HYDROGEN SULFIDE DETERMINATION IN BACTERIAL CULTURES AND IN CERTAIN CANNED FOODS

C. R. FELLERS, O. E. SHOSTROM AND E. D. CLARK

Laboratories of the Northwest Branch, National Canners Association, Seattle, Washington

Received for publication October 6, 1923

The determination of hydrogen sulfide in bacterial cultures has always been regarded as unsatisfactory. The recent Manual of Methods for pure culture study prepared by the Committee on Bacteriological Technic of the Society of American Bacteriologists (1923) gives a provisional method for observing hydrogen sulfide production only and states that certain weaknesses in the methods have been pointed out. Use is made of the lead acetate agar method as recommended by Burnet and Weissenbach (1915), Maymone (1917), Jordan and Victorson (1917), Kligler (1917) and Thompson (1921). Briefly the medium consists of a beef extract agar containing 30 grams peptone and 5 cc. of a 0.1 per cent basic lead acetate solution per liter (pH 7.2 to 7.6). An organism producing hydrogen sulfide when inoculated into this medium at a suitable temperature causes blackening of the medium. In general the more marked the intensity of blackening the greater the amount of hydrogen sulfide present. Obviously this procedure yields only qualitative results.

The only other attempt at even an approximate determination of hydrogen sulfide in bacterial cultures is the method used by Schardinger (1894), Dunham (1897), Redfield (1912), Myers (1920) and Tilley (1922). These investigators attempted to correlate hydrogen sulfide formation by water bacteria with the sanitary condition of the water.

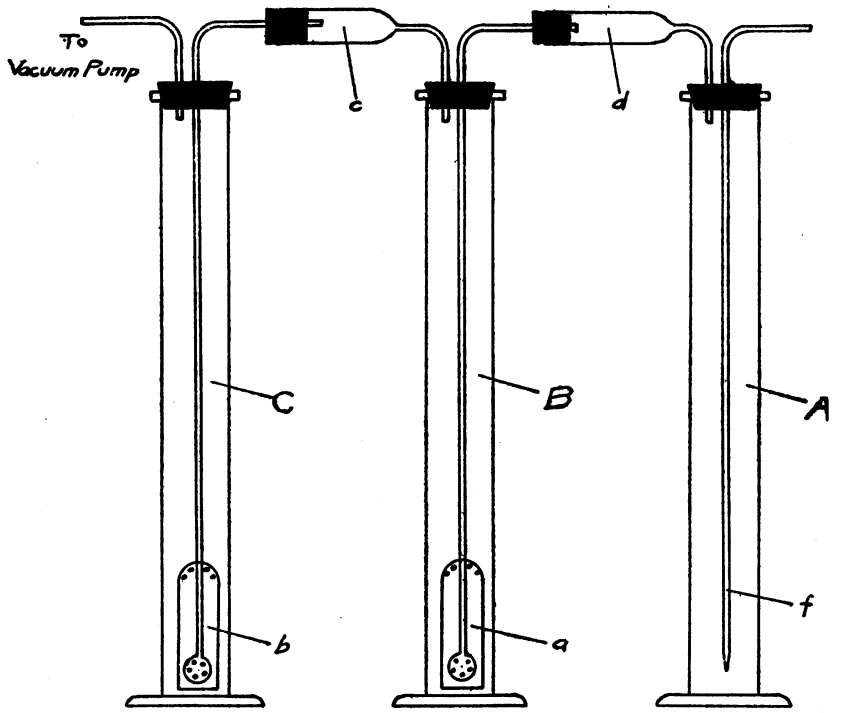
The technic of this method is as follows: A strip of filter paper moistened with a 10 per cent solution of neutral lead acetate is suspended in the mouth of a flask in such a manner that a given length is exposed to the action of any hydrogen sulfide formed. After incubation the approximate number of millimeters of lead acetate paper blackened is used as the basis of comparison. Obviously this method gives only approximate quantitative results.

