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Reduction of Nitrates by Bacteria
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AND
Consequent Loss of Nitrogen

BY ELLEN H. RICHARDS, A.M.
AND
GEORGE WILLIAM ROLFE, A.M.

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REDUCTION OF NITRATES BY BACTERIA AND CON-
SEQUENT LOSS OF NITROGEN.

BY ELLEN H. RICHARDS, A.M., AND GEORGE WILLIAM ROLFE, A.M.

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In the course of experiments upon the cycle of nitrogen in aqueous solution under different conditions, it was observed that in some cases a disappearance of nitrogen occurred for which no theory seemed to satisfactorily account. At length a series of tests was made by several consecutive classes of students, which, although liable to irregularities, as are all results obtained by inexperienced workers, were so far conclusive as to encourage further investigation, and two years later one of the instructors carried out a number of confirmatory tests, the results of which are given combined with the rest. It remained for us to complete the investigation and establish the point in question.

It has been repeatedly proved¹ that the cycle of nitrogen in natural surface waters is (1) albuminoid ammonia, (2) free ammonia, (3) nitrites, (4) nitrates, (5) again, albuminoid ammonia; but when feeding nitrates together with organic material to hasten growth it was observed that *nitrites* were the first and only products, and that they soon disappeared. The problem was, What became of the nitrogen, and under what conditions did this disappearance take place?

The nitrates obviously were not reduced to ammonia, although we know that by many chemical and electrical processes this change is quite possible. Neither was this loss of nitrogen from the solution an apparent one only, as was first suggested, due to defects in our analytical methods. The results of the Kjeldahl process did not include the missing nitrogen even in presence of a zinc-copper couple. The general thought, moreover, seems to be against the probability of such loss.

The data herein tabulated represent investigations made with twenty-five or more solutions, the majority of which were so prepared as to typify the condition of a water polluted with decaying organic matter and at the same time containing nitrates. Such a

¹ Among others, see the numerous papers on the purification of waters in the Reports of the State Board of Health of Massachusetts.

Rich. Coll.

solution could be obtained by the admixture of fresh sewage with its purified effluent. We may for convenience allude to these solutions as sewages, but wish it borne in mind, however, that they differ from a mixture of ordinary town sewage in three important characteristics—low mineral content, absence of urea, and presence in most of a small quantity of sugar; moreover, the organic matter at the outset was not as advanced in decomposition as that of even very fresh sewages. The mixture was originally designed to furnish a solution in which at the end of a given time there should be no dissolved oxygen.

The city tap water was used in the preparation of most of these solutions. Nitrates were furnished by potassium nitrate. With one or two exceptions, to be mentioned, fresh milk, in the proportion of one cubic centimeter to a liter, gave the necessary organic matter. Taking the average casein content of milk as 3.4 per cent., the theoretical total ammonia possible, provided none resulted from reduction of the nitrate, was therefore about 0.71 parts per 100,000.

As will be seen by the tabulated data, the general characteristics of the solutions in their successive stages of decomposition were practically identical, the principal variant being in time.

The white turbid solution quickly turned gray, became exceedingly offensive in odor, and evolved considerable gas. This condition reached its most pronounced stage in from four to ten days. The solution then began gradually to lose its turbidity, the evolution of gas practically ceased, and green growth began to appear, usually at the bottom. This growth rapidly increased on the glass until the entire bottle was thickly coated with green, the offensive odor meanwhile disappearing.

Turning to the chemical analyses, we find the following well-marked periods corresponding to these changes: (1) A rapid disappearance of nitrates, usually less than 10 per cent. remaining at the end of three days; (2) a correspondingly rapid increase in nitrites, (3) these usually reaching a maximum two or three days later, and (4) then in turn rapidly disappearing; (5) a slight reappearance of nitrites following the green growth; (6) gradual reappearance of very small quantities of nitrates.

