Determination of Hydrogen Sulfide in Fermentation Broths Containing SO₂

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A procedure for the determination of hydrogen sulfide in fermentation broths containing up to 100 μg of SO₂ per ml is described. The method involves the sparging of H₂S from the broth into a cadmium hydroxide absorption solution, the formation of methylene blue from the absorbed sulfide, and the measuring of this color spectrophotometrically. The use of cadmium hydroxide instead of zinc acetate, the common absorbent, substantially reduced the interference of SO₂ with the analysis.

Hydrogen sulfide is occasionally produced by yeasts during wine fermentation in amounts sufficient to impair quality (9). It has been difficult to determine the cause of hydrogen sulfide formation by yeast in wine fermentation broths largely because there has been no sufficiently sensitive and quantitative method for determining H₂S in systems containing SO₂. Amounts of 50 to 100 μg of SO₂ per liter are commonly added to musts and wines to inhibit spoilage organisms and prevent oxidation. The extremely sensitive and quantitative procedures for H₂S, which use either N,N-dimethyl-p-phenylenediamine (3, 4), spectrophotometry (7), or fluorimetry (2), show severe SO₂ interferences. However, SO₂ does not interfere with the semi-quantitative and relatively insensitive procedures based on the formation of lead sulfide (9, 10).

This report describes the adaptation of a procedure originally developed for measuring H₂S in the atmosphere (6) to the determination of H₂S in wine and other fermentation broths.

MATERIALS AND METHODS

Sparging apparatus. A diagram of the sparging apparatus used to remove the hydrogen sulfide from various broths is shown in Fig. 1. The sample chamber was made from 65/40 ball and socket stock, sealed at one end and calibrated for 20-ml samples. The acid storage cylinder was a commercial cylindrical addition funnel, and the absorption cylinder was a 25-ml graduated cylinder. The impingers were made from 6-inch (15.2 cm) Pasteur pipettes cut to fit the absorption cylinder. All joints were greased and wired to prevent loss of H₂S. Two such apparatus were used so that duplicate samples could be examined simultaneously.

Cadmium hydroxide absorption solution. The solution was prepared by dissolving 4.3 g of 3 CdSO₄·8 H₂O (or 3.4 g of CdCl₂) in about 500 ml of distilled water. The cadmium hydroxide was then precipitated by adding approximately 50 ml of 0.3 M NaOH. The resulting suspension was diluted to 1 liter and shaken immediately before each use. Photooxidation of cadmium sulfide (1) was not a problem in this procedure due to the short sulfide absorption times used before color development (10 min).

Methylene blue color reagents. An amine stock solution was prepared by dissolving 12 g of N,N-dimethyl-p-phenylenediamine into a solution made by adding 50 ml of concentrated sulfuric acid to 30 ml of distilled water. This stock solution was stored at 5 C. The amine test solution was prepared by diluting 25 ml of amine stock solution to 1 liter with 9 M H₂SO₄. The ferric chloride solution was prepared by dissolving 100 g of FeCl₃·8H₂O in 100 ml of water.

Sample solutions. A solution of sulfur dioxide (100 μg/ml) was prepared from reagent Na₂SO₃ and 0.001 M ethylenediaminetetraacetic acid (EDTA) solution. The EDTA prevented the oxidation of SO₂ (5). A stock sulfide solution was prepared by dissolving 0.7 g of Na₂S·9H₂O in 1 liter of water and was then standardized with an iodine-thiosulfate titration. Test solutions were prepared from freshly made dilutions of the stock solution.

Sparging procedure. A 20-ml sample was poured down the inside wall of the sample chamber. Samples were not pipetted to prevent loss of H₂S due to degassing. Then, 20 ml of freshly suspended Cd(OH)₂ was added to an absorption cylinder, and a new impinger was attached to the sparging apparatus and placed in the absorption cylinder. Five milliliters of concentrated H₂SO₄ were introduced into the sample chamber from the storage cylinder, and the samples were sparged with helium at a flow rate of 0.5 liter/min for 10 min.

Color development. After sparging, the impinger was disconnected but left inside the absorption cylinder, and 0.6 ml of the amine reagent was pipetted directly into the bore of the impinger. The ab-
Determination of H₂S

The sparging apparatus used for the determination of H₂S is shown in Fig. 1. The apparatus consists of a Helium gas inlet (A), a sample chamber (B), an acid storage cylinder (C), an impinger (D), and an absorption cylinder (E). The gas inlet allows the introduction of Helium gas into the sample chamber, where it is passed through the sample. The acid storage cylinder contains a solution of Cd(OH)₂ and is used to absorb any excess SO₂. The impinger is used to capture the H₂S gas, and the absorption cylinder contains a reagent to react with the H₂S.

