reproducibility and the response time. We tested this modification but were not able to obtain reliable quantitative results because of the non-linearity of the calibration graph [150]. This is consistent with the investigations of De Marco et al. [154]. Yu et al. [159] presented a modified sulfide microelectrode which was pretreated with HgCl₂ solution similarly to the procedure proposed by Dobcnik et al. [116]. The calibration of their microelectrode, as well as that of a commercial sulfide ISE used in their study, showed increasing slopes of the calibration curves with decreasing pH up to about 50 mV (pS²⁻)⁻¹ at pH 7.2. The authors interpret this behavior as a response of the sulfide ISE to HS⁻ and S²⁻, which is in contrast to the well-known theory for the Ag/Ag₂S electrode.

Another problem with the potentiometric sulfide ISE is the so-called super-Nernstian behavior. At low sulfide concentrations (< 1 µmol L⁻¹ [S(−II)], at pH 12.7), reducing conditions and high pH (e.g. by use of so-called sulfide antioxidant buffer, SAOB, with commercial macroelectrodes [115]) the slope of the E versus \text{ln}[S(−II)] calibration graph can show a significant deviation from -29 mV decade⁻¹ [119,160,161]. This is due to the reduction of Ag₂S under these conditions, which leads to the formation of elemental silver at the electrode surface [156,158].

Frevert and coworkers developed a so-called pH₂S sensor based on the Ag/Ag₂S electrode in conjunction with a pH glass electrode, thus avoiding a liquid junction reference [118-122,125]. Although, it was stated that the influence of ionic strength was reduced by 50% and that the sensor signal was pH independent at pH < 6, the basis of Frevert's sensor remains the Ag/Ag₂S electrode including all the above-mentioned problems and disadvantages.

In conclusion, Ag/Ag₂S-based sulfide sensors can be used for environmental analysis under near neutral to alkaline conditions in the water column as well as in sediments and biofilms with detection limits of ca. 1 µmol L⁻¹ for \text{[S(−II)]}. The construction of well-functioning Ag/Ag₂S-based sulfide sensors and calibration of such sensors is, however, complicated by the above-mentioned factors and this puts a limitation on the practical use of such sensors for in situ analysis. Nevertheless, such sensors have been used for laboratory [7,155,162] as well as in situ applications [117,163,164] in various aquatic systems (see section 5).

4.3 VOLTAMMETRIC SULFIDE SENSORS

Voltammetry is an electroanalytical method, which enables multi-species analysis by measuring oxidation or reduction currents of chemical species as function of the potential imposed to the electrode. The potential at which the electrode reaction occurs is primarily determined by the redox potential of the electron transfer, but it is additionally influenced by pH (when H₂O⁺ is involved in the redox reaction), complexation of the test species and its diffusion properties. A more detailed account of voltammetric techniques in environmental analysis in water and sediment is given by Buffle and Tercier-Waebel in Chapter 9 of this book. In the following, we only address some aspects relating to voltammetric determination of sulfide.

Various voltammetric techniques have been used with Hg electrodes to measure S(−II) in natural waters [110]. Recently, Brendel and Luther [165] used differential pulse polargraphy at an amalgamated gold electrode (tip diameter ~ 100 µm) for the direct measurement of S(−II) in sediment cores. First applications of this technique for the combined measurement of concentration gradients of sulfide, oxygen, I⁻, Fe(II), and Mn(II) in porewater of sediments have been reported [166,167]. Also, data from in situ profiling of sulfide and other chemical variables in sediments have been obtained by Reimers and Luther (cited in ref. [23]). These studies demonstrate that new information on the complex porewater chemistry of aquatic sediments can be obtained. However, voltammetry with bare electrodes in complex media can be problematic. In the following, we list a few concerns on the limitations of voltammetric techniques with respect to sulfide analysis and give some suggestions for improvement. Nevertheless, besides a need for technical optimizations the approach of performing in situ voltammetry with microelectrodes seems very promising and we regard the technique as having a large potential for the quantification of various redox species in natural systems, especially for fine scale analysis of iron and manganese species.