During all these changes the total nitrogen obtained as ammonia remained fairly constant,¹ while the tests for free and albuminoid am-

¹ Discrepancies in individual analyses are considerable, but this is to be expected, for, aside from the inexperience of the students, it is very difficult to get a representative sample from such large bottles thickly coated with a tenacious growth.

monia show a general conformity to the laws governing the cycle of nitrogen changes already referred to in this paper and fully discussed in a previous publication.¹ Stages (5) and (6) can be interpreted in the same way.

This conformity is also manifest in the tests made of solutions made with milk without the addition of nitrate. Moreover, the fact is at once established that the nitrate rapidly changes to nitrite, and almost as rapidly disappears. We do not, however, find practically any of this nitrogen of the nitrate revealed to us by our tests for ammonia. In fact, it not only does not conform to the laws of change observed in surface waters, but, as far as our examination of the solution goes, it has quite disappeared. At the same time the nitrogen from the milk proceeds in the regular cycle, at any period of which the various tests give a sum total of nitrogen approximating closely to that obtained from the original fresh milk solution. Tests made many months later confirmed the result.

The following tabulated data from tests on milk solutions made as described—one with nitrate, the other without—illustrate these nitrogen transformations clearly:

A. $\left\{ \begin{array}{l} \text{Milk, 8 cc.} \\ \text{City Water, 8 liters.} \end{array} \right.$

Date.	Age. Days.	AMMONIA.			Nitrogen as Nitrites.	Nitrogen. as Nitrates.
		Free.	Albuminoid.	Total.		
1891.						
March 13001 ²	000	.004
March 16	3	.002	000
March 17	4	.015	000	.007
March 18 ³	5	000	.035
March 23	10	.201	000	.010
March 30	17	.275	000	.020
April 6	24	.360	000
April 13	31	.332	.240	000	.032
April 20	38	.200	.140	000	.008

¹ "Nitrification and the Nitrifying Organism," by E. H. Richards and E. O. Jordan, Exp. Investigations by the Mass. Board of Health upon Purification of Sewage. Part II, 1890, p. 872.

² All figures express parts per 100,000 unless otherwise specified.

³ On this date a little soda was added to the solution, which was quite acid.

Green Growth.

April 29	47	.180	.160002
June 22	101	.003700000

B. { Milk, 8 cc.
 { KNO_3 , 2.50. (N = 4.33 parts per 100,000.)
 { City Water, 8 liters.

Date.	Age. Days.	AMMONIA.			Nitrogen as Nitrites.	Nitrogen as Nitrates.
		Free.	Albuminoid.	Total.		
1891.						
March 13	$\frac{1}{2}$	000	.330	000	1.500
March 16	3	.079	2.280
March 17	4	.159	3.040	.060
March 18	5	.216	1.600	.070
March 19	6	.330
March 20	7	.386420
March 23	10	.429050
March 30	17	.555009	.090
April 6	24	.680100
April 13	31	.320	.080444
April 20	38	.018	.110
April 25	42540	.080

Green Growth.

April 29	47	.009500	.075
May 2	50	.005	.251	.545	.333	.020
May 5	53333
May 13	61286	.012
June 22	1010002

Green growth was first noticed in both solutions on April 27.

In case of Solution A, where no nitrates were added, we can find no evidence that nitrites are formed from the decomposition and oxidation of the organic matter. Solution B, containing nitrates originally, on the other hand, shows two remarkable facts—practically the complete reduction of this nitrate to nitrite in less than four days, and the almost complete disappearance of the nitrite a few days later. After the green growth appears the nitrogen passes through the normal life-cycle, the ammonia figures declining as those of the oxidized nitrogen increase.

The tests on Solutions 3 and 4, given below, in general show similar results. It will be noted that the reduction progresses equally in both solutions, but that as soon as the smaller store of nitrate in Solution 4 is exhausted the resulting nitrites *totally disappear in about six hours*. Unfortunately, no tests were made on Solution 3 during the critical fifth and sixth days, so that the time of complete reduction is not exactly established. It will be seen, however, that the evidence is strong that it occurred on the afternoon of May 10. The relatively slower reduction of these solutions may be due to their frequent disturbance during the sampling for tests. The time of reduction, as we have said, is variable. Solutions started in March and April apparently show a greater rapidity of decomposition than those of June and July.

