ON THE NATURE OF AMMONIFICATION AND NITRIFICATION¹

By

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It has been found by many experiments on ammonification and nitrification that the rates of increase of ammonia and nitric acid produced at first rise very slowly, then very rapidly and later slowly again. If the rates are put in the form of a curve, the curve is very similar to that of an autocatalytic chemical reaction in which one of the products of reaction accelerates the reaction. It will be of interest, therefore, to study the nature of these microbiological chemical phenomena from the standpoint of autocatalysis.

THE QUANTITATIVE RELATION BETWEEN THE AMOUNT OF REACTION PRODUCTS AND THE TIME OF AUTOCATALYSIS

The differential equation which is characteristic of the initial stages of autocatalytic mono-molecular reaction is as follows:

$$\frac{dx}{dt} = k_1 x (a - x) \dots \dots \dots (1)$$

which expresses in mathematical symbols the fact that the velocity of the transformation is, at any instant, proportional to the amount of material which is undergoing change and to the amount of material which has already been transformed. If, however, the reaction has proceeded so far that the depressant effect of the products of the reaction upon its velocity is measurable, equation (1) becomes:

$$\frac{dx}{dt} = k_1 x (a - x) - k_2 x^2 \dots \dots \dots (2)$$

This equation can, however, be reduced to the same form as (1), only the constants have a somewhat different meaning, for equation (2) is obviously the same as the following equation:

$$\frac{dx}{dt} = \frac{k_1}{k_1 + k_2} x \left(\frac{k_1 + k_2}{k_1} a - x \right) \dots \dots \dots (3)$$

which is of the same form as equation (1) but instead of k_1 we have $k_1 + k_2$, and instead of a we have $\frac{k_1 + k_2}{k_1} a$.

¹ Received for publication, September 17, 1916.

Equation (3) may therefore be written:

$$\frac{dx}{dt} = kx(A - x) \dots\dots\dots (4)$$

where k and A are constants.

Integrating equation (4), we obtain:

$$\log \frac{x}{A - x} = Akt + C \dots\dots\dots (5)$$

where C is the constant of integration.

Now, it is evident that when $x = \frac{1}{2}A$, that is, when the reaction has proceeded half way to equilibrium, $\log \frac{x}{A - x} = 0$. Choosing C , therefore, so as to make the right-hand side of equation (5) vanish at the same time as the left-hand side we have:

$$\log \frac{x}{A - x} = Ak(t - t_1) \dots\dots\dots (6)$$

where t_1 is the time at which the reaction is half completed. This equation may obviously be written,

$$\log \frac{x}{A - x} = K(t - t_1) \dots\dots\dots (7)$$

If an increase of reaction products with time can be regarded as expressing, primarily, the progress of an autocatalytic reaction, equation (7) should hold good. Where x represents the amount of reaction product at the time t , and A represents the maximum or final amount of reaction product, t_1 is the time at which half this maximum amount has been attained, and K is a constant which must be determined from a known value of x at a given time t .

On differentiating equation (4) with respect to x , calling $\frac{dx}{dt} = v$ we obtain:

$$\frac{dv}{dx} = k(A - 2x) \dots\dots\dots (8)$$

Differentiating again we obtain:

$$\frac{d^2v}{dx^2} = -2k \dots\dots\dots (9)$$

The right-hand side of equation (8) vanishes and $\frac{d^2v}{dx^2}$ is negative

when $x = \frac{A}{2}$, hence $\frac{dx}{dt}$ is at a maximum when $x = \frac{A}{2}$. In other words the rate of increase of reaction products during a given period of reaction should be at a maximum when the reaction of that period is half completed.

APPLICATION TO AMMONIFICATION

I.

Lipman (2), working in collaboration with Blair, Owen and McLean, has carried out a series of determinations upon the daily increase of ammonia formed in ammonification. In his experiment, 80 gm. of soil, 20 gm. of sand, 0.5 gm. of calcium carbonate and dried blood equivalent to 465 mg. of nitrogen were used. Six c.c. of soil infusion, 12 c.c. of water and 60 per cent of moisture for organic matter were added. The mixture was kept in tumblers at room temperature. The amounts of ammonia nitrogen recovered are indicated in the following table and the accompanying curve. On examining the table in which the daily increase of ammonia nitrogen is indicated, choosing the points with which to determine the constants of the curve sufficiently far apart and in such a manner that each pair of points on the curve is separated by an interval of 2 days, we find that:

When time = 2 days, Weight of ammonia nitrogen = 8.11 mg.