In gas analysis however several quantitative methods have been used. Hydrogen sulfide may be quantitatively determined by Dupasquier's method, a measured quantity of gas being drawn through a solution of iodine to which some starch paste has been added. This method has also been used and improved upon by McBride and Weaver (1913) of the United States Bureau of Standards. They found that the volatility of the iodine introduced an error in this determination which could be obviated by absorbing the volatilized iodine in sodium thiosulfate. This improved method is somewhat like the one presented in this paper. Another method that has had considerable use is the passing of a measured quantity of gas through bromine water and determining the sulfur gravimetrically. This method is slow and the bromine is disagreeable to work with. There are other ways, such as the Fresenius gravimetric method, suggested for the determination of hydrogen sulfide in gas but these need not be mentioned here.

The advantages of a rapid volumetric method are obvious. Quantitative methods in bacteriology, particularly in pure culture studies, have been much neglected. Except as a qualitative test the lead acetate agar and paper strip methods must be considered inadequate and inaccurate. It is felt that any addition to quantitative methods along these lines is particularly welcome. With such a purpose in mind the method given in this paper for a rapid volumetric determination of hydrogen sulfide formed in bacterial cultures was developed. The problem of liberating the hydrogen sulfide from the solutions without splitting off any sulfur was the main difficulty to overcome. This was finally accomplished by an aeration

method. The liberated hydrogen sulfide was passed through standard iodine solution, the volatilized iodine collected in standard sodium thiosulfate solution, and finally the two solutions titrated with iodine or thiosulfate, depending upon which was present in excess.

Fig. I



APPARATUS

The apparatus employed is illustrated in figure 1; *A*, *B* and *C* are cylinders containing the sample, standard iodine and sodium thiosulfate solutions respectively; *a* and *b* are Folin absorption tubes; *c* and *d* are Folin drying tubes to prevent any spray from being carried over into cylinders *B* and *C*, or into the tube leading to the vacuum pump. A glass tube *f* with a small opening is used to cause considerable agitation

in the sample. Another cylinder may be used containing potassium hydroxide solution to absorb any hydrogen sulfide that might pass in along with the air from the laboratory. In our laboratory this was found to be unnecessary. It was also recognized that cork or rubber stoppers absorb small amounts of iodine, but the iodine solution was so weak and so little came in contact with the corks that no measurable error was introduced by the use of cork stoppers.

PROCEDURE

Introduce into cylinder *B* an excess of standard N/40 iodine solution. Then place sufficient N/40 sodium thiosulfate in cylinder *C* to absorb all of the iodine from the iodine solution during aeration (usually 10 or 15 cc. is ample). Add enough water to these two cylinders so that the level stands about 4 inches above the upper holes in the Folin absorption tubes. Into cylinder *A* introduce 100 cc. of the solution, culture, or suspension to be analyzed and acidify with phosphoric acid. An excess of phosphoric acid will do no harm. If the sample is known to contain a large amount of hydrogen sulfide an aliquot may be used, thereby eliminating the use of too great an amount of the iodine solution. Place connecting tubes in position at once, pass air through the apparatus slowly for two or three minutes, then more rapidly for at least fifteen minutes. The final rate and time of aeration depend upon the amount of frothing. By the addition of a drop or two of caprylic alcohol frothing can be prevented. Under most conditions fifteen minutes is sufficient to remove all of the hydrogen sulfide. The two solutions in *B* and *C* are then mixed, cylinders and bulbs being carefully washed to remove all of the reagents, and titrated with either iodine or sodium thiosulfate solutions, depending upon which is in excess, using starch as an indicator and making the necessary correction for the blank. The results are calculated as percentage of hydrogen sulfide, as milligrams per 100 cc. of sample, or as parts per million.

EXPERIMENTAL

Volatility and recovery of iodine by aeration

Since it was found, in preliminary experiments using only one cylinder, that a considerable error was caused by loss of iodine from the standard iodine solution, due to volatilization, a second cylinder containing standard sodium thiosulfate solution was used to absorb the iodine volatilized. By using this method of recovery a larger excess of standard iodine solution could be used.