3. $\left\{ \begin{array}{l} \text{Milk, 8 cc.} \\ \text{KNO}_3. \text{ (N = 4.33 parts per 100,000.)} \\ \text{City Water, 8 liters.} \end{array} \right.$

Date.	Age. Days.	AMMONIA.			Nitrogen as Nitrites.	Nitrogen as Nitrates.
		Free.	Albuminoid.	Total.		
1891.						
May 4, A.M.044	.270000	4.200
May 4, 4 P.M.003
May 6, A.M.	2	.068	.248400
May 7, A.M.	3	1.080	.080
May 7, 4 P.M.	2.000
May 8, A.M.	4	2.182	.050
May 11	7	.112	.294	.710	.900	.012
May 20	16	.384	.169000	.003

Green Growth.

May 28	24003	.002
June 4	31003	.002
June 22	49	.002830	.001	000
1892. February 27010	.340	.744	000
1893. January 2150

4. { Milk, 8 cc.
KNO₃. (N = 1.00 parts per 100,000.)
City Water, 8 liters.

Date.	Age. Days.	AMMONIA.			Nitrogen as Nitrites.	Nitrogen as Nitrates.
		Free.	Albuminoid.	Total.		
1891.						
May 4, A.M.032	.252	.605	000	1.000
May 6, 4 P.M.	2	.064	.294400
May 7, A.M.	3	1.080	.046
May 7, P.M.	000
May 8	40002	000
May 9	5	.080
May 11	7	.024	.300	.710	.0001	000
May 20	16	.240	.2020001	000

Green Growth.

May 28	240001	000
June 4	310003	000
June 22	49	000	.002
1892. June 29550

Solution No. 3 continued to support a vigorous growth of algae for over three years. On February 6, 1894, a Kjeldahl test gave .640 parts of ammonia in 100,000. Microscopical investigations

showed that organisms were present in the following numbers per cubic centimeter of solution :

<i>Cosmarium</i> ,	44,000.	<i>Protococcus</i> ,	42,000.
<i>Raphidium</i> ,	3,100.	Masses of Zoöglöea,	2,800.
<i>Scenedesmus</i> ,	23,000.	Infusoria (Monas),	250.

Taking as a commonly accepted fact that these nitrogen transformations which we have discussed are the result of *life* processes, presumably those of bacteria, several questions at once suggest themselves: Can these organisms live on nitrates alone? If not, were we to increase the organic matter present, as the milk, could we make their forces more energetic? Would these organisms thrive on all kinds of nitrogenous organic matter equally well as on milk? The following data were therefore collected. Solution 7, containing no organic matter other than that naturally present in the water, shows that reduction takes place very slowly and incompletely. Although the organisms were present, evidently some necessary food, such as that in milk, was lacking. The solution remained clear and free from odor during the month or more that this investigation was carried on.

7. $\left\{ \begin{array}{l} \text{KNO}_3. \text{ (N = 4.00 parts per 100,000.)} \\ \text{City Water, 2 liters.} \end{array} \right.$

Date.	Age — Days.	Nitrogen as Nitrites.	Nitrogen as Nitrates.
1891.			
May 13	4.000
May 15	2	.000
May 18	3	.000
May 20	7	.0004
May 28	15	.0006	4.000
June 22	40	.0025	2.000

Solutions 5 and 6 show the effect of increase of organic matter by use of more milk.

There is manifestly more rapidity of reduction; at the same time this increase of energy is not proportional to the food supply, showing that, while the latter has a marked influence, there are other limiting conditions.

5. { Milk, 6 cc.
 .KNO₃. (N = 4.00 parts per 100,000.)
 City Water, 2 liters.

