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DISULFUR MONOXIDE: PRODUCTION BY DESULFOVIBRIO

Warren P. Iverson

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DEPARTMENT OF THE ARMY
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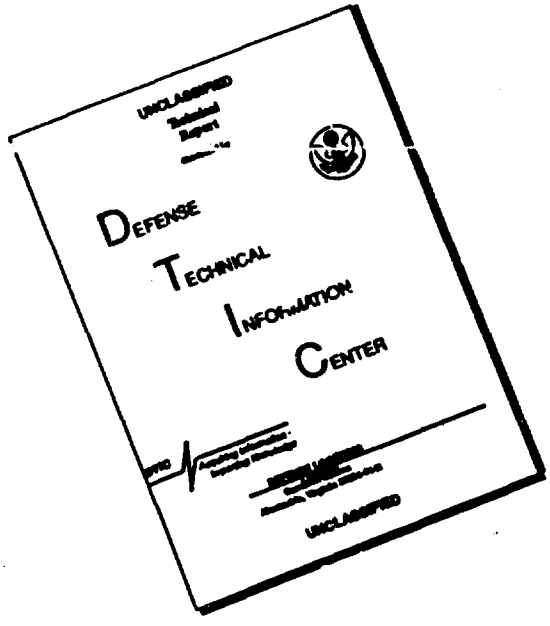

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TECHNICAL MANUSCRIPT 388

DISULFUR MONOXIDE: PRODUCTION BY DESULFOVIBRIO

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Special Operations Division
COMMODITY DEVELOPMENT AND ENGINEERING LABORATORY

Project 1C522301A061

May 1967

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DISULFUR MONOXIDE: PRODUCTION BY DESULFOVIBRIO

ABSTRACT

Desulfovibrio desulfuricans growing on agar surfaces produces a gas that appears to be identical to "Schenk's sulfur monoxide," later identified as disulfur monoxide. The gas stimulates surface growth of Desulfovibrio on an agar medium and is used by the cells as an electron donor for the reduction of benzyl viologen.

Previous investigations with Desulfovibrio desulfuricans (Mid-Continent strain A) had indicated the presence of a volatile substance or gas that was produced by this organism growing on plates of trypticase soy broth* plus 2% agar.[1] This gas was characterized by its ability to stimulate growth of the same organism on agar plates of API** medium[2] where growth was ordinarily very poor and also by its ability to be used by the organism as an electron donor for the reduction of benzyl viologen (BV). Violet (reduced) areas of the dye were produced in agar [2% agar plus 0.01 M tris(hydroxymethyl)aminomethane - HCl buffer, pH 7.0 ± 1, and 0.01% BV] around heavy concentrations of Desulfovibrio cells when petri plates of the agar were placed in contact with this gas in a helium or nitrogen atmosphere.

Attempts to identify this gas by gas chromatography were unsuccessful. When the volatile material was flushed with helium from a Brewer jar containing inoculated trypticase soy agar plates through a series of dry glass traps (cooled with NaCl-ice, methanol - dry ice, and liquid nitrogen successively), the gas, as indicated by the two biological properties described above, could be concentrated in the liquid nitrogen trap. An odor resembling that of H_2S was detected in the liquid nitrogen trap as well as from the outlet tube from this trap, which was immersed in water. The water produced blackening of lead acetate paper. The odor of H_2S could be detected, however, only infrequently from a Brewer jar containing inoculated plates of trypticase soy broth plus agar. A sample of the gas from the trap in liquid nitrogen was analyzed in the mass spectrometer but no sulfur compounds could be detected. Substances with mass peaks of 44, 28, and 16 corresponding to CO_2^+, N_2^+ or CO^+, and CH_4^+ were detected. CO_2 and CH_4 have previously been reported as metabolic products of Desulfovibrio.[3,4] Because the trap, cooled with liquid nitrogen, was evacuated to remove helium before analysis, the compound might have been removed. In an attempt to isolate the sulfide compound,

* Baltimore Biological Laboratories.

** American Petroleum Institute.

the biologically produced gas was passed (using helium as a carrier) through a series of three lead acetate traps (5% solution w/v) and then to the atmosphere by bubbling through water. No darkening in any of the three traps was noticed, but a strip of lead acetate paper placed in the water exposed to the air darkened in a minute or less. An odor resembling that of H_2S was also present. The gas appeared to form a sulfide only when in contact with oxygen.

A small amount of black precipitate was obtained in a week by placing a lead acetate solution (5% w/v) in a petri plate on top of 12 inoculated plates of trypticase soy broth plus 4 ml per liter sodium lactate plus agar in a Brewer jar with an atmosphere of hydrogen with traces of oxygen present. The precipitate was collected and the gas resulting from the addition of concentrated H_2SO_4 to the precipitate in nitrogen atmosphere stimulated growth on API agar (growth and blackening in 17 hours) and acted as a hydrogen donor for BV reduction.