To determine the amount of the error due to volatilization of iodine from its solution by aeration, known amounts of standard iodine and standard sodium thiosulfate were intro-

TABLE 1

Volatility and recovery of iodine by aeration

CYLINDER B	CYLINDER C						
N/40 iodine added	N/40 thiosulfate used to titrate excess iodine	N/40 sodium thiosulfate added	N/40 iodine used to titrate excess thiosulfate	N/40 iodine volatilized	N/40 iodine recovered	Iodine volatilized	Volatilized iodine recovered
(1)	(2)	(3)	(4)	(1)-(2)	(3)-(4)		
cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*
6.61	3.46	8.05	4.93	3.15	3.12	47.65	99.47
7.72	4.76	6.68	3.76	2.96	2.92	38.34	98.65
9.20	3.79	9.96	4.59	5.41	5.35	58.80	98.89

duced into cylinders *B* and *C* respectively, and air was drawn through the apparatus by means of a vacuum pump. After aerating for ten minutes the known solutions were titrated to determine the amount of iodine volatilized and recovered. The loss due to volatilization during aeration is large but inasmuch as the recovery is practically complete no appreciable error is introduced.

The data in table 1 represent only a few actual determinations taken at random, whereas a great number were made using varying amounts of iodine and thiosulfate as well as varying the time and speed of aeration. The results show that within a reasonable experimental error, the iodine recovery is complete.

Time required for the liberation of hydrogen sulfide from solutions by aeration

The length of time required for complete liberation of the hydrogen sulfide was determined by adding known amounts of a water solution of hydrogen sulfide, aerating for various periods of time, titrating the added iodine and sodium thiosulfate and also the hydrogen sulfide remaining in the solution. Table 2 gives one series of results out of the several made showing that fifteen minutes is a sufficient time for the liberation of the hydrogen sulfide.

TABLE 2

Time required for the liberation of hydrogen sulfide from solutions or cultures

TIME OF AERATION MINUTES	N/40 IODINE ADDED	N/40 $Na_2S_2O_3$ USED	IODINE N/40 USED BY THE H_2S	H_2S RECOVERED	H_2S ADDED	H_2S REMAINING AFTER AERATION	H_2S RECOVERED
	cc.	*cc.*	*cc.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
1	23.79	19.63	4.16	13.28	16.80	3.07	79.04
2	22.91	18.41	4.50	14.26	17.11	2.63	83.34
3	21.53	16.31	5.21	16.51	17.33	1.29	95.84
4	22.13	16.85	5.28	16.73	17.56	1.10	94.78
5	20.86	15.44	5.42	17.18	17.84	1.01	96.30
6	21.45	15.86	5.59	17.72	17.91	0.69	98.94
8	22.37	16.62	5.75	18.22	18.38	0.38	99.13
10	20.90	15.01	5.89	18.67	18.60	0.16	100.37
12	21.51	15.65	5.86	18.57	18.76	0.37	98.98
14	23.20	17.20	6.00	19.02	18.86	0.16	100.84
16	20.70	14.73	5.97	18.92	18.86	0.34	100.31
16	27.06	10.55	16.51	52.33	52.68	0.25	99.33
16	25.76	9.40	16.36	51.86	52.36	0.12	99.04
16	16.20	8.20	8.00	25.36	25.58	0.06	99.14
16	17.00	8.60	8.00	25.36	25.58	0.19	99.14

Effect of certain bacterial decomposition products on the liberation and recovery of hydrogen sulfide from solutions

Dunham's solution, together with known decomposition products, were mixed with hydrogen sulfide solution and aerated for fifteen minutes to determine if they would retard or interfere with the volatility of the hydrogen sulfide. In table 3 it will be seen that neither the media nor the known decomposition products have any noticeable effect on the liberation

or recovery of hydrogen sulfide, excepting large amounts of ammonia, and this of course is overcome by addition of acid. Large amounts of ammonia are however, seldom present in cultures or foods where this method is applicable.

