

XLIX.—*On Nitrification.* (Part II.)

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BEFORE describing the investigations made in the Rothamsted Laboratory since the last communication to the Society (*Trans. Chem. Soc.*, 1878, 44), it may be well to glance at the results recently obtained by other chemists.

It appears that A. Müller (*Land. Versuchs-Stat.*, **16**, 273; *Chem. Soc. J.*, 1873, 1267) was the first to advance the opinion that nitrification is due to the action of a ferment. He observed that pure solutions of ammonium salts or of urea were remarkably stable, while the ammonia of sewage and of certain well-waters rapidly changed into nitric acid. He suggests that the latter solutions probably contain a ferment which effects the transformation.

Two additional papers by Schloesing and Müntz require notice. The first (*Compt. rend.*, **85**, 1018; *Abstracts Chem. Soc.*, 1878, 163) adds another proof to those which they had before advanced, that nitrification is due to an organized ferment. They find that exposure for one hour to a temperature of 100° is sufficient to destroy the power of

nitrification. Soils thus treated were exposed to a current of air, purified by ignition, without nitrification taking place; the addition of a little unheated mould was sufficient to cause nitrification to recommence. The second paper (*Compt. rend.*, **86**, 892; *Abstracts Chem. Soc.*, 1878, 597) describes attempts to produce nitrification by seeding suitable mixtures with various moulds and mycoderms. They employed for this purpose *Penicillium glaucum*, *Aspergillus niger*, *Mucor mucedo* and *racemosus*, and *Mycoderma vini* and *aceti*. In no instance did they obtain nitrification. These low organisms are shown to feed on ammonia and nitric acid, and to convert their nitrogen into organic substance. It is stated that a growth of mould has been long known as injurious to the productiveness of a nitre-bed.

Experiments confirming the ferment theory of nitrification have also been published by F. Storer and others, but without any new light being thrown on the subject.

In my previous communication it was shown that weak solutions of ammonium chloride, containing small quantities of tartrate and phosphate of potassium, with an excess of calcium carbonate, could be readily nitrified by seeding them with a few drops of another solution which had already undergone nitrification. Nitrification being thus producible at will, an excellent opportunity seemed to be presented of studying the conditions under which it took place. Experiments in solutions appeared also to afford a simple and accurate mode of investigation: for though oxidation is probably far slower in a solution than in a porous soil, the solution has the great advantage of being readily made of any composition desired, and the changes which take place in it can be easily ascertained from time to time.

The primary objects of the present experiments were to ascertain the influence of light and temperature, and of variations in the composition and concentration of the solutions, on the process of nitrification. The attempt was also made to study the rate at which nitrification progresses, and the relation of the nitric acid produced to the ammonia consumed. Other points of interest have also been dealt with. The whole work is as yet confessedly imperfect. It appeared, however, better to publish the results already obtained than to wait till a complete answer could be given to these questions, especially as many of the results are now considerably more than a year old.

It must be borne in mind, while following the ensuing account, that several series of experiments were simultaneously in progress; the experience gained in one series could not therefore always be made use of in another series. In giving the history of the solutions, only the more important dates are mentioned, some intermediate qualitative testings being omitted. A summary of the conclusions arrived at will be given at the end of the paper.

First Series. Bottles A.

This series consisted of four bottles, the contents and behaviour of which have been described down to December 19, 1877, in the first report of these experiments.

The bottle AL2, which had been twice seeded with soil, and freely exposed to light, remained unnitrified after more than eleven months, when the experiment was concluded. A green growth had formed in this bottle. It will be recollected that the corresponding bottle kept in darkness (AD2) completely nitrified, and that from this bottle the two remaining bottles, AL and AD, were seeded.

Bottle AD, kept in darkness, had commenced nitrification when examined 22 days after seeding, and nitrification was completed by December 19. The corresponding bottle, placed in the window (AL), contained no nitric acid 49 days after seeding: no green growth was visible. On December 21 this bottle was placed in the dark cupboard and seeded afresh, by adding 1 c.c. from AD. Examined on February 21, the ammonia originally present was found to be completely nitrified.

Solution AD is the only one in this series in which the course of nitrification was regularly followed. The results obtained from the date of seeding to that of complete nitrification were as under.

Date.	Interval.	Nitrogen as nitric acid.	Nitrogen daily oxidised.
1877.			
October 15	—	per million.	per million.
November 6	22 days.	—	—
„ 19	13 „	0·7	—
December 3	14 „	2·0	0·10
„ 19	16 „	11·9	0·71
		20·1	0·51
Mean rate of action during 43 days			0·45

Seeding was thus followed by a period of inaction, which we may perhaps style the period of incubation. This is succeeded by a period of action, in which the activity increases up to a certain stage, and then diminishes again as the nitrification approaches completion. This mode of procedure will be found to hold good for all the subsequent examples of nitrification which have been fully studied.

In these first experiments the nitrifying germ was obtained from the soil of a fairy ring; no further recourse has been had to this original source of the ferment, all subsequent solutions being seeded from bottles AD or AL, or from some of their descendants.

In this series of experiments the effect of light is absolute in preventing nitrification.

Second Series. Bottles B.

The primary idea with which this series commenced was to ascertain whether the mycelium from a fairy ring would act as a nitrifying ferment. Eight wide-mouthed stoppered bottles of pale green glass were filled to the shoulder with a solution containing chloride of ammonium equal to 20·6 milligrams of nitrogen per litre, with a little sulphate of potassium; a small quantity of dry precipitated phosphate of calcium was also added to each bottle. Four of the bottles then received acid tartrate of potassium equal to 200 milligrams per litre. Four were also seeded with a fragment of mycelium, weighing in each case three- or four-tenths of a milligram. The mycelium was part of a stout sheet lying among the grass roots upon the surface of the turf. Heated on platinum foil it blackened, and evolved an odour of burning horn. The bottles now contained as follows:—

BD and BL,	general solution.	
BD2 and BL2,	„	and tartrate.
BD3 and BL3,	„	and mycelium.
BD4 and BL4,	„	tartrate and mycelium.

The BL's were placed on October 16, 1877, in an east window, and the BD's in a dark cupboard beneath the same window.

On November 5 a growth of mould was noticed in each of the bottles containing tartrate.

On November 9 some precipitated carbonate of calcium was added to bottles BD2 and BL2, and on November 19 the same was added to BD3 and BL3.

The solutions were tested from time to time up to December 19, but no nitric acid was found; all the bottles were then seeded with 1 c.c. of the nitrified solution AD; the solution was shaken before the portions used for seed were taken.

On January 10, 1878, nitrification was found to have commenced in BD2 and BD4, most progress having taken place in the first named.

On February 21 the nitrification of BD2 was found to be completed, all ammonia having disappeared. The solution, when examined by the indigo method, yielded 18·7 parts of nitrogen as nitrates per million.

On March 20 nitrification having made very little progress in BD4, some precipitated carbonate of calcium was added to the solution; the solution at this date contained a considerable amount of nitrous acid.

On April 24 the nitrification of BD4 was found completed; neither ammonia nor nitrous acid was present. The nitric acid found equalled 19·1 parts of nitrogen per million.