Another sample of the precipitate was similarly obtained and the gas was analyzed in the mass spectrometer. Mass peaks corresponding to H_2S^-, SO_2^-, and SO^- were found.

H_2S had no stimulatory effect on growth and no hydrogen donor ability. SO_2 similarly had no effect in stimulating growth on API agar, but very slight reduction of BV by Desulfovibrio cells did occur. "Sulfur monoxide," now established as S_2O by Meschi and Myers,[5] was prepared according to the method of Schenk[6] by passing an electrical discharge (about 5 kv) through SO_2 (generated by adding concentrated H_2SO_4 to anhydrous reagent grade Na_2SO_3) in a straight glass tube (74 cm long; 2.5 cm I.D.) with platinum electrodes sealed at each end at low pressure (2 to 3 mm). The S_2O was flushed with helium into an evacuated Brewer jar with inoculated plates of API agar and BV agar. Sufficient S_2O was produced after repeating the procedure seven or eight times so that growth was visible on the API medium within 17 hours, as well as evidence of reduction of BV.

The infrared (IR) spectra of SO_2 before and after electrical discharge are indicated in Figure 1, A and B. The absorption peaks for SO_2 at 1,155 cm^{-1} and 1,125 cm^{-1} were replaced by a single peak at 1,135 cm^{-1}. Peaks similar to those in Figure 1 B were also obtained by passing an electrical discharge through SO_2 in S vapor, using the apparatus described by Jones.[7] The gas appears to be stable in a dry glass trap, as evidenced by the appearance of the 1,135 cm^{-1} peak, for at least 24 hours. This stability is in agreement with the finding of Cordes and Schenk.[8]

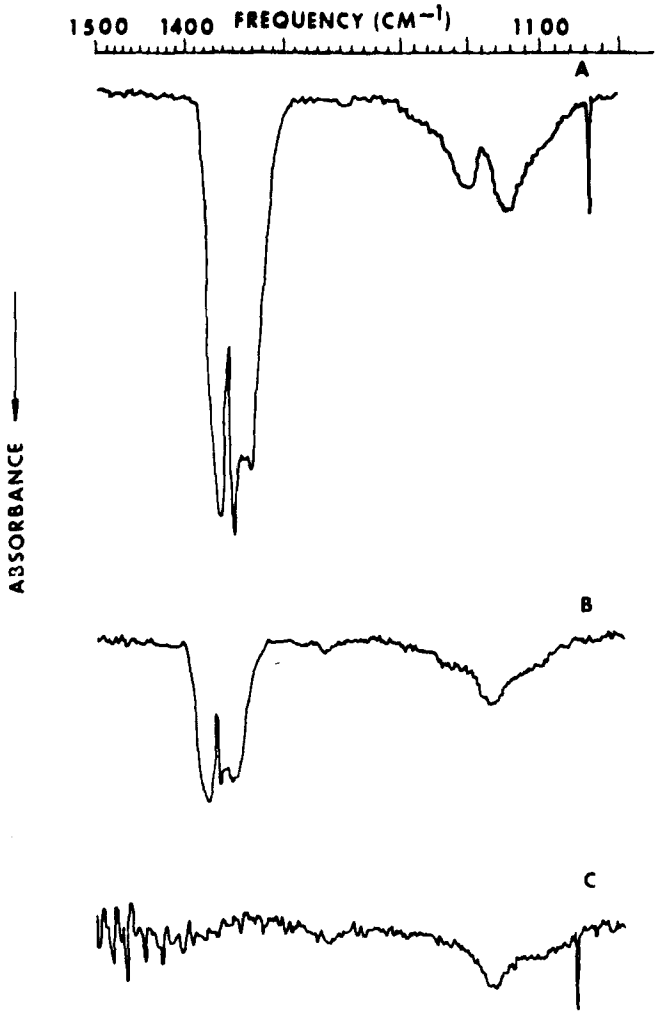

Figure 1. Infrared Absorption Bands. A. IR absorption bands of SO_2; path length 10 cm; NaCl windows. B. IR absorption bands of SO_2 after passage of electrical discharge; path length 10 cm; NaCl windows. C. IR absorption band of gas produced by Desulfovibrio collected in liquid N_2 trap; path length 10 cm; NaCl windows.

The IR spectrum of the gas produced by Desulfovibrio and collected in the liquid nitrogen-cooled trap is shown in Figure 1 C. A peak at 1,135 cm^{-1}, identical to the peak of the electrically discharged SO_2, is present. No bands for H_2S or SO_2 were found.

The IR spectra reported by Jones[7] for S_2O indicates bands at 1,165 cm^{-1} and 679 cm^{-1}. Blukis and Myers,[9] using frozen films, reported that the 679 cm^{-1} band disappears and the band at 1,165 cm^{-1} decreases in intensity and shifts to 1,300 cm^{-1} after heating the film to 280 K. On further examination it appears that the band found at 1,135 cm^{-1} and the one reported by Myers at 1,130 cm^{-1} are probably identical and are due to a decomposition product of S_2O, the band being caused by an S-O stretch.

