STUDIES ON SULFOFICATION AND THE SULFOFYING BACTERIA

BY

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TABLE OF CONTENTS

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INTRODUCTION

The importance of sulfur as one of the elements indispensable for plant growth has been noted by numerous investigators. It has been definitely proven that plants assimilate sulfur in the form of sulfates and recent studies of the sulfur content of plants have shown that many crops use considerable quantities of the element for their growth.

The fact that many cultivated soils have shown a rather low total sulfur content has led to the use of sulfur as a fertilizing material especially in the arid regions and interest has been aroused in the effects of sulfur and sulfur compounds on various soil conditions.

The occurrence, numbers and activities of microorganisms in the soil and their relation to the fertility problem have, in recent years, been the subject of numerous investigations. Some very interesting results have been secured from the study of the various factors affecting biological activities in the soil. Various soil treatments have been found to exert a marked influence on the numbers of microorganisms, and on the ammonifying,

nitrifying, nitrogen fixing, cellulose decomposing, sulfofying and crop producing powers of the soil.

Many workers are now of the opinion that certain biological phenomena are definitely correlated with the crop producing power of the soil. Correlations between the ammonifying and nitrifying efficiencies of soil, the bacterial content and the crop yields have been established.

Although scientists have shown that the oxidation of sulfur to sulfate in the soil or sulfofication is brought about chiefly by biological agencies, comparatively little work has dealt with the bacterial activities involved. Therefore studies of the effect of various fertilizer treatments and of other factors which may influence bacterial activities in the production of greater amounts of sulfates in the soil are of importance. It is also of interest to determine whether or not there is any relation between the sulfofying power of soils and their ability to produce crops.

The work reported in the following pages was planned to throw some light on the sulfofication process and its occurrence under various soil conditions and to study the organisms involved in the process.

REVIEW OF LITERATURE

Studies on Sulfofication.

The fact that sulfur is transformed into sulfate in the soil has long been known (36). More recently extensive studies of sulfur oxidation, and its relation to the production of soluble phosphates have been carried on at the New Jersey Agricultural Experiment Station (33 and 38).

Simon and Schollenberger (49) and Stephenson (51) found that the rate of sulfur oxidation was dependent on the degree of fineness of the fertilizing material. This result agreed with the finding of Kappen and Quensell (27).

Martin (37) recognized that environmental conditions play an important rôle in the oxidation of sulfur in the soil. Lipman (29) emphasized temperature, and Brown and Kellogg (11) showed that soil composition, treatment, texture, moisture content, temperature and aeration are all important factors.

Brown and Gwinn (8) reported that the transformation of sulfur to sulfate was more readily accomplished in the presence of rock phosphate than in its absence. Additions of phosphorus and manure were shown

to have increased sulfofication.

Shedd (47) made a comparative study of sulfur oxidation in the soil and in sand. That each soil has a definite sulfofying power has been demonstrated by Brown and Kellogg (11).

Halverson and Bollen (19) found that the application of sulfur to soil tended to increase the sulfur oxidizing efficiency of the soil. These authors further showed that there was a correlation between the sulfofying power and the sulfate content of the soil.

Pfeiffer and Blanck (41) found that the increase in the sulfate content of the soil was proportional to the amount of sulfur added.

Ames and Richmond (2), Brown and Johnson (10), and Brioux and Guerbet (12) reported that calcium carbonate stimulated sulfur oxidation in the soil.

Neller (39), and Haynes (20) showed that sulfur was oxidized more rapidly under alkaline conditions and that a high concentration of salts did not prevent sulfur oxidation. This is in accord with the results obtained by Thomas (28), and Samuels (46).

Sulfofication in Relation to Bacterial Activity.

Investigations have shown that when sulfur is added to the soil it is transformed into sulfate mainly through the influence of bacterial acitivities.

Brown and Kellogg (11) who first made a thorough study of sulfofication in soils showed that the sulfofying power of the soil was primarily a bacterial action.

Joffe (25) stated that the oxidation of sulfur in the soil, while chiefly brought about by the activities of the microorganisms, may be due, in part, to chemical reactions. This has been pointed out by Kappen and Quensell (27). However, Boullanger (4), and Demolon (13 and 14) emphasized the significance of the biological factor. They have shown that in sterilized soils the sulfur applied was not converted into sulfate as efficiently as in unsterilized soils.

Lipman (29) and his associates reported that elemental sulfur is oxidized by certain bacteria. Several types of sulfur oxidizing bacteria have been isolated and studied and the process of sulfofication has been shown to occur in soils.

Shedd (48) found that sulfofication did not

proceed as rapidly in uninoculated soil as when the soil was inoculated with sulfofying organism.

Gubin (18) suggested that different groups of sulfur organisms act on different sulfides. Among the iron sulfides, marcasite, and ferrous sulfides are oxidized more rapidly than pyrite.

Several investigators have also found that the process of sulfofication has some relation to the nitrifying bacteria and to the nitrogen cycle in the soil.

Boullanger and Dugardin (5) attributed the favorable action of sulfur on the soil to the stimulus produced on bacteria which were known to decompose nitrogenous matter with the production of ammonia.

Ames and Richmond (2), and Fife (15) stated that small amounts of elemental sulfur increased ammonification but the process of nitrification was retarded.

Brown (6) found that sulfur oxidation affected the activities of the nitrifying organisms. Calcium carbonate when added to the soil frequently stimulated sulfofication, and it also prevented the injurious effects of sulfur oxidation upon the nitrifying bacteria.

Pitz (42) stated that sulfur when applied to a silt loam soil increased the acidity of the soil and this tended to decrease the number of bacteria after a

certain period. He further reported an increase in ammonification which was accompanied by a parallel decrease in nitrate formation.

Brioux and Guerbet (12) studied the transformation of sulfur in the soil, and the effect of carbohydrates, peptones and other nitrogenous material on the process of sulfur oxidation. They found that carbohydrates had a retarding effect while peptone and certain other nitrogenous substances accelerated the process, so that in 30 days 82 percent of the sulfur added was found to have been oxidized to sulfate.

Lipman and Joffe (30), and Brown (6) have shown that dextrose prevented the rapid oxidation of sulfur. The elimination of dextrose from the medium led to a more rapid oxidation.

Sulfofication in Relation to Soil Acidity.

The results of investigations have shown that the development of a strong acidity is caused by the continued application of sulfur, especially where large amounts are applied to soils (41, 42 and 44).

Hibbard (21), and Lipman and Joffe (33) claimed that the increase in the acidity of the soil was due to the oxidation of the sulfur and phosphorus in the soil.

Tottingham and Hart (52) reported a gradual increase in the acidity in a garden soil composted with sulfur and rock phosphate. The acidity of the soil was found to be correlated with the amount of sulfur added. Similar results have been secured by Rudolfs (45), and Simon and Schollenberger (49).

Lipman, McLean and Lint (33), Neller (40), and Joffe and McLean (26) showed that applications of 200 or 500 pounds of sulfur did not very materially change the hydrogen ion concentration through the season. Higher applications caused a decided increase in hydrogen ion concentration after the fourth to eighth weeks.

Gardner, Noll, and Baker (16) stated that crop failures at the Pennsylvania Agricultural Experiment Station Farm were due mainly to strong acidity which developed as a result of the continued application of ammonium sulfate.

Adams (1) applied sulfur to Miami silt loam soil at the rate of 1,500, 2,000 and 3,000 pounds per acre and incubated the composts for a period of 30 days. The hydrogen ion concentration was found to have increased from 4.75 to 3.62 and 3.16 respectively.

Reimer and Tartar (43) believed that sulfur should not be applied to soils deficient in lime as it causes acidity to develop.

EXPERIMENTAL

This study was carried out on a series of plots under the five-year rotation system on the Agronomy Farm of the Iowa Agricultural Experiment Station. The soil is a typical Wisconsin drift and the plots include soils of the Carrington series.

The treatments of the plots are as follows:

Plot No.	Treatment
912	Check
913	Manure
914	Manure + lime
915	Manure + lime + rock phosphate
916	Manure + lime + superphosphate
917	Check
918	Crop residue
919	Crop residue + lime
920	Crop residue + lime + rock phosphate
921	Crop residue + lime + superphosphate
922	Check

Methods of Treatments.

The various fertlizing materials are applied in amounts which are ordinarily used in practice. Manure is added at the rate of 10 tons per acre, or 2,000 pounds per plot.

Lime is applied at the rate of 750 pounds per plot, or 7,500 pounds per acre. The application is

made in the spring preceding seeding of the clover crop.

Rock phosphate is applied at the rate of 2,000 ponds per acre. The application is generally made in the fall and the phosphate is plowed under.

The superphosphate is applied at the rate of 200 pounds per acre annually. It is usually applied in the spring and disced in.

The first sampling was made on August 19, 1927 and it was planned to sample every two weeks during the fall, winter and spring months. However, because of the unfavorable weather conditions this plan was not accomplished. The dates of the samplings appear in Table 1.

Methods of Sampling.

The soil samples were taken by removing about two inches of the surface soil from an area about six inches square. The soil was thoroughly stirred to a depth of from four to seven inches and the samples taken. Twenty small samples were drawn in this manner from each of the plots and well mixed to form composite samples which were then placed in mason jars.

The samples were immediately taken to the laboratory and tests were made of the numbers of molds and bacteria, the moisture content, the reaction, and

the sulfofying power of the soil. The quinhydrone electrode method was employed in the determination of the soil reaction.

Determination of the Number of Bacteria in the Soil.

The number of bacteria present in the soil was determined by plate method, using Brown's modified albumen agar which has the following composition:

Dextrose	10.00 gms.
Di-potassium phosphate (K_2HPO_4)	.50 gm.
Magnesium sulfate ($MgSO_4.7H_2O$)	.20 "
Ferric sulfate ($Fe_2(SO_4)_3$)	Trace
Egg albumen	15.00 gms.
Distilled water	1,000.00 c.c.

The reaction was adjusted to pH 7.0. Ten c.c. portions of the medium were tubed and sterilized in the autoclave at fifteen pounds pressure for about twenty five minutes.

Before sampling the soil, a physiological salt solution was prepared by adding 12.5 grams of sodium chloride per liter of distilled water. Eleven one-liter Erlenmeyer flasks containing 500 c.c. and fifty five 250 c.c. flasks containing 90 c.c. of the salt solution were prepared and sterilized.

Fifty grams of soil were added to each flask containing the 500 c.c. of salt solution and the suspensions were shaken for five minutes. The coarse soil particles were allowed to settle and by means of sterile pipettes, employed for each transfer, the following dilutions were made:

(a) 10 c.c. of the infusion in 90 c.c. sterile salt solution; dilution equals 1:100.
(b) 10 c.c. of (a) in 90 c.c. sterile salt solution; dilution equals 1:1,000.
(c) 10 c.c. of (b) in 90 c.c. of sterile salt solution; dilution equals 1:10,000.
(d) 10 c.c. of (c) in 90 c.c. sterile salt solution; dilution equals 1:100,000.

After shaking thoroughly, one c.c. portions of dilution (d) were transferred to each of five sterile Petri dishes. The ten c.c. of Brown's modified albumen agar were poured into each of the five dishes. The plates were incubated at room temperature for a period of one week, after which time the bacterial colonies were counted. The numbers of colonies secured on five plates were averaged.

Determination of the Number of Molds in the Soil.

Waksman's synthetic acid agar was used in the determination of the number of molds. The medium was prepared as follows:

Glucose	10.00	gms.
Peptone	5.00	"
KH_2PO_4	1.00	"
$MgSO_4.7H_2O$	0.50	"
Agar	25.00	gms.
Distilled water	1,000.00	c.c.

The different ingredients were dissolved in the water, and the reaction was adjusted to a pH of 4.0 by the addition of normal sulfuric acid.

Dilution (e) i.e. 1:10,000 was used for the determination of the mold content of the soil.

The method of procedure was the same as in the case of bacteria. The incubation period was three to four days. The results of the determinations of the numbers of bacteria, molds, soil reaction and moisture content are given in Tables I, II, III and IV respectively.

Results of the Determinations of the Number of Bacteria.

The results obtained in the bacterial counts on each of the eleven soils at the various samplings are shown in Table 1.

Before attempting to discuss the results it would seem advisable to point out the fact that the plots may be grouped into two groups on the basis of soil treatments. Plots 913, 914, 915 and 916 constitute the manure group. These two groups of plots may also be differentiated on the basis of the texture of the soil. It is to be noted, according to the soil map, that the soil in the manured plots is lighter in texture than that in the crop residue group.

In order to present the results more clearly, Fig. II was prepared to show graphically the actual numbers of organisms present in the same soils at the different dates of sampling. It will be noted that the fluctuation in bacterial numbers in each of the soils is considerable. This is especially true with the manured soils in which the rise and fall in the numbers were more evident than with the soils in the crop residue plots. The check plots, however, show a more or less regular curve for the rise and fall in the numbers of bacteria.

