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# A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria

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**Summary**

Dissolved sulfide was determined spectrophotometrically as a colloidal solution of copper sulfide. Calibration curves were linear. Maximal deviation error was below 5%. Sulfide precipitated as FeS was determined after acidification of the medium.

Key words: *Ferrous sulfide - Spectrophotometric sulfide detection - Standard preparation - Sulfide calibration curve*

## Introduction

Sulfide in cultures of sulfidogenic bacteria is usually determined by the methylene blue reaction [1, 2]. This method permits quantitative analysis of traces of dissolved sulfide (e.g., in groundwater) down to concentrations of micromoles per liter. Since this reaction takes about 20 min, the presence of dissolved sulfide in cultures of sulfate-reducing bacteria (SRB) is proved rapidly by its colloidal precipitation as CuS in a copper sulfate reagent [3, 4]. After its modification, this qualitative proof can be quantified photometrically, as described in this paper.

## Materials and Methods

The copper reagent consisted of HCl (50 mmol/l) and $CuSO_4$ (5 mmol/l). The reaction was:

$$CuSO_4 + H_2S \rightarrow CuS + H_2SO_4$$

*Procedure*

All culture vessels were completely filled. If not, the pH of the culture was increased to pH 10 to reduce loss of volatile $H_2S$ that escaped into the gas phase. Culture fluid (0.05 ml) was removed by syringe or pipette from the culture vessel while 1.95 ml copper reagent was magnetically stirred (1000 r.p.m.) in a 2 ml, 1 cm measuring cuvette. The culture fluid was rapidly injected into the stirring reagent. Immediately after mixing for 5 s, the absorbance was measured at 480 nm in a Bausch & Lomb Spectronic 21 photometer. The mixture of 0.05 ml culture liquid and 1.95 ml HCl (50 mmol/l) served as blank.

The magnetically stirred cuvette could be replaced by a test tube posed on a whirl mixer (1000 r.p.m.). In this case, 4 ml reagent and 0.1 ml culture fluid were used.

*Sulfide standard preparation*

Distilled water (200 ml) was boiled to remove dissolved oxygen. The hot water was cooled under $N_2$ as the gas phase (Hungate technique [5, 6]) and the vessel was sealed with a rubber septum. Five ml of this anoxic water was dispensed by syringe into several 14 ml $N_2$ filled, septum sealed test tubes (Hungate tubes, Bellco Glass Inc., Vineland, NJ, USA).

A washed crystal of $Na_2S \cdot 9H_2O$ was dissolved in anoxic water to a known concentration (50–100 mM). Five ml of this concentrated sulfide solution was transferred by syringe into the first of the tubes containing 5 ml anoxic water. After shaking, 5 ml of this mixture were transferred anaerobically into the second tube. By this means, an anoxic dilution series of dissolved sulfide was prepared. Since solutions of $Na_2S$ are alkaline (pH 12), volatile $H_2S$ in the gas atmosphere was negligible.

*Precipitated sulfide*

In culture media containing dissolved $Fe^{2+}$, the produced sulfide was partly precipitated to the vessel bottom as FeS. Five percent of the FeS-free culture liquid was removed from the completely filled vessel by syringe through the rubber septum and was replaced by 4 M HCl. The tube was shaken until all FeS dissolved and total sulfide was determined as described above. Undissolved sulfide was calculated by subtraction of dissolved sulfide from total sulfide.

## Results and Discussion

Aliquots of sulfate-reducing bacterial cultures, cultivated without gas phase in the medium of Widdel and Pfennig [7], when added (1:4, v/v) to the acidic $CuSO_4$ solution [1, 2], caused a dark brownish precipitation of CuS. Smaller amounts (1:40, v/v) of aliquots added to the moving copper reagent resulted in a colloidal CuS solution which remained stable for 20–40 s. During this time, the absorbance of the end product was measured at 480 nm wavelength.

At 480 nm, the reagent had the same optical density as distilled water. HCl (50 mmol/l) added by the culture aliquot served as blank. Calibration curves were linear up to an absorbance of 0.5 (Fig. 1). Therefore a factor could be used to calculate the sulfide concentration from the measured absorbance. Maximal obtained deviation from

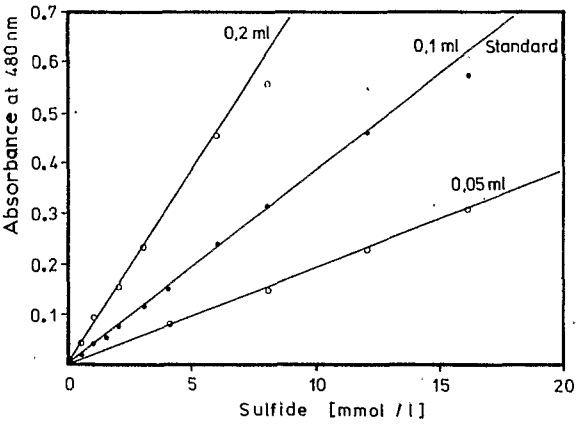


Fig.1. Calibration curves of sulfide determination. Sulfide standards were added to 4 ml moving copper reagent.

TABLE I

| | Methylene blue reaction | Copper sulfide reaction |
|---|---|---|
| Reaction time | 20 min | 0.5 min |
| Maximal error | below 5% | below 5% |
| Detection limit | 0.01 mM | 0.1 mM |
| Calibration curve | not linear | linear |
| Highly toxic residues | 100 ml *N,N*-dimethyl-1,4-phenylene-diamonium dichloride-containing solution | none |

the calculated straight regression line was less than 5%. Reproducible results were only obtained when the reagent and culture aliquot were mixed together while they were moving (magnetic stirrer or whirl mixer). Even intensive stirring of the reagent immediately after the culture liquid was added to the resting reagent, resulted in maximal deviation of about 15%.

Some culture media of SRB contain high amounts of ferrous iron salts (e.g., Postgate's medium [7]) which precipitate after bacterial growth as FeS; or dissolved iron is supplemented to trap hydrogen sulfide [8]. In the presence of elemental iron, a part of the sulfide produced also precipitates as FeS (corrosion experiments [9]). In these cases, only excess sulfide remains dissolved in the medium. After sedimentation of the FeS-flocks, the remaining dissolved sulfide was determined as above. After acidification of the culture liquid (see above), the FeS dissolved, and total produced sulfide could be determined. Compared to the methylene blue reaction [1, 2], the determination of dissolved sulfide in cultures of sulfate-reducing bacteria with the described method was more rapid with similar accuracy and no toxic products (Table 1).

No other anions usually present in bacterial cultures or biological environments precipitate with copper in acidic solution. Therefore this method may also be applied directly in natural habitats of sulfate-reducing bacteria (marine sediments).

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