The calculation is made by first subtracting the factor obtained by running the mixture of chemicals as above described, omitting the oil. The remainder r gives the rise in temperature due to the combustible; 73 per cent. of this is due to the heat of combustion, and 27 per cent. to the heat of combination of the chemical products, hence,

$$\frac{r \times 0.73 \times \text{wt. of water}}{\text{wt. of oil}} = \text{calories per kilo.}$$

A homogeneous mixture may result from simply shaking, and without the use of the wire. Indeed, some of the above results were so obtained, but in general it is better to mix by stirring as described.

It is a convenience to have a thoroughly triturated mixture of potassium persulphate and tartaric acid carefully adjusted to the proportions above indicated. It is then necessary to make but one weighing and, of course, 1.5 grams of the mixture are taken.

ZURICH, July, 1901.

STUDIES IN NITRIFICATION.[1]

BY J. G. LIPMAN.

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IN undertaking the work submitted here it was intended to study nitrification in its bearing to practical agriculture. The aim was to investigate in how far certain conditions influence the production of nitrates in the soil, in how far the application of the various fertilizers promote or retard nitrification, and in how far the moisture conditions modify this. Much work has already been done on the subject, notably by the French investigators, and in the light of our present knowledge we have come to modify many of the older methods of husbandry, so as to bring them in agreement with recent facts. Yet great as is the service rendered to agriculture by the research of the last two decades, the conclusions reached are by no means final in every case; nor have all of the questions raised by the work been satisfactorily answered. Much of our knowledge on nitrification is rather obscure; many questions still await solution. We know now that plants take up most of their nitrogen in the form of nitrate; we know that the conversion of organic nitrogen into ammonia, nitrite, and nitrate is effected by living organisms; we know, to a great extent, what conditions are favorable or injurious to the development of these

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[1] Abstract from Master's Thesis, Cornell University, 1900.

organisms, but we do not know just *how* the micro-organisms oxidize ammonia nitrogen to nitrite or nitrate nitrogen ; we are not yet certain as to the exact relations in the soil of the nitrifying, denitrifying, and, what Beijerinck[1] calls the "oligo nitrophile" organisms to one another ; we have much to learn as to the physiology and morphology of these organisms. Standing alone, neither chemistry nor bacteriology can solve the problem fully. If it is to be solved at all, as we hope it will, it will be by the combined efforts of chemistry and bacteriology.

It is not claimed that the work reported in the following pages is, all of it, strictly original. The same ground has, partly at least, been covered by others ; nevertheless, even in such cases the conditions and methods were not the same, and it is hoped that the results obtained will be of value to the student of agriculture. It should be stated here that this work was intended to be little more than preliminary. It was more than anything else a study of methods and conditions, only a preparation for a more critical and more extended study of the same phenomena, which it is hoped may be continued in the future.

Now as to the methods: For the determination of organic and of total nitrogen, the Kjeldahl and the Kjeldahl modified[2] methods were used. The Ulsch[3] and the phenolsulphonic acid[4] methods were used for the determination of nitrates. Of the different methods proposed for the determination of nitrites in the presence of nitrates the method first proposed by Griess[5] and known as the sulphanilic acid and naphthylamine method was selected because of its convenience and delicacy. Ammonia was determined by distillation with soda or magnesia, and titration against standard acid or by nesslerizing. For qualitative tests there were also used metaphenylenediamine for nitrites, and brucine and diphenylamine for nitrates.

For the study of nitrification three soils were used.

1. A calcareous sand containing

0.017 per cent. of total nitrogen,
0.00083 per cent. of nitrate nitrogen,
0.00003 per cent. of nitrite nitrogen.

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[1] *Centr. für. Bact.*, 1901, p. 561.

[2] Wiley's "Agricultural Analysis," Vol. II, 215.

[3] *Chem. Centr.*, **2**, 926 (1890).

[4] Mason's "Examination of Water," p. 44.

[5] *Ztschr. anal. Chem.*, **18**, 597; *Ztschr. angew. Chem.*, (1889), p. 656; *Bull. Soc. Chim.* [3], **2**, 347.

2. A loam soil from the college farm, Ithaca, N. Y., containing

0.201 per cent. of total nitrogen,
0.004 per cent. of nitrate nitrogen,
0.00002 per cent. of nitrite nitrogen.