Solution BD3 contained on February 21 no nitric acid, but when examined on June 25 it was found to be completely nitrified. BD has remained unnitrified during 16 months.

The bottles placed in the window were neglected, it being too hastily assumed that they would not nitrify. On June 25, it was found that BL2 had nearly finished nitrification; a trace of ammonia and a little nitrous acid were present. A small green growth covered the surface of the glass. In BL3 nitrification had begun; but here both ammonia and nitrous acid were in considerable quantity. The solutions were not again examined for ten months, at the end of which time no ammonia could be detected in BL3, but a considerable amount of nitrous acid was present. BL and BL4 have remained quite unnitrified.

The conclusions to be drawn from these experiments are important. In the first place, the mould which develops in a solution containing tartrates is shown to be incapable of effecting nitrification. In four of the bottles this growth of mould appeared; two of the bottles were kept in the dark 44 days, and one of these contained an excess of carbonate of calcium during the last 40 days, but no trace of nitric acid was produced. The ineffectiveness of the mycelium from the fairy-ring is less certainly proved, for in no case was it used in conjunction with carbonate of calcium. One bottle, however, was supplied with tartrate of potassium, and kept in darkness 64 days, and in this case a little nitric acid should have been found if the mycelium had the power of acting as a nitrifying ferment.

Turning to the results obtained after seeding with a ferment of known efficacy, we see that in no case is nitrification completed except where carbonate of calcium has been present. The bottle (BD) containing chloride of ammonium solution, supplied with phosphate of calcium and phosphate of potassium, contained only a trace of nitric acid after 16 months, though kept in darkness, and seeded with an active nitrifying ferment. When, in addition to these ingredients a little acid tartrate of potassium was supplied, a small amount of nitrification took place. Bottles BD2 and BD4 both contained tartrate of potassium, but only the first named contained carbonate of calcium when the seed was added. The ammonia in this bottle was found completely nitrified 64 days after seeding, while in BD4 but little nitrification had taken place in 84 days, and no progress had apparently been made during the last 20 days. When carbonate of calcium was added to this bottle nitrification became speedy, and was soon accomplished. The small amount of nitrification which took place before the addition of the carbonate of calcium was probably due to the decomposition of the organic potassium salt, which would produce a little carbonate of potassium serving the same function as the calcium

carbonate. I have elsewhere shown (*Chem. Soc. J.*, 1875, 968) that the growth of mould in a solution of acid tartrate of potassium results in a feebly alkaline solution.

Another point which comes out in a striking manner is the very small amount of organic carbon which is requisite for nitrification. Bottles BD3 and BL3 contained only the carbonaceous matter supplied by the minute quantity of mycelium, and by the small amount of seed solution, containing tartrates, introduced, with of course the minute quantity supplied by the distilled water and atmosphere. This quantity proves, however, sufficient to effect nitrification, though a very long period is occupied in its completion. Bottle BD3 showed no nitric acid 64 days after seeding; tested again in summer time, 124 days later, nitrification was found to be complete. At this last date BL 3 still contained much ammonia, but 10 months afterwards the ammonia had disappeared, though much nitrous acid was still present.

The influence of light in this series was not an absolute prevention of nitrification; the contents of two bottles nitrified, though exposed to light. Unfortunately the date when nitrification commenced is unknown, but in the case of BL3 it is evident that nitrification proceeded much more slowly than in the corresponding bottle kept in darkness.

Third Series. Bottles C.

This series consisted only of two bottles; it was designed to show more plainly the importance of a salifiable base for nitrification. Tartrates had generally been employed in the preparation of solutions for nitrification; they possess the great advantage of being without influence on the determination of nitric acid with indigo. Such salts could not, however, be employed without the introduction of some salifiable base, as the consumption of the tartaric acid by an organism must result in the formation of an alkaline carbonate. In the present case cane-sugar was used in place of a tartrate. The solution contained in 1 litre chloride of ammonium equal to 20·5 milligrams of nitrogen, 50 milligrams of cane-sugar, and a very little phosphate of potassium, gypsum, and sulphate of magnesium. Bottles C and C2 were partly filled with this solution; to the latter bottle some precipitated carbonate of calcium was added. Both bottles were seeded on December 21, 1877, with 1 c.c. from AD, and were then placed in a dark cupboard.

The action was very slow in commencing. On January 10, 1878, no nitric acid was found in either bottle. On February 21, nitrification was found to have commenced in C2, but no change was observed in C. On March 23 the nitrification in C2 was almost completed,

very little ammonia was present, and no nitrous acid. At the same date C contained a trace of nitrous acid. On March 27 the nitrification of C2 was found to be completed.

The experiment in bottle C was allowed to go on till April 18, at which time a small quantity of nitrous acid was present, but no significant amount of nitrification had taken place. Precipitated carbonate of calcium was now added to the contents of the bottle. On May 3 nitrification had considerably advanced, and on May 25 no ammonia was present.

A marked difference was observed between C2 and C respecting the production of nitrous acid. C2, which had carbonate of calcium from the first, seems to have produced no nitrous acid; while in the case of C the nitrification which occurred after the late addition of carbonate of calcium was wholly nitrous. On May 16 the nitrogen present as nitrous acid amounted to 18·1 per million. After nitrification was completed the nitrous acid gradually diminished in quantity, and on June 17 none was found. A similar, but less marked production of nitrous acid, was observed in the case of BD4 in the preceding series, in which, as in the present instance, nitrification was commenced in the absence of calcium carbonate.

In the case of C2 the course of nitrification was as follows:—

Date.	Interval.	Nitrogen as nitric acid.	Nitrogen daily oxidised.
		per million.	per million.
1877.			
December 21.....	—	—	—
1878.			
February 21.....	62 days.	1·1	—
March 12.....	19 „	13·1	0·63
„ 27.....	15 „	18·7	0·37
Mean rate of action during 34 days			0·52

The rate of nitrification is here very similar to that shown by solution AD.

The fact that the presence of a salifiable base is necessary for nitrification, which the results of both this and the preceding series of experiments illustrate, is one of great practical importance. It helps to explain why peat-waters, and soft waters generally, are usually free from nitrates. It throws new light on the well-known value of lime as a dressing for many soils, and especially on its importance in reclaiming peat-land, for which purpose lime is absolutely essential. When we understand that the nitrogenous humic matter of soils, which is apparently useless as plant food, can only be oxidised into

nitrate in the presence of a salifiable base, the function of chalk and lime in agriculture becomes better understood. Peat soils entirely consist of such nitrogenous humic matter, and till lime is applied it is probable that nitrification never takes place.

Fourth Series. Bottles D—G.

In this, the most extensive series among the present experiments, the design was—1. To ascertain the influence of temperature, by conducting the nitrification in baths the temperature of which was invariable. 2. To ascertain the effect of concentration of the solution, by nitrifying solutions of graduated strengths. 3. To determine the minimum proportion of organic carbon necessary for the nitrification of ammonia. 4. To study the rate of nitrification at fixed temperatures by repeated quantitative analyses of the solutions.