TABLE 3

Effect of media and certain decomposition products upon the liberation and recovery of hydrogen sulfide from solutions

DECOMPOSITION PRODUCTS ADDED	MEDIA ADDED 2.5 PER CENT PEPTONE	N/40 IODINE ADDED	N/40 $Na_2S_2O_3$ USED	N/40 IODINE USED BY THE H_2S	H_2S RECOVERED	H_2S ADDED	IODINE RECOVERED	H_2S RECOVERED
	cc.	*cc.*	*cc.*	*cc.*	*mgm.*	*mgm.*	*per cent*	*per cent*
	50	15.49	15.49	0	0	0	100.00	
	50	11.54	11.51	0	0	0	99.74	
	50	11.48	11.44	0	0	0	99.65	
	50	12.83	12.81	0	0	0	99.79	
Formic*, acetic, butyric, propionic, valeric acids, phenol, indol, skatol, alcohol, NH_4OH and NH_4Cl	50	14.24	14.19	0	0	0	99.64	
	50	11.83	11.74	0	0	0	99.24	
	50	12.73	12.68	0	0	0	99.61	
	50	12.93	12.87	0	0	0	99.49	
	50	17.43	9.02	8.01	25.36	25.58		99.14
	50	25.92	9.65	15.87	50.30	50.72		99.17
	50	49.35	7.82	41.23	130.69	130.92		99.82
	50	30.90	9.16	21.70	68.78	60.07		99.37
2 cc. of 30 per cent NH_4OH	50	19.98	8.75	11.23	35.59	28.21		128.1
	50	17.32	6.69	10.63	33.69	28.21		119.4
	50	18.75	7.61	11.14	35.30	28.21		125.1

* One-fourth cubic centimeter each of the acids and NH_4OH, 0.5 cc. alcohol and 0.1 gram of each phenol, indol, NH_4Cl and skatol were used.

Effect of acids and alkalies on the liberation of hydrogen sulfide from solutions

Dunham's Solution containing known amounts of hydrogen sulfide was treated with potassium hydroxide, aerated and titrated to determine the amount of iodine used up by the hydrogen sulfide. The same sample was then treated with

sufficient phosphoric acid (H_3PO_4) to make it distinctly acid and again aerated and titrated. Tabular results given in table 4

TABLE 4

Effect of acids and alkalies on the liberation of hydrogen sulfide from solutions

KOH OR H_3PO_4 ADDED	MEDIA ADDED 50 CC.	N/40 IODINE ADDED	N/40 $NO_2S_2O_3$ USED BY THE H_2S	IODINE USED UP BY THE H_2S	H_2S RECOVERED	H_2S ADDED	H_2S RECOVERED
	Series 1						
		cc.	*cc.*	*cc.*	*mgm.*	*mgm.*	*per cent*
KOH	Dunham's solution with 25 grams peptone	11.82	10.75	1.07	3.39	26.74	12.65
H_3PO_4	Dunham's solution with 25 grams peptone	18.61	11.35	7.26	23.01	26.74	86.05
Total				8.33	26.40	26.74	98.7
	Series 2						
KOH	Dunham's solution with 25 grams peptone	11.17	10.45	0.72	2.28	26.54	8.59
H_3PO_4	Dunham's solution with 25 grams peptone	18.03	10.45	7.58	24.02	26.54	90.12
Total				8.30	26.30	26.54	98.71
	Series 3						
KOH	Dunham's solution with 25 grams peptone	10.64	10.11	0.53	1.68	28.21	5.95
H_3PO_4	Dunham's solution with 25 grams peptone	18.56	10.24	8.32	26.37	28.21	93.48
Total				8.85	28.05	28.21	99.42

Peptone solutions and canned salmon when treated with hot 10 per cent potassium hydroxide solution liberated volatile sufur, which could be recovered by acidifying the solution and aerating as described. Due to the slowness of this reaction and the gradual hydrolysis of proteins and intermediate products, no definite endpoint is reached and the hydrogen sulfide recovered after acidifying and aerating is in no sense an indication of decomposition.

show that potassium hydroxide combines with the hydrogen sulfide and is finally liberated by acid treatment showing

TABLE 5

Milligrams of hydrogen sulfide produced by various bacteria in 100 cc. of 2.5 per cent "Difco" peptone media