When time = 4 days, Weight of ammonia nitrogen = 46.03 mg.

When time = 8 days, Weight of ammonia nitrogen = 164.87 mg.

When time = 10 days, Weight of ammonia nitrogen = 185.91 mg.

Applying equation (7) to these values we find:

$$\log \frac{8.11}{A - 8.11} = K (2 - t_1) \quad (10)$$

$$\log \frac{46.03}{A - 46.03} = K (4 - t_1) \quad (11)$$

$$\log \frac{164.17}{A - 164.17} = K (8 - t_1) \quad (12)$$

$$\log \frac{185.91}{A - 185.91} = K (10 - t_1) \quad (13)$$

From (10) and (11), by subtraction we obtain:

$$\log \frac{46.03 (A - 8.11)}{8.11 (A - 46.03)} = 2K \quad (14)$$

While from (12) and (13) we obtain:

$$\log \frac{185.91 (A - 164.17)}{164.17 (A - 185.91)} = 2K \quad \dots \dots (15)$$

Hence, from (14) and (15) we have:

$$\frac{46.03 (A - 8.11)}{8.11 (A - 46.03)} = \frac{185.91 (A - 164.17)}{164.17 (A - 185.91)} \quad \dots \dots (16)$$

Solving this equation for A we find:

$$A = 189.83$$

A convenient check upon the above calculation is afforded by the fact expressed in equations (8) and (9), namely that the rate of increase in x , that is, daily increase in weight, should be at a maximum when x ($=$ amount of ammonia nitrogen) $= \frac{1}{2}A$, in this case 94.94 mg.

Examining the table we find that:

At time $=$ 3 days, Weight of ammonia nitrogen $=$ 19.80 mg.	Daily increase $=$ 11.70 mg.
At time $=$ 4 days, Weight of ammonia nitrogen $=$ 46.03 mg.	Daily increase $=$ 26.23 mg.
At time $=$ 5 days, Weight of ammonia nitrogen $=$ 68.66 mg.	Daily increase $=$ 22.63 mg.
At time $=$ 6 days, Weight of ammonia nitrogen $=$ 123.23 mg.	Daily increase $=$ 54.57 mg.
At time $=$ 7 days, Weight of ammonia nitrogen $=$ 144.27 mg.	Daily increase $=$ 21.04 mg.
At time $=$ 8 days, Weight of ammonia nitrogen $=$ 164.87 mg.	Daily increase $=$ 20.60 mg.

Thus we see that when the amount of ammonia nitrogen is between 68.66 and 123.23 mg., the rate of increase in ammonia nitrogen is at a maximum. Hence we may conclude that the value of A deduced from equation (16) is the correct one.

Now we find from the curve that the amount of ammonia nitrogen is 94.94 mg. at 5.6 days. Hence equation (7) becomes for this case:

$$\log \frac{x}{189.83 - x} = K(t - 5.6) \quad \dots \dots (17)$$

Now, when $t = 1$ we have:

$$\log \frac{5.56}{184.27} = -4.6 K$$

$$K = 0.3305$$

Similarly:

$t = 2$	$K = 0.3753$
$t = 4$	$K = 0.3100$
$t = 5$	$K = 0.4097$
$t = 8$	$K = 0.3415$
$t = 10$	$K = 0.3810$

The average value of K from these values is 0.358, hence equation (7) becomes:

$$\log \frac{x}{189.83 - x} = 0.358 (t - 5.6) \quad (18)$$

By giving t the successive value 1, 2, 3, etc., we can calculate the corresponding theoretical value of x , that is, of the weight of ammonia nitrogen. In Table I and figure 1 the theoretical and experimental results are compared.

TABLE I
COMPARISON OF AMOUNT OF AMMONIA NITROGEN FOUND WITH THAT
CALCULATED BY FORMULA

Time, Days	Ammonia Nitrogen mg.	
	Found	Calculated
1	5.55	4.19
2	8.11	9.39
3	19.80	19.94
4	36.93	40.10
5	68.66	72.01
6	123.23	110.55
7	144.27	144.78
8	164.87	169.99
9	172.70	179.23
10	185.91	185.20

It will be seen that in nearly every case the observed and calculated values agree well.