The composition of the solutions employed will be gathered from the following table. The figures represent milligrams per litre; or, in other words, parts per million :—

Name of bottle.	Chloride of ammonium.	Rochelle salt.	Phosphate of potassium.	Sulphate of magnesium.
D	80	150	20	5
D2	80	150	20	5
D3	640	1200	160	10
D4	640	1200	160	10
E	80	150	20	5
E2	640	300	40	5
E3	640	600	80	5
E4	640	1200	160	10
F	80	150	20	5
F2	160	300	40	5
F3	320	600	80	5
F4	640	1200	160	10
G	80	150	20	5
G2	640	1200	160	10

It was intended that the D solutions should be kept at the temperature of the air; the E solutions at 20°; the F solutions at 30°; the G solutions at 40°. Two of the solutions employed, namely, the weakest and strongest, formed part of each of the sub-series; the behaviour of the same solution at different temperatures could thus be studied. In sub-series E there was a graduated proportion of organic carbon (tar-

taric acid) to ammonia. In sub-series F there was a graduation in the concentration of the solutions employed.

The solutions were placed in wide-mouthed pint bottles, filled to the shoulder. The bottles were all supplied with precipitated carbonate of calcium, and seeded with 1 c.c. from bottle AD on March 1, 1878. Those bottles which were to be kept at fixed temperatures were then placed in water-baths, maintained at a nearly uniform heat by means of a gas regulator immersed in the water. The regulator first employed was the ordinary one acting by the expansion of air; this was afterwards changed for Page's mercurial regulator, which proved far more safe and accurate. The bottles left at the temperature of the air were placed in a cool cupboard, the temperature of which was daily ascertained. All the bottles were protected from light.

The solutions were thoughtlessly seeded on a day when some hay was being moved in the laboratory. By March 5 all the stronger solutions in the bath had become more or less turbid from the growth of bacteria, the greatest turbidity being in E3 and F3. On March 12 the turbidity was found to have increased, and had extended to all the bottles. The turbidity was greater in proportion to the strength of the solutions. Thus in sub-series E and F the turbidity followed the order of the numbers, being greatest in E4 and F4. These two solutions, together with G2, had now a considerable film on the surface, and smelt like a rotten turnip.

On March 20 samples from F and E4 were sent to Professor Dyer, who kindly submitted them to a microscopical examination. E4 was reported as swarming with bacteria; F as containing some of these organisms. On April 1 the turbidity of the bottles in the baths had generally diminished, and continued to do so from this date. Bottles D3 and D4 were now the most turbid in the series, and held this position to the end of the experiment. Bottle G was remarkably clear.

In order to renew the air in the bottles the stoppers were removed for a few moments two or three times a week; the bottles were also necessarily opened from time to time when taking samples for examination; the possible entrance of germs was thus unavoidable.

Nitrification was not detected in any solution on March 5.

On March 12 nitrification was found to have made distinct progress in F, standing in the bath at 30°; no other solution showed change.

On March 18 only a trace of ammonia remained in F. Nitrification was found to have commenced in F2 and F3; also in E and E2, standing in the bath at 20°.

On March 20 the solution in bottle F4 showed a distinct amount of nitrous acid when acidified with sulphuric acid, and tested with a drop of starch solution containing iodide of potassium. Bottle E3 also contained a little nitrous acid.

On March 23 very little ammonia was found in E, and on March 25 nitrification was complete. Nitrification had now made rapid strides in E2, F3, F2, E3, and F4, the progress being apparently in the order named.

On March 27 nitrous acid was distinctly found in E4. All the solutions in the baths kept at 20° and 30° had now commenced nitrification, and the two weakest solutions had completely nitrified; no start had, however, been made in the bottles at 40°, or in those at the temperature of the air.

In examining these solutions with indigo a great disappointment was experienced. It was intended to study the varying intensity of nitrification from the beginning to the end of the process by means of frequent quantitative determinations with indigo. On examining solution F, after all ammonia had disappeared, a novel and very unsatisfactory reaction was obtained. On pouring the oil of vitriol into the mixture of solution and indigo, the action on the indigo was immediate, instead of occupying, as usual, an appreciable time. There was also no sharpness in the result, quantities of indigo distinctly different giving nearly the same final tint. The maximum quantity of indigo used was also far below that required if the ammonia which had disappeared had been converted into nitric acid. The explanation of these reactions was soon found; nitrous acid instead of nitric had been produced, and was the cause of these abnormal results. As the indigo method clearly failed in the presence of nitrous acid, the study of the course of nitrification was greatly interfered with.

Attention was now turned to the determination of nitrous acid by solution of permanganate of potassium. It was found that the solutions of tartrates, &c., employed for nitrification did not decolorise permanganate, even when acidified with sulphuric acid. Solutions which had nitrified, but which contained no nitrites, had also no effect on permanganate. It seemed, therefore, feasible to determine the quantity of nitrous acid formed by the use of this reagent. The solutions of permanganate employed were standardised from time to time with crystallised sulphate of iron and ammonium; 56 parts of this salt correspond to 1 part of nitrogen as nitrous acid. 1 c.c. of the permanganate used generally equalled about .00015 gram of nitrogen. The mode of determining was to place 10 c.c. of the solution in a small beaker, add some permanganate, then a few drops of dilute sulphuric acid, and then, as the colour disappeared, a further quantity of permanganate, till the tint produced by one drop was not wholly discharged in five minutes.

On April 2 solution F2 was found completely nitrified; abundance of nitrous acid was present. At this date G and G2 were seeded afresh; the first with 1 c.c. from Bottle F; the second with 1 c.c. from F4.

On April 6 a sad accident happened to the F bath (30°); an india-rubber of the gas regulator burst, and the temperature was found as high as 55° . Bottles F and F2 had been taken out of the bath when nitrification was completed, and escaped this disaster. The temperature series being thus broken, the E bath was raised from 20° to 30° , the latter temperature being thought more favourable to nitrification.

On April 8 signs of nitrification appeared for the first time in D and D2, the weak solutions which had been placed in the cool cupboard.

On April 24, no nitrification having appeared during 54 days in bottles G and G2 kept at 40° , the temperature of the bath was lowered to 30° .

On April 30 nitrification was found to have made considerable progress in bottles D and D2; and in striking contrast to the bottles in the baths, the nitrification which occurred was purely nitric, especially in the case of D, which gave a trace of nitrous acid only at the first starting of the oxidation. Solution D now contained, by experiment with indigo, 10.1 of nitrogen per million in the form of nitric acid, and solution D2, 6.4 parts. In all previous experiments on the effect of light the solutions had been exposed when freshly seeded, and before nitrification had become active; it seemed possible that a solution rapidly nitrifying might be better able to withstand the prejudicial influence of light than one in which the ferment was small in quantity, and had not yet entered on its active phase of existence. At this date bottle D, henceforth called DL, was placed in a south window, and at once exposed to bright sunshine; a similar bottle filled with water, and provided with a maximum thermometer, was placed beside it to mark the highest temperature to which it was exposed. Bottle D2 remained in the cool cupboard.