3. An artificial greenhouse soil rich in organic matter and coal ashes, and containing

0.0139 per cent. of nitrate nitrogen,
0.00008 per cent. of nitrite nitrogen.

The complete extraction of the nitrites and nitrates formed in the soil was one of the first problems encountered. A number of devices for accomplishing the purpose were tried with varying success. The final mode of procedure was as follows: 100 grams of soil were shaken in a 2-liter bottle, provided with a stopper, with 1 liter of distilled water. After shaking for five minutes the liquid holding the nitrites and nitrates in solution was filtered, about a gram of freshly precipitated and washed aluminum cream added to the filtrate and the latter shaken. It was then filtered again, 100 cc., representing one-tenth of the nitrates held in solution, carefully measured off for the determination of nitrates, and 100 cc. for the determination of nitrites.

THE SULPHANILIC ACID AND NAPHTHYLAMINE METHOD.

This is a very delicate method, being capable of distinguishing 1 part of nitrogen as nitrous acid in 1,000,000,000 parts of water. It is easy of manipulation and peculiarly well adapted for the determination of nitrites in soil leachings. The details of manipulation are: 100 cc. of the filtered and charified soil leachings are placed in a colorimeter, 1 cc. each of sulphanilic acid and naphthylamine solutions added, the contents well shaken and allowed to stand for thirty minutes. 100 cc. of distilled water are similarly treated in another colorimeter to which a known amount of standard sodium nitrite is also added. At the end of thirty minutes the depths of color are compared, and the nitrite content in the sample determined.

THE PHENOLSULPHONIC ACID METHOD.

The details of this method as observed in the work reported below are as follows: 100 cc. of the filtered and clarified soil leachings are placed in a 125–150 cc. porcelain evaporating dish, 0.3 cc. of a saturated sodium carbonate solution added, and evaporated to dryness on a water-bath. Two cc. of phenolsulphonic acid are then added to the dry residue, and the latter is thoroughly moistened with it. About 15 cc. of distilled water are then introduced into the dish, and then enough ammonia to render the whole distinctly ammoniacal. The contents of the dish are next washed into a 100 cc. colorimeter, filled up to the mark with distilled water, and the depth of color compared with a standard in another colorimeter. It is important that the standard should be treated in precisely the same way as the unknown, and where chlorine is present in considerable quantities it should be determined and an equivalent quantity added to the standard. The addition of sodium carbonate is necessary where free nitric acid or ammonium nitrate are present, otherwise low results will be obtained. Where many determinations are made it is advisable for the sake of saving time to prepare a standard color solution. This is made as follows: 25 cc. of standard potassium nitrate solution (0.7221 gr. per liter) are treated as described above, the yellow liquid diluted to 1 liter and kept in stock. Every cubic centimeter of this solution corresponds to 0.0025 mg. of nitrogen as nitrate. On the whole the method has been followed as given in Mason's "Examination of Water."

The method as thus employed was eminently satisfactory, and leaves nothing to be desired when the quantities of nitrate present are small, and when care is taken to treat the standard and the unknown in exactly the same way. When, however, the amount of nitrate nitrogen exceeds 25 mg. per liter, great care is required in the readings if accurate results are to be obtained, although with experience close determinations can be made with 40 to 50 mg. of nitrate nitrogen per liter. For solutions containing greater quantities than 50 mg. of nitrate nitrogen per liter the Ulsch method was used. Many difficulties were encountered while working with this method, and about 100 determinations were made of known solutions before results were obtained that were entirely satisfactory. It was found necessary to observe the following precautions to insure accurate results:

1. Blank determinations should be made to allow for the nitrogen in the reagents, especially for that in the iron dust.

2. Five grams of iron should be used to not more than 0.5 gram of potassium nitrate.

3. Nine to ten cc. of sulphuric acid (1 : 1) give the best results.

4. The flask during reduction should be stopped up with a rubber stopper through which passes a funnel tube containing a few pieces of glass moistened with sulphuric acid.

5. The flask during reduction should be heated gradually to boiling, and the solution boiled until the evolution of hydrogen is over.

6. The contents of the flask should be diluted to about 250 cc.

7. Distillation should be carried on not too rapidly for at least forty-five minutes and until 150 cc. of the distillate has been collected. It is best to carry on the distillation nearly to dryness.