Electrodes with a diameter of ~ 100 µm or larger are not microelectrodes (see also Chapter 9) and, therefore, the well-known advantages of microelectrodes such as measurement in low conductivity freshwaters and independence of stirring rate will not be fully realized. Such relatively large electrodes exhibit currents in the nA range, related to a local analyte consumption that may disturb local gradients and equilibria around the sensor tip. Microsensors in µm size range have a much smaller consumption of analyte (typical measuring currents in the pA range) and are the only ones to which spherical diffusion occurs. The advantageous measuring characteristics of microelectrodes are
obtained only when the dimensions of the sensor tip, where the reactions take place, are smaller than the thickness of the diffusion layer at the electrode surface, leading to a spherical or hemispherical diffusion field. [150]

One of the main advantages of using mercury as an electrode is its high overpotential for the reduction of water to form hydrogen. When it is coated onto substrates of high solubility such as Au or Ag, however, this advantage is decreased. Glassy carbon electrodes do not suffer from this problem, but the deposition of mercury is not very reproducible on this substrate [168,169]. Buffle and coworkers [170,171] have shown that iridium is by far the best substrate for mercury because of its low solubility in mercury (< 10^{-6} wt%) and its good wettability by Hg.

Another aspect to consider is the measurement of sulfide by oxidation at metal electrodes with pulse techniques. Shimizu et al. [172] wrote in 1981: “It may be necessary to extend the frequently employed pulse techniques to an electrochemical system forming a deposit or film at a solid electrode. A variation of the thickness of the deposited film with time may cause a change in double-layer capacity, and thus capacitive currents may still remain at the current sampling time”. In addition, in all cases double peaks are observed at high sulfide concentrations (>10-100 μmol L^{-1}) when Hg electrodes were used with DPP [108,109]. Canterford attributed this phenomenon to the formation of dense HgS films. Davison and Gabbett [173] observed similar problems with DPP when sulfide concentrations in natural waters exceeded 2 μmol L^{-1}, but at lower concentrations good linear calibration curves were obtained. Normal pulse polarography, in conditions which prevent surface accumulation, can be recommended for higher concentrations, while the reliable determination of sulfide at a very low concentration level is possible by the use of cathodic stripping techniques [107,111,112] or a.c. voltammetry [113] provided the accumulation time is short enough so that a multilayer film is never formed. The important point is that at high concentration the electrode must always be placed at $E < E_{pc}$ (to avoid oxidation of Hg) except during the analysis, where a fast positive scan must be used followed immediately by a return of the electrode potential to $E < E_{pc}$ (The reaction is: $S(-II) + Hg -> HgS + 2e^-$. At low sulfide concentration, the electrode can be put at $E > E_p$ for a given time to accumulate HgS, but not too long in order to avoid a multilayer film formation, and then a negative scan is used (the reaction is: $HgS + 2e^- -> Hg + S(-II)$) (see Chapter 9, section 5 for more details).

Electrochemical measurements at an unprotected electrode under complex environmental conditions are problematic because of fouling of the electrode (see Chapter 9) by natural waters, colloidal and particulate forms of organic and inorganic matter that can be reduced, oxidized or simply adsorbed onto the electrode surface. This often interferes with the electrode reaction of interest and drastically perturbs the voltammetric peaks, which can result in large analytical errors, hard to quantify. This is especially true in sediments exhibiting complex porewater composition and high concentrations and gradients of both inorganic and organic compounds. In some cases electrochemical conditioning between scans can help alleviate such interferences but reproducibility is rarely good and the presence of memory effects should be carefully checked.

The problems associated with in situ voltammetry with bare electrodes may also partly apply to bare potentiometric electrodes. Thus, for in situ applications a protection of the electrode surface by a membrane, permeable to the test analyte, is usually necessary (a detailed discussion of voltammetry on bare and membrane-covered electrodes is given in Chapter 9).