The recovery of H₂S from distilled water, sodium bisulfite solution, and wine is determined using this apparatus. Table 1 shows the recoveries of known amounts of sulfide added to distilled water. Throughout the range of 48 to 480 ng/ml, there was an average loss of 35 ng/ml. We were unable to find the source of this loss either in the apparatus or the procedure. However, in our analytical system, this loss was reproducible and constant and was corrected in analyses of samples containing H₂S. It is recommended that each investigator determine the loss for his particular analytical system. In the range of 500 to 5,000 ng/ml, the uncorrected recoveries of H₂S were 96 ± 9%.

There appeared to be two ways in which SO₂ affected the sulfide analysis. (i) SO₂ slowed the rate of methylene blue color formation, and (ii) SO₂ decreased the total amount of methylene blue which was formed. In the absence of SO₂, full color development occurred within 2 min after the addition of the reagents to the cadmium sulfide solution. However, total color development occurred only after 20 min when the samples contained 100 μg of SO₂ per ml (Fig. 2). Thus, allowing the samples to stand for 30 to 60 min after the addition of the reagents yielded the maximal methylene blue color when the samples contained approximately 100 μg of SO₂ per ml.

The original methylene blue procedure used for this analysis was modified so that (i) the sample was allowed to stand for 30 to 60 min after the addition of the reagents, and (ii) the blank was prepared by adding 0.6 ml of amine reagent to 20 ml of Cd(OH)₂ and diluting to 25 ml with water. Samples with optical densities greater than one were diluted with this blank.

RESULTS AND DISCUSSION

Experiments were carried out to determine the recovery of sulfide from distilled water, sodium bisulfite solution, and wine. Table 1 shows the recoveries of known amounts of sulfide added to distilled water. Throughout the range of 48 to 480 ng/ml, there was an average loss of 35 ng/ml. We were unable to find the source of this loss either in the apparatus or the procedure. However, in our analytical system, this loss was reproducible and constant and was corrected in analyses of samples containing H₂S. It is recommended that each investigator determine the loss for his particular analytical system. In the range of 500 to 5,000 ng/ml, the uncorrected recoveries of H₂S were 96 ± 9%.

Table 1. Recovery of sulfide added to distilled water

| Sample no. | Conc. of sulfide (ng/ml) | Actual recovery (%) | Calculated recovery (%) |
|------------|--------------------------|---------------------|-------------------------|
|            | Added  | Found | Loss |                    |                     |
| 1          | 480    | 450   | 30   | 93.8               | 101                 |
| 2          | 490    | 454   | 36   | 92.7               | 100                 |
| 3          | 464    | 400   | 64   | 86.2               | 94                  |
| 4          | 380    | 348   | 32   | 91.6               | 100                 |
| 5          | 324    | 300   | 24   | 92.6               | 87                  |
| 6          | 224    | 185   | 39   | 82.5               | 98                  |
| 7          | 122    | 90    | 32   | 74.0               | 102                 |
| 8          | 100    | 72    | 28   | 72.0               | 107                 |
| 9          | 80     | 46    | 34   | 57.5               | 101                 |
| 10         | 48     | 16    | 32   | 33.3               | 106                 |

* Average loss was 35 ng/ml and the average calculated recovery was 99.2 ± 6%.

* Sulfide found +35 ng/ml.
Table 2. Recovery of hydrogen sulfide added to a wine containing approximately 100 µg of SO₂ per ml

| Sample no. | Conc. of H₂S (ng/ml) | Recovery(%) |
|------------|---------------------|-------------|
|            | Initial             | Added       | Total       | Found      |
| 1          | 55                  | 96          | 151         | 149        | 99         |
| 2          | 55                  | 96          | 151         | 143        | 95         |
| 3          | 55                  | 180         | 235         | 210        | 89         |
| 4          | 55                  | 180         | 235         | 203        | 86         |
| 5          | 55                  | 300         | 355         | 321        | 90         |
| 6          | 55                  | 300         | 355         | 277        | 78         |
| 7          | 55                  | 460         | 515         | 431        | 84         |
| 8          | 55                  | 502         | 555         | 439        | 79         |

*Average recovery was 88 ± 2%.*

ml as compared to 83% recovery by using cadmium hydroxide.

Table 2 shows the recoveries of sulfide added at different levels to a Riesling wine which contained approximately 100 µg of SO₂. This young wine initially had approximately 55 ng of H₂S per ml. The average per cent recovery of H₂S added to this wine was 88 ± 2.

The use of cadmium hydroxide as an H₂S absorption solution greatly reduced the SO₂ interferences previously reported (9) for this type of analytical procedure. The application of this analysis to SO₂ containing fermentation broths permits studies on sulfide formation at concentrations near the odor threshold which is less than 0.1 ng/ml (8).

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