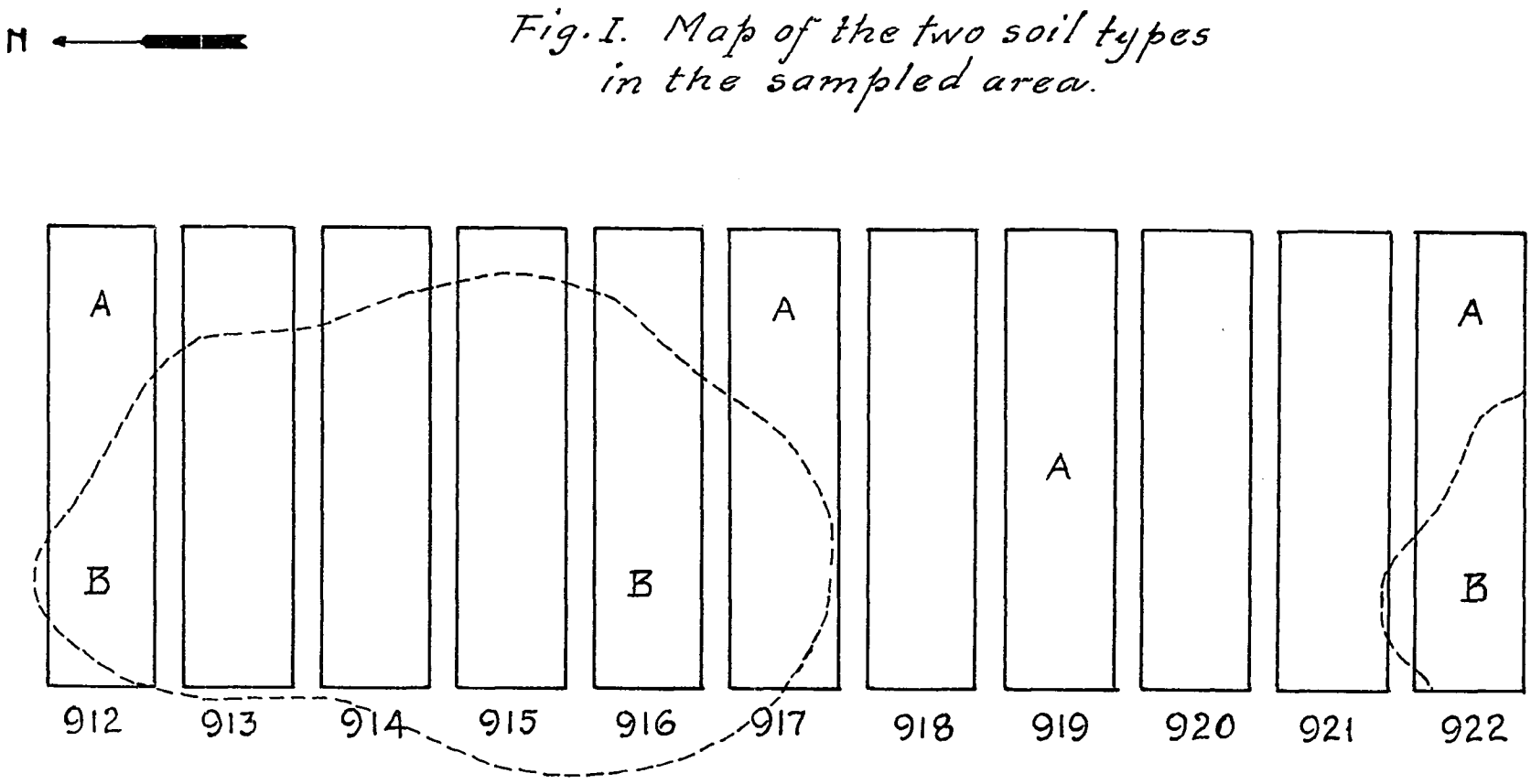


Fig. I. Map of the two soil types in the sampled area.

A= Carrington loam
B= Carrington fine sandy loam

TABLE I

Numbers of Bacteria Per Gram

Plot No.	Dates of sampling					
	Aug. 19 1927	Sept. 8 1927	Sept. 22 1927	Oct. 5 1927	Oct. 20 1927	Nov. 2 1927
912	2,285,000	2,830,000	3,170,000	2,985,000	1,780,000	2,275,0
913	4,730,000	6,410,000	3,405,000	5,270,000	2,220,000	3,290,0
914	2,610,000	3,629,000	2,825,000	2,935,000	1,305,000	4,070,0
915	4,930,000	6,550,000	2,955,000	4,560,000	1,760,000	3,720,0
916	5,420,000	6,640,000	4,670,000	5,960,000	2,875,000	4,140,0
917	3,375,000	3,890,000	2,730,000	3,410,000	1,572,000	1,885,0
918	4,240,000	4,760,000	2,585,000	3,750,000	1,910,000	2,660,0
919	3,495,000	4,340,000	4,356,000	3,710,000	1,669,000	3,330,0
920	4,140,000	5,060,000	3,856,000	3,840,000	2,089,000	2,890,0
921	4,460,000	5,450,000	4,160,000	5,390,000	1,632,000	3,025,0
922	2,875,000	3,080,000	3,445,000	3,980,000	1,650,000	2,275,0
Av.	3,960,000	4,774,460	3,468,820	4,162,730	1,860,190	3,050,9

TABLE I

Numbers of Bacteria Per Gram of Air-Dry Soil

Dates of sampling						Average
. 22 : 7	Oct. 5 : 1927	Oct. 20 : 1927	Nov. 28 : 1927	Dec. 23 : 1927	Jan. 7 : 1928	
,000	2,985,000	1,780,000	2,275,000	3,180,000	3,375,000	2,735,000
,000	5,270,000	2,220,000	3,290,000	2,760,000	5,900,000	4,248,000
,000	2,935,000	1,305,000	4,070,000	3,070,000	6,080,000	3,315,000
,000	4,560,000	1,760,000	3,720,000	3,590,000	6,890,000	4,378,400
,000	5,960,000	2,875,000	4,140,000	3,790,000	6,750,000	5,155,600
,000	3,410,000	1,572,000	1,885,000	3,540,000	3,730,000	3,016,000
,000	3,750,000	1,910,000	2,660,000	3,086,000	4,760,000	3,471,400
,000	3,710,000	1,669,000	3,330,000	3,979,000	4,050,000	3,616,200
,000	3,840,000	2,089,000	2,890,000	3,770,000	4,330,000	3,748,000
,000	5,390,000	1,632,000	3,025,000	3,299,000	4,680,000	4,012,000
,000	3,980,000	1,650,000	2,275,000	3,525,000	4,520,000	3,168,750
,820	4,162,730	1,860,190	3,050,990	3,326,270	5,005,910	

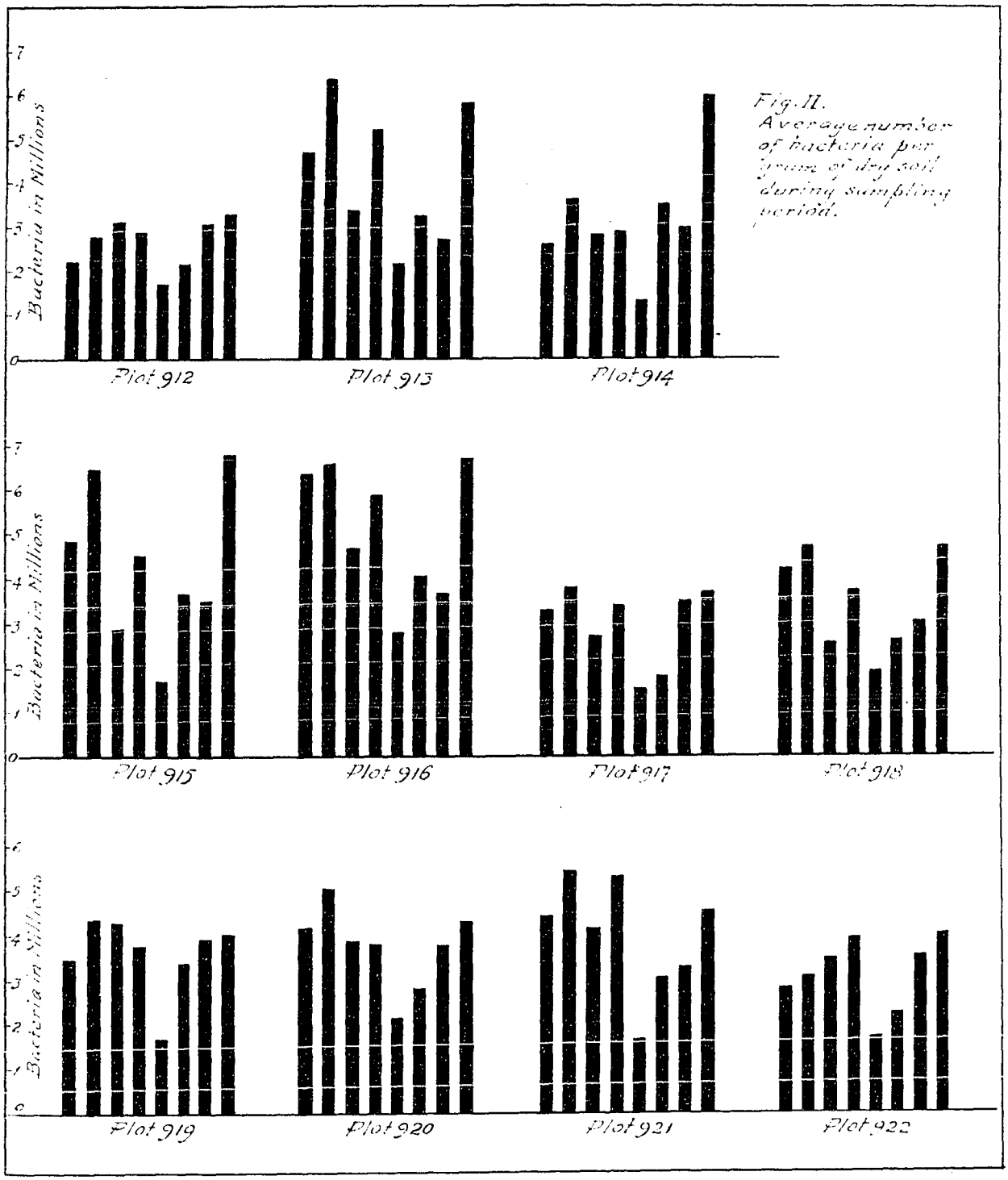


Fig. II.
Average number of bacteria per gram of dry soil during sampling period.

Considering the averages of the bacterial counts in all the soils at each sampling as shown in Table I, it may be of interest to note that the numbers varied considerably. For instance, on August 19, 1927 when the first sampling was made there were 3,960,045 bacteria per gram of soil. This was followed by an increase on September 8. At the third sampling there was a decrease followed by an increase at the fourth sampling. At the fifth sampling which was made on October 20, the bacterial numbers had dropped considerably. After that time the number gradually increased, and on January 7, 1928 when the last sampling was made there were 5,005,910 bacteria per gram of soil. This suggests that seasonal condition and temperature affect to a marked extent the number of bacteria in the soil.

The data also indicate that the bacterial numbers present in the soil varied with the soil treatments. The averages of the numbers in each of the eleven soils at the end of the experiment are shown in Fig. IV.

Comparing the bacterial counts in the soils in the manured plots, it will be noted that the lime, manure and superphosphate treated plot (916) showed the largest number of bacteria. Plot 915 which received applications of manure, lime and rock phosphate was second highest.

The soil in plot 913 which was treated with manure alone showed a greater number than the soil in plot 914 which received manure and lime. In this case, it would appear that the addition of lime to the soil in plot 914 did not seem to influence the bacterial development.

An examination of Table V shows that the addition of lime to the soil did not cause any large variation in the soil reaction as evidenced by the hydrogen ion concentration determination. It will also be noted that the soil in plot 913 which received manure alone showed an average pH of 6.06 while that receiving the manure and lime treatments (914) had an average pH of 6.03. The hydrogen ion concentration, therefore, although the differences were not very significant, may, with several other factors not studied, be considered to have influenced the variation in the bacterial counts in the soils of these plots.

Comparing the bacterial counts in each of the soils with relation to the treatments, it was found that the three check plots, 912, 917 and 922 showed lower numbers than any of the treated plots. The soils from plot 916 to which manure, lime and superphosphate were applied contained a greater number of bacteria than that from plot 921 which was treated with crop residues, lime

and superphosphate. Likewise, the soil in plot 915 which was treated with crop residues, lime and rock phosphate contained greater bacterial numbers than the soil in plot 920 which received an application of crop residues, lime and rock phosphate.

The soil from plot 914 treated with manure and lime showed a smaller number of bacteria than that from plot 919 which was treated with crop residues and lime. The difference, however, was not very significant. The soil in plot 913 with a treatment of manure alone was found to contain more bacteria than the soil in plot 918 which received an application of crop residues alone.

When the soils from the two groups of plots, the manure and the crop residue plots are compared it is noted that the former showed a greater number of bacteria than the latter. It appears therefore that the manure had a greater effect on bacterial numbers than the crop residues exerted. This finding is in accord with the results secured by Brown (7) and Greaves and Carter (17).

Numbers of Molds.

The results of the determination of the numbers of molds are presented in Table II. From Fig. III which presents graphically the averages of the mold counts secured from each of the soils at the different samplings, it appears that in most cases the numbers fluctuated without much regard to seasonal conditions. When the mold counts of each of the eleven soils at the conclusion of the tests are compared it is noted that the soil treatments did not seem to have a direct influence on the rise and fall of the numbers.

The check soil, plot 917, showed greater numbers than either of the soils from plot 914 and 920 which were treated with manure and lime, and crop residues, lime and rock phosphate respectively.

Comparing the mold contents with the bacterial counts obtained on each of the soils, there seems some relationship between the development of these two groups of organisms. In Fig. IV, it appears that there is a rather definite ratio between the numbers of molds and bacteria. For instance, the increase in the number of bacteria in each of the manured soils is accompanied by an increase in the mold content. In the crop residue treated soils, however, it is noted that as the numbers

TABLE II

Numbers of Molds Per Gram of Air-Dry

Plot No.	Dates of Samplin					
	Aug. 19 1927	Sept. 8 1927	Sept. 22 1927	Oct. 5 1927	Oct. 20 1927	Nov. 192
912	315,000	271,900	240,100	210,000	189,000	227,
913	499,000	376,000	361,900	285,900	335,000	306,
914	369,900	275,000	239,000	228,200	206,500	302,
915	350,000	331,800	428,000	344,500	384,900	431,
916	375,000	303,000	412,000	472,000	380,000	379,
917	348,500	279,000	395,500	286,000	281,200	341,
918	512,000	343,100	303,000	341,000	314,500	289,
919	470,000	303,500	290,100	292,000	289,000	264,
920	526,600	348,500	330,000	214,500	307,900	266,
921	435,000	379,000	348,500	284,000	315,000	267,
922	276,900	285,800	255,500	398,000	263,900	238,
Av.	388,900	363,290	325,600	314,200	296,980	303,

TABLE II

mbers of Molds Per Gram of Air-Dry Soil.