4.4 AMPEROMETRIC HYDROGEN SULFIDE SENSORS

Most electrochemical gas sensors have a gas permeable membrane, through which the analyte can diffuse into an inner electrolyte compartment, where the electrochemical reactions take place under well-defined conditions. Consequently, gas sensors often exhibit much better measuring characteristics in terms of stability and, especially, selectivity as compared with potentiometric and voltammetric sensors. Gas sensors are well suited for in situ analysis and especially Clark-type oxygen microelectrodes have proven to be excellent tools for environmental analysis (see Chapter 1) [174, 175].

An amperometric detection principle for the determination of dissolved hydrogen sulfide in aquatic systems was developed by Jeroschewski and coworkers. [143-148] and several macrosensors for H$_2$S were realized. In collaboration with our group, a new H$_2$S microsensor based on this amperometric measuring principle was developed [6,149,150]. As the sensor principle is relatively new in comparison with potentiometric and voltammetric sulfide sensing, we describe the new H$_2$S microsensor in some more detail below.

The sensor design is based on the Clark principle, i.e. the sensor consists of electrodes in an electrolyte filling an inner compartment, which itself is separated from the analyte solution by a gas permeable membrane (silicone). All electrodes (working electrode, guard electrode, and counter-electrode; Figure 5A) are made of platinum and are placed in a glass casing made of a Pasteur pipette, that is tapered to a tip diameter of a few micrometers and sealed with a thin silicone membrane. The working and guard electrode have a tip diameter of a few micrometers and are prepared by electrochemical etching in concentrated KCN solution. For the sake of mechanical stabilization, electrical insulation towards the guard electrode and minimization of the active electrode surface, the working electrode is coated with a highly resistive glass except for the very tip of a few micrometers. The analyte, H$_2$S, diffuses through the gas permeable membrane of the casing and is electrochemically determined inside the sensor.

The direct oxidation of sulfide to elemental sulfur at platinum electrodes leads to an inactivation of the electrode surface [176,177]. Hence, the Clark
principle was improved by using a redox mediator (hexacyanoferrate(III)),
which oxidizes sulfide to sulfur before it reaches the charged Pt surface. This
reaction forms hexacyanoferrate(II) that diffuses to the Pt microanode, where it
is reoxidized to hexacyanoferrate(III) (Figure 5B). A more detailed reaction
scheme is as follows [149]:

Anode reactions:

\[
H_2S + 2[Fe(CN)_6]^{3-} \rightleftharpoons 2[Fe(CN)_6]^{4-} + S^{0} + 2H^+ \quad \text{(chemical step)} \quad (19)
\]

\[
2[Fe(CN)_6]^{4-} \rightleftharpoons 2[Fe(CN)_6]^{3-} + 2e^- \quad \text{(electrochemical step)} \quad (20)
\]

Cathode reaction:

\[
2[Fe(CN)_6]^{3-} + 2e^- \rightleftharpoons 2[Fe(CN)_6]^{4-} \quad \text{(electrochemical step)} \quad (21)
\]

Brutto reaction:

\[
H_2S + 2[Fe(CN)_6]^{3-} \rightleftharpoons 2[Fe(CN)_6]^{4-} + S^{0} + 2H^+ \quad (22)
\]

A potential difference, \(\Delta E_{\text{pol}}\) (Figure 6), between +80 and +150 mV is imposed
between the working or guard electrode and the counter-electrode. The guard
electrode serves to shield the measuring electrode from reduced components,
hexacyanoferrate(II) is so high that the potential of the counter-electrode is shifted to very negative potentials, the sensor lifetime ends and the inner solution must be changed. The latter is, however, seldom practicable with H₂S microsensors. This is only one of several parameters determining the lifetime of the microsensor, which is typically 2–8 weeks. More details of sensor characteristics are discussed elsewhere [6,149,150].

The H₂S microsensor exhibits a linear response from ~0.1 μmol L⁻¹ to >1–2 mmol L⁻¹ H₂S, with a sensitivity of 0.2–3 pA (μmol L⁻¹)⁻¹. The detection limit is ~0.1–1 μmol L⁻¹ H₂S, depending on the actual sensitivity of the sensor and the accuracy of the amperemeter, which is used in connection with the H₂S microsensor. We recommend the use of picomperemeter meters with a resolution of 0.1–1 pA, with the possibility for polarizing both measuring and guard electrode. Such meters are commercially available (e.g. from Unisense Aps, Denmark).

