123. THE NITROGEN CYCLE IN BIOLOGICAL SYSTEMS

1. SOME CONDITIONS AFFECTING THE DISTRIBUTION OF NITROGENOUS COMPOUNDS DURING TREATMENT OF SEWAGE BY THE ACTIVATED SLUDGE PROCESS

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IN methods of treatment of sewage, the object is usually to reduce the amount of readily oxidizable matter, so that the treated effluent does not cause serious depletion of the dissolved O_2 content of a river or stream into which it is discharged. In the activated sludge process, after preliminary screening and sedimentation, the sewage is vigorously aerated for several hours in admixture with activated sludge. The mixture withdrawn from the aeration tanks is allowed to settle, the treated effluent is discharged, and a proportion of the settled activated sludge is returned to the aeration tanks and used again. Usually the process is continuous and the period of aeration is controlled by adjusting the rate of flow of the sewage.

Activated sludge is prepared by aeration of sewage and consists largely of bacterial cells, some alive, some dead [Wooldridge & Standfast, 1933], together with smaller amounts of granular material. Though it is known that nitrification of the ammoniacal N in sewage usually occurs during aeration with activated sludge, and that nitrite is frequently detected as an intermediate compound, there is little precise knowledge of the conditions which determine the course of the N cycle in the process under consideration. The object of the present investigation was to obtain a clearer understanding of this problem.

Of the N in the settled sewage employed in the experimental work described in this paper, usually about 90% was present as ammonia-N,¹ the balance occurring in organic form: the N in the sludge was present almost entirely in organic form.

Vigorous aeration of sewage with biologically active sludge results in a more or less rapid disappearance of the ammonia-N from the liquid phase. Under usual conditions, part of this ammonia-N is converted into organic form and becomes incorporated in the sludge, while the balance is oxidized to nitrite and then to nitrate. Examination of published results from activated sludge plants in English towns has shown that the fraction of the ammonia-N in the crude sewage which is subsequently found as nitrite or nitrate in the treated effluent varies within very wide limits; sometimes there is no oxidized N in the final effluent.

¹ Except where otherwise stated, no distinction is made here between ammonia- and ammonium-N.

Experimental technique

The settled sewage used in these experiments was supplied from the Finchley Sewage Disposal Works, through the kindness of the Borough Engineer. The activated sludge was made in the laboratory and was maintained in an active condition by continuous aeration and daily additions of sewage. Usually, mixtures for experimental work were taken within a period of 5–60 min. after the addition of fresh sewage to the stock of activated sludge.

In the main, the experiments were concerned with the changes in the nitrogenous compounds during aeration of mixtures of sewage and activated sludge, either alone or after addition of N compounds.

In the first experiments, bubbles of air were passed through 2-l. mixtures of crude sewage and activated sludge, to which nitrogenous compounds were sometimes added, in Winchester bottles at laboratory temperature: the proportion of activated sludge was between 10 and 20% by volume (Tables 1, 2, 4–7). Samples of 100 ml. of the mixture were withdrawn at intervals; the liquid was separated from the sludge by centrifuging, and the two phases were analysed separately. In experiments of not more than 3 days' duration, no account was taken of the slight losses of water due to evaporation since these necessitated a correction of less than 1% in the amount of total N.

In further experiments, mixtures of sewage and sludge containing different amounts of added nitrogenous compounds were aerated continuously with ammonia-free air for several days in 500 ml. flasks at laboratory temperature (Tables 3, 8). The distribution of the nitrogenous compounds was determined at the beginning and end of the experiments. Any ammonia liberated from the mixtures and removed in the current of air was collected and estimated. The liquid and sludge were separated by filtration and were analysed separately.