On May 15 nitrification was completed in D2; no nitrous acid was present. Analysed by indigo it gave 20.0 parts of nitrogen per million existing as nitric acid. DL when examined with indigo was found to have made no advance; it decolorised the same amount of indigo as on April 30th. A trace of nitrous acid was present. The maximum thermometer showed that the temperature had once reached 35° ; this temperature was attained on May 12. The prejudicial effect of light is strikingly shown by this experiment.

Bottle DL was now placed in a dark bath at 30° , with a view to discover whether the nitrifying germs had been killed by exposure to light. On June 3 nitrification was found to be complete; a large amount of nitrous acid was present. On June 17 a determination with permanganate showed the quantity of nitrous acid to equal 9.0 of nitrogen per million; the nitrification in the bath had thus been wholly nitrous.

The bottle was kept in the bath, and on July 8 the nitrous acid was found to have disappeared.

On May 18, nitrification was first observed in solutions D3 and D4, the strong solutions in the cool cupboard; nitrous acid was present in each case. At the same date G and G2 were seeded for the third time, 1 c.c. from bottle AL being added to each. The ferment had apparently been killed in these solutions by long exposure to the temperature of 40°; this is at least the natural conclusion from the fact that they had remained 24 days at the favourable temperature of 30° without nitrification taking place.

On May 25 nitrification was found to be complete in the case of E3 and E4, nitrous acid had also disappeared. In E2 ammonia was still abundant.

On May 30 nitrification had commenced both in G and G2; most progress had been made in the former.

On June 7 solution E2 was found to be completely nitrified; nitrous acid as well as ammonia had disappeared.

On June 17 nitrification was completed in G; only a trace of nitrous acid was present.

On July 30 solution G2 had completely nitrified; nitrous acid was absent.

On October 4 solution D3 was found to be completely nitrified; nitrous acid had disappeared. In the duplicate bottle D4, a large quantity of nitrous acid and a little ammonia were present.

On October 15 no ammonia was found in D4; nitrous acid was still large.

The history of all the seeded bottles has now been sketched; two bottles which remained unseeded require a short notice. The solutions in question were the duplicates of F2 and E3. They were placed in new bottles, which had been simply rinsed with distilled water, but not chemically cleaned and heated in the water-bath, as was the case with the other bottles used in this series. The solutions also were not heated before being placed in the bottles. The bottles were preserved in a dark cupboard.

On March 9 some carbonate of calcium was added to F2', but none to E3'.

On May 25 no ammonia was found in F2', but a considerable amount of nitrous acid was present. Nitrification was thus completed in 85 days, without artificial seeding.

On May 6, 1879, bottle E3' was examined. It contained nitric acid equal to 30.8 of nitrogen per million; no nitrous acid was present. Rather less than $\frac{1}{5}$ th of the ammonia present had thus nitrified in 14 months without artificial seeding, and in the absence of lime, tartrate of potassium was however present.

We have now completed the diary of these experiments, and must next attempt to summarise their teaching.

One of the objects with which the experiments were planned was to ascertain how strong a solution of chloride of ammonium could be nitrified; this object was not attained, as the strongest solution used (640 milligrams per litre) was completely nitrified.

It has been made tolerably evident that bacteria are incapable of producing nitrification. Bacteria occurred in bottle G, and swarmed in G2, yet these two bottles were maintained 54 days at 40°, and 24 days at 30°, without nitrification commencing. Bacteria were also extremely abundant in bottles D3 and D4; yet these solutions remained 78 days at a mean temperature of 10° before nitrification commenced. These experiments at least prove that bacteria may flourish in ammoniacal solutions of favourable composition without producing nitrification.

Some light has also been thrown on the limits of temperature at which nitrification is possible. It has been shown that in the case of solutions the temperature must be below 40°; but the maximum temperature at which nitrification will take place has not been ascertained. The record of temperature also affords evidence that nitrification will proceed with some briskness at 10°, and probably at still lower temperatures.

The effect of different temperatures on the duration of the two periods of incubation and of action, in the case of solutions varying both in concentration and composition, is shown by the following tables. The periods observed in some of the earlier experiments are given together with the more recent results.

Periods of Incubation.

Approximate strength of solutions. Milligrams NH ₄ Cl per litre.	Temperature 7°-10°.		Temperature 20°.		Temperature 30°.	
	Bottle.	Days.	Bottle.	Days.	Bottle.	Days.
80	AD	22*	E	17	F	11*
	D	31	—	—	G	12*
	D2	38	—	—	—	—
160	—	—	—	—	F2	17
320	—	—	—	—	F3	17*
640	D3	78	E 2	17*	F4	19
	D4	78	E 3	19	—	—
	—	—	E 4	26	—	—

The periods marked with an asterisk are known to be somewhat

longer than the truth, nitrification having made some little progress before its commencement was observed.

With solutions of the same strength and composition—as the weak solutions D, D2, E, F, and G, or the strong solutions D3, D4, E4, and F4,—the period of incubation is considerably shortened by a rise in temperature. The extreme periods observed with the weak solutions are 38 days in the case of D2 (mean temperature 7°·6), and about 10 days in the case of F (temperature 30°). With the strong solutions the extremes are 78 days in the case of D3 and D4 (mean temperature 10°), and 19 days in the case of F4 (temperature 30°). The period of incubation is distinctly shorter at 30° than at 20°, as we see by comparing E with F, and E4 with F4.

As the solutions become stronger the period of incubation evidently tends to lengthen.

Periods of Action.

Approximate strength of solutions. Milligrams NH ₄ Cl per litre.	Temperature 10°-16°.		Temperature 20°.		Temperature 30°.	
	Bottle.	Days.	Bottle.	Days.	Bottle.	Days.
80.....	AD	43	E	7	F	7†
	BD2	42	—	—	—	—
	C	37	—	—	—	—
	C2	34	—	—	—	—
	D2	37	—	—	—	—
160.....	—	—	—	—	F2	15*
640.....	D3	139	—	—	G2	61
	D4	147	—	—	E2	81
	—	—	—	—	E3	66
	—	—	—	—	E4	59

Some of these periods are probably somewhat longer than the truth, nitrification having ceased before the fact was ascertained. On the other hand the period marked with a dagger is too short, as a part of the time accredited to incubation was really taken up by action.

The period of action becomes much shorter as the temperature rises; but it is not clear that in this respect 30° gives a better result than 20°, the only comparative experiment being rather in favour of the lower temperature. The extreme periods in the case of weak solutions of the same composition were 37 days in the case of D2 (mean temperature 12°·0), and 7 days in the case of E (temperature 20°). With the strong solutions we have 147 days for D4 (mean temperature 16°·2), and 61 days for G2 (temperature 30°).

The period of action in solutions of different strengths maintained

at the same temperature varies apparently pretty directly with their degree of concentration. Thus nitrification in F occupied about 8 days; in F2, a solution of twice the strength, it occupies 15 days, or perhaps rather less; while in G2 and E4, eight times the strength of F, the period is 61 and 59 days. It must be recollected that in the case of E2, E3, and E4, the first 19 days of nitrification took place at 20°, and not at 30°.