II

Applying the same method to another observation of Lipman's upon the daily increase of ammonia nitrogen in ammonification in which dried blood equivalent to 310 mg. of nitrogen was added, and using the same points ($t = 2, 4, 8$ and 10) on the curve to determine A , we find:

$$A = 133.13$$

Hence the agreement between the theoretical and actual curves of increase of ammonia nitrogen is very good, for when $x = \frac{1}{2}A = 66.57$ the daily increment has reached its maximum.

On the curve we find 5.6 days corresponding to 66.57 mg. of ammonia nitrogen. Hence the equation becomes for this case:

$$\log \frac{x}{133.13 - x} = K (t - 5.6)$$

From all of the observed value we find that the average value of K is 0.3352, hence the equation becomes:

$$\log \frac{x}{133.13 - x} = 0.3352 (t - 5.6)$$

As before, giving the successive value 1, 2, 3, etc. for t , we can compute the corresponding theoretical value of x . In Table II the theoretical and observed results are compared.

TABLE II
COMPARISON OF AMOUNT OF AMMONIA NITROGEN FOUND WITH THAT
CALCULATED BY FORMULA

Time, Days	Ammonia Nitrogen mg.	
	Found	Calculated
1	4.24	3.72
2	6.08	7.78
3	15.13	15.77
4	31.07	30.00
5	51.15	51.11
6	81.07	76.75
7	98.31	99.39
8	108.27	115.06
9	119.36	124.14
10	128.51	128.78

III

Applying the same method to another experiment of Lipman's in which dried blood equivalent to 155 mg. of nitrogen was added and using the points ($t = 3, 5, 6$ and 8) on the curve to determine A , we find:

$$A = 82.10$$

$$\frac{A}{2} = 41.05$$

The daily increment of ammonia nitrogen is at a maximum between 25.52 and 54.04 mg. of ammonia nitrogen. Hence the value of A deduced from the equation is the correct one. The time to produce 41.05

mg. of ammonia nitrogen is 5.6 days. Hence the equation becomes for this case :

$$\log \frac{x}{82.10 - x} = K (t - 5.6)$$

By calculating the value of *K* from the observations at *t* = 1, 2, 3, 4, 7 and 8 we find, respectively, 0.2927, 0.3461, 0.3698, 0.4134, 0.3889 and 0.3781. The average of these values is 0.3651. Hence the equation becomes :

$$\log \frac{x}{82.10 - x} = 0.3651 (t - 5.6)$$

TABLE III
COMPARISON OF AMOUNT OF AMMONIA NITROGEN FOUND WITH THAT CALCULATED BY FORMULA

Time, Days	Ammonia Nitrogen mg.	
	Found	Calculated
1	3.34	1.68
2	4.41	3.79
3	8.13	8.21
4	14.70	16.96
5	25.52	30.91
6	51.04	47.90
7	63.73	62.76
8	73.06	72.44
9	55.10	77.10
10	61.53	80.10

Now giving *t* the successive value 1, 2, 3, etc., we find the following theoretical value of *x*.

The discrepancy in the last two values is evidently due to the accompanying nitrification in which ammonia formed undergoes change to nitric acid.

APPLICATION TO NITRIFICATION

I

Warington (3) has made an experiment upon the growth of nitric acid in nitrification. By the application of the method above described to his result we find :

$$\log \frac{1.1}{A - 1.1} = K (62 - t_1)$$

$$\log \frac{9.6}{A - 9.6} = K (77 - t_2)$$

$$\begin{aligned} \log \frac{13.1}{A - 13.1} &= K (81 - t_1) \\ \log \frac{18.7}{A - 18.7} &= K (96 - t_1) \\ \frac{9.6 (A - 1.1)}{1.1 (A - 9.6)} &= 15 K \\ \frac{18.7 (A - 13.1)}{13.1 (A - 18.7)} &= 15 K \\ \frac{9.6 (A - 1.1)}{1.1 (A - 9.6)} &= \frac{18.7 (A - 13.1)}{13.1 (A - 18.7)} \\ A &= 19.2 \\ \frac{A}{2} &= 9.6 \\ t_1 &= 77. \end{aligned}$$

Then the equation becomes:

$$\log \frac{x}{19.2 - x} = K (t - 77)$$

By calculation of K from the observations at $t = 62, 81$ and 96 we have, respectively, $0.0811, 0.0830$ and 0.0828 . The mean of these values is 0.0823 . Hence the equation becomes:

$$\log \frac{x}{19.2 - x} = 0.0823 (t - 77)$$

Now giving t the value of $62, 77, 81$ and 96 we can calculate their corresponding theoretical value of x , as will be seen in Table IV in which the observed and calculated values of x are compared.