Adsorption of ammonium ions by activated sludge

Some experiments were carried out in which 2.5 l. of a mixture of settled sewage and activated sludge were aerated for periods of 24 hr. Samples of 100 ml. of the mixture were withdrawn periodically and, after centrifuging for 5 min. to remove suspended matter, the concentrations of ammonia- and of nitrite- and nitrate-N in the liquid were determined. The adsorbed ammonium-N in the sludge was determined by the method of Olsen [1929], in which the sludge, after having been thoroughly washed with water, was added to 50 ml. *N* KCl, the pH value was adjusted to 1.0 with HCl and the mixture was made up to 200 ml. with distilled water. After shaking for 1 hr., the mixture was filtered and the ammonia-N in the filtrate was determined (Table 1). The results, which

Table 1. *Adsorption of ammonium ions by activated sludge during aeration of mixtures of sewage and activated sludge*

The figures refer to mg. N in 100 ml. of the mixture

Period of aeration	Liquid			Moist solid adsorbed NH ₄ -N
	NH ₃ -N	NO ₂ - + NO ₃ -N	NO ₂ -N	
At start	4.3	0.0	—	0.3
30 min.	4.2	0.1	+	0.6
2 hr.	3.5	0.6	+++	0.4
4 hr.	2.4	1.4	+++	0.5
6 hr.	1.4	2.2	+++	0.2
8 hr.	0.6	3.4	+++	0.2
24 hr.	0.1	3.9	—	0.1

+ Indicates a weak reaction and +++ a strong reaction for nitrite.

agree with those of Heukelekian [1936], show that the amount of ammonium-N adsorbed on the sludge was greatest shortly after addition of fresh sewage and decreased as the treatment proceeded; the amount adsorbed at any time was small.

The evidence for Cambier's conclusion [1920], that adsorption of ammonium ions from sewage by activated sludge may be considerable, rests upon the rapid disappearance of ammonia from sewage, without any corresponding nitrification, after the sewage had been in contact with sludge for 15 min.

Distribution of nitrogenous compounds during aeration of mixtures of sewage and activated sludge

(a) *Mixtures in which no nitrification occurred.* In some experiments in which 2-l. mixtures of sewage and activated sludge were aerated in Winchester bottles, there was an approximately exponential diminution in the concentration of the ammoniacal N in the liquid phase, accompanied by a corresponding increase in the N content of the sludge, but no oxidation to nitrite or nitrate occurred (Table 2). The addition of ammonium sulphate before aeration did not alter this type of change during the experiments. When potassium nitrite or nitrate was added to the mixture of sewage and sludge, there was a

Table 2. *Distribution of nitrogenous compounds during aeration of non-nitrifying mixtures of sewage and activated sludge, with added nitrogenous compounds*

The figures refer to mg. N in 100 ml. of mixture

Period of aeration	Liquid					Moist solid Total N	Total N in both phases
	NH ₃ -N	NO ₂ -N	NO ₃ -N	Organic	Total N		
				N			
(a) No addition							
At start	4.3	0.0	0.0	0.2	4.5	31.2	35.7
2 hr.	3.8	0.0	0.0	0.2	4.0	31.1	35.1
5 hr.	3.3	0.0	0.0	0.5	3.8	31.5	35.3
1 day	1.4	0.0	0.0	0.2	1.6	34.0	35.6
2 days	0.2	0.0	0.0	0.3	0.5	35.0	35.5
3 days	0.0	0.0	0.0	0.2	0.2	35.7	35.9
(b) N added in the form of ammonium sulphate							
At start	7.8	0.0	0.0	0.2	8.0	30.9	38.9
2 hr.	7.0	0.0	0.0	0.5	7.5	—	—
5 hr.	6.8	0.0	0.0	0.2	7.0	32.7	39.7
1 day	4.5	0.0	0.0	0.5	5.0	—	—
2 days	3.3	0.0	0.0	0.1	3.4	36.0	39.4
3 days	2.8	0.0	0.0	0.2	3.0	36.3	39.3
6 days	1.8	0.0	0.0	0.3	2.1	36.3	38.4
(c) N added in the form of potassium nitrite							
At start	3.7	2.4	0.0	0.2	6.3	27.6	33.9
2 hr.	3.3	1.0	0.0	0.1	4.4	27.5	31.9
5 hr.	2.9	0.2	0.0	0.1	3.2	28.2	31.4
1 day	0.7	0.0	0.0	0.1	0.8	30.5	31.3
2 days	0.0	0.0	0.0	0.1	0.1	32.0	32.1
3 days	0.0	0.0	0.0	0.0	0.0	32.3	32.3
(d) N added in the form of potassium nitrate							
At start	3.4	0.0	2.5	0.4	6.3	27.9	34.2
2 hr.	3.2	1.1	0.3	0.2	4.8	27.7	32.5
5 hr.	3.1	0.2	0.0	0.2	3.5	28.6	32.1
1 day	0.6	0.0	0.0	0.1	0.7	31.2	31.9
2 days	0.2	0.0	0.0	0.1	0.3	31.8	32.1
3 days	0.0	0.0	0.0	0.1	0.1	31.8	31.9