Dates of Sampling						
22	Oct. 5 1927	Oct. 20 1927	Nov. 28 1927	Dec. 23 1927	Jan. 7 1928	Average
0	210,000	189,000	227,500	273,000	349,000	259,500
0	285,900	335,000	306,100	253,000	385,000	350,273
0	228,200	206,500	302,500	239,000	374,500	279,325
0	344,500	384,800	431,000	247,000	371,900	380,837
0	472,000	380,000	379,000	263,500	270,000	356,850
0	286,000	281,200	341,000	274,200	410,000	326,925
0	341,000	314,500	289,000	240,000	405,000	343,450
0	292,000	289,000	264,000	261,000	345,000	313,075
0	214,500	307,900	266,000	251,800	351,000	299,537
0	284,000	315,000	267,000	261,500	316,000	350,750
0	398,000	263,900	238,500	238,500	309,000	283,262
0	314,200	296,980	303,055	254,770	373,310	

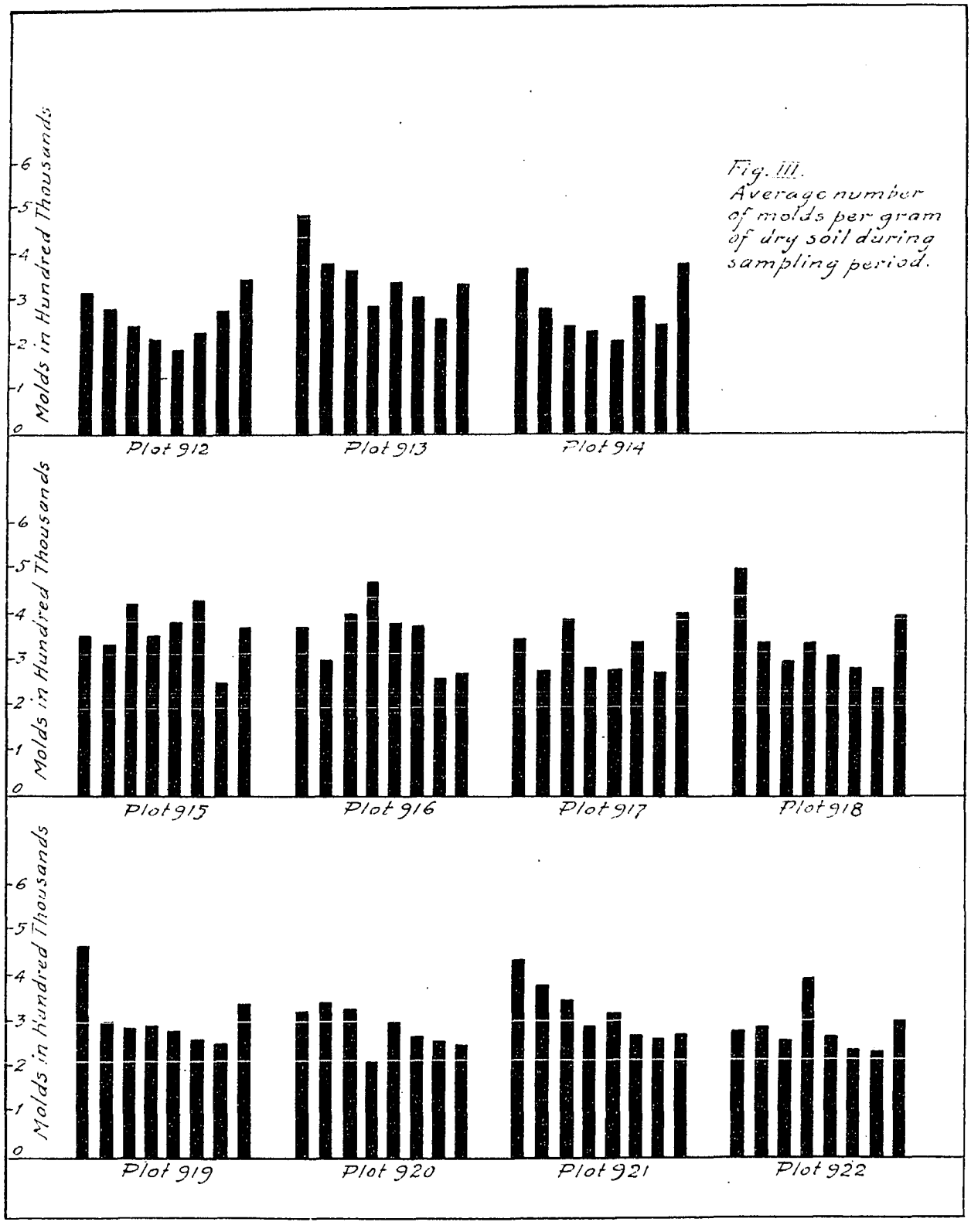


Fig. III.
Average number of molds per gram of dry soil during sampling period.

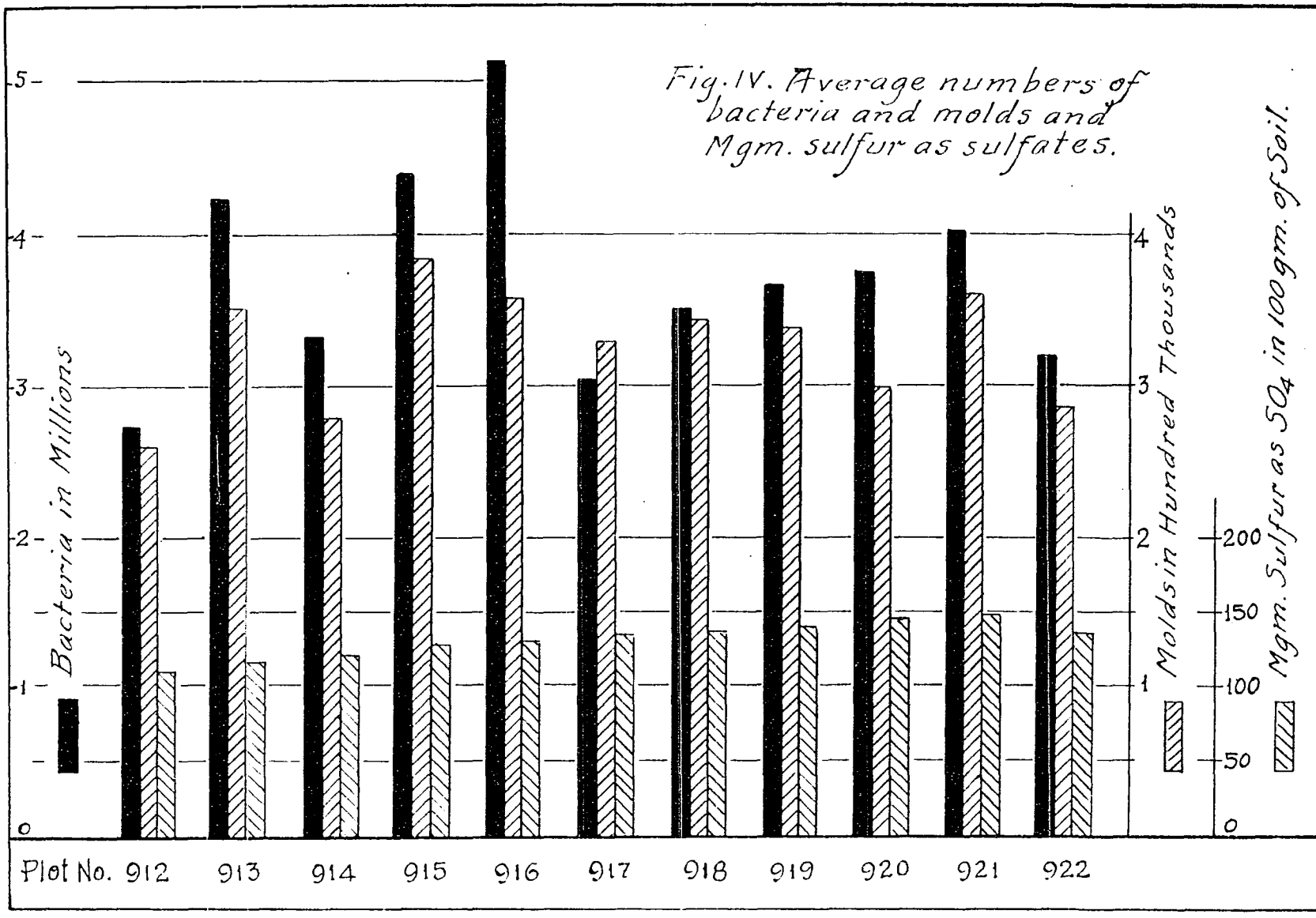


Fig. IV. Average numbers of bacteria and molds and Mgm. sulfur as sulfates.

of bacteria increased, the molds decrease. In the manured soils there was greater development of both groups of organisms than in the crop residue treated soils.

Moisture Determinations.

Table III shows the results obtained from the determinations of the moisture content of the soils of the various plots. The data indicate that there was no great difference in the amounts of moisture in the manured and the crop residue treated soils. The manured soils show a somewhat lower moisture content and this difference was probably due to the fact that the soils in the manured plots are lighter in texture than those in the crop residue plots. The addition of manure would be expected to increase the water holding capacity of the soils but did not bring about sufficient influence to offset the effect of the difference in texture.

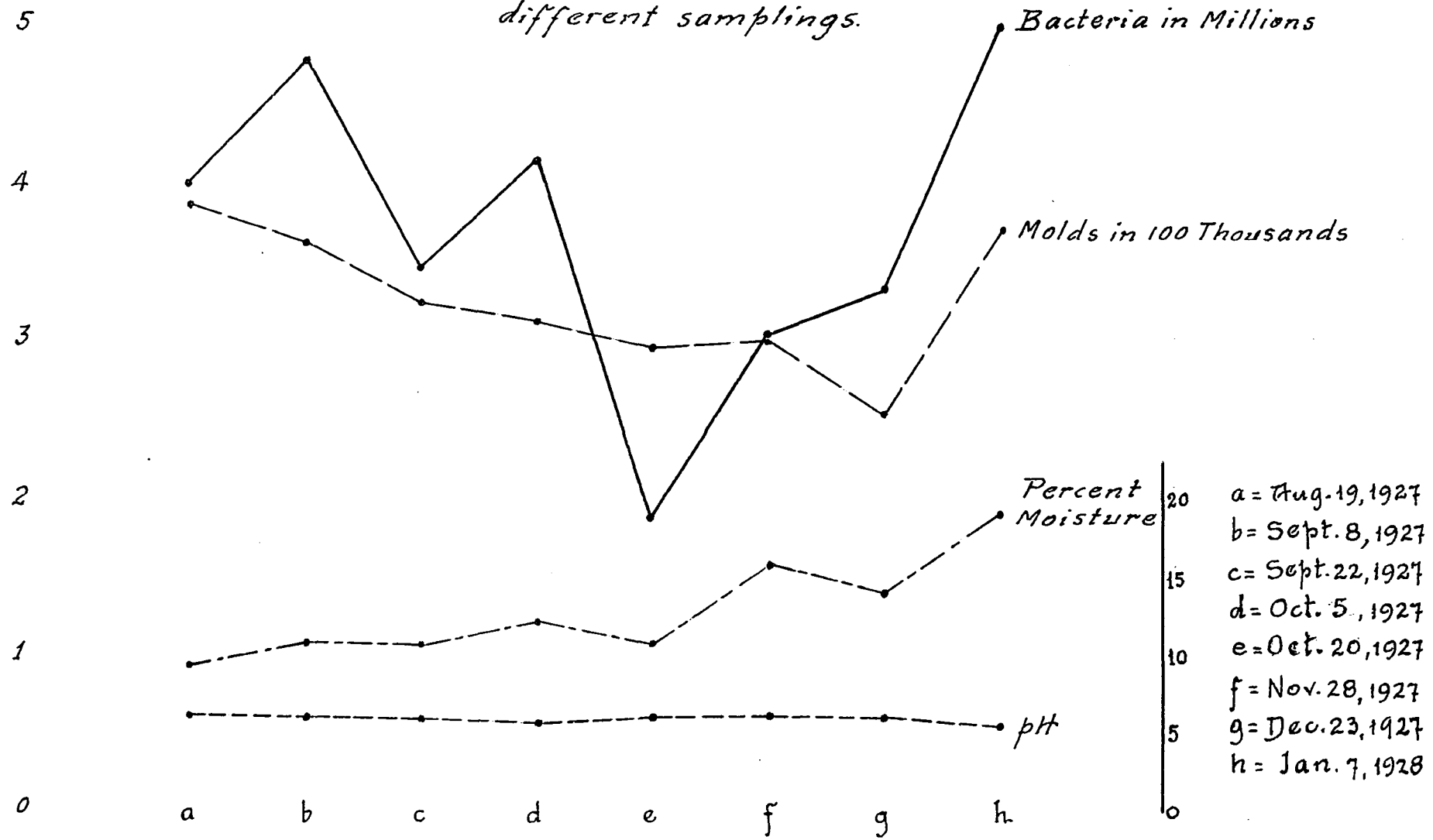
According to these results the moisture content of the soil influences the bacterial development to a large extent. Fig. V shows that the numbers of bacteria decreased with decrease in the moisture content of the soils in the various plots. The increase in the numbers of bacteria were also in most cases accompanied

TABLE III

Moisture Content of the Soils.
(Percent)

Plot No.	Dates of Sampling								
	Aug. 19: 1927	Sept. 8: 1927	Sept.22: 1927	Oct. 5 : 1927	Oct. 20: 1927	Nov. 28: 1927	Dec. 23: 1927	Jan. 7: 1928	Average
912	8.70	8.70	9.80	11.10	11.10	13.65	13.65	16.30	11.64
913	7.54	11.10	6.40	9.80	11.10	17.65	14.90	20.50	12.38
914	8.70	12.80	8.70	12.70	10.70	16.30	15.65	19.65	13.15
915	9.80	11.10	9.80	11.10	9.80	16.30	12.40	20.50	12.60
916	11.10	8.70	8.70	12.40	11.10	14.90	14.90	20.50	12.80
917	12.40	11.10	13.70	9.80	12.40	17.65	14.90	20.50	14.06
918	8.70	11.10	12.40	13.65	12.40	16.30	13.65	19.00	13.40
919	9.80	12.40	12.40	12.40	11.10	14.90	13.65	19.00	13.21
920	8.70	12.40	13.70	13.65	9.80	16.30	14.98	17.65	13.40
921	8.70	11.10	12.40	12.40	8.70	16.30	13.65	17.65	12.61
922	7.40	9.80	11.10	13.65	9.80	13.65	13.65	19.00	12.38
Av.	9.22	10.94	10.80	12.06	10.72	15.81	14.18	19.11	

Fig. V. Average numbers of bacteria and molds, moisture and reaction of all soils at different samplings.



by an increase in moisture content. The number of molds, on the other hand, fluctuated without regard to the moisture in the soil. In fact a gradual decrease in the mold counts occurred from the first to the fourth samplings while there was an increase in the moisture content.

Hydrogen Ion Concentration Determinations.

The results of the hydrogen ion determinations as measured by the quinhydrone electrode method are given in Table IV. The data show that there was no significant variation in the reaction of the soils in each of the eleven plots.

Comparing the average results on the different dates of samplings, it is noted that the seasonal effects were not very marked. All the manured soils, plots 913, 914, 915 and 916 showed an average pH of 6.09, as compared with 6.01 for the crop residue treated soils, plots 918, 919, 920 and 921 for the entire sampling period. It would appear therefore that the reaction of these soils was not definitely correlated with the development of bacteria and molds in the soils.