Date.	Age. Days.	AMMONIA.			Nitrogen as Nitrites.	Nitrogen as Nitrates.
		Free.	Albuminoid.	Total.		
1891.						
May 4080
May 6, A.M.	2526	1.00
May 6, P.M.	2.182
May 8, A.M.	40003
May 8, P.M.0002	000
May 9, A.M.	5	.080
May 11	7	000	000

Green Growth.

May 28	23333	.005
June 4	31364	.010

6. { Milk, 10 cc.
 KNO₃. (N = 4.00 parts per 100,000.)
 City Water, 2 liters.

Date.	Age. Days.	AMMONIA.			Nitrogen as Nitrites.	Nitrogen as Nitrates.
		Free.	Albuminoid.	Total.		
1891.						
May 4092
May 6, A.M.	2741
May 6, P.M.073
May 7, A.M.	3	3.243
May 8, A.M.	4	000
May 8, P.M.	000	000
May 9, A.M.	5	.080	1.500
May 11	7	.666	1.232	000	000

Green Growth.

May 29	25	000	000
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Green growth was first noticed in No. 5 on May 22, in No. 6 on May 26.

Aëration of milk solutions by means of aspirators produced no difference in their behavior other than a slight retardation of the reducing action. Evidently the air could not be retained long enough by the solution to be of any use to the organisms. This is conformable with the results obtained by Dr. Drown on the aëration of natural waters.¹

II. { Milk, 8 cc.
KNO₃ Solution, 80 cc. (N = 4.00 parts per 100,000.)
City Water, 8 liters.

Date.	Age. Days.	AMMONIA.			Nitrogen as Nitrites.	Nitrogen as Nitrates.
		Free.	Albuminoid.	Total.		
1891.						
June 23355	.500	.000	4.000
June 24, 12 M.	1007
June 24, 5 P.M.022
June 25	2	.112	2.272	1.000
June 30	7	.236	4.000

II A. Same solution as II, but aërated continuously.

Date.	Age. Days.	AMMONIA.			Nitrogen as Nitrites.	Nitrogen as Nitrates.
		Free.	Albuminoid.	Total.		
June 23355	.500	.000	4.000
June 24	10025
June 25	2	1.600	2.000
June 30	7	.258	4.000

Another solution was made up in a precisely similar way as No. II, except that a portion of Solution No. 3 was used in its preparation, with the intention of seeding it with organisms already developed.

¹ "Effect of the Aëration of Natural Waters," by Dr. Thomas M. Drown, in the Twenty-Third Annual Report of the State Board of Health of Massachusetts. Also, page 479 of the Twenty-Sixth Report of the same Board in an article on "Filtration of Sewage," by George W. Fuller.

12. { Milk, 8 cc.
KNO₃ Solution, 80 cc. (N = 4.00 parts per 100,000.)
City Water, 7.5 liters.
Solution 3, .5 liter.

Date.	Age. Days.	AMMONIA.			Nitrogen as Nitrites.	Nitrogen as Nitrates.
		Free.	Albuminoid.	Total.		
1891.						
June 23303	.700	.0001
June 24, A.M.	10002
June 24, 5 P.M.0018
June 25	2	.048	1.740	2.000
June 30	7	.004	4.000

12 A. Same solution, except aerated continuously.

Date.	Age. Days.	AMMONIA.			Nitrogen as Nitrites.	Nitrogen as Nitrates.
		Free.	Albuminoid.	Total.		
1891.						
June 23	000	.303	.700	.0001
June 24	10007
June 25	2800	3.000
June 30	7	4.500

We have so far made but few investigations as to the nature of the food required by these organisms.

Solutions have been made with one cubic centimeter of blood per liter instead of milk. These solutions changed but little in appearance even after a month's standing. The chemical tests show that the reduction proceeded very slowly; in short, the organisms present, if they thrived on the blood, did not apparently obtain oxygen by tearing apart the nitrate.