The small consumption of H₂S by the microsensor in the test water minimizes the corresponding concentration gradients and, therefore, the effect of external convection at the sensor tip. The sensor signal in stagnant water exhibits a decrease by <1–2% of the value measured in vigorously stirred water. In addition, there is no disturbance of sulfide equilibria at the microsensor tip. Therefore, the sensor signal, for a given total sulfide concentration, at various pH, can be predicted from the protonation equilibria of the sulfide system (equation (3)). The only significant interfering agent is SO₂, which is not important in most natural waters. As the electrolyte of the H₂S microsensor is photodegraded, we recommend shielding the inner electrolyte-filled compartment from bright light by painting the outer sensor casing with an optically dense paint with a good adhesion to glass, e.g. a black enamel paint containing xylene as the solvent.

5 SULFIDE AND H₂S MICROSENSOR APPLICATIONS IN AQUATIC SYSTEMS

Most studies of sulfide in the water column are still based on spectrophotometric methods (see Section 3.1) and relatively few measuring devices equipped with sulfide sensors have been used for profiling the pelagic environment. As a representative example, Eckert et al. [125] described a measuring device equipped with a potentiometric Ag/Ag₂S electrode, that was used to monitor the vertical sulfide distribution in the hypolimnion of a stratified lake.

Water sampling and subsequent sulfide analysis or monitoring with large sulfide sensors can in principle provide sufficient resolution for applications in the water column. The consumption of H₂S may, however, be high and stirring effects may therefore be important. Furthermore, with water sampling the risk of degassing or oxidation by O₂ during sampling is very high. In situ measurements are therefore important. It is even more so in the porewaters of sediments and biofilms, where sulfide cycling is of major importance, steep concentration profiles of sulfide and other redox species prevail and must be determined [5–7]. Nevertheless, porewater extraction from defined sediment strata followed by spectrophotometric (Section 3.1) or chromatographic (Section 3.2) analysis are still the most widely used methods for sulfide analysis of sediments in biogeochemistry, despite the inherent limitations in spatial and temporal resolution, and the necessity for sample destruction by this method.

During the last decade a slowly increasing number of studies have used microsensors for measuring sulfide species in benthic systems. Besides the advantages of microsensors over macrosensors in terms of e.g. signal stability, response time and low stirring sensitivity, they are also ideal tools for monitoring steep sulfide gradients owing to their negligible analyte consumption and small mechanical disturbance of the sediment or biofilm matrix. Hence relatively fast and repetitive measurements in practically undisturbed environmental samples become possible, from which detailed information about sulfide producing and consuming processes can be obtained. Below, we list some examples of such sulfide microsensor applications in natural biofilms and sediments, both in the laboratory and in situ (Figures 7 and 8).

In spite of the many practical problems with Ag/Ag₂S microsensors discussed in section 4.2, they have been used successfully in many different systems ranging from wastewater biofilms [7,159], sediments and cultures of microorganisms [117,162,179] to microbial mats growing under extreme environmental conditions in hot springs [180] and hypersaline lakes [155,181]. Ag/Ag₂S microsensors were also the first sulfide microsensors to be deployed on benthic lander instruments for autonomous in situ profiling of sediments and microbial mats near hydrothermal vents in the deep sea [23,164].