TABLE IV

Hydrogen Ion Concentration of the Soils
(pH)

Plot No.	Dates of Sampling								Average
	Aug. 19 1927	Sept. 8 1927	Sept. 22 1927	Oct. 5 1927	Oct. 20 1927	Nov. 28 1927	Dec. 23 1927	Jan. 7 1928	
912	6.20	5.90	6.00	5.90	6.00	5.90	5.90	5.80	5.95
913	6.00	6.20	6.00	6.00	6.00	6.20	6.10	6.00	6.06
914	6.00	6.20	6.00	6.00	6.10	6.00	6.00	5.90	6.03
915	5.90	6.20	5.90	6.10	6.20	6.10	6.10	6.00	6.06
916	6.20	6.40	6.20	6.20	6.00	6.20	6.30	6.10	6.20
917	6.00	6.20	6.20	6.00	6.00	6.20	6.20	6.00	6.10
918	6.30	6.00	6.00	6.00	5.90	6.00	6.00	5.80	6.00
919	6.20	6.20	6.20	5.90	5.90	6.00	6.00	5.80	6.03
920	6.00	5.90	6.20	6.80	6.10	5.90	5.80	6.00	5.96
921	6.30	6.00	6.00	6.00	6.00	6.00	5.90	6.00	6.03
922	6.30	6.00	5.90	6.00	6.00	5.90	6.00	5.90	6.00
Av.	6.13	6.11	6.06	5.91	6.02	6.04	6.03	5.57	

TABLE V

Average Results of Moisture, Reaction, Numbers of Microorganisms, and Sulfate Determinations at All Samplings.

Plot No.	Percent H_2O	pH	Numbers of microorganisms: Bacteria	Numbers of microorganisms: Molds	Mgm. sulfur as sulfates *: A	Mgm. sulfur as sulfates *: B
912	11.64	5.95	2,735,000	259,500	109.16	132.72
913	12.38	6.06	4,248,000	350,237	117.88	137.54
914	13.15	6.03	3,315,500	279,325	120,36	139.54
915	12.60	6.06	4,378,400	380,837	125.22	143.80
916	12.80	6.20	5,155,600	356,850	128.17	144.95
917	14.06	6.10	3,016,500	326,925	129.85	146.35
918	13.40	6.00	3,471,400	343,450	131.50	152.13
919	13.21	6.03	3,616,200	313,075	134.90	156.24
920	13.40	5.96	3,748,000	299,537	142.69	157.27
921	12.61	6.03	4,012,000	350,750	143.61	157.26
922	12.38	6.00	3,168,750	283,262	137.84	156.75

* A, 1 gm. of elemental sulfur used in the treatment.
B, 4 gms. of sodium thiosulfate used in the treatment.

Sulfofication Experiments.

The method used in the sulfofication experiments was as follows:

Six one-hundred gram portions of each of the soils under investigation were placed in tumblers. Two tumblers were used as checks. To each of two tumblers, one gram of elemental sulfur was added. In the last two tumblers the soils were each treated with four grams of sodium thiosulfate. The tumblers were covered with tin covers and incubated at room temperature for a period of six weeks at a moisture content of fifty percent saturation.

For the determination of the sulfates the 100 grams of soil which had been air-dried were transferred to an Erlenmeyer flask and water added to bring the total volume up to 500 c.c. The flask was fitted with a rubber stopper and shaken for 30 minutes. One gram of aluminum chloride was added and the flask was shaken for five minutes. The coarser particles were allowed to settle and the soil was filtered, the clear filtrate being collected in a 250 c.c. Erlenmeyer flask. From 1 to 10 c.c. of the filtrate from the treated soil were taken and the volume was made up to 100 c.c. In the case of

the check, 100 c.c. were used and about 0.05 gram of barium oxalate powder was added. The flask was shaken for one to two minutes, and allowed to stand for 15 minutes with occasional shaking. The amount of sulfates was determined by the use of a sulfur photometer.

Results of the Experiments on Sulfofication.

The average results presented in Table VI are expressed in milligrams of sulfur as sulfate per 100 grams of air-dry soil. The results in column A were secured with the elemental sulfur treatment and column B with the sodium thiosulfate application.

A study of the figures presented in the test shows that all the soils in the variously treated plots differed in their sulfofying efficiency. A greater sulfofying power was observed with the use of sodium thiosulfate than with the elemental sulfur. In the later discussion of the results, unless otherwise stated, only the figures given in column A are considered.

Comparing the amounts of sulfates which were produced in each of the eleven soils at the end of the sampling period, it is evident that the soil treatments exerted an influence on the sulfofying power of the soil. Considering only the manured plots, it will be noted that

TABLE

Sulfofication

Plot No.	Milligrams of sulfur as sulfate per 100								
	Aug. 19, 1927		Sept. 8, 1927		Sept. 22, 1927		Oct. 5, 1927		Oct.
	A*	B*	A	B	A	B	A	B	A
912	42.00	62.25	62.13	94.88	108.13	115.50	109.00	144.38	109.25
913	79.88	100.30	95.50	115.88	92.50	99.63	103.69	116.88	109.75
914	71.50	100.02	74.50	105.25	87.13	94.38	93.13	108.13	106.00
915	76.75	116.00	83.63	120.88	89.88	104.63	106.63	115.00	108.50
916	89.88	124.38	84.88	92.50	108.13	118.13	102.50	112.50	105.75
917	100.25	111.88	95.63	124.38	103.63	113.13	104.25	116.25	116.25
918	100.88	151.00	98.13	110.00	104.25	115.88	109.75	116.75	115.00
919	101.38	126.25	95.75	130.00	113.75	119.38	114.00	118.50	118.75
920	115.88	124.38	115.88	128.13	109.38	114.13	115.00	119.00	119.25
921	130.00	137.13	121.88	148.75	103.50	109.38	119.50	123.00	126.00
922	102.50	121.00	110.00	130.00	104.25	145.63	124.50	133.80	124.75
Av.	91.90	115.85	94.36	118.24	103.14	113.61	111.04	120.38	126.59

* A - Elemental sulfur treatment.
B - Sodium thiosulfate treatment.
** Results given are the average of duplicates.

TABLE VI

Sulfofication Experiments

s sulfate per 100 grams of soil from various plots **											
5, 1927	Oct. 20, 1927		Nov. 28, 1927		Dec. 23, 1927		Jan. 7, 1928		Average		
B	A	B	A	B	A	B	A	B	A		
144.38	109.25	126.00	130.50	155.00	143.13	167.38	169.13	196.25	109.16	13
116.88	109.75	115.00	137.75	138.75	150.63	187.25	173.38	226.63	117.88	13
108.13	106.00	143.00	146.75	155.13	182.75	201.38	201.13	209.00	120.36	13
115.00	108.50	119.25	159.88	178.00	191.38	204.88	185.13	191.75	125.22	14
112.50	105.75	126.25	161.50	168.50	189.00	207.75	188.13	209.63	128.72	14
116.25	116.25	118.75	137.63	154.88	184.25	203.13	196.88	228.38	129.85	14
116.75	115.00	119.50	138.00	160.63	184.25	218.75	201.75	225.50	131.50	15
118.50	118.75	124.25	156.13	177.38	196.25	226.88	183.25	227.25	134.90	15
119.00	119.25	132.18	154.25	169.75	201.75	233.75	210.12	236.88	142.69	15
123.00	126.00	140.25	132.00	157.38	207.38	216.90	208.88	225.38	143.61	15
133.80	124.75	133.50	131.50	142.00	206.88	220.88	214.38	227.25	137.84	15
120.38	126.69	137.27	144.17	159.76	185.24	208.06	193.83	217.53		

TABLE VI

ation Experiments

er 100 grams of soil from various plots **									
Oct. 20, 1927		Nov. 28, 1927		Dec. 23, 1927		Jan. 7, 1928		Average	
A	B	A	B	A	B	A	B	A	B
109.25	126.00	130.50	155.00	143.13	167.38	169.13	196.25	109.16	132.72
109.75	115.00	137.75	138.75	150.63	187.25	173.38	226.63	117.88	137.54
106.00	143.00	146.75	155.13	182.75	201.38	201.13	209.00	120.36	139.54
108.50	119.25	159.88	178.00	191.38	204.88	185.13	191.75	125.22	143.80
105.75	126.25	161.50	168.50	189.00	207.75	188.13	209.63	128.72	144.95
116.25	118.75	137.63	154.88	184.25	203.13	196.88	228.38	129.85	146.35
115.00	119.50	138.00	160.63	184.25	218.75	201.75	225.50	131.50	152.13
118.75	124.25	156.13	177.38	196.25	226.88	183.25	227.25	134.90	156.24
119.25	132.18	154.25	169.75	201.75	233.75	210.12	236.88	142.69	157.27
126.00	140.25	132.00	157.38	207.38	216.90	208.88	225.38	143.61	157.26
124.75	133.50	131.50	142.00	206.88	220.88	214.38	227.25	137.84	156.75
126.59	137.27	144.17	159.76	185.24	208.06	193.83	217.53		

the soil from the manure, lime and superphosphate treated plot (916) was capable of producing the greatest amount of sulfate. Plot 915 treated with manure, lime and rock phosphate, showed a greater sulfofying power than the soil from the manure and lime treated plot (914). With the manure application alone, plot 913, the sulfofying power was the lowest. This relation between the soil treatments and the sulfur oxidizing power of the soils in the manured plots was found to correspond with that found for the soils from the crop residue treated plots.

A further consideration of these figures in comparison with those in Table 1, shows that there is a correlation between the sulfofying power of the soil and bacterial numbers present. For instance, all the soils in each of the two groups of plots, with the exception of that in plot 914, showed a relation between bacterial numbers and the sulfate produced.

Comparing the two groups of plots, it will be noted that in general the crop residue treated soils produced more sulfate than the manured soils regardless of the larger bacterial numbers in the latter. The relative increases in the amounts of sulfates produced in the soil from the crop residue plots did not correspond with an increase in the numbers of bacteria.

This being the case, it would seem that the textural difference in the soils may possibly have played an important part in stimulating the sulfur oxidizing action of the organisms in the soil.

Considering the relation of mold content to sulfate production in the soil, it is apparent that the numbers of molds did not run parallel with the amount of sulfates produced. In fact the number of molds in the soil fluctuated without regard to sulfate production.

Perhaps one of the most interesting things which may be noted from the results is the relation of mold and bacterial development in the soil to sulfate production. In Fig. IV, the results secured from the manured plots showed that as the relation between the amount of sulfate produced and the number of molds increased, the relative number of bacteria in proportion to the number of molds also increased. In the crop residue treated soils, however, this relationship did not appear.

The Influence of Calcium Carbonate and Dextrose on Sulfofication.

This experiment was undertaken in order to determine the effect of calcium carbonate and dextrose on the sulfofying power of the soil.

The soil used in this experiment was secured from the manure, lime and superphosphate treated plot (916). The soil was air-dried, sieved and treated with the materials as indicated in Table VII. At the end of the incubation period, the amount of sulfur as sulfate was determined using the photometric method. The results, calculated in milligrams of sulfur as sulfate in 100 grams of air-dry soil, are given in Table VII.

Results.

From the average figures of the duplicate treatments presented in Table VII, it may be noted that there were significant variations in the amount of sulfate produced.

The tests using sodium thiosulfate showed that when dextrose was applied 115.25 mgm. of sulfur as sulfate were produced, while the soil without the dextrose produced 173.20 mgm. The dextrose gave, therefore, a decrease of 57.95 mgm. There is certainly a definite

TABLE VII

The Effect of 0.3 Gram of Calcium Carbonate on the Oxidation of Sodium Thiosulfate and Sulfur in Soil Treated with One Percent of Dextrose.

Lab. No.	Treatment				Mgm. sulfur as sulfate in 100 grams of air-dry soil.	
	$Na_2S_2O_3$	Sulfur	Dextrose	$CaCO_3$		Av.
1	Check	----	----	----	Trace	
2	"	----	----	----	"	Trace
3	4 gms.	----	----	----	172.30	
4	"	----	----	----	174.10	173.20
5	"	----	1 gm.	----	114.62	
6	"	----	"	----	115.87	115.25
7	"	----	"	0.3 gm.	210.82	
8	"	----	"	"	203.56	207.19
9	----	1 gm.	----	----	62.35	
10	----	"	----	----	59.33	60.34
11	----	"	1 gm.	----	103.25	
12	----	"	"	----	108.25	105.75
13	----	"	"	0.3 gm.	161.38	
14	----	"	"	"	166.38	163.88

retarding influence of dextrose on the oxidation of sulfur in the soil when tested with sodium thiosulfate. It may be noted that this observation is in accord with the conclusions of Brown (6), and Lipman and Joffe (30). These authors claim that dextrose prevents the rapid oxidation of sulfur in the soil.

The soil which was treated with dextrose and calcium carbonate showed a production of 207.19 mgm. of sulfate or an increase of 91.94 mgm. over the 115.25 mgm. produced in the soil treated with dextrose alone. The difference in the amount of sulfates produced with these two different treatments is certainly due to the influence of the addition of calcium carbonate on the sulfofying power of the soil, or to its effect in overcoming the injurious influence of the dextrose.

Lime, according to the findings of Brown and Johnson (10), and Neller (39) increased the sulfofying power of the soil.

The results secured with the elemental sulfur run parallel with those obtained with sodium thiosulfate application, but the figures are smaller.

Influence of Sulfur and Lime Applications on the Nitrifying Power of the Soil.

In the review of the literature it was noted that sulfur additions had been observed to cause a retarding influence on the nitrifying efficiency of the soil. This experiment was carried out in order to determine the effects, if any, of various additions of sulfur on the nitrifying power of the soil. It was also considered desirable, if it be true that sulfur applications exert a toxic effect on the nitrifying power of the soil, to determine whether or not the addition of lime would be of value in preventing the toxic action of sulfur.

Using soil from plot 918 (crop residue plot) cultures were prepared in the same way as in the sulfofication experiment. The treatments are shown in Table VIII. After the incubation period which lasted for four weeks the nitrogen as nitrate was determined colorimetrically by the use of the phenoldisulfonic acid method. The results which are expressed as milligrams of nitrogen as nitrate in 100 grams of air-dry soil are given in Table VIII.

TABLE VIII

The Effect of Different Amounts of Sulfur With and Without Lime on the Nitrifying Power of the Soil.

Lab. No.	Treatment: 30 mgm. N. $(NH_4)_2SO_4$	Sulfur Gram	Calcium Carbonate	Mgm. nitrogen as nitrate in 100 gms. of air-dry soil.	Av.
1	Check	----	----	6.88	
2	"	----	----	6.66	6.77
3	30 mgm.N.	----	----	16.00	
4	"	----	----	15.40	15.70
5	"	0.025	----	10.50	
6	"	"	----	10.80	10.65
7	"	0.050	----	9.75	
8	"	"	----	9.52	9.64
9	"	0.100	----	9.09	
10	"	"	----	9.52	9.31
11	"	0.500	----	8.69	
12	"	"	----	8.12	8.41
13	"	1.000	----	7.84	
14	"	"	----	7.91	7.38
15	"	2.000	----	6.66	
16	"	"	----	6.89	6.78
17	"	0.025	210 mgm.	18.18	
18	"	"	"	17.65	17.92
19	"	0.050	"	12.50	
20	"	"	"	12.10	12.30
21	"	0.100	"	11.42	
22	"	"	"	11.77	11.60
23	"	0.500	"	9.09	
24	"	"	"	8.90	9.00
25	"	1.000	"	8.00	
26	"	"	"	8.23	8.12
27	"	2.000	"	7.69	
28	"	"	"	7.25	7.47

Results.