Solution 8, containing in addition to the blood four parts of sugar per 100,000, showed no marked difference in behavior or reduction of nitrate, although the tests show slightly more nitrite.

1. { Blood, 8 cc.
KNO₃. (N = 4.00 parts per 100,000.)
City Water, 8 liters.

Date.	Age. Days.	AMMONIA.			Nitrogen as Nitrites.	Nitrogen as Nitrates.
		Free.	Albuminoid.	Total.		
1891.						
May 4052	.458	3.11	.000	4.000
May 4, 4 P.M.0003
May 6	2	.0720003	4.000
May 8	40075
May 9	5	.560003
May 20	16	1.454013	2.500
May 28	24020
June 22	49040	1.200

8. { Blood, 2 cc.
KNO₃. (N = 4.00 parts per 100,000.)
Grape Sugar, .080 gram.
City Water, 2 liters.

Date.	Age. Days.	AMMONIA.			Nitrogen as Nitrites.	Nitrogen as Nitrates.
		Free.	Albuminoid.	Total.		
1891.						
May 13000	4.000
May 15	20002
May 18	5136
May 19	6200
May 20	7200	3.500
May 28	15190
June 22	40028	1.200

These solutions showed no green growth for many months, and at no time was this growth anything but scanty.

A few solutions were prepared from materials in themselves practically sterile, distilled water and evaporated milk. These solutions were inoculated with liquid from old solutions; one with a solution

originally made with milk, another with a blood solution, a third with a solution containing milk and glycerine (to be described later), while the fourth contained no admixture of old solutions. Bacteriological cultures were made from these solutions from time to time. The tables show the immense multiplication of these organisms, and also give the results of some Kjeldahl tests at the end of twenty-two days, which, while they undeniably show that the original total ammonia figures have not changed perceptibly, emphasize the difficulty of getting exact representative samples from the bottles, a trouble already referred to. The figures for the oxygen consumed of Solution 13 are also instructive, and will be referred to later.

13. { Milk diluted from evaporated milk, 8 cc.
 { Distilled Water, 8 liters.

Date.	Age. Days.	AMMONIA.			Oxygen Consumed.	Bacteria per cc.
		Free.	Albuminoid.	Total.		
1892.						
March 3016	.356	.696	4.326	104
March 4	1	2.802	3,000
March 5	2	2.348
March 7	4	2.307
March 15	12	{ 1.081 f.589	150,000
March 25	22	.240	{ .206 f.072	{ .630 f.150 f.652 f.384

f = filtered.

14. 2 liters of same solution, but with addition of 50 cc. of No. I. (Blood solution.)

Date.	Age. Days.	TOTAL AMMONIA.		Bacteria per cc.
		Filtered.	Unfiltered.	
March 3	262
March 4	1	(3 molds) 800
March 15	12	1,260,000
March 25	22	{ .300 .348	{ .700 .670	

15. 2 liters of same solution as 13, but with the addition of 50 cc. of No. 2. (Glycerine and milk solution.)

Date.	Age. Days.	TOTAL AMMONIA.		Bacteria per cc.
		Filtered.	Unfiltered.	
1892.				
March 3	147 (34 moulds)
March 4	1	2,800
March 15	12	1,300,000
March 25	22	{ .150 .188	{ .650 .788	

16. 2 liters of same solution, but with the addition of 50 cc. of No. 3. (Milk solution.)

Date.	Age. Days.	TOTAL AMMONIA.		Bacteria per cc.
		Filtered.	Unfiltered.	
March 3	291 (3 moulds)
March 4	1	1,400
March 15	12	1,108,000
March 25	22	{ .140 .140	{ .550 .572	

A few of the conditions affecting the disappearance of this nitrogen as a result of the decomposition of the nitrates have been investigated. The experiments on this line did not, however, throw any light on what becomes of the element. Is it that this nitrogen does not take any part in the life history of the plant forms, and therefore has it no influence on the final result as we interpret it? Is it rejected by all the busy organisms which successively play their parts in this well-marked life-cycle? Is it stored away in some form which has escaped the scrutiny of the chemist, or has it passed off in some gaseous excretion?

