

PART. IV.—FACTORS INFLUENCING THE RATE OF OXIDATION.

By S. Ranganathan and Roland V. Norris.

In the foregoing Part Kotwal has described certain experiments on intensive nitrification as carried out in suspensions of 'activated sludge.' These experiments had as their object (1) to study the rate of nitrification producible in such circumstances and (2) to ascertain the concentration of nitrate attainable by these methods. The work was interrupted before the second of these factors could be investigated and this aspect of the case has, therefore, been taken up by the present authors, and extended to a study of certain other factors bearing on the process of nitrification.

The method employed by Kotwal consisted essentially in aerating suspensions of activated sludge in large cylinders, ammonium salts being added from time to time in suitable proportions.

In the beginning of the present investigation the same procedure was followed and the work begun with two nitrifying solutions A and B handed over by Kotwal and which already contained nitrate equivalent to 2.17 and 1.08 per cent. respectively of calcium nitrate.

The first object being to ascertain the concentration of nitrate attainable, ammonium nitrate was used as the source of ammonia instead of ammonium sulphate. By this means, assuring equal rates of nitrification, the accumulation of nitric nitrogen would be twice as rapid as when ammonium sulphate was the source of ammonia.

The ammonium nitrate was dissolved in water, a suitable amount of calcium carbonate (usually equivalent to the amount of acid to be produced) added and the suspension mixed with 50 c.c. of supernatant liquid taken from the aeration cylinder. The mixed solution thus prepared was then slowly added through a dropping funnel to the aeration vessel, the liquor in the latter being aerated for not less than twelve hours a day by means of compressed air. Further additions of ammoniacal solution were made only when the previous amount added had been fully oxidised. Moreover the volume added at any one time was kept constant until evidence was forthcoming that nitrification was taking place with increasing rapidity when the dose was augmented. In practice it was found that as a rule not less than three days should elapse before such an increase was made. The

concentration of the ammoniacal solution was also increased from time to time. The solution initially used contained 17.14 gms. ammonium nitrate per litre equivalent to 0.3 gm. of ammoniacal nitrogen per 100 c.c.

Periodical determinations of the nitrate-content of the reaction mixtures were made by means of the modified Devarda method (*J. Ind. Eng. Chem.*, 1920, **12**, 352). A correction was made for loss of ammonia due to the aeration, such ammonia, if any, being caught in suitable traps.

When the experiment was begun on March 27th the two aeration mixtures A and B were nitrifying respectively 150 mgs. and 90 mgs. of ammoniacal nitrogen in 24 hours. The rate in A containing the higher nitrate-content, however, soon began to fall off while that in B remained unimpaired with the result that after one month the amount of nitrogen oxidised was only 1.65 gms. as against 2.66 gms. in B. The concentration of nitrate was at the end of the period equivalent to 3.02 per cent. calcium nitrate in A and 1.98 in B. The concentration of the ammoniacal solution was increased at this stage to 28.57 gms. ammonium nitrate per litre or 0.5 gm. ammoniacal nitrogen per 100 c.c. Nitrification proceeded normally for another two weeks when the rate began to slacken in both cylinders. The decrease became steadily more marked, and in June the two solutions could oxidise only 25-30 mgs. ammoniacal nitrogen per day. At the beginning of July the concentration of calcium nitrate was 3.40 per cent. and 2.70 in A and B respectively, while the two solutions which previously had been able to nitrify 50 c.c. of the ammoniacal solution in 24 hours could only with difficulty oxidise 10 c.c. of the same solution in this space of time; after a further two months only 5 c.c. were oxidised daily. The concentration of nitrate was then 4.79 per cent. in A and 4.55 in B.

Shortly before this stage trouble was caused by the development of green algae in the nitrifying vessels. Attempts were made to check this in the first place by adding copper sulphate (1 in 50,000). Control experiments, however, indicated that even in this high dilution nitrification was checked and this method was not further used. It was ultimately found that the simple device of excluding light by covering the cylinders with black paper was quite efficacious in preventing such growths.

Apart from this difficulty, however, nitrification continued only for a few weeks more, and in the middle of December the process came to a standstill when the nitrate concentration in the two flasks was 5.34 per cent. and 5.56 respectively. This concentration seems in fact to be the highest that can be attained under these conditions of working, using activated sludge alone without adding other salts.

Attention has already been drawn to the fact that the rate of nitrification steadily decreased as the concentration of nitrate in the reaction mixture increased, solution A, e.g., nitrifying 150 mgs. ammoniacal nitrogen in 24 hours when the nitrate concentration was 2.17 per cent. and only 5 mgs. when there was 5.26 per cent. calcium nitrate present. The phenomenon is clearly seen in the following table :—

TABLE I.