Considering the averages of the results of the duplicate determinations on all the samples, as shown in the table, it will be noted that the sulfur applications tended to cause a depressing effect on the nitrifying power of the soil.

It may also be observed that larger amounts of sulfur brought about greater decrease in nitrate production. Increasing the amount of the sulfur applications, however, did not seem to bring about a proportional decrease in the amount of nitrates produced.

The addition of sulfur to the soil, as pointed out by Martin (37), Neller (40), and Reimer and Tartar (43) leads to an increase in soil acidity due to the oxidation of the sulfur to sulfuric acid. Lipman, McLean and Lint (33) also showed that the larger applications of sulfur caused a decided increase in the hydrogen ion concentration of the soil. The oxidation of elemental sulfur in the soil, as shown by Ames and Richmond (2), and Brown (6) depressed the activities of nitrifying bacteria.

If the suggestions of these authors are accepted, it would seem, therefore, that the decrease in the amount of nitrate produced, which indicates a

retarded nitrifying power of the soil, may be attributed to the depressing effect of the acidity produced from the oxidation of the sulfur on the activities of the nitrifying organisms.

The sulfur-lime treated soils were found to be generally higher in their nitrifying power, as evidenced by the greater amount of nitrate produced, than those in which sulfur was applied alone. It may be noted, however, that when the sulfur applications were increased, the amount of calcium carbonate added had very little effect upon the rate of nitrate production in the soil. The increase in the amount of nitrate produced is undoubtedly due to the stimulating influence of calcium carbonate upon the activities of nitrifying organisms in the soil, or to a removal of injurious acidity. Brown (6) showed a definite influence of lime in reducing the injurious effects of additions of sulfur on the nitrifying bacteria.

The Effect of the Various Amounts of Sulfur on the Nitrifying and Sulfofying Powers of the Soil.

In the previous experiment it was indicated that the oxidation of sulfur in the soil may limit the nitrifying process because of an increase in soil acidity. The following test was planned to determine the relative influence of the application of various amounts of elemental sulfur, with and without the addition of lime, upon the nitrification and sulfofication processes in the soil.

In this study soil taken from plot 921 that has been treated with crop residues, lime and superphosphate was used. The cultures were prepared in the usual way and the various treatments were made. The cultures were each provided with 30 milligrams of nitrogen as ammonium phosphate and were incubated at room temperature for a period of five weeks at a moisture content of fifty percent saturation.

After incubation, the hydrogen ion concentration of each of the soils was determined. The photometric method was employed for the sulfate determination and the phenoldisulfonic acid method was used in the determination of nitrates. The results are

given in Table IX.

Results.

From the figures presented in the table, it will be noted that increasing the sulfur applications brought about corresponding increases in the sulfate production and in the hydrogen ion concentration, which were not proportional to the amounts of sulfur applied.

The rate of sulfur oxidation in the soil varied with the sulfur applications. It was found that sulfofication occurred most vigorously and efficiently when sulfur was added in the smallest amount; and where it was applied in the largest amount the relative amount of sulfur oxidized was the smallest (6.11 percent). The results showed further that when 25 milligrams of elemental sulfur were used, 46 percent was oxidized. The addition of 100 and 500 milligrams of sulfur to the soil brought about a much smaller percent oxidized but a similar amount for the two additions (19.23 and 19.06 percent respectively). A further increase in the sulfur application caused a marked decrease in the rate of oxidation. When one and two grams of sulfur were added, the relative amounts

TABLE IX

The Effect of the Various Amounts
Lime on the Nitrifying and Sulfo

Lab. No.	Treatment: $(NH_4)_3PO_4$ 30 mgm.	Elemental Sulfur	$CaCO_3$ 210 mgm.	*: Hydrogen ion Concentrations	Av.	Increase in: the average: of pH values: Percent	Mg su
1	Check	---	---	6.10		---	Tr
2	"	---	---	6.06	6.08	---	
3	$(NH_4)_3PO_4$	---	---	6.18		---	
4	"	---	---	6.00	6.09	---	
5	"	0.025 gm.	---	5.20		---	11
6	"	" "	---	5.25	5.23	14.29	12
7	"	0.050 "	---	4.88			12
8	"	" "	---	4.93	4.91	19.38	12
9	"	0.100 "	---	4.38			19
10	"	" "	---	4.76	4.57	24.96	19
11	"	0.500 "	---	3.08			95
12	"	" "	---	3.28	3.16	48.12	95
13	"	1.000 "	---	3.32			98
14	"	" "	---	3.12	3.22	47.13	98
15	"	2.000 gms.	---	3.18			120
16	"	" "	---	3.17	3.18	47.78	123
17	"	0.025 gm.	$CaCO_3$	6.36			17
18	"	"	"	6.33	6.35	-.43	17
19	"	0.050 "	"	6.33			19
20	"	" "	"	6.23	6.23	-.23	19
21	"	0.100 "	"	5.93			--
22	"	" "	"	5.88	5.91	3.12	20
23	"	0.500 "	"	5.85			100
24	"	" "	"	5.85	5.85	3.94	98
25	"	1.000 "	"	5.25			136
26	"	" "	"	----	5.25	13.79	134
27	"	2.000 gms.	"	4.58			140
28	"	" "	"	4.33	4.45	26.93	123

* Figures represent pH.

TABLE IX

of the Various Amounts of Sulfur With and Without the Nitrifying and Sulfofying Powers of the Soil

*: Increase in: the average: of pH values:		Mgm. sulfur as sulfate in 100 grams soil		Average sulfur oxidized	Mgm. nitrogen as nitrate in 100 grams soil		Average nitrogen oxidized
Av.	Percent		Av.	(Percent)		Av.	(Percent)
	---	Trace		---	6.34		
6.08	---	"	Trace	---	6.42	6.38	---
	---	"		---	18.18		
6.09	---	"	Trace	---	19.60	18.89	---
	---	11.25		---	17.65		
5.23	14.29	12.25	11.75	47.00	15.40	16.53	13.20
		12.74			12.50		
4.91	19.38	12.74	12.74	25.50	-----	12.50	10.00
		19.20			10.50		
4.57	24.96	19.25	19.23	19.23	9.75	10.13	8.10
		95.75			8.33		
3.16	48.12	95.00	95.38	19.06	8.33	8.33	6.66
		98.75			7.69		
3.22	47.13	98.75	98.75	9.88	7.84	7.77	6.62
		120.63			7.14		
3.18	47.78	123.75	122.19	6.11	7.06	7.10	5.68
		17.35			20.00		
6.35	-.43	17.50	17.43	67.70	18.18	19.09	15.27
		19.00			16.60		
6.23	-.23	19.25	19.13	36.25	16.00	16.30	13.04
		-----			15.40		
5.91	3.12	20.10	20.10	20.10	14.30	14.85	11.88
		100.00			12.50		
5.85	3.94	98.25	99.13	19.82	11.75	12.13	9.70
		136.00			9.75		
5.25	13.79	134.35	135.18	13.52	9.09	9.42	7.53
		140.35			8.33		
4.45	26.93	123.00	131.68	6.58	8.30	8.32	6.65

oxidized did not vary materially.

From the results it seems evident that while the soil showed a definite sulfofying efficiency, the sulfur oxidation seemed to reach a maximum with certain amounts of sulfur, and when this point was reached, a further addition of sulfur depressed the sulfofying power of the soil.

While the process of nitrification in the soil occurred in spite of the increasing development of soil acidity due to the sulfur oxidation, it decreased gradually as sulfofication and soil acidity increased. The most favorable condition for nitrification was found when sulfur was added in the smallest amount. There was a gradual decrease in the amount of nitrate produced as the amount of sulfur added was increased.

The addition of calcium carbonate not only stimulated the oxidation of sulfur in the soil but also tended to reduce the depressing influence of the acidity on the nitrification process.

DISCUSSION AND SUMMARY

In this work studies have been made on sulfofication, the numbers of bacteria and molds, the reaction and the moisture content of soil from plots variously treated.

The data in Table 1 indicate that bacterial umbers varied with the soil treatments. The occurrence of bacteria in the soil as illustrated in Fig. II, was also influenced by seasonal conditions.

Comparing the two groups of plots, the manured and the crop residue plots, it appeared that there was a greater number of bacteria in the manured soils than in the crop residue treated soils. The results seemed to indicate that the manure increased the bacterial numbers more than did the crop residue treatment.

The study of the data presented in Table II, and shown graphically in Fig. III, shows that the soil treatments and the seasonal conditions did not seem to have a very significant influence on the mold occurrence in the soil.

When a comparison is made of the mold and the bacterial counts secured from each of the manured soils, it is found that there is a rather definite relation

between the development of these two groups of organisms.

The data given in Tables III and IV, and shown graphically in Fig. V, indicate that the moisture content of the soils exerted a greater influence on the bacteria than it did on the molds. The hydrogen ion concentration of the soils studied did not show a definite influence on either the molds or the bacteria.

The results of the sulfofication studies show that the soils had a definite sulfofying efficiency.

Comparing the results on the manured and the crop residue treated soils, it may be noted that there was greater sulfofication in the soils treated with the crop residues than in those receiving manure although the latter had greater numbers of bacteria. This difference has been attributed in part, at least, to the textural condition of the soils. It may also be seen that the numbers of molds did not run parallel with the amount of sulfates produced. Still a greater sulfofying power of the soil was observed when phosphate materials were added. In both groups of plots, it was found that the rock phosphate had somewhat less effect than the superphosphate; the difference, however, was not very pronounced.

A definite correlation between the numbers of molds and bacteria and the sulfate production has also

been shown. In the manured plots it was found that increases in the sulfate production were accompanied by increases in the numbers of bacteria. The numbers of molds did not increase with sulfate production however. Dextrose was found to have a retarding influence on sulfofication in the soil. Lime, when added to the soil, increased the sulfofying power and reduced the retardation brought about by the dextrose.

The nitrifying power of the soil was found to be appreciably depressed by the addition of sulfur. Greater decreases in the nitrate production occurred with the larger sulfur applications. The addition of sulfur increased the acidity of the soil and this retarded the nitrification process.

The rate of sulfur oxidation in the soil varied with the amounts of sulfur added. A marked decrease in the rate of sulfofication was brought about by too large an addition of sulfur.

CONCLUSIONS

1. The soils studied showed a definite sulfofying efficiency which varied with the different soil treatments.

a. Manure treatments appeared to cause a slight increase in sulfofication.

b. Lime applications apparently brought about a small increase in the sulfofying efficiency of the soil.

c. The phosphate treatments caused a still greater increase in the sulfofying power of the soil; superphosphate seemed to be slightly more effective than rock phosphate.

2. A correlation was found between the sulfofying power of the soils and the numbers of bacteria present.

3. The numbers of molds in the soils were not correlated with the sulfate production.

4. Dextrose, when added to the soils retarded the process of sulfofication.

5. The rate of sulfur oxidation in the soil was influenced by the various amounts of sulfur added in the tests.

6. Sulfofication was most vigorous in the presence of the smallest sulfur applications.

7. The application of sulfur to the soil brought about an increased hydrogen ion concentration.

8. The addition of sulfur retarded the process of nitrification. The highest concentrations of sulfur used did not inhibit the process.

9. Lime not only increased the sulfofying power of the soil but also tended to decrease the injurious effect of dextrose on sulfofication.

STUDIES ON THE SULFOFYING BACTERIA

During the last few years, considerable attention has been directed to the subject of sulfur oxidation in the soil. Some workers have claimed that sulfofication is purely a chemical process, while others believe that it is brought about, mainly, as a result of microbiological activities in the soil.

Several investigators have succeeded in isolating from the soil, some of the organisms concerned in the process of sulfofication. Studies have been made also of some of the factors that tend to stimulate or retard the bacterial activities which bring about the production of sulfates in the soil.

At the present time, it is quite generally accepted that biological phenomena, rather than chemical processes are of the most significance in the oxidation of sulfur and its complex organic compounds, to sulfates, in which form the sulfur is available to plants.

HISTORICAL

The sulfur bacteria are those bacteria which are capable of obtaining the energy which is needed for their growth from the oxidation of sulfur or its

compounds. A classification based on the physiological characteristics of these organisms has been proposed by Waksman (54).

Among the earliest investigators of sulfur oxidation were Beijerinck (3), and Jacobsen (23 and 24). These two authors described two sulfur oxidizing organisms, Thiobacillus denitrificans and Thiobacillus thioparus which were able to oxidize elementary sulfur, sulfides and thiosulfates.

Winogradsky (61) made a study of the biological oxidation of sulfur. He claimed that hydrogen sulfide could be used as a source of sulfur which in turn was oxidized in the presence of moisture to sulfuric acid.

Waksman and Joffe (59) isolated from the sulfur-floats-soil composts which have been developed by Lipman, McLean and Lint (29), McLean (36), and Lipman, Waksman and Joffe (35), the organism, Thiobacillus thiooxidans which was found to oxidize elemental sulfur to sulfuric acid very rapidly.

Studies of the physiological activities of the organism, Thiobacillus thiooxidans, have also been reported by Joffe (25), Starkey (50), Waksman (55, 56 and 57), and Waksman, Wark, Joffe and Starkey (60).

METHODS OF ISOLATION

In the attempt to isolate in pure culture some of the sulfur oxidizing organisms methods suggested by a number of investigators have been followed.

For the preliminary studies, medium 1 as shown below was prepared and the reaction was adjusted to pH 6.0.

1. Modified medium developed for the growth of Thiobacillus thiooxidans.

Sodium thiosulfate ($Na_2S_2O_3$) -------	5.00 gms.
Potassium nitrate (KNO_3) ----------	1.00 gm.
Calcium chloride ($CaCl_2$)----------	0.25 "
Magnesium chloride ($MgCl_2$) ---------	0.10 "
Monopotassium phosphate (KH_2PO_4) ---	3.00 gms.
Distilled water ----------	1,000.00 c.c.

One hundred c.c. portions were placed in 250 c.c. Erlenmeyer flasks and sterilized. Each flask was inoculated with 2 grams of fresh soil obtained from plot 916, which received a treatment of manure, lime and superphosphate. The cultures were incubated at room temperature for a period of 7 days.

With the addition of 2 percent agar to the above solution, a solid medium was prepared. Several plates were poured and in 6 to 7 days, a few bacterial colonies and molds began to appear on the plates. With the aid of a platinum loop, transfers from the bacterial

colonies were made into tubes of fresh nutrient media.

Afetr 2 to 3 days all of the cultures showed signs of growth. One c.c. of the inoculated nutrient medium was placed in 10 c.c. of sterile distilled water, shaken and fresh flasks of the same solution (medium 1) were inoculated.