8. The greatest care should be taken that no rubber connections are exposed to the hot ammoniacal vapors.

In the first series of investigations the soil was sifted through a 7 mm. mesh sieve to remove pebbles and coarse particles, and about 3 pounds of that soil were placed in each of the 6-inch flower pots used. The latter were placed in porcelain saucers and water was added from below, it having been found from experience that this is the best way of preventing packing. Even with this method of watering it was necessary to stir the soil occasionally. While the experiment lasted the attempt was made to keep the soil at about 25 per cent. of its water content, that is, 3 pounds of air-dry soil to 1 pound of water. It was found, however, that under ordinary conditions it was very difficult to do it.

SERIES A.

No.	Sodium chloride. Gram.	Per acre.		Ammonium sulphate. Gram.
		Per cent.	Pounds.	
1	....	...	...	0.641
2	0.136	0.01	300	0.641
3	0.272	0.02	600	0.641
4	0.544	0.04	1200	0.641
5	1.360	0.1	3000	0.641
6	2.720	0.2	6000	0.641

This series was started on November 16th, and the moisture conditions were kept constant as far as was possible. The soil used was a clayey loam from the college farm. On January 10th, the six pots of the series were leached. The water ran through very slowly, and it took three days for about 1000 cc. of leachings to accumulate. At the end of that time the last portions of the leachings still gave a reaction for nitric acid with brucine, but none with diphenylamine. The color of the leachings indicated that where more salt was used more organic matter was dissolved out of the soil; and, moreover, where the larger quantities of salt were used the water ran through more rapidly. The leachings were then evaporated at 80° to dryness, the residue taken up with about 50 cc. of distilled water, filtered, washed and diluted to 100 cc. One-half of this was used for the determination of ammonia, the other half for the determination of nitrites and nitrates by the Ulsch method. After deducting the ammonia nitrogen and that of the blanks the following quantities of nitrite and nitrate nitrogen were obtained:

SERIES A.

No.	Mg.
1	20.89
2	16.06
3	18.60
4	18.86
5	24.47
6	24.21

In Series C, a coarse calcareous sand was used. Like the loam of Series A, it was sifted through a 7 mm. mesh sieve, and 3.5 pounds of that sifted sand were placed in each pot. In this series it was proposed to study the influence of stirring on nitrification.

SERIES C.

No.	Ammonium sulphate. Gram.	
13	....	Stirred every day.
14	0.641	" " "
15	0.641	" " three days.
16	0.641	" " " "
17	0.641	" " seven "
18	0.641	" " " "

To insure nitrification a quantity of rich garden soil was shaken up with some water, and 5 cc. of the latter were added to each of the pots. The experiment lasted from November 21st until January 26th. At the end of that time the soils were leached, the water running through much more readily than it did in Series A. In a few hours leaching was complete, the last portion giving a very faint reaction with brucine. The leachings were evaporated in large dishes at 80° and treated as in Series A. The quantities of nitrite and nitrate nitrogen obtained by the Ulsch method were:

No.	Mg.
13	traces
14	traces
15	traces
16	traces
17	1.65
18	1.53

In Series D, the same quantities of calcareous sand were used as in Series C. Like the latter it was inoculated with 5 cc. of extract from a rich garden soil. The scheme of experiment was as follows :

SERIES D.

No.	Ammonium sulphate. Gram.	Monopotassium phosphate. Grams.
19	1	0.017
20	1	0.170
21	1	1.700
22	1	0.017
23	1	0.170
24	1	1.700

The experiment began on November 25th, and ended on January 29th. As in Series A and C the Ulsch method was used, and the treatment in determining the nitrogen content of the different pots was exactly the same as in the preceding series. The following quantities of nitrite and nitrate nitrogen were obtained.

No.	Mg.
19	3.06
20	8.15
21	lost
22	5.86
23	2.55
24	4.34

In Series B the effect of sulphur on nitrification was studied. The subject has some practical significance, for quantities up to 400 or 500 pounds per acre have been used to prevent scab on potatoes. The soil used in this series of experiments was the same as in Series A, and, namely, a clay loam from the college farm, connected with Cornell University. The experiment began on November 21st and ended on January 13th. During that time the soils were kept moist, and at about 25 per cent. of water content. In this series the determinations of nitrite and nitrate nitrogen were made by the color methods : the former by the

Griess sulphanilic acid and naphthylamine method and the latter by the phenolsulphonic acid method. For the analytical data 100 grams of soil were taken from each pot after the entire mass had been carefully mixed, placed in a 2-liter bottle, 1 liter of distilled water added, and the contents shaken for five minutes. The water now holding the nitrates in solution was filtered, clarified with aluminum cream, and again filtered. The second filtrate now perfectly clear and colorless was placed in a stoppered flask after 100 cc. each were first drawn off for the determination of nitrates and nitrites respectively.