The series E2, E3, and E4 comprises the solutions containing an increasing proportion of Rochelle salt and phosphate of potassium. The solution containing the smallest proportion of these salts (E2) is the first to commence nitrification, while that containing the largest proportion (E4) is the last to make a start; the presence of saline matter thus apparently hinders the commencement of nitrification. The period of action follows, however, a reverse order, being shortest in E4 (59 days), where the supply of organic carbon and phosphates was largest, and longest in E2 (81 days), where these necessary constituents were present in one-fourth the quantity.

The proportion of organic carbon to nitrogen in E2 is approximately as 3 : 10; this is evidently sufficient for the requirements of nitrification. The minimum proportion of carbon necessary for nitrification has not yet been determined. The proportion present in the case of experiments BD3 and BL3 was probably far smaller than in E2.

We next turn to the evidence afforded by these experiments as to the rate at which nitrification proceeds in different stages of its progress, and the different character it assumes under different circumstances. To do this it will be necessary to tabulate the results of the periodical examinations of the solutions most thoroughly studied.

Bottle.	Date.	Interval.	Tem- perature.	Nitrogen per million			
				As ammonia.	As nitrous acid.	As nitric acid.	Daily oxidised.
D2 ..	1878. March 1	—	—	20·8	—	—	—
	April 8	38 days	6°·9	abundant	trace	trace	—
	" 30	22 "	10°·0	abundant	none	6·4	0·30
	May 15	15 "	13°·2	none	none	20·0	0·91
Mean rate of action during 37 days at 11°·3							0·54

Bottle	Date.	Interval.	Tem- perature.	Nitrogen per million			
				As ammonia.	As nitrous acid.	As nitric acid.	Daily oxidised.
D3 ..	March 1	—	—	166·9	—	—	—
	May 18	78 days	9°·3	abundant	considerable	—	—
	June 17	30 "	13°·0	"	24·5	—	0·82
	July 8	21 "	17°·5	"	50·8	—	1·25
	" 29	21 "	17°·8	"	76·8	—	1·24
	Sept. 5	38 "	17°·0	"	93·4	—	0·44
	" 19	14 "	15°·5	"	5·2	—	—
	Oct. 4	15 "	13°·0	none	none	143·7	—
Mean rate of action during 139 days at 15°·8.....							1·03
D4 ..	March 1	—	—	166·9	—	—	—
	May 18	78 days	9°·3	abundant	trace	—	—
	June 17	30 "	13°·0	"	16·2	—	0·54
	July 8	21 "	17°·5	"	43·4	—	1·30
	" 29	21 "	17°·8	"	73·4	—	1·43
	Sept. 5	38 "	17°·0	"	108·3	—	0·92
	" 19	14 "	15°·5	"	120·6	—	0·88
	Oct. 4	15 "	13°·0	distinct	116·2	—	—
	" 12	8 "	15°·3	none	100·4	—	—
	" 15	3 "	} 9°·1	"	79·4	—	—
	Nov. 4	20 "		"	none	158·0	—
Mean rate of action during 147 days at 15°·7							1·07

We have here three examples of ammoniacal solutions nitrified at the temperature of the air. The first is a weak solution; the last two are duplicates of eight times greater strength.

In the case of the weak solution the character of the nitrification is purely nitric, no nitrous acid being formed except a minute quantity at the commencement. The same result was obtained in all the earlier experiments with weak solutions of normal composition nitrified in the dark at low temperatures. In the case of bottle C, however, a weak solution from which carbonate of calcium was long withheld, it will be recollected that the nitrification was purely nitrous.

With the stronger solutions the character of the nitrification is very different. Large quantities of nitrous acid are at first produced, and the nitrification is probably for some time wholly nitrous, though this cannot be proved, as no quantitative determinations of the total nitrous and nitric acids were made.

The manner in which nitrification concludes is not always the same. With D3 the nitrous acid probably disappeared before all the ammonia

had been consumed, as the latter was still considerable in quantity on September 27. If this be so, the last stage of the nitrification was purely nitric in character. With D4, on the other hand, the nitrous acid survives the ammonia, and gradually changes into nitric. The period of action in all such cases has been reckoned from the disappearance of the ammonia.

The mean rate of oxidation is about twice as great in the strong solutions as in the weak, the former having, however, the more favourable temperature. A gradual increase in the rapidity of the action up to a certain point, followed by a decline towards the end, is not shown in the case of D2, but appears distinctly (if we may trust the determinations of nitrous acid as expressing the whole action) in solutions D3 and D4.

We turn now to the results obtained at higher temperatures.

There is only one experiment (E) in which nitrification was entirely conducted at the temperature of 20° . In this case the character of the action was decidedly nitrous, but no quantitative determination of nitrous acid was made. The nitrous acid continued present for one or two weeks after all ammonia had disappeared. The temperature during this time was maintained at 20° .

In the similar solution (F) nitrified at 30° , the character of the reaction was purely nitrous. The solution when nitrified was placed in a cool cupboard. After 36 days it was found to contain 18.0 parts of nitrogen per million in the form of nitrous acid, the amount originally present as ammonia being 20.9 parts. The bottle was then placed again in the bath at 30° . After nine days the nitrogen as nitrous acid had fallen to 10.6 per million. Examined two weeks later the nitrous acid had entirely disappeared.

In solution F2 the reaction was again purely nitrous. The solution when nitrified was placed in a cool cupboard. After 22 days the nitrogen as nitrous acid amounted to 39.9 per million, the amount originally present as ammonia being 41.7 per million. The bottle was then returned to the bath at 30° , but after 36 days still contained 35.6 of nitrogen as nitrous acid. Examined about two weeks later the nitrous acid had disappeared.

Experiments F3 and F4 were spoilt, as already mentioned, by the accident to the bath containing them. Determinations of nitrous acid were made in both on their removal from the bath. The rate of progress up to this date is therefore known.

In G2 one determination of nitrous acid was made. In this solution the nitrous acid disappeared before the ammonia.

The mean daily rate of oxidation in these partially studied solutions was as follows :—

Bottle.	Temperature.	Interval.	Nitrogen per million daily oxidised.
E	20°	Whole period of action	2·89
F	30°	" "	2·50
F2	30°	" "	2·66
F3	30°	First 20 days	2·96
F4	30°	First 17 days	3·71
G2	30°	First 18 days	3·79

As the numerical results obtained in the case of solutions E2, E3, and E4 are somewhat more complete, it will be better to give them before discussing the subject.