rapid diminution, during aeration, in the concentration of oxidized N; only a small portion of this diminution could be accounted for by an increase in the organic N. In these experiments, added nitrite was not converted into nitrate, but nitrite was always detected during aeration of mixtures to which nitrate had been added. The increase in the amount of N in the moist solid was approximately the same as the decrease in the amount of ammonia-N in the liquid

Table 3. *Distribution of nitrogenous compounds during aeration of non-nitrifying mixtures of sewage and activated sludge, with added nitrogenous compounds*

No additions were made in the experiments marked with an asterisk (*). The air entering the system was ammonia-free (passage through sulphuric acid) and the ammonia liberated from the flasks and carried in the air current was collected and estimated: the amount of ammonia-N thus liberated did not exceed 0.2 mg. for any series of flasks except series (b).

The figures refer to mg. N per 125 ml. of mixture and, except in series (b), are the means of duplicate experiments.

Before aeration					After aeration					Loss of N
Organic N	NH ₃ -N	NO ₂ -N	NO ₃ -N	Total N	Organic N	NH ₃ -N	NO ₂ -N	NO ₃ -N	Total N	
(a) N added in the form of ammonium sulphate: 2 days' aeration										
12.4	10.5	0.1	0.0	23.0	14.9	7.9	0.0	0.0	22.8	0.2
12.4	3.7	0.1	0.0	16.2	12.6	3.2	0.0	0.0	15.8	0.4
12.4	2.3	0.1	0.0	14.8	12.4	1.6	0.0	0.0	14.0	0.8
*12.4	1.4	0.1	0.0	13.9	13.1	0.2	0.0	0.0	13.3	0.6
(b) N added in the form of ammonium sulphate: 14 days' aeration										
7.6	32.1	0.0	0.0	39.7	9.6	27.0	0.0	0.0	36.6	3.1
7.6	25.9	0.0	0.0	33.5	10.0	23.9	0.0	0.0	33.9	0.4 (gain)
7.6	19.7	0.0	0.0	27.3	10.6	15.4	0.0	0.0	26.0	1.3
7.6	13.4	0.0	0.0	21.0	10.8	9.0	0.0	0.0	19.8	1.2
7.6	7.2	0.0	0.0	14.8	11.3	2.6	0.0	0.0	13.9	0.9
7.6	4.1	0.0	0.0	11.7	11.1	0.1	0.0	0.0	11.2	0.5
*7.6	1.0	0.0	0.0	8.6	8.9	0.0	0.0	0.0	8.9	0.3 (gain)
*7.6	1.0	0.0	0.0	8.6	8.5	0.0	0.0	0.0	8.5	0.1
Gross loss										6.4
N liberated at NH ₃										6.4
N unaccounted for										0.0
(c) N added in the form of potassium nitrite: 2 days' aeration										
12.3	2.4	8.7	0.0	23.4	11.7	0.9	5.0	0.0	17.6	5.8
12.3	2.4	2.2	0.0	16.9	12.4	0.6	0.5	0.0	13.5	3.4
12.3	2.4	0.9	0.0	15.6	12.9	0.2	0.0	0.0	13.1	2.5
*12.3	2.4	0.0	0.0	14.7	12.2	1.2	0.0	0.0	13.4	1.3
(d) N added in the form of potassium nitrite: 14 days' aeration										
14.2		20.4	0.0	34.6	12.4	0.0	0.0	0.9	13.3	21.3
14.2		10.2	0.0	24.4	11.1	0.0	0.0	0.6	11.7	12.7
14.2		4.1	0.0	18.3	10.8	0.0	0.0	0.0	10.8	7.5
*14.2		0.0	0.0	14.2	11.2	0.0	0.0	0.0	11.2	3.0
(e) N added in the form of potassium nitrate: 2 days' aeration										
12.1	2.9	0.0	9.0	24.0	12.7	2.1	5.8	0.0	20.6	3.4
12.1	2.9	0.0	2.3	17.3	14.3	2.1	0.0	0.0	16.4	0.9
12.1	2.9	0.0	0.9	15.9	14.1	2.0	0.0	0.0	16.1	0.2 (gain)
*12.1	2.9	0.0	0.0	15.0	12.9	2.2	0.0	0.0	15.1	0.1 (gain)
(f) N added in the form of potassium nitrate: 14 days' aeration										
16.0		0.0	66.9	82.9	13.9	0.0	0.0	11.7	25.6	57.3
16.0		0.0	33.5	49.5	13.8	0.1	0.0	5.1	19.0	30.5
16.0		0.0	13.5	29.5	13.4	0.0	0.0	1.3	14.7	14.8
*16.0		0.0	0.0	16.0	15.7	0.0	0.0	0.0	15.7	0.3