The plates were incubated at 28°C, and were examined daily for the appearance of bacterial colonies. After 8 days a few small bacterial colonies and several molds appeared. After 14 days the plates showed many molds with only very few bacterial colonies. Platings of these colonies have been made but several attempts to cultivate the organism on agar plates were unsuccessful.

Having failed to isolate any sulfur oxidizing organisms in the preliminary test, it was decided to prepare a sulfur-soil compost. This compost was made up by mixing 10 grams of elemental sulfur with 100 grams of soil, and incubating at room temperature at optimum moisture for a period of six weeks.

The medium suggested by Trautwein (53), medium 2, was employed.

2. Trautwein (53) medium.

Sodium thiosulfate	2.00 gms.
Potassium nitrate	1.00 gm.
Ammonium chloride	0.10 "
Sodium bicarbonate	1.00 gm.
Magnesium chloride	0.10 "
Disodium phosphate	0.20 "
Distilled water	1,000.00 c.c.

The solution, in 100 c.c. portions in 250 c.c. Erlenmeyer flasks, was unsterilized. Each flask was inoculated with an infusion made from the sulfur-soil compost and incubated at 28°C. Examinations of the cultures were made every day and in 7 to 8 days there was a good growth of the organisms. Subinoculations were made using the same solution, sterilized. At various times during the period of incubation, the cultures were examined for motility. Hanging drops were prepared and the organisms in mixed cultures were observed to be active. The examination of the organisms from smears which were stained gentian violet showed that beside the small bacilli which were present in greater numbers, there was a large celled organism. There seemed to be two distinct types of bacilli, which were somewhat different in size.

Several plates were again poured. Transfers were made from individual colonies into fresh lots of

the medium. The several subcultures made resulted in the isolation of two organisms, cultures 60 and 400. Tests for the presence of sulfate in the solution during the subculturing process showed that the organisms isolated in pure culture were sulfur oxidizers. A third organism was isolated later, culture 800.

Bacterial suspensions from each of the three pure cultures were plated using medium 1. The dilution was 1:1,000. The plates were incubated at room temperature for a period of 8 days. Characteristics of the colonies were noted as follows:

Culture 60. Size of the colonies. Ranging from 0.3 to 0.6 mm. in diameter with a dark spot at the center of each of the colonies; transparent with the exception of the spot which is rather opaque. The colonies are circular and flat.

Culture 400. Size of the colonies. Ranging from 0.3 to 1 mm. in diameter, with a dark brown spot found at the center of each colony. A yellowish color occurs around the spot; the edge of each colony is a little paler. The colonies are circular and flat.

Culture 800. Size of the colonies. Ranging from 0.5 to 2 mm. in diameter; slightly brownish in color, and are uniformly colored. Circular and very slightly raised.

Repeated attempts to grow the organisms on plates were unsuccessful and the stock cultures were maintained in liquid media.

SULFOFICATION STUDIES WITH PURE CULTURES

In order to determine whether or not the organisms isolated in pure culture were sulfur oxidizers, the following experiment was conducted.

Experiment 1. Sand as a medium.

In this experiment fifty grams of well washed white sand were weighed into 250 c.c. Erlenmeyer flasks. To each of the flasks were added 10 c.c. of a solution, medium 3, of the following composition:

3. Medium.

Sodium thiosulfate	2.00 gms.
Potassium nitrate	1.00 gm.
Sodium bicarbonate	1.00 "
Magnesium chloride	0.10 "
Di-potassium phosphate	0.20 "
Distilled water	1,000.00 c.c.

The reaction of the medium was adjusted to pH 7.0. The flasks were sterilized in the autoclave at 15 pounds pressure for about 30 minutes, then two flasks were each inoculated with 5 c.c. of a bacterial suspension

of pure culture 60; two flasks with culture 400, and two flasks with the culture 800. The flasks were incubated at room temperature for a period of 42 days. After incubation sulfates were determined by the sulfur photometric method. The results are shown in Table X.

TABLE X.

Studies on the Oxidation of $Na_2S_2O_3$ by Pure Cultures in Sand.

Lab. No.	Culture Number	Mgm. sulfur as sulfate	Average
1	Check	Trace	
2	"	"	Trace
3	60	2.95	
4	60	2.63	2.79
5	400	3.10	
6	400	3.52	3.31
7	800	1.32	
8	800	1.48	1.40

It may be seen from the above table that the sulfur in the $Na_2S_2O_3$ was oxidized by the microorganisms in pure cultures. The greatest oxidation occurred with culture 400 which produced 3.31 mgm. of sulfur as sulfate. Culture 60 was second with 2.79 mgm., and culture 800, gave 1.40 mgm. of sulfur as sulfate. The hydrogen ion concentration of the cultures after incubation was not

determined hence no conclusion can be drawn regarding the relation of sulfur oxidation to change in the reaction of the medium.

Experiment 2. Solution culture.

In this experiment, a solution of the same composition as that used with the sand, but with flowers of sulfur instead of sodium thiosulfate was prepared. All the ingredients except the sulfur were dissolved in 1,000 c.c. of distilled water and the reaction was adjusted to pH 7.0. The solution was divided into 100 c.c. portions in 250 c.c. flasks and sterilized. Several one-gram portions of elemental sulfur were weighed, wrapped in paper and also sterilized. Inoculations were made as before, using 5 c.c. of bacterial suspensions and the elemental sulfur was added to each flask. Table XI shows the results of the tests.

TABLE XI

Studies on the Oxidation of Sulfur
by Pure Cultures in Solution.

Lab. No.	Culture Number	Mgm. sulfur as sulfate	Average
1	Check	Trace	
2	"	"	Trace
3	60	1.42	
4	60	1.60	1.51
5	400	1.74	
6	400	2.02	1.88
7	800	0.98	
8	800	1.20	1.09

The above data show that elemental sulfur was oxidized by the organisms in pure culture. Culture 60, after 6 weeks incubation gave 1.51 mgm. of sulfur as sulfate. Culture 400 produced 1.88 mgm. of sulfate, showing a somewhat greater sulfofying efficiency than culture 60. Culture 800 produced 1.09 mgm. of sulfate.

Experiment 3. The Effect of Varying Concentrations of Sodium Thiosulfate on the Oxidizing Power of Culture 60.

The same medium employed in the sand culture experiment above was prepared with the different amounts of sodium thiosulfate as shown in Table XII. The solution was divided into 100 c.c. portions in 250 c.c. flasks and

sterilized. Each flask was inoculated with 5 c.c. of the bacterial suspension and incubated for 45 days after which time the cultures were analyzed for sulfates. The results are given in Table XII.

Results.

From the results presented in Table XII, it is evident that the rate of sulfofication varied with the amount of sodium thiosulfate applied. With the addition of 0.025 gm. of the salt to the medium there was a production of 1.86 mgm. of sulfur as sulfate. When 0.05 gm. or twice as much as the original amount of salt was added, 2.03 mgm. of sulfur as sulfate were obtained or an increase of 0.17 mgm. With the 0.10 gm. sodium thiosulfate application, there was a slight decrease in sulfates formed and with 0.30 gm. a further decrease occurred. However in these cases the differences were small.

When 0.50 gm. of sodium thiosulfate was added to the medium there was a production of 3.63 mgm. of sulfate. Gradual increase in sulfate produced occurred as the concentration of sodium thiosulfate was increased up to the 3.0 gm. addition. With 5.0 gm. there was a decided decrease in the amount of sulfate.

TABLE XII

The Effect of Varying Concentrations of $Na_2S_2O_3$ on the Oxidizing Power of Culture 60.

Lab. No.	Treatment	Mgm. sulfur as sulfate	Average
1	Check	Trace	
2	"	"	Trace
3	0.025 gm. $Na_2S_2O_3$	2.06	
4	" " "	1.67	1.86
5	0.050 " "	1.96	
6	" " "	2.10	2.03
7	0.100 " "	1.80	
8	" " "	2.03	1.92
9	0.300 " "	1.82	
10	" " "	1.95	1.88
11	0.500 " "	----	
12	" " "	3.63	3.63
13	0.750 " "	3.82	
14	" " "	----	3.82
15	1.000 " "	4.62	
16	" " "	5.20	4.91
17	1.500 " "	6.16	
18	" " "	7.03	6.59
19	3.000 " "	9.03	
20	" " "	8.27	8.65
21	5.000 " "	1.30	
22	" " "	1.02	1.16

The results show that the amount of sulfates produced with two exceptions increased in proportion to the concentration of sodium thiosulfate until 5.0 grams were added. With this high concentration of the salt there was a depressing influence on the sulfur oxidizing power of the organism.

Experiment 4. The Effect of Various Concentrations of $Ca(NO_3)_2$ With and Without 1.0 Percent Dextrose and 0.3 Gram $CaCO_3$ on the Oxidation of $Na_2S_2O_3$ by Culture 60.

Using the same procedure employed in the above experiment a test was made of the sulfur oxidizing power of culture 60 with varying concentrations of calcium nitrate with and without dextrose and calcium carbonate. The different treatments and the results are shown in Table XIII.

Results.

The data shown in Table XIII show that calcium nitrate decreased the sulfur oxidizing efficiency of the organism. With the addition of 0.025 gm. of calcium nitrate to the medium in the presence of dextrose there was a production of 6.82 mgm. of sulfate. The application of greater amounts of calcium nitrate caused a gradual decrease in the amount of sulfate produced.

TABLE XIII

The Effect of Various Concentrations of $Ca(NO_3)_2$ With and Without One Percent Dextrose and 0.3 Gram $CaCO_3$ on the Oxidation of $Na_2S_2O_3$ by Culture 60.

Lab. No.	Treatment: $Na_2S_2O_3$ Gram	Dextrose Percent	$Ca(NO_3)_2$ Gram	$CaCO_3$ Gram	Mgm. sulfur as sulfate in 100 c.c. solution.	Av.
1	Check	----	----	----	Trace	
2	"	----	----	----	"	Trace
3	0.5	1.0	0.025	----	6.45	
4	"	"	"	----	7.20	6.82
5	"	"	0.050	----	6.15	
6	"	"	"	----	5.95	6.05
7	"	"	0.100	----	4.93	
8	"	"	"	----	5.75	5.34
9	"	"	0.500	----	5.13	
10	"	"	"	----	4.30	4.75
11	"	"	1.000	----	1.35	
12	"	"	"	----	0.95	1.25
13	"	"	0.025	0.30	7.00	
14	"	"	"	"	----	7.00
15	"	"	0.050	"	6.68	
16	"	"	"	"	6.95	6.82
17	"	"	0.100	"	6.17	
18	"	"	"	"	6.85	6.51
19	"	"	0.500	"	6.13	
20	"	"	"	"	6.95	6.54
21	"	"	1.000	"	5.50	
22	"	"	"	"	6.35	6.93
23	"	----	0.025	"	7.95	
24	"	----	"	"	8.50	8.28
25	"	----	0.050	"	7.75	
26	"	----	"	"	9.25	8.50
27	"	----	0.100	"	7.25	
28	"	----	"	"	----	7.25
29	"	----	0.500	"	6.70	
30	"	----	"	"	5.55	6.13
31	"	----	1.000	"	4.75	
32	"	----	"	"	3.75	4.25

When 0.3 gm. of calcium carbonate was added, it was noted that the depressing influence of the calcium nitrate was somewhat reduced.

It may also be observed from the results that greater amounts of sulfate were formed in cultures in which calcium carbonate was used in the absence of dextrose. Calcium carbonate, therefore, may stimulate sulfofication while dextrose tends to depress the sulfur oxidizing power of the organism.

Experiment 5. The Effect of Various Concentrations of $Na_2S_2O_3$ on the Oxidizing Power of Culture 400.

These tests were made following the procedure employed in Experiment 2. Table XIV gives the results.

Results.

An examination of the results in Table XIV shows that the rate of sulfur oxidation in the medium varied with the different concentrations of sodium thiosulfate.

With the smallest amount of sodium thiosulfate, 0.025 gm., there was a production of 3.71 mgm. of sulfate. A gradual decrease in sulfate occurred as the concentration of sodium thiosulfate was increased up to 0.30 gram.

TABLE XIV

The Effect of Various Concentrations of $Na_2S_2O_3$ on the Sulfofying Power of Culture 400.

Lab. No.	Treatment	Mgm. sulfur as sulfate in 100 c.c. solution	Average
1	Check	Trace	
2	"	"	Trace
3	0.025 gm. $Na_2S_2O_3$	3.57	
4	" " "	3.85	3.71
5	0.050 " "	3.37	
6	" " "	3.41	3.39
7	0.100 " "	2.88	
8	" " "	2.83	2.86
9	0.300 " "	----	
10	" " "	2.75	2.75
11	0.500 " "	4.96	
12	" " "	4.91	4.93
13	0.750 " "	5.46	
14	" " "	5.30	5.38
15	1.000 " "	6.76	
16	" " "	6.66	6.71
17	1.500 " "	8.19	
18	" " "	8.33	8.26
19	2.000 " "	11.92	
20	" " "	11.35	11.64

When 0.5 gm. of the salt was added, there was an increase and 4.93 mgm. of sulfates were formed. Further increases in sodium thiosulfate up to 2.0 grams increased the sulfate production.

Experiment 6. The Effect of Various Concentrations of Dextrose on the Oxidation of Sodium Thiosulfate by Cultures 60, 400 and 800.

The plan of this experiment is similar to those already performed. Medium 2 was used. The concentration of the medium was adjusted to pH 7.0.

During the progress of the experiment, observations of the cultures and tests for the presence of nitrite in the medium were made. Observations of the cultures are presented on pages 71 and 72. The results of the determinations of sulfates are shown in Table XV.

Results.

The figures in Table XV indicate that dextrose has a decided influence on the oxidizing power of the organisms.

With culture 60, it was observed that smaller applications of dextrose gave the largest amounts of sulfates. With the addition of 0.025 gm. of dextrose there was a production of 8.73 mgm. of sulfur as sulfate. When the amount of dextrose applied was doubled, there

Observations During the Progress of Experiment 6.

This Experiment Was Started October 18, 1928.

Culture 60.

October 25, 1928. The medium in all of the inoculated flasks showed indications of turbidity. There was no sign of the presence of nitrite.

November 5, 1928. Turbidity of medium in all of the inoculated flasks was observed. There was no sign of the presence of nitrite.

November 18, 1928. All inoculated flasks were turbid, and the turbidity became more apparent as the amount of dextrose was increased. The medium in the checks did not show sign of turbidity.

Culture 400.

October 25, 1928. All inoculated flasks became turbid. There was no sign of the presence of nitrite.

November 5, 1928. Inoculated flasks, turbid. No sign of the presence of nitrite.