ORGANISM	TIME OF INCUBATION		
	Forty-eight hours	Ninety-six hours	One hundred forty-four hours
Bact. typhi (Hopkins)	0	0	3.54
Bact. paratyphosum A, strain I	0	0	0
Bact. paratyphosum A (Weinzirl)	0	0	0
Bact. paratyphosum B (Cascade)	5.34	5.84	8.48
Bact. paratyphosum B (Rowland)	6.42	5.30	5.56
Bact. paratyphosum B (Jordan)	6.30	6.08	7.20
Bact. enteritidis, strain I	2.70	3.45	4.66
Bact. enteritidis, strain II	2.86	7.40	7.90
Bact. dysenteriae (Flexner)	0	0	0
Bact. dysenteriae (Shiga)	0	0	0
Bact. suipestifer	0	0	0
Human feces	10.20	19.12	21.22
Bact. coli	14.06	12.18	13.74
Bact. cummunior	16.04	12.16	16.44
Bact. cloacae	0	0	1.32
Bact. aerogenes	0	0	0
Bact. lactis-viscosus	0	0	0
Bact. alkaligines	0	0	0
Bact. acidi-lactici	0	0	0
P. vulgaris	6.64	6.92	12.04
Bact. capsulatum	0	0	0
Msp. comma	0	0	0
B. mycoides	0	0	0
B. mesentericus	0	0	0
B. megatherium	0	0	0
B. thermoidifferens	0	0	0
B. centrosporus	0	0	0
Thermophile from canned corn	0	0	0
B. aerothermophilus	0	0	0
B. subtilis	0	0	0
B. albolactus	0	0	0
B. flexus	0	0	0
B. cohaerens	0	0	0
B. terminalis	0	0	0
B. vulgatus	0	0	0
B. circulans	0	0	0
B. cereus	0	0	0
B. mesentericus flavus	0	0	0
B. pseudo-tetanicus	0	0	0

TABLE 5—*Continued*

ORGANISM	TIME OF INCUBATION		
	Forty-eight hours	Ninety-six hours	One hundred forty-four hours
Skatol liberating clostridium (Fellers)	1.64	33.72	34.54
Ps. flurorescens	0	0	0
Bact. aurescens	0	0	0
Erw. carotovora	0	0	0
Thiothrix janiculatus	0	0	0
Erythrob. prodigiosus	0	0	0
Mic. albus	0	0	0
Sar. lactis-acidi	0	0	0
Sar. ventriculi	0	0	0
Sar. lutea	0	0	0
Staph. albus	0	0	0
Sacch. ellipsoideus	0	0	0
Asperigillus flavus	0	0	0
Penicillium sp?	0	0	0
Torula from clams	0	0	0

the necessity of an acid reaction. Due to the presence of free hydrogen sulfide in the cylinder above the solution, the potassium hydroxide did not combine with the total amount of hydrogen sulfide added. The small amount remaining unfixed by the alkali was absorbed by the iodine solution during aeration and accounts for the small per cent recovered before addition of the acid.

Quantitative determination of hydrogen sulfide produced by various bacteria, yeasts and molds in Dunham's solution containing 2.5 per cent "Difco" peptone

Various bacteria, yeasts and molds were inoculated into 300 cc. of Dunham's media using "Difco" peptone and incubated at 37°C. for one hundred and forty-four hours. Aliquots of 100 cc. were withdrawn at forty-eight hour intervals by means of sterile pipettes, transferred to the apparatus shown in figure 1, acidified, aerated and the amount of hydrogen sulfide liberated determined by titration as described under determination.

Witte's and Armours' peptone were also used but proved unsatisfactory because upon aerating media before inoculation a considerable reduction of the iodine took place. Sterilization of the media for fifteen minutes at 10 pounds pressure in an autoclave liberated hydrogen sulfide from Witte's and Armours' peptone but not from Difco.