Influence of Nitrate-concentration on Rate of Nitrification.

Period of observation	Experiment A		Experiment B	
	Average speed of nitrification	Percentage calcium nitrate	Average speed of nitrification	Percentage calcium nitrate
March	150	2.17	90	1.06
April	45	2.69	77.7	1.61
May	30	3.08	44.3	2.46
June	25	3.40	28	2.73
July	24	4.08	25	3.19
August	17	4.46	25	3.66
September	21.8	4.61	24	4.37
October	16.8	5.08	24	4.93
November	8.3	5.34	24	5.4

It is noteworthy that the fall in activity is much more marked in A, which initially was nitrifying the more vigorously of the two.

Influence of Salts on Nitrification.

Though nitrification had for all practical purposes ceased in the above experiments this was not due to the death of the nitrifying organisms, but was apparently caused either by a paralysing effect of the high concentration of nitrate, or by the lack of some essential nutrient material in the mixture.

Other experiments having indicated the beneficial action of iron salts and phosphates in moderate concentration, an experiment was carried out to ascertain whether nitrification could be again set up in one of the mixtures used above by adding such salts.

Two one-litre aeration cylinders were therefore prepared and equal quantities of the solution from the previous experiments (i.e., containing already 5.5 per cent. calcium nitrate) placed in each. To them was added in small quantities at a time one of the following solutions :—

- (1) 1,000 c. c. water containing 28.57 gms. ammonium nitrate, 37.71 gms. calcium carbonate and 1 per cent. ferrous sulphate.
- (2) As above, but containing 1 per cent. potassium phosphate instead of ferrous sulphate.

In both cases nitrification was resumed with fair vigour, but the rate again fell after about two weeks and then continued very slowly for the next three months, about 2 c. c. of the solution being oxidised per 24 hours. The final concentrations of nitrate obtained were 7.55 and 7.44 per cent. respectively and in no experiment have we been able to exceed this concentration.

It has been suggested that ammonium nitrate is less favourable for nitrification than ammonium sulphate, the nitrate ion exercising a paralysing action on the multiplication of the organisms. An experiment similar to that just described was therefore carried out using a nutrient solution containing ammonium sulphate. To obviate the disadvantages of using calcium carbonate as the neutralising agent, namely, deposition of calcium sulphate, a solution of ammonium carbonate containing ammoniacal nitrogen equal in amount to that in the ammonium sulphate solution was added between two additions of the sulphate solution. No improvement was, however, effected by this procedure and it was not found possible to obtain more than 7.5 per cent. of calcium nitrate.

It is of interest to note that in the above experiments it was never possible to obtain a quantitative oxidation of ammoniacal nitrogen to nitric nitrogen, the highest figure obtained being a 96 per cent. conversion.

Nutrition-requirements of the Nitrifying Organisms.

A mixed culture of the nitrite and nitrate-producing organisms was isolated by frequent sub-culturing of an actively nitrifying solution into Winogradsky's medium. After six sub-cultures the growth was inoculated on the surface of sterile gypsum blocks placed in the above medium and a mixed culture of nitromonas and nitrosomonas obtained.

This mixed culture was utilised to inoculate fresh suspensions of activated sludge in subsequent experiments in order that nitrification might be more rapidly induced.

Influence of added phosphates.—Two 2-litre cylinders each containing 500 c.c. of well aerated sludge (settlement in 15 minutes) and 1,500 c.c. tap-water were arranged for aeration as in the previous experiments. To the one was added in suitable quantities from time to time a solution containing in 1,000 c.c. of distilled water the indicated grams of the following substances:—Ammonium nitrate, 28·57, calcium carbonate, 35·71, sodium potassium tartrate, 5·00, potassium phosphate, 2·50, and magnesium sulphate, 1·00. To the second cylinder was added the same solution without the phosphate.

From the beginning of the experiment, the phosphate solution nitrified more than double the amount of ammoniacal nitrogen, as compared with the control. The experiment continued for about three months, the results being recorded in the following table:—

TABLE II.

Influence of Phosphate on Rate of Nitrification.