Schenk[10] and Murthy[11] reported that $S_2O_3^{--}$, S^{--}, S were produced by "SO" or S_2O (because the properties previously attributed to the presence of SO in the discharge product are shown to be due to S_2O) when it reacted with alkaline solutions. It was of interest that (0.02%) $Na_2S_2O_3$ incorporated in the API agar also stimulated growth and acted as an electron donor for the reduction of BV.

The possibility is suggested that the organisms may be producing SO as an intermediate in the reduction of sulfate from trypticase soy broth (7 to 8 mg SO_4^{--} as the barium salt per gram dry medium):

$$\overset{6+}{SO_4^{--}} \longrightarrow \overset{4+}{SO_3^{--}} \longrightarrow \overset{2+}{SO} \longrightarrow \overset{0}{S} \longrightarrow \overset{2-}{S^{--}}$$

SO is quite unstable, decomposing within 3 seconds, and probably forms S_2O immediately according to the following reaction: $3\ SO \longrightarrow S_2O + SO_2$.[5] SO_2, however, was not detected in the traps during these observations.

A large number of extracellular, irregularly shaped particles was found among the cells of Desulfovibrio that were reducing BV on agar in the presence of the gas produced by growth of the organism on trypticase soy broth agar plates. Using the technique described by Skerman,[12] in which sulfur is extracted and recrystallized from pyridine, a large number of rhombic crystals were found among the cells after several hours, suggesting that the organisms might have been producing sulfur. It cannot be too strongly stressed that until the complete products of the reaction of the gas with the agar are known, it would be impossible to state whether sulfur is produced by the organisms or by a direct breakdown of S_2O with water.[11]

It was previously mentioned that the gas has an odor resembling that of H_2S in contact with air. Cordes and Schenk[13] also observed a similar odor from their "sulfur monoxide." Murthy[11] reported S^{--} as a reaction product with 2N NaOH.

It thus appears that the gas S_2O, or its decomposition product, is produced by Desulfovibrio growing on agar plates and that it may be an intermediate product in the reduction of sulfate.

LITERATURE CITED

1. Iverson, W.P. 1966. Growth of Desulfovibrio on the surface of agar media. Appl. Microbiol. 14:529-534.

2. Allred, R.C. 1958. Methods used for the counting of sulfate reducing bacteria and for the screening of bacteriocides. Prod. Mon. 22:32-34.

3. Postgate, J. 1959. Sulfate reduction by bacteria. Annu. Rev. Microbiol. 13:505-520.

4. Sisler, F.D.; Zobell, C.E. 1951. Hydrogen utilization by some marine sulfate-reducing bacteria. J. Bacteriol. 62:117-127.

5. Meschi, D.J.; Myers, R.J. 1956. Disulfur monoxide: I. Its identification as the major constituent in Schenk's "sulfur monoxide." J. Amer. Chem. Soc. 78:6220-6223.

6. Schenk, P.W. 1933. Uber das Schwefelmonoxyd: I. Mitteilung. Z. Anorg. Allg. Chem. 211:150-159.

7. Jones, A.V. 1950. Infra-red and ultraviolet spectra of sulphur monoxide. J. Chem. Phys. 18:1263-1268.

8. Cordes, H.; Schenk, P.W. 1933. On sulfur monoxide. Trans. Faraday Soc. 30:31-32.

9. Blukis, U.; Myers, R.J. 1965. Disulfur monoxide: III. Its infra-red spectrum and thermodynamic functions. J. Phys. Chem. 69:1154-1156.

10. Schenk, P.W. 1951. Über das Schwefelmonoxyd: XII. Reaktionen des Schwefelmonoxyds in Wasser. Z. Anorg. Allg. Chem. 265:169-179.

11. Murthy, A.R.V. 1952. Studies on the chemical behavior of some compounds of sulfur: I. Production of disulfur monoxide by the combustion of sulfur in oxygen at low pressure. Proc. Indian Acad. Sci. 36A:388-401.

12. Skerman, V.B.D. 1959. A guide to the identification of bacteria. The Williams & Wilkins Company, Baltimore, Md. 228 p.

13. Cordes, H.; Schenk, P.W. 1933. Über das Schwefelmonoxyd: II. Darstellung und Eigenschaften von Reinen Schwefelmonoxyd. Z. Anorg. Allg. Chem. 214:33-43.

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13. ABSTRACT

Desulfovibrio desulfuricans growing on agar surfaces produces a gas that appears to be identical to "Schenk's sulfur monoxide," later identified as disulfur monoxide. The gas stimulates surface growth of Desulfovibrio on an agar medium and is used by the cells as an electron donor for the reduction of benzyl viologen.

14. Key Words

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