The scheme of experiment was as follows :

SERIES B.

No.	Sulphur. Per cent.	Sulphur. Gram.	Per acre. Pounds.	Ammonium. sulphate. Gram.
7	....	....	...	0.641
8	0.005	0.068	150	0.641
9	0.01	0.136	300	0.641
10	0.02	0.272	600	0.641
11	0.04	0.544	1200	0.641
12	0.1	1.360	3000	0.641

The quantities of nitrite and nitrate nitrogen found were :

No.	Nitrite. Mg.	Nitrate. Mg.
7	0.31	31.99
8	0.38	24.43
9	0.40	25.24
10	0.36	30.37
11	0.27	22.54
12	0.13	13.50

The results obtained in the above series were highly unsatisfactory. Instead of obtaining a gain in nitrate nitrogen there was a distinct loss. In Series A and B, where loam soil was used, there was present at the beginning of the experiment, as shown by the color method, 0.004 per cent. of nitrate nitrogen, and hence there was present in the 1350 grams of soil used 54 mg. of nitrogen as nitrate. At the end of the experiment the highest quantity of nitrate nitrogen found in any one sample in Series A was 24.47 mg. In other words, there was a loss of almost 30 mg. of nitrate nitrogen, and the results obtained, if they indicate anything at all, show that where sodium chloride was used at the rate of 3,000 and 6,000 pounds to the acre the loss of nitrate nitrogen was diminished. The deoxidation of the nitrates might be ascribed here to denitrifying bacteria, and the fact that more nitrate was found where more sodium chloride was used would indicate that the latter prevented, to some extent, the denitrifying processes.

In Series B there was also a destruction of nitrates, but in this case the loss was the greatest where the greatest quantities of sulphur were used. Thus where sulphur was applied at the rate of 600 pounds to the acre the nitrate nitrogen found at the end of the experiment was 30.37 mg., while in the pots where the application was 1,200 and 3,000 pounds to the acre the nitrate nitrogen found at the end of the experiment was 22.54 mg. and 13.50 mg. respectively. In this case the nitrites and nitrates seemed to correspond to some extent, for in pots 11 and 12, where the least quantities of nitrate were found, there were also found the least quantities of nitrites. It is possible that the oxidation of large quantities of elementary sulphur in the soil may cause the destruction of nitrates without the intervention of micro-organisms. This point will bear further investigation.

In Series C and D, 1800 grams of air-dry soil were placed in each pot, and according to analysis by the color methods this air-dry sand contained 0.00083 per cent. of nitrate nitrogen, and 0.00003 per cent of nitrite nitrogen. In other words, there were present in each pot at the beginning of the experiment 14.94 mg. of nitrate nitrogen and 0.54 mg. of nitrite nitrogen. At the end of the experiments the nitrates and nitrites had practically disappeared in the soils of Series C, for in the samples stirred every day and every three days there were only traces of oxidized nitrogen present, while in the samples stirred once in seven days there was found about 1.5 mg. of oxidized nitrogen.

In Series D, containing the same amount of nitrates and nitrites as Series C, there was also a loss of oxidized nitrogen, except that the disappearance of the nitrates was not as complete as in the other case. The difference can hardly be attributed to either the ammonium sulphate or to the acid potassium phosphate, for there appears to be no correspondence in the quantities of nitrate nitrogen found, and the different amounts of the substances used.

Whatever the value of the results obtained in the above four series, they certainly were not a success as far as the production of nitrates is concerned. Whether the loss of nitrates was due to insufficient aeration, or whether the constant variation in the moisture conditions was the cause of it, is hard to decide at present. It became apparent, however, that some other arrangement would be necessary in order that nitrification might be secured. Valuable data on the subject have been secured by a number of investigators who kept their soils in an atmosphere saturated with moisture. Acting accordingly, it was decided to study nitrification on 100 gram samples of soil placed in 750 cc. Erlenmeyer flasks.