These questions led to further experiments, with two objects in view: (a) To make this nitrogen appear as albuminoid ammonia in the solution; (b) to find if this nitrogen existed as a gaseous product, excreted by the organisms or rejected by them. The first series of investigations is as yet far from complete.

The only successful experiment in this line was made by adding two cubic centimeters of glycerine per liter of the usual milk and

nitrate solution. The results indicate that a part of the nitrogen was fixed. The nitrate and nitrite figures are in general like those of similar solutions without the glycerine. The solution, however, as far as its external behavior was noted, acted in a radically different manner. Large quantities of gas, apparently carbon dioxide, were given off. This active fermentation began about the eleventh day and continued for over a month, the solution being quite sour, with an odor of yeast. The organisms which developed in this solution in great abundance are a bright red, and have retained their color for five years.

2. $\left\{ \begin{array}{l} \text{Glycerine, 16 cc.} \\ \text{Milk, 8 cc.} \\ \text{KNO}_3 \text{ (N = 4.00 parts per 100,000.)} \\ \text{City Water, 8 liters.} \end{array} \right.$

Date.	Age. Days.	AMMONIA.			Nitrogen as Nitrites.	Nitrogen as Nitrates.
		Free.	Albuminoid.	Total.		
1891.						
May 4052	.011	.695	.000	4.100
May 4, 4 P.M.0003
May 6	2	.108400
May 7	3	1.080	.800
May 8	4	2.650
May 9	5	1.750
May 20	16000	.020
May 29	250005	.002
June 14.	310003	.003
June 21.	48	.007	1.665	.0001	.000

No green growth has appeared in this solution.

In the investigation of the gaseous products of the decomposition much more work has been done.

On March 13, 1894, three milk solutions were made up with potassium nitrate after the usual manner. Two of these were so arranged that the gas evolved could be collected over mercury, any air other than that dissolved in the water of each bottle being carefully excluded. The composition of the gases dissolved in the water of

each bottle was determined by their removal from a standard volume by means of a Sprengel pump and their examination in a Frankland gas apparatus. The gases evolved were also determined¹ and finally those dissolved in the resulting sewage. A few chemical tests were made of the contents of the third bottle during the decomposition. The same work was repeated in June, 1895, the apparatus being so modified that chemical tests might be made of the solutions at the same time that the gaseous products were being investigated. The results as arranged are self-explanatory, and show at once what has become of the nitrogen of the nitrate.² Two of the investigations show that within less than 3 per cent. this nitrogen can be accounted for. The third shows that 7 per cent. more nitrogen is evolved than can be accounted for by the decomposition of the nitrate, a discrepancy we cannot explain. However, it is clearly demonstrated that practically all the nitrogen of the nitrate is freed as the elementary gas.

The rapid decrease in "oxygen consumed"³ is also shown to be the result of the early escape of carbon as the dioxide, and makes it clear that this test is useless as a measure of pollution, however valuable it may be as an aid in ascertaining the nature of the organic matter present.

18. *Experimental Data.*⁴ { Potassium Nitrate: .3125 gram per 1,000 cc. of City Water.
(Nitrogen = 4.33 parts per 100,000.)
Milk: 1 cc. per 1,000 cc. of City Water.
Three bottles were filled:
- A, 8,580 cc.
B, 8,660 cc.
C, 8,380 cc.

To each bottle had been previously added 10 cc. of an old milk solution (3).

Chemical tests were made on Bottle C. Gas evolved by A and B was collected over mercury.

Started March 9, 1894, 2.15 P.M. Putrefaction was very rapid. All gas was evolved by March 17.

¹ By Hempel's Method.

² It has already been shown by previous investigators that nitrogen gas is sometimes a product of decomposition. (See recent article by George W. Fuller in Twenty-Sixth Annual Report of State Board of Health of Massachusetts, pp. 472, 522, 581.)