Bottle.	Date.	Interval.	Tem- perature.	Nitrogen per million			
				As ammonia.	As nitrous acid.	As nitric acid.	Daily oxidised.
E2 ..	1878. March 1	—	—	166·9	—	—	—
	" 18	17 days	20°	abundant.	considerable	—	—
	April 6	19 "	"	"	abundant	—	—
	" 9	3 "	30°	"	80·6	—	3·66
	" 17	8 "	"	"	99·4	—	2·35
	May 3	16 "	"	"	122·5	—	1·44
	" 17	14 "	"	"	135·3	—	0·91
	" 30 June 7	13 8 "	" "	" "	distinct none	none "	— 182·5
Mean rate of action during 81 days							2·06*
E3 ..	March 1	—	—	166·9	—	—	—
	" 20	19 days	20°	abundant	distinct	—	—
	April 6	17 "	"	"	abundant	—	—
	" 9	3 "	30°	"	63·1	—	3·16
	" 17	8 "	"	"	87·4	—	3·04
	May 3	16 "	"	"	58·6	—	—
	" 17 " 25	14 8 "	" "	" "	considerable none	none "	— 170·7
Mean rate of action during 66 days							2·53*
E4 ..	March 1	—	—	166·9	—	—	—
	" 27	26 days	20°	abundant	distinct	—	—
	April 6	10 "	"	"	abundant	—	—
	" 9	3 "	30°	"	48·4	—	3·72
	" 17	8 "	"	"	84·7	—	4·54
	May 3	16 "	"	"	137·0	—	3·27
	" 17 " 25	14 8 "	" "	" "	considerable none	132·1 none	— 169·5
Mean rate of action during 59 days							2·83*

In all these experiments made at high temperatures, whether with weak or strong solutions, the character of the action is decidedly nitrous. In F2 the character is proved to have been wholly nitrous throughout, and in the other solutions the same was probably the case, at least till near the end of the nitrification. It appears, therefore, that at high temperatures, or, in the case of strong solutions, at low temperatures, the character of the nitrification is at first solely nitrous, while in the case of weak normal solutions at low temperatures nitric acid only is produced. Is this difference to be attributed to the more rapid consumption of oxygen at high temperatures and in strong solutions, coupled with the smaller solubility of oxygen in warm liquids, or is the nitrous character of the action mainly due to the bacteria present, whose avidity for oxygen, and power of reducing nitrates to nitrites have been already studied? That the production of nitrous acid is due to some limitation in the quantity of available oxygen hardly seems a perfectly satisfactory explanation; at least the amount of oxygen consumed seems to have been at times far less than that which the amount of action at other times proved to be available. Thus the oxygen consumed by the warm strong solutions was clearly equally available for the cold strong solutions, yet the latter daily consumed less than half this quantity, though still producing only nitrous acid. The production of nitrous or nitric acid seems to depend in part upon the condition of the ferment. Of this we have had an illustration in the case of bottle C, and further evidence will be forthcoming.

In the solutions nitrified at moderately high temperatures we have fresh illustrations of the different manner in which nitrification may conclude. It would seem that when the ammonia disappears before the nitrous acid is converted into nitric, the nitrites left in the solution are very slow in undergoing change. When, however, the oxidation of the nitrites sets in before the ammonia has been all consumed, the conversion into nitrates is accomplished with surprising rapidity. This view has not yet however received an experimental proof.

The rate of oxidation in these solutions is seen to be from two and a-half to five times greater than in the case of those nitrified at a lower temperature. The rates marked with an asterisk are calculated from the ammonia disappeared, the remainder from the nitrogen found to be oxidised. The former method will give results a little higher than the latter. Solutions of different strengths, but of similar constitution, appear to nitrify at 30° at almost the same rate, the daily oxidation being apparently a little greater in the stronger solutions. The variation in the rate of oxidation as the action advances is best seen in the case of E2 and E4. It must be recollected, however,

that the figures in these cases simply represent oxidation to nitrous acid. If we assume that the hydrogen of the ammonia is oxidised at the same time as the nitrogen, then 1 mol. of nitrogen will require 3 mols. of oxygen to produce nitrous acid, and 4 mols. to produce nitric acid.

We have, lastly, to consider the evidence afforded as to the relation which the nitric acid produced bears to the ammonia originally present. The results obtained in earlier series will be here included.

The determinations of the quantity of nitric acid formed were made by means of indigo, the absence of nitrous acid being previously ascertained by testing with iodide of potassium and starch. A number of determinations were also made by the Crum-Frankland method. These, as already described (p. 376 of this volume), gave at first results far below the truth, but when the use of sulphate of silver was abandoned the results agreed fairly with those obtained by indigo. The following are the comparative determinations made after silver was omitted:—

Nitrogen as Nitric Acid per Million of Solution.

Bottle.	By indigo.	As nitric oxide.	Bottle.	By indigo.	As nitric oxide.
C2	18·7	18·8	E2	182·5	185·5
D2	20·0	19·6	E3	170·7	171·5
D3	143·7	140·1	E4	169·5	170·0
E	20·2	18·5	—	—	—

In some cases the quantity of nitric acid found per million exceeded the ammonia originally present. This was due to the concentration of the solutions by evaporation, and was most perceptible where the nitrification had been long continued, and at a high temperature. To obtain a correction for this error determinations of chlorine were made in the nitrified solutions, from which the amount of concentration which had taken place since the commencement could easily be calculated.

In the following table the proportion of nitrogen originally present as ammonia has been corrected for the error due to concentration in all cases marked with an asterisk. The nitric acid determinations given are, for uniformity, those obtained with indigo, except in the case of D4, which was analysed solely by the Crum-Frankland method:—

Bottle.	Nitrogen per million		Nitrogen obtained as nitric acid for 100 as ammonia.
	Originally present as ammonia.	Obtained as nitric acid.	
BD2	20·5	18·7	91·2
BD4	20·5	19·1	93·2
C	20·5	19·1	93·2
C2	20·5	18·7	91·2
D2	20·9	20·0	95·7
D3	169·7*	143·7	84·7
D4	170·5*	158·0	92·7
E	20·9	20·2	96·7
E2	191·9*	182·5	95·1
E3	179·8*	170·7	94·9
E4	182·5*	169·5	92·9
Mean of 10, excluding D3			93·7

In no case do we obtain the whole of the ammonia in the form of nitric acid. A part of the nitrogen has doubtless become a constituent of the living organisms—the nitrifying ferment and bacteria—present; whether the whole loss is thus to be accounted for, it is impossible to say. In the case of E2, E3, and E4, where increasing proportions of tartrates and of bacteria were present, the product of nitric acid is greatest where the organic matter is least, and least where the tartrates and bacteria were most abundant.

In BD2 and BD4, a small growth of mould in the earlier stages may account for some loss of nitrogen. In C and C2 the indigo determinations may be somewhat below the truth owing to the presence of sugar. I am at a loss to account for the serious deficiency observed in D3, which is not shared by the duplicate experiment D4.

Fifth Series. Bottles H.

This short series of experiments related solely to the influence of light. It had been found that a temperature of 40° was inimical to nitrification, it appeared therefore possible that the effect of sunshine might be due in part to the high temperature which it produced. This idea received strength on finding that a pint bottle of water, placed behind a south window, and exposed to four hours of bright sunshine in July, rose in temperature 17°, and stood at the end of the four hours at 40°·2.

Experiments were then made with a screen of alum-water, and with one filled with a strong solution of sulphate of aluminium. The screen consisted of a glass photographic bath, a foot square, with a space 1 inch in width for fluid; this was filled with the heat-absorbing solution, and placed against the window. The bottles placed behind

the screen were protected from any side radiation by screens of white paper. It was found that after full exposure to bright sunshine, a bottle behind a screen of sulphate of aluminium was $5^{\circ}4$ lower in temperature than one unprotected. Another bottle behind the screen, but protected entirely from light, stood, however, 6° lower than that receiving filtered light; the screen was thus only partially effective.