Period	Phosphate cylinder		Control	
	c. c. of amm. solution oxidised	Percentage of calcium nitrate	c. c. of amm. solution oxidised	Percentage calcium nitrate
1st month	150	...	75	...
2nd "	135	0·499	47	0·393
3rd "	118	0·813	54	0·512
Total	403	0·813	176	0·512

It is quite evident from the above figures that the phosphate exercised a very beneficial influence. This may be brought about by either (1) direct stimulation of the nitrification process, or (2) increase in the number and vigour of the nitrifying organisms. While the latter factor cannot be excluded there appears to be a good deal of evidence to suggest that the phosphate has a direct accelerating influence on the process of nitrification though the manner in which this is exercised is not clear. The point is being further examined.

Influence of Other Salts.

The influence of various salts on the rate of nitrification of soils has been investigated with varying results by a number of different workers. Of such salts, calcium and manganous sulphates have seemed the most promising and an experiment was therefore carried out to ascertain whether similar results would be obtained in culture solutions by using these salts.

Four 1-litre aeration cylinders were therefore set up each containing 250 c. c. of well aerated sludge and 750 c. c. tap-water. To one cylinder, serving as a control, was added at intervals a suitable amount of nutrient ammonium nitrate solution while to the other three cylinders was added a similar solution containing 1 per cent. of calcium, ferrous or manganous sulphate. As before, additions of the ammoniacal solution were only made when the previous dose had been nitrified. All four solutions were aerated for the same period each day and the nitrate nitrogen estimated at frequent intervals.

During the first week the rate of nitrification in the control was slightly slower than in the other three cylinders. This, however, was only a temporary effect and after a further short period the solution containing ferrous sulphate was the only one which was ahead of the control, the other salts exhibiting a retarding influence. The concentration of ferrous sulphate required to produce the above acceleration was small and higher amounts ultimately checked the rate of nitrification. The critical strength appeared to be about 0.07 per cent. ferrous sulphate, higher concentrations being toxic. The above results are illustrated by the figures given in Table III, showing the nitrification brought about at two stages of the experiment.

TABLE III.

Salt added	Early stage			Later stage		
	Percentage salt in re-action mixture	Percentage calcium nitrate	Relative ratio	Percentage salt in re-action mixture	Percentage calcium nitrate	Relative ratio
Control	...	0.377	100	..	1.115	100
CaSO ₄	0.035	0.254	67.4	0.048	0.828	74.3
FeSO ₄	0.074	0.48	127.3	0.088	1.058	94.7
MnSO ₄	0.051	0.353	93.5	0.055	0.926	83

It is obvious that in a culture solution the organisms are highly sensitive to the action of such salts. In soil experiments much higher

concentrations have been successfully employed. In such cases, however, absorption of the salts undoubtedly takes place and the actual concentration of the salt in the soil solution at any particular moment would probably be quite low. Further experiments with lower concentrations of ferrous sulphate are therefore indicated.

Influence of Surface.

The rapid rate at which oxidation is brought about by activated sludge is due to two causes. In the first place the surface at which oxidation can take place is very large owing to the finely divided state of the sludge; and secondly the oxidisable material, probably absorbed on the sludge particles, is brought into immediate contact with relatively large quantities of oxygen by the aeration employed.

It was not therefore surprising to find that the rate of nitrification in an activated sludge suspension could be increased by adding substances such as silt or charcoal which increased the area of the oxidation surface. Preliminary experiments with silt, animal charcoal and ordinary charcoal indicated that animal charcoal assisted the process to the greatest extent. The influence of this substance and of the aeration is shown in the following experiment:—Six aeration cylinders were set up in the usual way, four containing 500 c.c. of sludge and 1,500 c.c. of distilled water and two 2,000 c.c. of distilled water. Animal charcoal in the proportion of 1 gm. charcoal per 100 c.c. of solution was added to four of the cylinders as shown in the schedule below. A nutrient solution of ammonium nitrate was added in the usual way for nitrification. Aeration was given to three only of the flasks as indicated in Table IV which summarises the results obtained.

TABLE IV.

Composition of mixture	Treatment	A		B	
		Percentage calcium nitrate	Relative efficiency	Percentage calcium nitrate	Relative efficiency
1. Sludge alone (control)	Aerated	0.523	100	0.815	100
2. Sludge alone	Not aerated	0.038	7.3	0.044	5.4
3. Sludge and animal charcoal	Aerated	0.818	156.4	1.112	136.5
4. Sludge and animal charcoal	Not aerated	0.038	7.28	0.044	5.4
5. Animal charcoal alone	Aerated	0.412	78.8	0.668	82
6. Animal charcoal alone	Not aerated	0.104	19.9	0.168	22.8

The importance of the aeration is strikingly demonstrated by the figures given above, as was anticipated, and the effect of adding animal charcoal is highly interesting. In the first place it is clear that animal charcoal alone was able to bring about the oxidation, for even

in the non-aerated flask there was a definite production of nitrate. It is of interest, moreover, to note that in the absence of aeration, animal charcoal alone gave better results than animal charcoal with sludge. This was doubtless due to the fact that the limited amount of oxygen available under such conditions was utilised by the organisms in the sludge and hence was not available for the oxidation of ammonia. It may be mentioned that in all the flasks which were not aerated nitrites were present. No trace of these was found where aeration was given.