(Table 2). In the experiments with sewage alone and with added ammonia-N, the total N in the mixtures remained nearly constant during aeration for 3 days. In the experiments in which nitrite or nitrate was added, the total N decreased rapidly during aeration by an amount approximately equivalent to the decrease in the content of nitrite or nitrate.

In other experiments, mixtures of 25 ml. sewage and activated sludge with 100 ml. water, to which different quantities of nitrogenous compounds had been added, were aerated continuously for several days in 500 ml. flasks, and the distribution of N was determined at the beginning and end of each experiment. Typical results are given in Table 3. In the experiments in which ammonium sulphate was added, the ammonia-N lost from the liquid phase during aeration was converted almost quantitatively into organic N in the sludge, but in Exp. *b* there was a loss of ammonia from the mixtures to which large amounts of ammonium sulphate had been added: some ammonia-N remained in the liquid phase after 14 days. When potassium nitrite or nitrate was added to the mixtures of sewage and activated sludge, there was a loss of N during aeration: the loss appeared to be due to the escape of N_2 and no ammonia was detected in the escaping gases. The amount of N thus lost increased as the concentration of added potassium nitrite or nitrate was increased. In these experiments, no indication of the formation of hyponitrous acid or of any other likely intermediate compound was obtained, except for the production of variable quantities of nitrite with mixtures containing added nitrate. In separate experiments in which mixtures of potassium nitrite, sewage and activated sludge were allowed to stand without aeration but under aerobic conditions the gas evolved was almost pure N_2 and contained no N_2O or other combustible gases.

(*b*) *Mixtures in which nitrification occurred.* The results of experiments in which nitrite or nitrate was formed during aeration of mixtures of sewage and activated sludge, with added nitrogenous compounds, are given in Table 4. The total N content of the mixtures remained approximately constant during aeration in all cases. In mixtures with no added substances or containing added ammonium sulphate, a large proportion of the ammonia-N in the liquid phase was oxidized to nitrate, with the intermediate formation of nitrite. On some occasions, as indicated by the resorcinol-potassium periodate test [Corbet, 1934],¹ there were indications of the formation of small quantities of hyponitrite.

When potassium nitrite was added in an amount approximately equivalent to the ammonia-N already present in the sewage, the concentration of nitrite remained nearly constant during aeration until the whole of the ammonia-N had disappeared from the sewage and had been converted into organic N in the sludge; the nitrite was then oxidized to nitrate. The fact that the synthetic or protein-forming stage normally precedes that of nitrification was known to Adeney in 1895.

Effect of the carbon/nitrogen ratio

Many workers have attempted to explain the results of experiments with sewage or soil in terms of the carbon/nitrogen (C/N) ratio of the system under examination, and it might be suggested that, in the experiments described in this

¹ As Sundara Rao *et al.* [1938] have made certain criticisms against this test, it may be pointed out that it was not claimed that it was "delicate" or "specific", and it was clearly stated that hydroxylamine, borates and bicarbonates behaved similarly. This test is useful for distinguishing between hyponitrite and nitrite as, despite the statement made by the Indian investigators, the latter does not give a positive reaction.