On July 13, 1878, three pint bottles of pale-green glass were two-thirds filled with a weak solution of normal composition; the solution had been prepared on March 1, and formed part of the preceding series; it had not yet received carbonate of calcium, or seed. Nitrification had already commenced in this solution (compare duplicate bottles F2' and E3', last series), though this fact was not at first known. The three bottles now received carbonate of calcium, and each was seeded with 5 c.c. from bottle G. They remained five days in a dark cupboard, and on July 18 bottle HD was placed behind the screen, protected from all light by a double covering of paper; bottle HL was also behind the screen, but fully exposed to the light passing through it; bottle HLL stood in the same window, but exposed to full sunshine. The subsequent history of these solutions will be gathered from the following table:—

Date.	Interval.	Bottle HD.		Bottle HL.		Bottle HLL.	
		Ammonia.	Nitrogen as nitrous acid per million.	Ammonia.	Nitrogen as nitrous acid per million.	Ammonia.	Nitrogen as nitrous acid per million.
1878.	—	—	—	—	—	—	—
July 13	—	—	—	—	—	—	—
„ 18	5 days	—	7·9	—	2·8	—	9·6
„ 29	11 „	considerable	5·2	—	6·9	—	11·4
Aug. 5	7 „	none	none	trace	8·8	considerable	14·0
Sept. 4	30 „	—	—	—	6·9	trace	20·1
„ 19	15 „	—	—	—	6·9	—	19·2
Oct. 5	16 „	—	—	—	6·9	—	19·2
1879.	—	—	—	—	—	—	—
April 17	163 „	—	—	—	2·6	—	12·2

All these solutions were fully half nitrified when placed in the window; more nitric and less nitrous acid had been produced in HL than in the others. Nitrification was soon completed in all the solutions: that in the dark nitrified first; that behind the screen of sulphate of aluminium very soon after; that exposed to unfiltered light decidedly later. The chief point of interest is the exclusively nitrous character of the action in both bottles exposed to light, and the extraordinary permanence of the nitrites formed. A green growth was

observed in HL on September 4, and in HLL on April 17. The total nitric acid produced in HD was equal to 22·4 parts of nitrogen per million.

These experiments again leave the effect of light very uncertain.

Sixth Series. Bottles J—K.

This series was also mainly devoted to studying the influence of light; some experiments on the possibility of nitrification without seeding being included.

Five bottles, J, J2, JD, JL, and JLL were partly filled with a normal solution, the constituents of which were in similar proportion to that used for E3 in the fourth series of experiments, but of one-fourth the strength. Three other bottles, KD, KL, and KLL were partly filled with a perfectly similar solution, in which acid tartrate of potassium was used in place of Rochelle salt, and sulphate of calcium in place of sulphate of magnesium. Carbonate of calcium was added to every bottle.

For the first time in these experiments the bottles with their solutions were all maintained for one hour at the temperature of boiling water to destroy any germs that might be present. On the next morning, August 11, 1878, the stoppers were removed, 2 c.c. from bottle AL added as seed to all the bottles save J and J2, and the mouths of the bottles plugged with baked cotton wool. Bottles J, J2, JD, and KD were placed in a dark cupboard, and bottles JL, KL, and JLL, KLL, after 24 hours' darkness, were placed in the south window, the two former behind the screen of sulphate of aluminium solution, the two latter not thus protected. The bottles were all of white glass.

The course of nitrification in bottles JD and KD, kept in darkness at the temperature of the air, was as follows:—

Date.	Interval.	Bottle JD.			Bottle KD.		
		Ammonia.	Nitrogen as nitrous acid per million.	Nitrogen daily oxidised per million.	Ammonia.	Nitrogen as nitrous acid per million.	Nitrogen daily oxidised per million.
1878.							
Aug. 11	—	—	—	—	—	—	—
Sept. 5	26 days	abundant.	6·9	—	abundant	6·9	—
„ 19	14 „	„	18·3	0·81	„	17·5	0·75
Oct. 4	15 „	„	24·4	0·41	„	24·4	0·46
„ 21	17 „	distinct	34·1	0·57	distinct	34·9	0·62
Nov. 4	14 „	trace	35·8	0·12	trace	37·6	0·20
Dec. 16	42 „	none	37·6	—	none	38·4	—
1879.							
Mar. 13	87 „	„	37·6	—	„	38·4	—

We have here the remarkable result of a wholly nitrous action, in which the nitrites produced show no disposition whatever to pass into nitrates. The nitrous acid formed was nearly equal to the whole nitrogen originally present as ammonia, namely 41·4 per million.

Bottles JL, JLL, KL, and KLL, exposed to light, failed entirely to nitrify. A minute trace of nitrous acid was early found in JL and KLL, but this never increased. In JL and JLL a green growth developed, the germs being probably introduced when these bottles were opened on September 19. In KL and KLL no growth has appeared beyond a few minute mycelial threads. Bottle KL was placed in a dark bath at 30° on April 16th, nitrification commenced after a time, and was completed by June 16th; if therefore no fresh germs were accidentally introduced, it would appear that the ferment had not been killed by exposure to light. The nitrification was purely nitrous.

Bottle J, which had never been seeded, was allowed to remain untouched till April 29, 1879; the cotton-wool was then removed and the solution examined. No nitrification whatever had taken place. The solution was perfectly clear. More than eight months had thus elapsed without any commencement of nitrification, though all the conditions were favourable, excepting the absence of seed.

Bottle J2 was covered only with muslin; unfiltered air had, therefore, free access. A luxuriant growth of mould soon appeared, but no nitrification took place. The air of the laboratory doubtless contained nitrifying germs, as soil sampling was in progress for many weeks, but mould, as already mentioned, is apparently inimical to nitrification.

Seventh Series. Bottles L.

The behaviour of nitrites in the presence of a nitrifying ferment had appeared in several of the preceding experiments to be subject to great irregularity; in some solutions they had rapidly oxidised into nitrates, while in others they exhibited a remarkable degree of permanence. With the hope of throwing some light on the subject, a few experiments were now made on the nitrification of nitrites.

One gram of commercial potassium nitrite was dissolved in a litre of water, and divided among four bottles. Bottle LD contained this solution without addition. Bottles LD2, LD3, and LL, received in addition the tartrates, phosphates, and carbonate of calcium usually employed in preparing a nitrifiable solution. All the bottles were then heated for one hour to 100°. On the next day, August 11, 1878, LD3 and LL were seeded with 1 c.c. from bottle AL; the bottles LD, LD2, and LD3 were then placed in a dark cupboard, and bottle LL in an east window.

The bottles were examined from time to time up to October 4 (54 days), but no diminution in the quantity of nitrite was in any case apparent; on the contrary, a decided increase was observed in bottle LD2, the nitrate present in the commercial salt being possibly reduced to nitrite. At this date LD3 and LL were freshly seeded with 1 c.c. from bottle D3, in which a rapid conversion of nitrites into nitrates had just been effected. This fresh seeding proved most effectual; the whole of the nitrous acid quickly disappeared from LD3, while the corresponding solution in the light suffered little change. The numerical results were as follows:—

Date.	Interval.	Nitrogen as nitrous acid per million.			
		Bottle LD.	Bottle LD2.	Bottle LD3.	Bottle LL.
1878.					
August 11	—	143·2	143·2	143·2	143·2
September 20 . .	40 days	143·2	157·2	146·7	141·5
October 4	14 „	143·2	154·5	143·2	139·7
„ 21	17 „	141·5	153·7	104·8	136·2
November 11 . .	21 „	142·3	151·9	47·2	136·2
December 17 . .	36 „	140·6	143·2	none	136·2
1879.					
March 12	85 „	139·9	141·6	„	126·1

Numerous patches of dark-brown mould developed in bottle LD2, and in bottle LL a green growth commenced after the examination on September 20; the slow diminution of nitrite in these two cases is probably due to the production of vegetable matter.