Table 5 needs but little comment. All strains of *Bact. paratyphosum* B produced hydrogen sulfide, while none of the strains of *Bact. paratyphosum* A and only a few of the common

TABLE 6

Comparison of commercial peptones for hydrogen sulfide liberation by bacteria

	ARMOUR'S PEPTONE			WITTE'S PEPTONE			DIFCO PEPTONE		
	Forty-eight hours	Ninety-six hours	One hundred forty-four hours	Forty-eight hours	Ninety-six hours	One hundred forty-four hours	Forty-eight hours	Ninety-six hours	One hundred forty-four hours
Feces	16.86	29.46	47.54	32.12	5.70	4.75	10.20	19.12	21.22
Bact. cloacae	0	5.04	25.86	23.16	7.40	0.00	0	0	1.32
Bact. aerogenes	0	6.06	32.84	12.62	0.00	0.00	0	0	0
B. mycoides	0	0	33.32	11.68	8.64	0.00	0	0	0
Bact. enteritidis	45.56	Lost	40.56				2.70	3.45	4.66
Bact. coli	16.86	29.46	47.54	33.28	16.58	3.12	14.06	12.18	13.74
Bact. communior				10.00	0.00	0.00	16.04	12.16	16.44
Skatol liberating clostridium	25.08	19.08	14.0	32.7	29.28	22.58	1.64	33.72	34.54
Bact. typhosum	24.85	13.20	23.98				21.71	16.00	29.48

organisms were able to produce any. Yeasts and molds all gave negative amounts. A skatol liberating clostridium (Fellers) gave a very large amount of hydrogen sulfide. None of the 17 species of sporeforming aerobic bacteria produced hydrogen sulfide from Difco peptone. Likewise the non-proteolytic cocci and non-sporing rods did not possess this ability. The colon-typhoid and proteus groups together with the proteolytic anaerobes are the organisms capable of liberating hydrogen sulfide from "Difco" peptone. The use of this test in

studying the biochemical properties of microörganisms should aid materially in their identification and classification.

Both Armour's and Witte's peptone were used for purposes of comparison with Difco peptone but proved to be unsatisfactory. This was particularly true in the case of Witte's peptone. Both these brands when heated in 2.5 per cent concentration in an autoclave at 10 pounds pressure for ten minutes produced considerable hydrogen sulfide. Witte's peptone was more unstable in this respect than Armour's. Media containing either Armour's or Witte's peptone when sterilized, aerated and titrated with the standard iodine thiosulfate solution were found to neutralize considerable iodine, the amount varying greatly in several tests. A pink color was often present which interfered with the titration. Media containing these two brands of peptone and inoculated with bacteria gave discordant results in several tests.

As a result of this work the authors believe "Difco" peptone gives most reliable results, followed by Armour's. Witte's peptone is not suitable for quantitative hydrogen sulfide determination.

Quantitative determination of hydrogen sulfide in canned salmon and other canned foods

Salmon canned at various stages of decomposition, normal commercial canned salmon, swelled and normal cans of other food products, were treated the same as the bacterial cultures to determine their hydrogen sulfide content.

The method was found to be entirely applicable to the determination of hydrogen sulfide in various food products. Preliminary experiments showed satisfactory recoveries of hydrogen sulfide from salmon, gooseberries and shrimp, which had been artificially impregnated with known amounts of the gas. It was thought that some hydrogen sulfide might be formed during the decomposition of salmon or shrimp flesh but none was found either in the experimental or commercial samples examined. It is entirely probable however that where

certain hydrogen sulfide liberating bacteria are present in decomposed foods, hydrogen sulfide would be found. The authors believe that its presence in most cases indicates decomposition.