Table 4. *Distribution of nitrogenous compounds during aeration of nitrifying mixtures of sewage and activated sludge, with added nitrogenous compounds*

The figures refer to mg. N in 100 ml. of the mixture

Period of aeration	Liquid					Moist solid Total N	Total N in both phases
	NH ₃ -N	NO ₂ -N	NO ₃ -N	Organic N	Total N		
	(a) No addition						
At start	1.5	0.0	0.5	0.5	2.5	13.6	16.1
2 hr.	1.2	0.2	0.4	0.1	1.9	14.6	16.5
5 hr.	0.6	0.4	0.6	0.2	1.8	14.7	16.5
1 day	0.0	0.0	1.3	0.2	1.5	15.1	16.6
2 days	0.0	0.0	1.6	0.3	1.9	14.7	16.6
3 days	0.0	0.0	1.8	0.1	1.9	14.7	16.6
(b) N added in the form of ammonium sulphate							
At start	6.7	0.0	0.0	0.9	7.6	9.6	17.2
2 hr.	6.7	0.1	0.3	0.6	7.7	9.8	17.5
5 hr.	6.7	0.3	0.5	0.3	7.8	9.7	17.5
1 day	5.0	1.2	1.4	0.1	7.7	9.7	17.4
2 days	4.4	1.3	1.9	0.1	7.7	9.6	17.3
3 days	3.4	0.1	4.0	0.2	7.7	9.5	17.2
(c) N added in the form of potassium nitrite							
At start	2.3	2.1	0.0	0.6	5.0	5.7	10.7
2 hr.	2.3	2.1	0.0	0.4	4.8	5.7	10.5
5 hr.	1.5	2.4	0.0	0.4	4.3	6.5	10.8
1 day	0.0	2.2	0.3	0.1	2.6	8.5	11.1
2 days	0.0	0.0	1.9	0.0	1.9	8.4	10.3
3 days	0.0	0.0	2.0	0.0	2.0	8.4	10.4

paper, the extent of nitrification was dependent on the C/N ratio of the mixtures. Some experiments were carried out in which mixtures of sewage and activated sludge were aerated and the effect of alterations in the ratio of organic C to total N in the liquid phase was investigated.

In the first experiments, 2 l. of a mixture of settled sewage and activated sludge were aerated for a period of 23 hr.; the activated sludge was then allowed to settle and the supernatant liquid was siphoned off. Settled sewage, or on some occasions settled sewage mixed with an equal volume of an aqueous solution of glucose, was then added to the sludge remaining in the aeration vessel to bring the volume of the mixture to 2 l., the mixture was stirred and a sample was withdrawn for analysis; the mixture was then aerated for 23 hr. This daily cycle of operations was repeated in one experiment for 27 days and in another for 30 days. The sample withdrawn after each addition of fresh liquid was filtered through filter paper and the quantities of organic C and total N were determined in the filtrate and in the moist solid; in addition, the concentration of ammoniacal N was estimated in the filtrate. Nitrate-N was determined in the supernatant liquid withdrawn after settlement of the activated sludge at the end of each period of aeration. The results obtained are given in Table 5.

In mixtures of settled sewage containing glucose, the C/N ratio was, of course, much higher than in settled sewage alone. The C/N ratio in the activated sludge which had been aerated for several periods of 23 hr. in admixture with diluted settled sewage containing added glucose was, however, not greatly different from the ratio after aeration of the sludge in admixture with settled sewage alone. The C/N ratio of activated sludge thus appeared to be relatively unaffected by the composition of the liquid with which it was aerated. When the sludge

Table 5. *Effect of the carbon/nitrogen ratio of the liquid phase during aeration for 23 hr. of mixtures of activated sludge and sewage or sewage containing added glucose*