These experiments illustrate the great stability of nitrites in the absence of a nitrifying ferment, and the power of light to prevent the action of a ferment shown by experiment to be perfectly suitable. The great interest of these experiments is perhaps, however, the distinction which they show may exist between two nitrifying ferments. The ferment used as seed on August 11 was quite ineffective for converting nitrites into nitrates; the same ferment was used on the same date for the experiments of the sixth series. Here it proved capable of changing ammonia into nitrous acid, but the nitrites formed remained unconverted into nitrates, thus affording a striking corroboration of the purely nitrous character of this sample of ferment. On the other hand, a ferment taken from a solution rapidly converting nitrites into nitrates was found to exhibit the same nitric character when added to another solution of nitrite.

The account of the experiments made at Rothamsted is now completed. The novelty of the subject can be my only excuse for giving

in detail many experiments, the results of which are rather suggestive than conclusive.

It may be useful to summarise, as briefly as possible, the results arrived at.

1. A solution of ammonium chloride, fully supplied with plant-food, will not nitrify if germs be excluded (J).

2. A solution of ammonium chloride containing phosphate of calcium and sulphate of potassium, but no organic salt or carbonate of calcium, will not nitrify even when seeded (BD).

3. A solution of chloride of ammonium, supplied with phosphates and sulphates, potassium, calcium, and magnesium, with cane-sugar, will not nitrify even when seeded (C).

4. A solution of ammonium chloride, containing tartrate of potassium, in addition to phosphates and sulphates, may nitrify when seeded; but the nitrification takes place very slowly, the salifiable base required being furnished by the gradual decomposition of the tartrate (BD4).

5. Nitrification takes place speedily only when an excess of a salifiable base, as carbonate of calcium, is present. (Compare BD2 with BD4, and C2 with C).

6. Nitrification may occur in solutions in which calcium salts are apparently absent (E3).

7. A proportion of organic carbon (as tartrate) to nitrogen (present as ammonium chloride), equal to 3 : 10 by weight, is sufficient for the purposes of nitrification, and probably a smaller proportion of carbon would suffice (E2, BD3, and BL3).

8. Solutions containing as much as 640 milligrams of chloride of ammonium per litre can be completely nitrified. The limit of concentration, up to which nitrification is possible, is not yet ascertained.

9. Nitrification is not produced by the growth of mould which takes place in a solution containing tartrates (J2 and BD2).

10. Nitrification is not produced by the growth of bacteria; at least bacteria may flourish in solutions of composition suitable for nitrification without nitrification taking place (D3, D4, and G2).

11. Light certainly hinders nitrification; this is shown by every experiment save one (HL). In 12 experiments out of 13, nitrification is prevented, or greatly delayed by exposure to light, or rather to alternate light and darkness. Evidence is, however, at present wanting that the nitrifying ferment is killed by light (DL and KL). In examples of nitrification in light, the nitrites were abundant, even in weak solutions, and very permanent (BL3, HL, and HLL). Further investigation is required.

12. Nitrification will not take place at a temperature of 40°. Prolonged exposure to this degree of heat apparently destroys the ferment (G and G2).

13. The addition of a small quantity of a nitrified solution to an ammoniacal solution of suitable composition is not immediately followed by a perceptible action; a period of rest, often of considerable length, precedes the active work of the ferment. Whether this apparent rest was simply due to the extremely small quantity of ferment added, which till it had greatly multiplied could produce no appreciable effect, or whether the ferment, which was taken in a large majority of cases from an old solution, was really in the passive state, which has been proved to occur in the life history of many low organisms, is at present quite undetermined.

14. An increase in the concentration of the ammoniacal solution is attended by a lengthening of the period of incubation.

15. An increase of temperature, within certain limits, greatly reduces the length of the period of incubation; this period is shorter at 30° than at 20° .

16. The period of actual nitrification, which succeeds the period of incubation, increases in length with the concentration of the ammoniacal solution, and at a fixed temperature the length of this period varies pretty nearly with the degree of concentration, strong solutions taking, however, in proportion to their strength, rather less time than weak solutions; thus, a solution of double strength will require rather less than a double time for its nitrification. Further evidence is necessary to establish this rule.

17. The period of actual nitrification is diminished greatly in length by a rise in temperature. It is not yet proved, however, that this period is shorter at 30° than at 20° .

18. It follows from 16 and 17 that the average rate of oxidation increases up to a certain point with a rise of temperature, and is also somewhat increased with increasing concentration of the solution.

19. The rate of oxidation is not uniform throughout the process of nitrification; it begins slowly, increases in rapidity, and after reaching a maximum again diminishes towards the close. Further evidence is required on this head.

20. The product of nitrification is not uniform; sometimes nitrous, and sometimes nitric acid is produced. A purely nitric fermentation has occurred only in the case of cold dilute solutions nitrified in the dark. In the case of strong solutions, or of nitrification at elevated temperatures, or in the light, the nitrification has been wholly or chiefly nitrous. Cold dilute solutions, in which nitrification has been long checked by the absence of a salifiable base, also assume a nitrous fermentation when a base is introduced (C). Further investigation is needed under this head.

21. It does not appear that the production of nitrous acid is due to a deficiency of available oxygen; at least this is not a sufficient expla-

nation for all the facts. The production of nitrous or nitric acid seems to depend in part on the condition of the ferment. The subject is as yet very obscure.

22. The nitrites which are produced during nitrification sometimes pass into nitrates with astonishing rapidity during the final stage of the process, and before the ammonia has entirely disappeared. Under other circumstances, they remain unchanged for a long time after all ammonia has been consumed. Light is apparently a condition which prevents nitrites from turning into nitrates (BL3, HL, HLL, and LL). This, like the preceding question, is at present very obscure.

23. The nitrifying ferment in certain conditions seems only capable of producing nitrous acid, and is unable, even in the dark, of converting nitrites into nitrates (JD, KD, and LD3).

24. The whole of the ammonia originally present is not finally obtained as nitric acid; the largest proportion so obtained has been about 96 per cent.; the average of 10 experiments is 93.7 per cent. In one exceptional experiment the product was only 84.7 per cent.

25. A solution of nitrite of potassium will remain, at least for a long time, without change. The addition of tartrates, phosphates, and carbonate of calcium does not produce change. But if to the solution of nitrite, containing the above-named admixtures, a small quantity of a solution be added in which nitrites have lately changed into nitrates, the oxidation of the nitrite is rapidly effected. This conversion of nitrites, by means of an added ferment, takes place apparently only in the dark.