³ See Table, p. 51.

⁴ We are indebted to Professor A. H. Gill for valuable advice, as well as the facilities for carrying on this work.

Bottle C.

Date.	Age. Days.	Nitrogen as Nitrites.	Nitrogen as Nitrates.
1894.			
March 9, 2.15 P.M.	4.33
March 10, 10 A.M.	1	2.80
March 12	3	0.25
March 13	4	1.500
March 15	6	000
March 17	8	000
March 21	12	Signs of green growth at bottom of bottle.	

Gases Dissolved in City Tap Water used in making up Solutions ($t = 11^{\circ} C.$).

	Bottle A.	Bottle B.	Per cent. by Volume.
Nitrogen (by difference) . . .	201.80 cc. ¹	203.70 cc.	73.21
Oxygen	72.91	73.58	26.43
Carbon Dioxide	1.00	1.01	.36

Gases Evolved in First Four Days of Putrefaction.

	BOTTLE A.		BOTTLE B.	
	Volume.	Per cent.	Volume.	Per cent.
Nitrogen	76.91 cc.	93.75	52.49 cc.	96.37
Oxygen37	.45	000
Carbon Dioxide	2.10	2.56	1.99	3.63
Methane	2.66	3.24	000
Nitrous Oxide	000

No more gas was collected from Bottle A, owing to breakage of connection.

¹ All gas volumes are calculated at standard temperature and pressure.

Gases Evolved March 14-17. Bottle B.

	FIRST PORTION.		SECOND PORTION.		Total.
	Volume.	Per cent.	Volume.	Per cent.	
Nitrogen	80.87 cc.	97.58	149.56 cc.	97.81	282.92 cc.
Carbon Dioxide	1.31	1.54	2.23	1.46	4.54
Methane73	.88	1.11	.73	1.84

18. *Experimental Data* (concluded). Gas Dissolved in Resulting Sewage.

	Volume.	Per cent.
Nitrogen	212.80 cc.	71.24
Carbon Dioxide	76.58	25.64
Oxygen	9.34	3.12

Total Nitrogen Gas Produced in Milk Solution.—292.02 cc. = 4.23 parts per 100,000, or 97.57 per cent. possible nitrogen from dissolved nitrate.

19. *Experimental Data.* Solution prepared and inoculated exactly like Solution 18. Two bottles filled:

A, 8,600 cc.
B, 9,010 cc.

Solution prepared June 19, 1895, 4.30 P.M. Bottle A.

Date.	Age. Days.	Total Ammonia.	Nitrogen as Nitrates.	Remarks.
1895. June 19803	4.33	Putrefaction proceeding very slowly.
June 21	270	
July 2	13	.864	000	No signs of green growth.

Bottle B.

1895. June 19830	4.33	
June 21	250	
July 2	13	000	No signs of green growth.

Practically no gas was evolved from either bottle until after the third day. The gas evolution from both bottles was much slower than in the experiment of the previous year, Bottle B producing notably more gas than Bottle A.

19. *Experimental Data* (concluded). Gases Dissolved in City Tap Water used in making up Solutions ($t = 22.5^{\circ} \text{C.}$).

	Bottle A.	Bottle B.	Per cent. by Volume.
Nitrogen	122.7 cc.	128.5 cc.	69.86
Oxygen	44.87	46.99	25.57
Carbon Dioxide	8.05	8.44	4.57

Gases Evolved in Putrefaction.

	Bottle A.	Per cent. by Volume.	Bottle B.	Per cent. by Volume.
Nitrogen	195.80 cc.	98.42	235.20 cc.	98.85
Carbon Dioxide	3.14	1.58	2.73	1.15
Carbon Monoxide	000	000
Methane	000	000
Oxygen	000	000	...

Gases Dissolved in Resulting Sewage.