Period of aeration (days)	Weight of glucose added to 2 l. of mixture (g.)	Composition of liquid phase from filtration of sample of mixture of activated sludge and sewage before aeration			Composition of moist solid phase (i.e. sludge) from filtration of sample of mixture of activated sludge and sewage before aeration			Composition of mixture of activated sludge and sewage before aeration			Soluble nitrate-N after aeration of mixture for 23 hr. as % of soluble ammonia-N at beginning of aeration
		Organic carbon (mg. per 50 ml.)	Total N (mg. per 50 ml.)	C/N ratio	Organic carbon (mg. per 50 ml.)	Total N (mg. per 50 ml.)	C/N ratio	Organic carbon (mg. per 50 ml.)	Total N (mg. per 50 ml.)	C/N ratio	
Experiment A											
1	0	1.2	1.0	1.2	—	10.7	—	—	11.7	—	56
2	0	2.1	1.2	1.8	52.5	10.5	5.0	54.6	11.7	4.7	91
6	1.0	9.4	1.0	9.4	59.9	11.4	5.3	69.3	12.4	5.6	22
8	1.0	13.1	0.9	14.5	71.0	12.2	5.8	84.1	13.1	6.4	0
11	1.0	10.8	0.7	15.4	78.7	13.1	6.0	89.5	13.8	6.5	0
14	1.0	15.3	0.8	19.1	69.9	12.6	5.5	85.2	13.4	6.4	0
17	0	4.4	2.4	1.8	61.2	11.7	5.2	65.6	14.1	4.6	32
20	0	2.0	2.5	0.8	53.4	10.7	5.0	55.4	13.2	4.2	73
23	0	2.6	1.2	2.2	53.6	10.6	5.1	56.2	11.8	4.8	80
27	0	4.7	2.6	1.8	51.9	11.1	4.7	56.6	13.7	4.1	72
Mean = 5.3											
Experiment B											
1	0	—	—	—	47.3	7.8	6.1	—	—	—	100
2	0	—	—	—	41.0	6.7	6.1	—	—	—	100
3	0	—	—	—	35.6	5.7	6.3	—	—	—	85
6	0.25	—	—	—	33.8	5.9	5.7	—	—	—	71
8	0.5	—	—	—	32.3	5.8	5.6	—	—	—	100
10	0.5	—	—	—	36.4	7.0	5.2	—	—	—	0
13	1.0	—	—	—	32.3	9.1	3.6	—	—	—	0
15	1.0	—	—	—	61.1	9.7	6.3	—	—	—	0
18	0	—	—	—	68.8	12.2	5.6	—	—	—	64
21	0	—	—	—	59.4	11.5	5.2	—	—	—	67
24	0	—	—	—	51.0	11.0	4.6	—	—	—	77
27	0	—	—	—	41.4	8.4	4.9	—	—	—	80
30	0	—	—	—	47.2	10.0	4.7	—	—	—	85
Mean = 5.6											

There is no significant difference between the means of the C/N ratio of the moist solids in the two experiments, for $n=20$, $t=0.876$ and P lies between 0.4 and 0.3 [v. Fisher, 1934; Corbet & Wooldridge, 1936]. In the present investigation, differences between means are regarded as significant only when $P < 0.01$.

was aerated in admixture with settled sewage alone, a large proportion of the N in the sewage appeared as nitrate after each period of aeration. When settled sewage containing added glucose was used, oxidation to nitrate ceased but began again when settled sewage only was used. This is attributed to the fact that, in the presence of an excess of carbonaceous material available for bacterial growth requirements, soluble ammonia-N is utilized in the formation of insoluble organic nitrogenous compounds in the sludge; on the other hand, any ammonia-N in excess of the available carbonaceous material present is oxidized to nitrate.

Biochemical oxygen demand and bacterial counts

Determinations of the biochemical oxygen demand, by the method described by Wooldridge & Standfast [1933], and of counts of viable bacteria were made on the liquid phase during experiments in which mixtures of sewage and

activated sludge were aerated. An early and rapid O_2 uptake occurred, whether or not nitrate was formed during aeration of the mixtures, and the O_2 uptake was accompanied by a rapid increase in the numbers of bacteria (Tables 6 and 7).

Table 6. *Biochemical oxygen demand, bacterial counts and distribution of nitrogenous compounds in a mixture of sewage and activated sludge during aeration: no nitrate formed*

The figures for N and oxygen refer to mg. per 100 ml. and the bacterial numbers to colonies per 1.0 ml. of liquid.

Period of aeration	Liquid					Moist solid Total N	Total N in both phases
	NH ₃ -N	NO ₂ -N	NO ₃ -N	Organic N	B.O.D.		
Biochemical oxygen demand correlated with the disappearance of ammonia							
At start	2.4	0.0	0.0	0.3	17.2	22.5	25.2
2 hr.	1.6	0.0	0.0	0.4	10.0	23.5	25.5
5 hr.	1.0	0.6	0.0	0.1	3.6	23.8	25.5
1 day	0.4	0.0	0.0	0.1	4.1	25.1	25.6
2 days	0.0	0.0	0.0	0.4	1.2	25.6	26.0