FLASKS.

In Series I, 100 grams of calcareous sand were weighed off and placed in each of the flasks 1–10. The scheme of experiment was as follows:

Flasks.	Ammonium sulphate. Mg.	Water. cc.	Soil extract. cc.
1	100	8	1
2	100	8	1
3	100	9	1
4	100	9	1
5	100	10	1
6	100	10	1
7	100	11	1
8	100	11	1
9	100	12	1
10	100	12	1

The soil extract was obtained by shaking a quantity of rich greenhouse soil, in which nitrification was active, with some distilled water. As will appear from the above table the experiment was carried on in duplicate, and the conditions in the different sets were the same except the moisture content. The latter varied from 8 cc. in 1 and 2 to 12 cc. in 9 and 10. The experiment was begun on February 13th and ended on April 11th, when the contents of all the flasks except that of flask 1 were analyzed. In case of No. 1 the contents of the flask were analyzed on March 5th in order to determine whether nitrification was taking place. During the experiments the flasks were kept in the dark and tightly stoppered, so that no loss of water could take place. The nitrites and nitrates were determined by the color methods and the following amounts found:

	No.	Nitrate nitrogen. Mg.	Nitrite nitrogen. Mg.
March 5th	1	1.37	0.0025
April 11th	2	20.00	0.0124
“ 11th	3	18.91	0.9100
“ 11th	4	18.82	0.4000
“ 11th	5	18.82	0.0258
“ 11th	6	14.27	4.5710
“ 11th	7	15.87	0.0930
“ 11th	8	16.65	0.1600
“ 11th	9	19.80	0.0151
“ 11th	10	19.95	0.0121

The analysis of the soil before nitrification showed it to contain 0.017 per cent. of total nitrogen, 0.00083 per cent. of nitrate nitrogen, and 0.00003 per cent. of nitrite nitrogen. Hence the 100 grams of soil contained, at the beginning of the experiment, 17 mg. of total nitrogen (most of it in fossil remains and practically unavailable for immediate nitrification), 0.83 mg. of nitrate nitrogen, and 0.03 mg. of nitrite nitrogen. To this there were added 100 mg. of ammonium sulphate, containing 21.21 mg. of ammonia nitrogen. It appears, then, that practically all of the ammonia nitrogen was nitrified, and that within the stated limits the different amounts of water affected nitrification but little. Thus from February 13th to March 5th, only 1.37 to 0.83 mg. of nitrate were formed, while from March 5th to April 11th (only thirty-six days), more than 18.5 mg. of ammonia nitrogen were oxidized. Furthermore, the comparatively large amounts of nitrite nitrogen in some cases show that nitrification was still incomplete, or that the nitrates already formed were being reduced again. It will be noticed that in No. 6 where the amount of nitrate nitrogen is the least, the amount of nitrite nitrogen is the greatest, namely, 4.57 mg., an unusually large amount.

On April 30th the soil leachings of this series, that had been kept in stoppered flasks, were again analyzed for nitrates, 100 cc. being taken for each determination as previously. In this case duplicate determinations were made. There was found in :

	Duplicate determinations.		
No.	Mg.	Mg.	Mean Mg.
1	16.80	16.65	16.72
3	18.35	18.72	18.53
4	16.15	16.10	16.12
5	18.52	18.32	18.42
6	12.12	12.25	12.18
7	17.42	17.20	17.31
8	17.30	16.90	17.10
9	15.47	15.62	15.54
10	13.16	13.40	13.28

The liquid in the stoppered flasks remained clear, still it is quite evident that there was a reduction of nitrates between April 11th and April 30th, for in all cases but two (7 and 8) the second analysis showed less nitrates than the first. In 9 and 10 the difference was considerable, and in 5 the difference but slight.