November 18, 1928. All inoculated flasks exhibited turbidity; white milky precipitate was observed to be present at the bottom of the flasks.

Culture 800.

October 25, 1928. Flasks having 0.025 gram of dextrose showed traces of nitrite. More apparent indications of the presence of nitrite were observed in those flasks which contained 0.5 and 1.0 gram of dextrose. Higher amounts of dextrose showe traces of nitrite.

November 5, 1928. More apparent indication of the presence of nitrite has been noted.

November 18, 1928. White jelly mass of round colonies were floating on the surface of the medium; muddy residue, dark brown in color was found at the bottom of the flasks, especially in those with heavier applications of dextrose.

TABLE XV

The Effect of Various Concentrations of Dextrose on the Oxidation of Sodium Thiosulfate by Cultures 60, 400 and 800.

Lab. No.	Treatment Dextrose Gram	Culture 60 Mgm. sulfur as sulfate	Culture 60 Av.	Culture 400 Mgm. sulfur as sulfate	Culture 400 Av.	Culture 800 Mgm. sulfur as sulfate	Culture 800 Av.
1	Check	Trace		Trace		Trace	
2	"	"	Trace	"	Trace	"	Trace
3	0.025	8.71		5.15		0.93	
4	"	8.75	8.732	5.00	5.075	0.87	0.890
5	0.050	10.15		2.78		1.41	
6	"	-----	10.150	2.82	2.800	1.35	1.380
7	0.100	7.55		1.71		1.72	
8	"	7.65	7.600	1.80	1.755	1.67	1.695
9	0.500	8.92		1.92		1.72	
10	"	8.95	8.936	1.95	1.935	1.74	1.730
11	1.000	2.10		1.52		2.10	
12	"	2.15	2.125	1.48	1.500	2.08	2.090
13	2.500	1.53		0.49		1.40	
14	"	1.60	1.575	0.52	0.505	1.36	1.380

were 10. mgm. sulfate produced (flask No. 5) or an increase of 1.42 mgm. Flask No. 6, the duplicate of flask No. 5, was contaminated so that it was discarded. As the amount of dextrose was increased, a decided decrease in sulfate production resulted except when 0.5 gm. of dextrose was applied when there was an increase in sulfates produced. The decrease in sulfates produced became more significant as the concentration of dextrose was increased.

All the flasks inoculated with culture 400 showed in all cases smaller amounts of sulfate than those inoculated with culture 60. The greatest amount of sulfur as sulfate was obtained where the smallest amount of dextrose was applied. With the increasing application of dextrose there was a very gradual decrease in the sulfate production except when 0.5 gm. of dextrose was applied when 1.935 mgm. of sulfate were produced or an increase of 0.18 mgm. of sulfate over that formed in the presence of 0.1 gm. of dextrose.

In the flasks where culture 800 was used the increasing concentration of dextrose caused an increase in the sulfate produced. The sulfate production, however, did not increase proportionally with the amount of dextrose applied. The greatest amount of dextrose application caused a decrease in the amount of sulfate production.

The results in general showed that various concentrations of dextrose did not cause a similar influence on the oxidizing power of the organisms.

Experiment 7. The Effect of Varying the Hydrogen Ion Concentration on the Sulfofying Power of the Organisms.

In this experiment culture flasks were prepared in the usual way with Waksman's solution.

4. Waksman (56) medium.

Sodium thiosulfate	5.00	gms.
Ammonium chloride	0.10	gm.
Calcium chloride	0.25	"
Magnesium chloride	0.10	"
Monopotassium phosphate	3.00	gms.
Distilled water	1,000.00	c.c.

Potassium sulfide was used in place of the sodium thiosulfate. The pH values of the solutions are seen in Table XVI.

During the progress of the experiment observations of the characteristics of the inoculated medium were made.

Results.

From Table XVI, it may be seen that the oxidizing power of the organisms was influenced by the different hydrogen ion concentrations of the medium.

TABLE XVI

The Effect of Various Hydrogen
of the Medium on the Sulfofying Po

Lab. No.	Culture No.	Initial pH	After seven days				After five weeks			
			pH		p.p.m. sulfur as sulfate		pH		p.p.m. sulfu as sulfate	
				Av.		Av.		Av.		A
1	60	7.84	7.89		3.05		7.80		26.50	
2	60	7.84	7.82	7.85	2.60	2.82	7.72	7.76	24.75	25.
3	60	4.56	4.00		12.75		3.30		38.95	
4	60	4.56	4.25	4.12	14.00	13.37	3.02	3.16	40.30	38.
5	60	3.03	2.95		15.35		2.40		39.40	
6	60	3.03	2.78	2.86	16.92	16.13	3.05	2.72	36.50	37.
7	400	7.84	7.20		3.65		6.20		30.50	
8	400	7.84	7.40	7.30	2.95	3.30	6.75	6.47	32.15	31.
9	400	4.56	4.20		10.35		3.50		33.25	
10	400	4.56	4.15	4.18	9.20	9.77	3.00	3.25	35.00	34.
11	400	3.03	3.04		10.50		3.00		36.05	
12	400	3.03	3.00	3.02	9.15	9.82	3.35	3.16	38.00	37.
13	800	7.84	7.80		3.05		7.80		10.30	
14	800	7.84	7.80	7.80	2.95	2.97	7.60	7.70	9.20	9.
15	800	4.56	5.00		2.03		4.08		2.02	
16	800	4.56	4.60	4.80	1.82	1.92	4.40	4.24	1.90	1.
17	800	3.03	3.02		2.05		3.00		1.03*	
18	800	3.03	3.00	3.01	2.50	2.28	--	3.00	0.20*	0.

* Showed indications of contamination.
The medium became darker in color.

TABLE XVI

Various Hydrogen Ion Concentrations
the Sulfofying Power of the Organisms.

er five weeks			After twelve weeks			After seventeen weeks		
	p.p.m. sulfur as sulfate		pH		p.p.m. sulfur as sulfate		pH		p.p.m. sulfur as sulfate	
Av.		Av.		Av.		Av.		Av.		Av.
	26.50		7.80		40.00		7.80		40.50	
7.76	24.75	25.62	7.82	7.81	38.25	39.12	7.82	7.81	41.30	40.90
	36.95		2.25		38.00		--		--	
5.16	40.30	38.62	2.20	2.22	41.50	39.75	2.17	2.17	42.40	42.40
	39.40		2.40		48.30		2.00		46.50	
2.72	36.50	37.95	2.00	2.20	48.25	48.28	1.96	1.98	47.25	46.87
	30.50		6.04		43.50		6.64		44.50	
6.47	32.15	31.32	5.52	5.78	43.75	43.62	5.62	6.13	45.30	44.90
	33.25		3.00		60.30		2.16		59.00	
3.25	35.00	34.12	2.76	2.88	61.60	60.95	2.17	2.16	62.00	60.50
	36.05		2.05		53.00		2.05		54.35	
5.16	38.00	37.02	--	2.05	--	53.00	--	2.05	--	54.35
	10.30		7.80		23.50		7.79		25.50	
7.70	9.20	9.75	7.60	7.70	24.00	23.75	--	7.79	--	25.50
	2.02		6.20		22.50		6.16		23.50	
1.24	1.90	1.96	--	6.20	--	22.50	--	6.16	--	23.50
	1.03*		--		--		--		--	
5.00	0.20*	0.63	--	--	--	--	--	--	--	--

n Ion Concentrations
Power of the Organisms.

lfur te Av.	After twelve weeks: pH		After twelve weeks: p.p.m. sulfur as sulfate		After seventeen weeks: pH		After seventeen weeks: p.p.m. sulfur as sulfate	
		Av.		Av.		Av.		Av.
	7.80		40.00		7.80		40.50	
25.62	7.82	7.81	38.25	39.12	7.82	7.81	41.30	40.90
	2.25		38.00		--		--	
58.62	2.20	2.22	41.50	39.75	2.17	2.17	42.40	42.40
	2.40		48.30		2.00		46.50	
57.95	2.00	2.20	48.25	48.28	1.96	1.98	47.25	46.87
	6.04		43.50		6.64		44.50	
51.32	5.52	5.78	43.75	43.62	5.62	6.13	45.30	44.90
	3.00		60.30		2.16		59.00	
54.12	2.76	2.88	61.60	60.95	2.17	2.16	62.00	60.50
	2.05		53.00		2.05		54.35	
57.02	--	2.05	--	53.00	--	2.05	--	54.35
	7.80		23.50		7.79		25.50	
9.75	7.60	7.70	24.00	23.75	--	7.79	--	25.50
	6.20		22.50		6.16		23.50	
1.96	--	6.20	--	22.50	--	6.16	--	23.50
	--		--		--		--	
0.63	--	--	--	--	--	--	--	--

Observations in Connection with the Experiment on the Effect of Various Hydrogen Ion Concentrations of the Medium on the Sulfofying Power of the Organisms.

At the end of the incubation period (120 days) the following observations were made of the different cultures.

Culture 60. Flasks inoculated with culture 60 were turbid. The turbidity became more and more apparent as the period of incubation progressed. Granules were found at the bottom of the flasks.

Culture 400. The effects on the medium with culture 400 were practically the same as those with culture 60. A difference in the degree of turbidity of the two cultures was noted. The solution inoculated with 400 was more turbid than that with 60.

Culture 800. The medium inoculated with culture 800 showed a brownish color, with but little turbidity of the medium.

When culture 60 was used to inoculate the medium which had an initial pH of 7.84, it was found that after 7 days of incubation there were 2.82 p.p.m. of sulfate produced. The pH value of the medium remained practically the same. The greatest increase in the sulfate produced occurred after five weeks of incubation. This maximum increase was accompanied by a slight increase in the hydrogen ion concentration of the medium.

With an initial pH of 4.56, it was found that the oxidizing power of culture 60 was greater than when it was grown in the same medium having a pH of 7.84. The results show that after 7 days of incubation there were 13.37 p.p.m. of sulfates produced. After 5 weeks, 38.62 p.p.m. of sulfates were found. The medium became more acid and some contamination appeared in one of the flasks as the incubation period was prolonged.

Using 3.03 as an initial pH of the medium, culture 60 produced 16.13 p.p.m. of sulfates after 7 days, whereas the same organism when grown in the same medium having a pH of 4.56 produced only 13.37 p.p.m. of sulfates. With the use of the most favorable reaction, the process of sulfur oxidation continued very rapidly over a period of 12 weeks of incubation

at which time there were 48.28 p.p.m. of sulfates produced. Prolonging the period of incubation to 17 weeks resulted in a decrease of 1.41 p.p.m. of sulfates over the amount present at 12 weeks.

The results showed that in most cases the sulfate production and the hydrogen ion concentration of the medium did not run parallel. The rate of sulfate production decreased gradually as the period of incubation was increased.

Culture 400, appears to be more efficient in oxidizing sulfur than culture 60. It may be noted from the results that at the end of 7 days incubation, 3.30 p.p.m. of sulfates were produced in the medium having an initial pH of 7.84. There was an increase in the hydrogen ion concentration of the medium at the same time.

After 5 weeks of incubation, 31.32 p.p.m. of sulfates were secured or an increase of 28.02 p.p.m. of sulfates. There was also an increase in the pH of the medium. The maximum amount of sulfate production and the highest hydrogen ion concentration were noted after 12 weeks of incubation. After this time there was but a little increase in the sulfate produced, and a decrease in the pH of the medium was noted.

When the pH of the medium was adjusted to 4.56

it was observed that the amounts of sulfate formed by culture 400 were greater than those secured in the same medium with a pH of 7.84. It might also be of interest to note that the increase in the hydrogen ion concentration of the medium and the amount of sulfate produced were parallel till the end of 12 weeks after which time a decrease in sulfates was noted.

When the medium had a pH of 3.03, it favored normal development of the organisms. It may be noted that after 7 days of incubation, 9.82 p.p.m. of sulfates were produced. The pH of the medium was practically the same. After 5 weeks 37.02 p.p.m. of sulfates were found or an increase of 27.20 p.p.m. of sulfates. This increase in the amount of sulfates led to an increase in the hydrogen ion concentration of the medium. As the period of incubation was prolonged some of the cultures showed contamination and these caused difficulty in the sulfate and hydrogen ion concentration determinations.

In the case of culture 800, it was noted a pH of 7.84 in the medium was more favorable for its growth and activity than the acid medium. As may be seen from the results the higher acidity of the medium may prove toxic for this particular organism.

MORPHOLOGICAL, PHYSIOLOGICAL AND CULTURAL CHARACTERISTICS OF THE ORGANISMS

The morphological features of the organisms have been studied from smears mostly secured from liquid cultures. Smears were stained with carbol fuchsin, methylene blue, or gentian violet and Gram stain. The dried preparations were mounted in Canada Balsam under a cover glass.

Morphology.

Culture 60. The organism was rod shaped in stained mounts. The sizes were 0.68 x 2.10 μ when 24 hours old; 0.55 x 2.06 μ when 48 hours old; and 1.09 x 2.10 μ when 72 hours old. The cell was readily stained with the laboratory stains.

Culture 400. Small rods; sizes varied as: 0.48 x 1.48 μ when 24 hours old; 0.31 x 0.92 μ when 48 hours old; and 0.53 x 0.93 μ when 72 hours old. They were stained readily.

Culture 800. The organisms varied from ovoid to rod; sizes were 1.97 x 2.61 μ when 24 hours old; 1.84 x 2.33 μ when 48 hours old; and 2.05 x 2.36 μ when 72 hours old.

Physiology.

The physiological characteristics of the organisms have been studied. The reduction of nitrates, shown in tabular form on pages 71 and 72 and the changes in the reaction of the media in which the organisms have been grown were recorded. The organisms were also inoculated in azolitmin milk to detect the acid formation.

Studies on the cultural characteristics of the organisms were made by growing them in gelatin stabs to determine the liquefaction of gelatin. They were also grown on different solid media. Washed agar was employed in the preparation of the agar media.

The Various Media Used in the Study of the Cultural Characteristics of the Organisms.

1. Nutrient agar.

Distilled water	1,000.00 c.c.
Liebig's meat extract	3.00 gms.
Peptone	10.00 gms.
Agar	15.00 gms.

2. Dextrose agar.

Distilled water	1,000.00 c.c.
K_2HPO_4	0.20 gm.
Dextrose	10.00 gms.
Agar	15.00 gms.

3. Starch-Nitrate agar.

Bouillon	1,000.00 c.c.
KNO_3	1.00 gm.
Starch	5.00 gms.
Agar	10.00 gms.

4. Casein agar.

Distilled water	1,000.00 c.c.
Casein	10.00 gms.
N/1 NaOH	7.00 c.c.
Agar	10.00 gms.

5. Asparagin-dextrose agar.

Distilled water	1,000.00 c.c.
K_2HPO_4	0.50 gm.
$MgSO_4$	0.20 gm.
Asparagin	1.00 gm.
Agar	15.00 gms.