Voltammetric S(–II) microsensors (section 4.3) have been applied in several studies of marine sediment, both in the laboratory and in situ [23,166,167]. Although promising, these sensors still need improvement and further development (see Chapter 9). The most recently developed amperometric H₂S microsensor (section 4.4) has already found several laboratory applications in waste water, freshwater and marine biofilms and sediments [6,178,182,183], and the H₂S microsensor now seems to be used more frequently than the Ag/Ag₂S microsensor. First in situ applications were recently performed in coastal sediments and near shallow water hydrothermal vents [184,185]. The H₂S microsensor seems a favorable alternative to the Ag/Ag₂S microsensor in most applications where pH < 8.5, whereas for studies of more alkaline waters and sediments the Ag/Ag₂S microsensor is still the best choice. Above all, with the H₂S microsensor it is now possible to study sulfide distribution and dynamics at fine scale in acid environments [6]. The fast response time of this sensor has also allowed the first reliable estimates of anoxicogenic photosynthesis from measurements of sulfide dynamics around experimental light–dark shift events [186].
Figure 7. Examples of laboratory (A,B) and in situ (C) applications of Ag/AgCl microelectrodes. (A,B) Measurements of oxygen and sulfide in a biofilm and modeled reaction rates for sulfide production and consumption (redrawn from Kühl and Jørgensen [7] by permission of American Society for Microbiology). (C) Measurements of sulfide, oxygen, pH and temperature in a microbial mat subject to advective porewater transport; data were obtained in situ at a hydrothermal vent at 2 km water depth with a small measuring module controlled by the deep-sea research submersible Alvin. Reprinted with permission from Nature [164]. Copyright (1992) Macmillan Magazines Limited

Figure 8. Examples of laboratory (A,B) and in situ (C) applications of H₂S microsensors. (A) Oxygen and sulfide measurements in acid (pH < 5) lake sediment. (B) Oxygen and sulfide turnover rates modeled from the microprofiles. (C) In situ measurements of oxygen, sulfide, pH and temperature in a sediment near a shallow-water (7 m) hydrothermal vent (Milos, Greece). Redrawn from Kühl et al. [6] by permission of InterResearch, and from ref. [178]
6 SUMMARY AND DIRECTIONS FOR FUTURE RESEARCH

Several sensor techniques are available for direct sulfide analysis in the aquatic environment. Especially microsensors are well-suited for this purpose owing to their minimal consumption of the analyte, fast response times and their ability to measure with minimal invasion the distribution and dynamics of sulfide at high spatial and temporal resolution. Every analytical technique, however, has its advantages and drawbacks, which should be carefully considered for each application. Therefore, we see a need for a detailed experimental comparison of currently available sulfide microsensor techniques. Preferentially, such a comparison should involve measurements not only in defined sulfide solutions but also in defined gradients of sulfide and pH. The latter, would give a much better idea about how the various microsensors affect the analyte gradient itself, and, therefore, how applicable the various techniques are for fine scale measurements in environmental analysis.

Another complicating factor when working with in situ sulfide analysis is the instability of dilute sulfide solutions, which requires careful and strictly anaerobic calibration procedures under environmental conditions. For this, more detailed studies of sensor performance as a function of environmental variables such as temperature, salinity, and hydrostatic pressure are required. Besides the use of sulfide standards in traditional dosimetric calibration procedures, new devices may also simplify calibration of sulfide sensors [45].

At the moment, no reliable optical sulfide sensors are available for environmental analysis [20]. However, with the rapid development of optical sensor technology in recent years [19,187,188] optical sulfide sensors will probably become available. Also, advanced gel sampling techniques are becoming available for in situ measurements in complex natural systems (Chapter 9). Biosensors for sulfide species have already been developed [141], and with the recent development of new measuring and construction principles for microbiosensors by Revsbech and coworkers (Chapter 6), much better sulfide biosensors can now be realized.

Even if suitable and well-characterized sensors exist, it is a major undertaking to transfer the technology from the laboratory to the field in order to perform in situ environmental analysis. An adaptation of the sensors for special measuring platforms needs to be realized and first successful deployments of such measuring systems for in situ measurements of sulfide have been reported in the literature [92,144,156,159].

In conclusion, with the present array of sulfide sensors, detailed studies of the sulfur cycle can be performed in waters, biofilms and sediments. It must be emphasized, however, that the most suitable techniques have to be carefully chosen and optimized for each application. The largest gap in our ability to characterize the sulfide equilibria in nature is presently the lack of suitable sensors for measuring HS⁻, which is the most abundant sulfide species in most natural aquatic environments.

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