Bacterial counts correlated with the disappearance of ammonia

Period of aeration	Liquid			Bacterial counts on heart agar after 48 hr. at 34°
	NH_3 -N	NO_2 -N	NO_3 -N	
At start	3.8	0.0	0.0	1,120,000
2 hr.	3.7	0.0	0.0	3,575,000
5 hr.	3.4	0.0	0.0	2,046,000
1 day	1.6	0.6	0.0	944,000
2 days	0.2	0.2	0.4	343,000

Table 7. *Biochemical oxygen demand, bacterial counts and distribution of nitrogenous compounds in a mixture of sewage and activated sludge during aeration: nitrate formed*

The figures for the N and oxygen refer to mg. per 100 ml. and the bacterial numbers to colonies per 1.0 ml. of liquid.

Biochemical oxygen demand and bacterial numbers correlated with the disappearance of ammonia

Period of aeration	Liquid				Bacterial counts on heart agar after 48 hr. at 34°
	NH_3 -N	NO_2 -N	NO_3 -N	B.O.D.	
At start	3.1	0.0	0.0	14.0	1,393,000
2 hr.	2.4	0.1	0.4	4.4	7,864,000
5 hr.	1.8	0.1	1.0	2.8	1,027,000
1 day	0.0	0.0	2.3	0.8	309,000
2 days	0.0	0.0	2.8	1.2	58,000

Wooldridge & Standfast [1936] showed that the oxidations effected during treatment of sewage by the activated sludge process were dependent on the presence of bacterial enzymes. In the present investigation, it was found that heat sterilization of the mixtures containing added potassium nitrite arrested the processes of nitrification and protein synthesis (Table 8).

Table 8. *Distribution of nitrogenous compounds during aeration of sterilized mixtures of sewage and activated sludge, containing added potassium nitrite*

The figures refer to mg. N contained in the flasks
N added in the form of potassium nitrite: 5 days' aeration

Before aeration				After aeration				Loss of N
Organic and NH ₃ -N	NO ₂ -N	NO ₃ -N	Total N	Organic and NH ₃ -N	NO ₂ -N	NO ₃ -N	Total N	
4.7	0.1	0.1	4.9	4.0	0.0	0.1	4.1	0.8
4.7	0.1	0.1	4.9	3.5	0.1	0.1	3.7	1.2
4.7	2.3	0.1	7.1	4.1	2.4	0.0	6.5	0.6
4.7	4.4	0.1	9.2	4.0	4.5	0.1	8.6	0.6
4.7	6.6	0.1	11.4	4.8	6.1	0.0	10.9	0.5
4.7	8.7	0.1	13.5	4.3	8.3	0.0	12.6	0.9

Effect of the concentration of ammonia-nitrogen on the changes during aeration of mixtures of sewage and activated sludge

As the two main reactions discussed in this paper have ammonia as an initial reactant, it was important to determine whether the concentration of ammonia present at any time favoured one reaction while inhibiting the other. Meyerhof [1916, 1917] showed that the oxidation of ammonia to nitrite and of nitrite to nitrate, in the presence of nitrite- or nitrate-forming bacteria, only takes place below certain limiting concentrations of ammonium (0.1 *N*), or nitrite (> 0.5 *N*) ions respectively. In the experiments described in this paper, the concentration of ammonia-N never exceeded the value which, according to Meyerhof, would permit formation of nitrite to proceed at 70 % of the optimum rate. Moreover, in two parallel experiments in which equal amounts of ammonium sulphate were added to mixtures of sewage and activated sludge, in one experiment at the beginning of the period of aeration and in the other experiment in small instalments during aeration over a period of several days, the distribution of the N compounds at the end of the experiments was approximately the same. It is clear, therefore, that in the experiments in which no nitrification occurred during aeration of mixtures of sewage and sludge, the concentration of ammonium ions was not responsible for the absence of nitrification. Nevertheless it appears that concentrations of ammonia-N even below the limiting value found by Meyerhof can exert a marked inhibitory effect on the course of the reactions concerned with synthesis of protein. Table 9, which has been prepared

Table 9. *Relation between the concentration of ammonium-nitrogen in sewage and its conversion into organic nitrogen*

The figures are calculated from the data given in Table 3 (b)

	NH ₃ -N in liquid in mg. per 125 ml. of mixture						
	1.0	4.1	7.2	13.4	19.7	25.9	32.1
% of NH ₃ -N in liquid phase, converted to organic form	100.0	85.4	51.4	23.9	15.2	9.3	6.2
Increase in organic N in mixture in mg.	1.0	3.5	3.7	3.2	3.0	2.4	2.0

from the data given in Table 3 (b), shows that after aeration of a mixture of sewage and sludge for 14 days, the whole of the ammoniacal N was transformed into organic form only in the mixtures containing no added ammonium salt.