	Bottle A.	Per cent. by Volume.	Bottle B.	Per cent. by Volume.
Nitrogen	217.40 cc.	77.80	227.50 cc.	75.54
Carbon Dioxide	58.03	20.75	68.73	22.82
Oxygen	4.09	1.45	4.95	1.64

Total Nitrogen Gas Produced in Sewage of Bottle A.—290.5 cc. = 4.24 parts per 100,000, or 97.92 per cent. possible nitrogen from dissolved nitrate.

Total Nitrogen Gas Produced in Sewage of Bottle B.—334.2 cc. = 4.66 parts per 100,000, or 107.6 per cent. of the nitrogen gas possible from nitrate dissolved.

It is somewhat hazardous to theorize as yet on the chemistry of these changes, especially with our limited knowledge of the nature of the powerful life agencies at work in nature's laboratory through the

medium of bacteria. A natural inference would be that ammonium nitrate is formed, which breaks up into water and nitrogen gas. On the line of this theory the thought at once occurs, can we not introduce some substance in itself not readily decomposed by the processes of fermentation which, through the subtle reaction of bacterial life, might be made to form substituted ammonia products or their analogues which, breaking up into stable compounds, would result in fixing the nitrogen?

The addition of glycerine to the milk solutions was suggested to us. The results in this case certainly point to the fixing of some of the nitrogen, but further investigations, which we have in hand, are necessary to prove this beyond all doubt.

The conclusion may be stated thus: Whenever nitrates are added to decomposable organic matter not sterile, under such conditions that the growth of the bacteria requires more oxygen than the solution affords, the plants will take it from the nitrates, setting free nitrites, which in time are decomposed, setting free nitrogen.

Therefore, a very nice balance exists between the organic matter to be decomposed, the oxygen at hand, and the plants to do the work.

A clear understanding of these relations is of great importance to the agriculturist and especially to the sewage farmer, since it is easy to lose all the nitrogen once gained by an imprudent addition of food. In fact, to save nitrates already in the soil the sewage must be so applied that the conditions are to the highest degree favorable for contact with the air, as, for instance, in very thin layers. Aëration by the passage of air through the sewage in bulk is quite useless, however thorough the process. Again, even under the most favorable conditions it will be seen that the success of nitrogen storage is exceedingly dubious. The inference is that the most feasible way of economizing nitrogen is to feed it to the growing green plant¹ only as fast as it can be assimilated, rather than to attempt to prepare the soil in advance and risk having the element thrown back in its primal state into the atmosphere by the excretory processes of hordes of oxygen-loving bacteria. Even in spite of aëration certain fermentative organisms seem to prefer to take their supply of oxygen from

¹ George W. Fuller, Twenty-Sixth Annual Report of the State Board of Health of Massachusetts, p. 493.

its nitrogen combinations. Hence loss of nitrogen is liable to occur, if it is not inevitable, when a solution containing certain kinds of decomposing organic matter is added to a soil or water already nitrified.

In this connection the recent paper by George W. Fuller in the *Twenty-Sixth Annual Report of the State Board of Health of Massachusetts*, with its rich store of valuable analytical data, is most suggestive. We have already referred in footnotes to the conclusion of the author concerning the effect of aëration, the loss of nitrogen, and the influence of green growth on this loss as corroborative of our own. Can we not, on the other hand, point to the results of our investigations as shedding light on several points touched on in this work? For instance, is not the greater efficiency of coarse material over fine in sewage purification accounted for by the fact that less nitrogenous matter is retained to stimulate growth of reducing bacteria as well as by the explanation of more efficient air supply (p. 515)?

Again, cannot some of the irregularities of the working of water filters (p. 637) and the variation in nitrates in the Merrimac River water (p. 651) also be made clear if we consider that, during the time in which the nitrates were diminished, the food supply (sewage) was increased, resulting in the decomposition of the nitrates previously existing?

Certainly the fixing of nitrogen in a water or soil in a form available for higher vegetation is a problem requiring further consideration. In a subsequent paper we shall consider some of these points in question.