TABLE IV
COMPARISON OF AMOUNT OF NITROGEN AS NITRIC ACID FOUND WITH THAT CALCULATED BY FORMULA

Time, Days	N as HNO ₃ per million	
	Found	Calculated
62	1.1	1.06
77	9.6	9.60
81	13.1	13.08
96	18.7	18.69

Owing to the fact that the experiment was made at intervals too infrequent to enable us to determine A , the number in parenthesis, therefore, was measured by the author from the curve and added.

The agreement between the observed and calculated values is quite good.

II

Applying the same method to another result of nitrification by Warington we find also that the theoretical values of x nearly coincide with those of observation. Using the points ($t = 22, 35, 52$ and 65) on the curve to determine A , we have:

$$A = 25.3$$

$$\frac{A}{2} = 12.7$$

$$t_1 = 50.$$

Then the equation becomes:

$$\log \frac{x}{25.3 - x} = K (t - 50)$$

Giving t all of the observed values the average value of K is calculated to be 0.0501. Hence the equation becomes:

$$\log \frac{x}{25.3 - x} = 0.0501 (t - 50)$$

Now giving t the values of 22, 35, 49, 52 and 65 we find that the corresponding theoretical values of x are 0.96, 3.81, 11.92, 14.10 and 21.49, respectively. In Table V the observed and calculated values are compared:

TABLE V
COMPARISON OF AMOUNT OF NITROGEN AS NITRIC ACID FOUND WITH THAT CALCULATED BY FORMULA

Time, Days	N as HNO_3 per million	
	Found	Calculated
22	0.7	0.96
35	2.0	3.81
49	11.9	11.92
(52)	14.2	14.10
65	20.1	21.49

III

Applying the same method to one other experiment on nitrification obtained by Lemmermann, Fischer and Heintz (1), we find good agree-

ment between the values of observation and calculation. Using the points ($t = 0, 14, 28$ and 42) on the curve for the determination of A we have:

$$A = 15.53$$

$$A$$

$$= 7.77$$

$$2$$

$$t_1 = 13.$$

Assuming that the values obtained are correct, the equation becomes:

$$\log \frac{x}{15.53 - x} = K (t - 13)$$

Now when $t = 0$ we have:

$$\log \frac{4.70}{10.83} = -13 K$$

whence $K = 0.0279$, similarly from the observations at $t = 14, 28, 42$ and 49 we find, respectively, $K = 0.0236, 0.0353, 0.0316$ and 0.0345 . The average value of K from these observations is 0.0305 , hence the equation becomes:

$$\log \frac{x}{15.53 - x} = 0.0305 (t - 13)$$

Now giving t the values of $0, 14, 28, 42, 49$ and 77 , we can compute the corresponding theoretical value of x . In Table VI the theoretical and experimental values are compared.

TABLE VI
COMPARISON OF AMOUNT OF NITRATE NITROGEN FOUND WITH THAT
CALCULATED BY FORMULA

Time, Days	Nitrate Nitrogen mg.	
	Found	Calculated
0	4.70	4.70
14	7.97	8.03
28	11.96	11.51
42	13.85	13.74
49	14.69	14.36
77	14.26	15.35

The agreement between calculated and actual values of nitrate nitrogen is as perfect as in the previous cases.

SUMMARY

The preceding discussion may be summarized as follows:

1. The processes of ammonification and nitrification are autocatalytic chemical reactions.

2. In ammonification and nitrification the maximum increase of ammonia and nitric acid in a unit of time occurs when the total amount of production due to these processes is half completed.

3. Increases of ammonia and nitric acid in the processes are in accordance with the formula

$$\log \frac{x}{A-x} = K (t - t_1)$$

where x is the amount of ammonia and nitric acid which has been produced at time t , A is the total amount of ammonia and nitric acid produced during the process, K is a constant and t_1 is the time at which half of the total amount of ammonia and nitric acid is produced.

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