It is well known that certain acid fruits like gooseberries, cherries, apples and rhubarb when canned readily corrode the container, giving rise under favorable conditions to the forma-

TABLE 7

Hydrogen sulfide content of certain normal and abnormal canned foods

DESCRIPTION OF SAMPLE	ODOR	H_2S PER KILO OF SAMPLE
		mgm.
Red salmon one day old before canning.....	Normal	0
Red salmon two days old before canning....	Normal	0
Red salmon three days old before canning....	Stale	0
Red salmon four days old before canning.....	Slightly tainted	0
Red salmon five days old before canning....	Tainted	0
Red salmon six days old before canning.....	Putrid	0
Red salmon (normal).....	Normal	0
Chinook salmon (normal).....	Normal	0
Coho salmon (normal).....	Normal	0
Pink salmon (normal).....	Normal	0
Chum salmon (normal).....	Normal	0
Shrimp (swelled).....	Slightly tainted	0
Shrimp (normal).....	Normal	0
Gooseberries (swelled)	Hydrogen sulfide	366.0
Gooseberries (swelled).....	Hydrogen sulfide	90.0
Gooseberries (swelled).....	Hydrogen sulfide	81.0
Gooseberries (swelled).....	Hydrogen sulfide	61.0
Gooseberries (normal).....	Normal	0
Gooseberries (normal).....	Normal	0
Gooseberries (normal).....	Normal	0
Gooseberries (normal).....	Normal	0
Gooseberries (normal).....	Normal	0

tion of hydrogen gas. Where easily reducible sulfur compounds are present hydrogen sulfide may also be formed. This is undoubtedly what occurred in the canned gooseberries showing hydrogen sulfide reported in table 7. These samples had all been sprayed shortly before canning with a lime-sulfur mixture to prevent mildew. Normal gooseberries contained no volatile sulfur.

SUMMARY

The method as described is simple, rapid, easily executed, and reliable for the quantitative determination of the hydrogen sulfide formed in bacterial cultures and in canned foods containing free hydrogen sulfide. The hydrogen sulfide volatilized from acid solutions with air, is drawn through standard iodine; the volatilized iodine absorbed by passing through standard sodium thiosulfate; and the hydrogen sulfide determined by titration. Care must be exercised in washing the cylinders and bulbs after aeration and correction applied for starch indicator used. Standard apparatus is used throughout and no large quantities of reagents required. Fifteen minutes aeration of the culture or suspension being tested was sufficient to recover from 99 to 100 per cent of the hydrogen sulfide present.

Ordinary bacterial decomposition products such as volatile fatty acids, phenols, indol, skatol, alcohol and small quantities of ammonia did not affect the liberation of hydrogen sulfide from cultures or food suspensions. Twelve out of 53 microorganisms tested as to their hydrogen sulfide liberating properties in "Difco" peptone gave positive results. Armour's and Witte's peptone gave inconsistent results and in general proved unsatisfactory for this work. In general the non-proteolytic types of bacteria including the cocci, aerobic sporing rods, yeasts and inactive water and soil bacteria fail to liberate hydrogen sulfide from proteins. Certain members of the colon and proteus groups, *Bact. paratyphoid* B, *Bact. enteritidis* and a proteolytic anaerobe produced the greatest amounts of hydrogen sulfide.

The method may be used satisfactorily to determine volatile sulfur in foods. Canned gooseberries which had been sprayed while immature with lime-sulfur spray, contained large quantities of hydrogen sulfide. Under the conditions of the experiment no hydrogen sulfide was liberated from decomposed salmon or shrimp.

REFERENCES

BURNET AND WEISSENBACH 1915 Compt. rend. Soc. Biol., **78**, 565.
Committee on Bacteriological Technic, Soc. Amer. Bact., Methods of Pure Culture Study, 1923, p. A32.
DUNHAM 1897 Jour. Amer. Chem. Soc., **19**, 591.
JORDAN AND VICTORSON 1917 Jour. Inf. Dis., **21**, 554.
KLIGLER 1917 Amer. Jour. Pub. Health, **7**, 805.
MAYMONE 1917 Arch. Sci. Med., **4**, 202.
McBRIDE AND WEAVER 1913 Technologic Papers of the U. S. Bur. Standards, No. 20.
MYERS 1920 Jour. Bact., **5**, 231.
REDFIELD 1912 Hydrogen sulfide production, etc. Thesis for Ph.D. degree, Cornell.
SCHARDINGER 1894 Centralbl. Bakt., **16**, 853.
THOMPSON 1921 Jour. Med. Res., **42**, 383.
TILLEY 1922 Jour. Bact., **8**, 15.