In Series II it was decided to study the effect of sodium chloride on nitrification. One hundred grams of loam from the college farm, containing 201 mg. of total nitrogen and 4 mg. of nitrate nitrogen, were placed in each flask. The latter was kept in the dark and tightly stoppered from February 14th until April 16th. Flask No. 11 cracked when the experiment began, and nitrification in it stopped as soon as the soil became dry. The following is the scheme of the experiment:

Flask.	Ammonium sulphate. Mg.	Water. cc.	Soil extract. cc.	Sodium chloride. Mg.
19	50	11	1	..
20	50	11	1	..
21	50	11	1	10
22	50	11	1	10
23	50	11	1	50
24	50	11	1	50
25	50	11	1	100
11	50	11	1	100

Each flask had also one-half gram of calcium carbonate added to it. When analyzed on April 16th, the following amounts of nitrate and nitrite nitrogen were obtained:

	Nitrate. Mg.	Nitrite. Mg.
19	27.77	0.0154
20	27.82	0.030
21	25.97	0.014
22	25.65	0.015
23	23.88	0.015
24	23.80	0.022
25	22.72	0.030
11	10.67	0.020

As stated above, flask No. 11 was not under the conditions of the experiment, for it lost its moisture long before the experiment was completed. Otherwise the results are uniform, and while there is a distinct diminution in the amount of nitrates produced as the amount of salt is increased, still the differences are not very great, and from this experiment we must conclude that quantities of salt up to 0.1 per cent. or 3,500 pounds to the acre, while they retard nitrification, do not stop it entirely.

In Series III it was proposed to study the influence of ferrous salts on nitrification, since in low-lying lands these salts occur at times in considerable quantities. Ferrous sulphate was selected for the purpose. As in Series II, 100 grams of loam soil were used, and besides the substances mentioned in the table below each flask had also 0.5 gram of calcium carbonate added to it to furnish enough available base for the free acid formed. Following is the scheme of the experiment:

Flask.	Ammonium sulphate. Mg.	Water. cc.	Soil extract. cc.	Ferrous sulphate. Mg.
12	50	11	1	..
13	50	11	1	10
14	50	11	1	10
15	50	11	1	50
16	50	11	1	50
17	50	11	1	100
18	50	11	1	100

The experiment began on February 14th and ended on April 14th. The conditions were the same as those in Series II. Flask No. 16 was found to be badly cracked on February 27th and was, therefore, analyzed on that day. On April 14th the following amounts of nitrate and nitrite nitrogen were found.

No.	Nitrate. Mg.	Nitrite. Mg.
12	28.62	0.012
13	33.32	0.020
14	33.32	0.015
15	25.00	0.045
16[1]	5.00	....
17	25.00	0.023
18	25.00	0.024

There was apparently but little influence of the ferrous salt on nitrification, although the larger quantities used seemed to retard it a little. In the case of flask No. 16 there was scarcely any nitrification in the thirteen days between February 14th and February 27th, which again brings out the fact that the period of incubation lasts for some time.

On May 6th the nitrates were redetermined in Series II, and the following amounts of nitrate nitrogen found:

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[1] Analyzed February 27th.

Flask.	Duplicate determinations. Mg.	Mg.	Mean. Mg.
12	27.55	27.82	27.68
13	28.92	29.07	28.99
14	34.45	34.50	34.42
15	29.17	29.22	29.19
16	....	....	....
17	30.87	31.30	31.08
18	34.17	34.01	34.09

The general tendency in this case seems to have been a gain in nitric nitrogen. As it is, the amounts of ferrous sulphate used had not the power to stop nitrification.

In Series V equivalent quantities of calcium carbonate and calcium sulphate were compared as to their influence on nitrification. The scheme of experiment was as follows:

Flask.	Water. cc.	Soil extract. cc.	Ammonium sulphate. Mg.	Calcium carbonate. Grams.
28	13	1	100	0.5
29	13	1	100	0.5
36	13	1	100	1
37	13	1	100	1
38	13	1	100	2
39	13	1	100	2
				Calcium sulphate.
40	13	1	100	0.7555
41	13	1	100	0.7555
42	13	1	100	1.511
43	13	1	100	1.511
44	13	1	100	2.2665
45	13	1	100	2.2665

The experiment lasted from February 20th until April 20th, the conditions being the same as in the other series. It was intended to compare flasks Nos. 28 and 29 with the flasks of this series, since the conditions were the same. The amounts of nitrate and nitrite nitrogen were as follows:

Flask.	Nitrate, duplicate determinations. Mg.	Mg.	Mean. Mg.	Nitrite. Mg.
28	32.77	....	32.77	0.035
29	38.81	....	38.81	0.033
36	27.60	27.92	27.76	0.054
37	42.05	41.72	41.88	0.038
38	31.55	32.07	31.81	0.049
39	20.27	19.90	20.08	0.039
40	19.30	19.55	19.42	0.049
41	18.72	18.72	18.72	0.036
42	16.82	16.82	16.82	0.024
43	19.52	19.98	19.75	0.035
44	20.62	20.35	20.48	0.040
45	18.52	18.22	18.37	0.036

Where equivalent amounts of gypsum were used the nitrification was, on the whole, fairly uniform, and slighter than in the flasks where calcium carbonate was used. The agreement among the latter is not, however, all that might be expected. Thus, for instance, there were only 27.76 mg. of nitrate nitrogen found in flask No. 36, while in flask No. 37, treated in exactly the same way, there were found 41.88 mg. of nitrogen as nitrate. What the difference is due to is difficult to decide with certainty, although in dealing with micro-organisms such striking, and apparently unexplained, differences occur. On May 2nd, flasks Nos. 37 and 38 were again analyzed for nitrates, and the following amounts found.

Flask.	Duplicate determinations. Mg.	 Mg.	Mean. Mg.
37	48.07	47.65	47.86
38	36.98	37.04	37.01

In flask No. 37 there was a gain of from 41.88 to 47.86, almost 6 mg.; in flask No. 38 there was a gain of from 31.81 to 37.01 mg., or more than 5 mg. of nitric nitrogen.

In Series VI it was proposed to study the relation of large quantities of organic matter to nitrification. Linseed meal, containing, as analyzed by the Kjeldahl method, 4.51 per cent. of nitrogen, was used for the purpose. The following is the scheme of the experiment:

Flask.	Water. cc.	Soil extract. cc.	Calcium carbonate. Gram.	Linseed meal. Grams.
26	13	1	0.5	..
27	13	1	0.5	..
46	13	1	0.5	0.5
47	13	1	0.5	0.5
48	13	1	0.5	1.0
49	13	1	0.5	1.0
50	13	1	0.5	3.0
51	13	1	0.2	3.0

Flasks Nos. 26 and 27 being carried on under the same conditions as the flasks of Series VI, it was intended to place them also as blanks in Series VI. As reported elsewhere, however, they were cracked at the beginning of the experiment. The experiment in question lasted from February 20th until April 21st. Analyses for nitrate and nitrite nitrogen showed:

	Nitrate, duplicate determinations.			
Flask.	Mg.	Mg.	Mean Mg.	Nitrite. Mg.
46	31.42	32.55	31.83	0.046
47	29.52	29.44	29.48	0.053
48	38.57	39.17	38.87	0.051
49	33.70	33.94	33.82	0.071
50	....	....	0.14	0.010
51	....	....	0.0013	0.002

It appears that more nitrate was formed where 1 per cent. of organic matter was present than where only 0.5 per cent. was present. On the other hand, but traces of nitrates were found at the end of the experiment in flasks Nos. 50 and 51, where 3 per cent. of organic matter was used.

RAPID METHOD FOR THE VOLUMETRIC DETERMINATION OF MOLYBDENUM STEEL.

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WEIGH 0.5 gram of sample in a platinum crucible of about 100 cc. capacity, adding 2 cc. of sulphuric acid (sp. gr. 1.58), and 12 cc. of water. When thoroughly dissolved, which may be assisted by heating, evaporate over the Bunsen burner until white fumes are given off, cool, add 30 grams fused potassium hydrogen sulphate, and gently raise the temperature to a bright heat, holding it at this temperature until the sample is thoroughly fused, which usually will require ten to fifteen minutes.

Care is required in both evaporating and fusing the sample that no particles be carried over the top of the crucible, by the water being driven off too swiftly and thereby causing a spattering, or by fusing too briskly, the gas given off by the potassium hydrogen sulphate either carrying particles away, or causing the fusion to foam over the top.

When thoroughly fused, run the fusion around the side of the crucible and allow to cool. When cold, place the crucible and fusion in a No. 5 beaker containing 500 cc. of *hot* water, and keep it nearly boiling until the fusion is dissolved and the solution has become transparent; then wash the crucible with water into the beaker containing the solution.

Cool the solution to normal temperature and transfer to a liter flask, rinsing the beaker with water, and add 100 cc. ammonia