These experiments bear out the results of Warburg and others in so far as they demonstrate the power of animal charcoal to bring about biological oxidations. Working with cystine, Warburg showed that animal charcoal alone could oxidise this substance to ammonia and sulphuric acid. He did not however obtain any further oxidation of the ammonia to nitrite or nitrate such as has been observed in the present case.

Optimum Reaction for Nitrification.

It is generally known that nitrification proceeds more rapidly in alkaline than in acid media. To order to ascertain the extent to which changes in reaction would influence nitrification under the experimental conditions employed in the present investigation the following experiment was carried out. A series of nine flasks was prepared, each containing 5 c.c. of a dilute solution of 2 per cent. ammonium carbonate. Each flask was inoculated with 1 c.c. of an actively nitrifying culture and the volume made up to 100 c.c. The pH of the mixtures was approximately 8.0. In eight of the flasks the reaction was now adjusted in order to obtain pH values of 7.6, 7.2, 6.8, 6.4, 6.0, 5.8, 5.6 and 5.4.

The flasks were frequently shaken, samples being removed every six hours and tested for ammonia and nitrites; the absence of both indicates complete nitrification. The results obtained are given in Table V.

TABLE V.

Flask No.	pH	Time required for complete nitrification
1	8.0	28 hrs.
2	7.6	18 "
3	7.2	18 "
4	6.8	48 "
5	6.4	60 "
6	6.0	72 "
7	5.8	120 "
8	5.6	Not complete in 10 days
9	5.4	

It is clear that nitrification proceeded well when the pH was between 8.0 and 6.0. In more acid reactions the rate diminishes rapidly and nitrification was completely checked when the acidity was greater than 5.8. The optimum reaction is between 7 and 8, that is to say just on the alkaline side of the neutral point.

Requirement of Calcium Carbonate for Efficient Nitrification.

It has been the contention of some investigators, notably Fischer (*Landw. Versuchs Stat.*, 1909, 70, 335-342) that thrice or even more times the theoretical amount of calcium carbonate is necessary for efficient nitrification. To find out whether this was really the case, the following experiments were conducted. Ten 125 c.c. conical flasks each containing 25 c.c. of a dilute ammonium nitrate solution (8 grams in 500 c.c.) were inoculated with 1 c.c. each of an actively nitrifying culture. Flasks I and II received no calcium carbonate; II and VII received 0.5 gm. each, the amount theoretically necessary to neutralise the acid resulting from the nitrification of the 25 c.c. of the above dilute ammoniacal solution. To flasks III and VIII was added exactly twice the theoretical amount, namely, 1 gram; to IV and IX twice, and to V and X four times (i.e., 2.0 gms.) the theoretical amount of calcium carbonate. The volume in each flask was made up to 100 c.c. The flasks were then divided into two series, the first series being analysed after one week for ammoniacal and nitric nitrogen while the second series was examined after two weeks.

The figures obtained however indicated that no advantage at all resulted by increasing the amount of calcium carbonate above that theoretically required to neutralise the acid produced in the oxidation; on the other hand high amounts tended to bring about a loss of nitrogen and were therefore detrimental.

SUMMARY AND CONCLUSIONS.

1. The speed of nitrification decreased with increased concentration of nitrate.
2. With dilute solutions of ammonium salts and with calcium carbonate in quantity necessary to neutralise the resulting acid, but employing no nutrient salts, a concentration of 5.56 per cent. of calcium nitrate was obtained.
3. The maximum concentration of calcium nitrate obtained in this laboratory in culture solutions was 7.5 per cent.
4. Only about 96 per cent. of the nitrogen added was recovered as nitric nitrogen.

5. The influence of potassium phosphate, with ferrous, calcium and manganous sulphates has been studied; as also the effect of surface and aeration on the process of nitrification.

6. The theoretical quantity of calcium carbonate required to neutralise the acid produced is quite sufficient for efficient nitrification.

7. The nitrifying organisms thrive in media of pH ranging from 5.8 to 8.0, but function best when the pH lies between 7.0 and 8.0.

*Department of Bio-Chemistry,
Indian Institute of Science,*

Bangalore.

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