6. Saccharose agar.

Distilled water	1,000.00 c.c.
Saccharose	10.00 gms.
KH_2PO_4	1.00 gm.
$MgSO_4$	0.20 gm.
Agar	15.00 gms.

7. Potato agar.

Distilled water	1,000.00 c.c.
Dextrose	20.00 gms.
Agar	30.00 gms.
Potato	200.00 gms.

8. Potato plugs.

Liquefaction of Gelatin.

Nutrient gelatin was prepared. Medium 1 was used with gelatin instead of agar.

Culture Number	October 17, 1928.	October 25, 1928.	November 3, 1928.
60	An indication of liquefaction was observed; infundibulum.	No change, i.e no further progress in liquefaction.	No change.
400	A good sign of liquefaction was apparent; stratiform.	Same as during the first observation; stratiform.	Liquefaction is more advanced; about one third of the medium has been liquefied.
800	No sign of liquefaction. Villous growth of the organism was observed.	Same as in the first observation.	No liquefaction; growth is more prominent and extended down to about two cm. from the surface of the medium.

Examination of Milk and the Detection of Acid Forming Bacteria.

Azolitmin milk medium was prepared by the standard method. The tubes were wach inoculated with 1 c.c. of the bacterial suspensions.

Experiment began October 5, 1928

Dates	Culture 60	Culture 400	Culture 800
October 7: 1928.	There was a slight change of color of the medium. A pink color was detected.	Practically no change of color.	No color change observed.
October 14: 1928.	No further change of medium was observed.	At this time a pinkish color of the medium was observed.	Just a slight change of color of the medium, especially at the surface was observed; somewhat light pink in color.
October 21: 1928.	No further change.	No further change.	No further change.
November 4: 1928.	No change.	No change.	No change.
November 12, 1928.	No change.	No change.	No change.

Growth Characteristics of C

Characteristics noted	Nutrient Agar	Dextrose Agar	Starch-Nitrate-Agar	Casein Agar	Asp D
1. Growth	Moderate	Moderate	Abundant	Scanty	Mo
2. Forms of growth	Filiform	Filiform	Slightly spreading	Filiform	Fi
3. Elevation	Flat	Flat	Slightly raised	Flat	Sli rai
4. Luster	Glistening	Glistening	Glistening	Dull	Glis
5. Surface	Smooth	Smooth	Smooth	Contoured	Smo
6. Optical Characters	Opaque	Opaque	Opaque	Slightly opaque	Opa
7. Chromogenesis	Creamy in color	Milky	Light brown along the streak	Milky white	Whi
8. Odor	Odor resembling a decaying meat	Odorless	Odor same as rotting meat	Odorless	Odo
9. Consistency	Butyrous	Butyrous	Butyrous	Butyrous	But
10. Medium	No change	No change	Color of the medium the same; liquid is found at the bottom of tube	Color of the medium the same; liquid is found at the bottom of tube	

nracteristics of Culture 60

Casein Agar	Asparagin-Dextrose	Saccharose Agar	Potato Agar	Potato Plugs	Nutrient Broth
Scanty	Moderate	Very very scanty; or just an indication of growth; No other observation was made.	No growth of the organism was observed.	No growth was noted.	Medium become cloudy after 12 hours; a good sign of growth was observed.
Filiform	Filiform				
Flat	Slightly raised				
Dull	Glistening				
Contoured	Smooth				
Slightly opaque	Opaque				
Milky white	Whitish				
Odorless	Odorless				
Butyrous	Butyrous				
Color of the medium the same; liquid is found at the bottom of tube					

Growth Characteristics of Cultu

Characteristics noted	Nutrient Agar	Dextrose Agar	Starch-Nitrate-Agar	Casein Agar	Asp De
1. Growth	Very good	Moderate	Good	Good	G
2. Forms of growth	Filiform at first, then spreading on surface of medium	Filform	Spreading	Spreading	Fil
3. Elevation	Slightly raised	Slightly raised	Flat	Flat	Ra
4. Luster	Glistening	Glistening to dull	Glistening	Glistening and more opaque at streak	Glis
5. Surface	Smooth	Slightly raised	Smooth	Smooth	Smo
6. Optical Characters	Opaque	Opaque	Opaque	Opaque	Op
7. Chromogenesis	None	None	Brownish along line of streak	None	
8. Odor	Resembles a rotting meat	Odorless	Odor of a decaying meat	Odorless	Odo
9. Consistency	Butyrous	Butyrous	Butyrous	Butyrous	But
10. Medium	No change	No change	No change in color of medium; liquid is found at the bottom of tubes	No change in color of medium; liquid is found at the bottom of tubes	No c chan the

cteristics of Culture 400

Casein Agar	Asparagin-Dextrose	Saccharose Agar	Potato Agar	Potato Plugs	Nutrient Broth
Good	Good	Good	Moderate after 3 days; Good growth 14th day	Fair	Growth, rapid; became more apparent at the end of 12 hours. The liquid was cloudy; white precipitate at the bottom of test tube was noted.
Spreading	Filiform	Filiform	Filiform	Spreading	
Flat	Raised	Slightly raised	Slightly raised	Slightly raised	
Glistening and more opaque at streak	Glistening	Glistening	Glistening	Glistening	
Smooth	Smooth	Smooth	Smooth	Smooth	
Opaque	Opaque	Opaque	Opaque	Opaque	
None	None	Whitish in color	Cream color to brownish	Brownish	
Odorless	Odorless	Odorless	Odorless	Odorless	
Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	
No change in color of medium; liquid is found at the bottom of tubes	No apparent change in the medium	No change	Medium was slightly changed to very light blue after 14th day	Medium greyish, especially at the place of growth	

Growth Characteristics of Cultu

Characteristics noted	Nutrient Agar	Dextrose Agar	Starch Nitrate-Agar	Casein Agar	Asparag Dextr
1. Growth	Moderate	Moderate	Scanty	Moderate	Scanty
2. Forms of growth	Filiform	Filiform	Filiform	Filiform	Filifo
3. Elevation	Slightly raised	Flat	Flat	Slightly raised	Raised
4. Luster	Glistening	Glistening to dull	Glistening	Glistening	Gliste
5. Surface	Smooth	Contoured	Contoured	Contoured	Contou
6. Optical Characters	Opaque	Opaque	Opaque	Opaque	Opaque
7. Chromogenesis	Greenish in color	Greenish	Greenish	Greenish	Light green
8. Odor	Odorless	Odorless	Odorless	Odorless	Odorle
9. Consistency	Butyrous	Butyrous	Butyrous	Butyrous	Butyro
10. Medium	No change	No change	No change; liquid present at the bottom of tube	No change	No cha

racteristics of Culture 800

Casein Agar	Asparagin Dextrose	Saccharose Agar	Potato Agar	Potato Plugs	Nutrient Broth
Moderate	Scanty	Good growth	Good growth	Scanty	Growth, rapid; dark brown precipitate found at the bottom of of tubes. Turbidity of the medium was very apparent.
Filiform	Filiform	Filiform	Filiform	Filiform	
Slightly raised	Raised	Raised	Raised	Slightly raised	
Glistening	Glistening	Glistening	Glistening to dull	Glistening	
Contoured	Contoured	Contoured	Contoured	Slightly contoured	
Opaque	Opaque	Opaque	Opaque	Opaque	
Greenish	Light green	Dark green	Dark green	Light green	
Odorless	Odorless	Odorless	Odorless	Odorless	
Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	
No change	No change	No change	No change	No change	

DISCUSSION AND SUMMARY

Pure cultures of the three sulfur oxidizers were isolated by methods which have been suggested by previous investigators, and tests were made to determine whether or not the organisms were sulfofiers.

The results presented in Tables X and XI showed that the organisms were capable of oxidizing the sulfur contained in sodium thiosulfate and also elemental sulfur.

An examination of Table XII shows that the rate of sulfofication varied with the amount of sodium thiosulfate applied. The amounts of sulfates produced with two exceptions increased in proportion to the concentration of sodium thiosulfate added until 5.0 grams were applied. A depressing influence on the sulfur oxidation was noted when high concentrations of the salt were added.

The data shown in Table XIII indicate that calcium nitrate decreased the sulfofying efficiency of the organisms. A gradual decrease in the amount of sulfates produced occurred with the greater concentrations of calcium nitrate. Calcium carbonate appeared to have a stimulating effect on sulfofication, while dextrose tended to depress the process.

The study of the data given in Table XIV shows

that different concentrations of sodium thiosulfate caused variations in the rate of sulfur oxidation in the medium. A gradual decrease in sulfates produced was found as the concentration of the salt was increased from 0.025 gram up to 0.30 gram. The application of 0.5 gram up to 2.0 grams brought about an increasing amount of sulfates.

The results given in Table XV showed that various concentrations of dextrose did not cause a similar effect on the sulfur oxidizing power of the organisms.

When culture 60 was grown in the medium with different hydrogen ion concentrations, it was found that in most cases the sulfate produced and the pH values of the medium were not parallel.

Culture 400 was observed to be more efficient in oxidizing sulfur than culture 60, and a pH of 3.03 seemed to be more favorable for the normal development of the organism than any other reaction tested.

A pH value of 7.84 for the medium was found to be the best for the growth of culture 800. Greater acidity in the medium was somewhat toxic to this particular organism.

The morphological and cultural characteristics

of the three organisms isolated in pure culture were studied. The physiological activities were also investigated to some extent. The reduction of nitrates and the changes in the pH values of the media used in the above studies were recorded. Culture 800 was observed to be able to reduce nitrate to nitrite, while the other organisms did not have this power.

The study of the liquefaction of gelatin showed that culture 400 appeared to be able to liquefy gelatin, culture 60 was able to show only a very slight liquefaction, and culture 800 was not capable of liquefying the gelatin.

Various solid media were used in the study of the growth characteristics of the organisms.

CONCLUSIONS

1. The isolation and study of three sulfur oxidizers called Cultures 60, 400 and 800 have been reported.

2. Of the three sulfofiers, culture 400 appeared to have characteristics which seemed identical with the descriptions given by Waksman (55), and Waksman and Joffe (59). For this reason it may be

assumed that this organism (400) is Thiobacillus thiooxidans.

3. Before organisms 60 and 800 are classified further studies of their characteristics must be made.

4. The organisms isolated were capable of oxidizing elemental sulfur and sodium thiosulfate.

5. The rate of sulfofication was influenced by the varying amounts of sulfur and sulfur compounds used in the tests.

6. Increasing concentrations of sodium thiosulfate tended to depress the sulfur oxidation.

7. Calcium nitrate appeared to retard sulfofication.

8. Dextrose had a retarding influence on the oxidation of sulfur.

9. Calcium carbonate had a stimulating effect on the activities of the organisms.

10. The varying concentrations of the medium gave different results for the sulfofying efficiency of the organisms.

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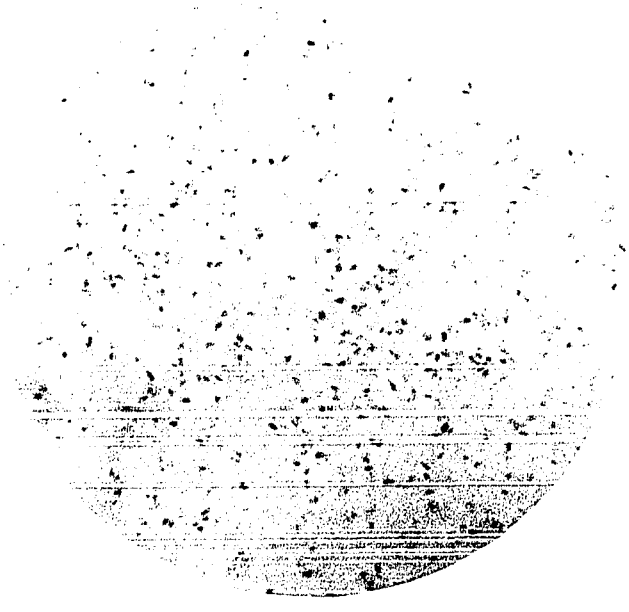
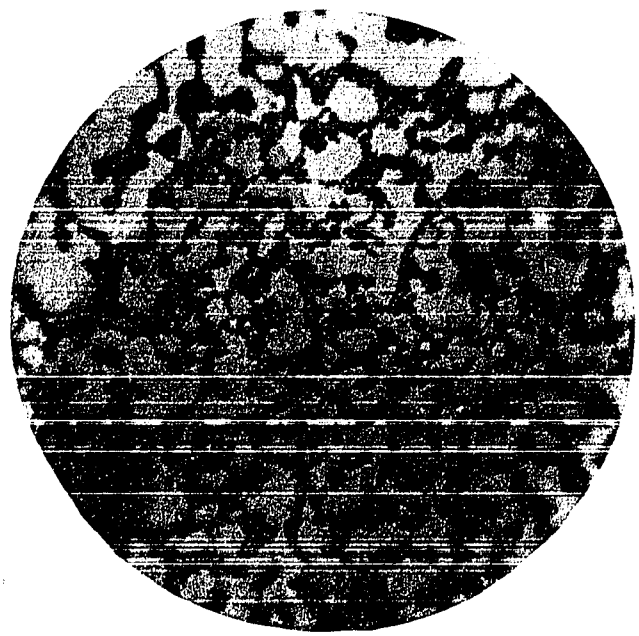


Culture 60, stained with gentian violet. Magnification 1,050.



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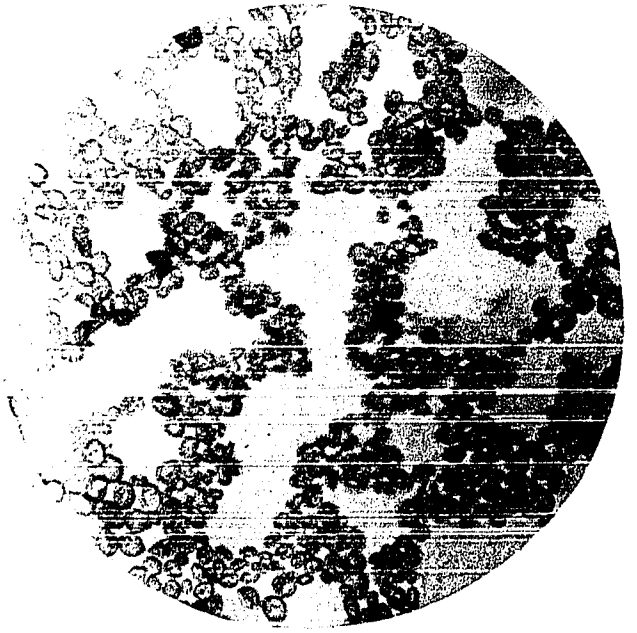

Culture 800, stained with gentian violet. Magnification 1,050.