In the mixture in which the greatest increase occurred in the amount of organic N, only about 50 % of the ammoniacal N in the liquid phase was converted into organic form. In this experiment the excess of ammoniacal N not converted into organic form remained unaltered in the liquid and was not oxidized to nitrite or nitrate.

Knight [1936] has pointed out that amino-acids strongly inhibit the respiration and proliferation of *Nitrosomonas* and *Nitrobacter*. Amino-acids were not detected in measurable amounts during the present investigation when mixtures of sewage and activated sludge were aerated for periods up to 13 days. When Beesley's medium¹ was inoculated with small amounts of activated sludge, nitrite was always produced, showing that organisms capable of converting ammonia into nitrite were present, even in mixtures of sewage and activated sludge in which no nitrification occurred during aeration. Nitrite was also formed when Beesley's medium containing appreciable amounts of sterilized sludge was similarly inoculated. The inhibition of nitrification during aeration of some of the mixtures was thus not due to the absence of the appropriate organisms, or to the presence of excessive quantities of organic matter in the sludge; nor could it have been due to O₂ lack.

Methods of analysis

Ammonia-N was estimated by distillation into standard acid. The sum of the nitrite- and nitrate-N was obtained by the method entailing simultaneous reduction with Devarda's alloy and distillation of the ammonia thus formed into standard acid. Nitrite-N was determined by the Griess-Ilosvay colorimetric method. In the absence of nitrite or nitrate, estimation of the ammonia- and organic-N was effected on a separate sample by the usual modified Kjeldahl method, involving the addition of potassium and copper sulphates.

Tests showed that when nitrite was present, the total N in sewage material could not be estimated by pre-treatment with reduced iron and sulphuric acid, followed by the usual digestion by the modified Kjeldahl method. Analysis of mixtures of sewage and sludge containing nitrite and/or nitrate was effected satisfactorily in the following manner. The mixture was filtered and the organic N in the well-washed solid was determined by the modified Kjeldahl method; on an aliquot portion of the filtrate were estimated first the ammonia, then the sum of the nitrite- and nitrate-N (by reduction with Devarda's alloy) and, finally, the total N. When only small amounts of soluble organic nitrogenous material were present in the liquid phase, as was usually the case, the magnesia and Devarda's alloy were removed by filtration, after distillation, and the filtrate was analysed for organic N as usual: when organic N was present in quantity, the Kjeldahl digestion was carried out with the whole of the mixture remaining after distillation with Devarda's alloy.

SUMMARY

An experimental study has been made of the distribution of N compounds during treatment of sewage by the activated sludge process.

In some experiments, part of the ammoniacal N in the liquid phase was transformed into organic form in the sludge during aeration of the mixture; the remainder of the ammoniacal N was oxidized, usually at a later stage, to

¹ Beesley's medium contains 0.95 g. (NH₄)₂SO₄, 0.2 g. KH₂PO₄, 0.2 g. NaCl, 0.066 g. MgSO₄, 7H₂O, 20.0 g. CaCO₃, and 1 l. distilled water.

nitrite and/or nitrate and remained in solution in the liquid. There was little or no loss of gaseous N.

In other experiments the whole of the ammoniacal N in the liquid was converted into organic form in the sludge during aeration. When nitrite or nitrate was added to such non-nitrifying mixtures of sewage and sludge, large losses of gaseous N₂ occurred.

Nitrification was inhibited when glucose was added to the sewage, but began again shortly after addition of glucose was discontinued. The carbon/nitrogen ratio of the activated sludge was relatively unaffected by such addition of glucose with the sewage.

The absence of nitrification was not due to the absence of the appropriate micro-organisms, or to the presence of excessive amounts of ammonia-N.

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