

Investigations on nitrification and denitrification in dune sand

1. Introduction

In artificial recharge of ground water, water from a surface source is stored underground before being extracted. The utilization of this process in Holland for the public water supply of some coastal regions implies the infiltration of pretreated water from the river Rhine into the coastal sandy subsoil. The rate of infiltration varies from 0.1 to 0.4 m.day⁻¹. It has been pointed out by Huisman and Van Haaren [1] that underground storage offers a reliable and aesthetic drinking water.

During its passage through the subsoil the quality of the river water is improved. Field studies by Leeftang [2] in the coastal region near Castricum revealed that organic matter is removed in all subsequent sections of the aquifer, albeit that purification is most extensive in the first 1 to 2 m.

Oxygen, nitrate and ammonia are important inorganic constituents of the river water. Oxygen and nitrate are being consumed in the mineralisation process and will thus favour the quality of the extracted water. Ammonia may be oxidized in the nitrification process under conditions of a plentiful supply of oxygen prevalent in the upper reaches of the aquifer. The presence of ammonia may thus result in the consumption of oxygen and the formation of nitrate. The nitrate is transported to the lower levels where it can be consumed by denitrifying bacteria under the condition of anaerobiosis. Just as ammonia is liable to be removed by nitrifying bacteria, it may be adsorbed on negatively charged soil colloids due to its positive charge, so that it is probable that ammonia will not move easily down through the soil. The mineralisation of nitrogenous organic compounds, on the other hand, leads to the formation of ammonia. Whether oxygen is of influence on its release in dune sand is a question which is of practical importance in the infiltration of river water. Minor quantities of ammonia have been traced by Leeftang [2] in recovered groundwater.

Investigations in the field by Leeftang [2] and Baars [3], lysimeter experiments by Maschhaupt [4], Kolenbrander [5] and many others and the innumerable laboratory investigations which have been carried out have all contributed to our present knowledge regarding the biological processes in soils. Little information is available, however, regarding the factors influencing nitrification and denitrification in dune sand.

2. Literature

The term nitrification usually applies to the oxidation of ammonia to nitrite and nitrate by autotrophic bacteria.

These bacteria are incapable of growing on organic matter. Meijerhof has formulated the oxidation of ammonia as follows:



This oxidative reaction supplies energy for the synthesis of cell material from carbon dioxide. From the stoichiometry of the nitrification reaction follows that for the complete oxidation of 1 mg NH₄-N to nitrate 4.57 mg of oxygen are required. Montgomery and Borne [6], in considering the oxygen balance of a nitrifying system, have assumed that the oxygen uptake is 4.35 mg instead of 4.57 mg, on account of the reduction of carbon dioxide for cell synthesis. From a summary on growth-rates of nitrifying bacteria presented by Downing et al. [7] it appears that the rates vary considerably with the type of medium as well as with temperature. The shortest generation time, however, is long compared with that of many heterotrophic bacteria.

The effect of oxygen on nitrification has been investigated by Greenwood [8], using the Warburg technique. He has found that at an oxygen content of about 0.1 p.p.m. the respiration rates were about half the rates in the presence of excess of oxygen. The effect of dissolved oxygen has also been studied by Downing et al. [7], using the dropping mercury electrode. They have found that nitrification in full-scale activated sludge plants ceased when dissolved oxygen contents fell below about 0.5 p.p.m.

In the mineralisation of organic matter nitrate may be used by different groups of facultative aerobic microorganisms instead of oxygen under the condition of anaerobiosis. This is called nitrate respiration. Depending upon the microorganisms and the conditions, nitrate may be reduced only to nitrite or to more reduced forms of nitrogen. If molecular nitrogen or nitrous oxide are the products of nitrate respiration, the process is known as denitrification. Wijler and Delwiche [9] have reported that the evolution of nitrous oxide varies widely from species to species. With mixed soil flora the production of N₂O was favoured at a neutral to alkaline pH.

Denitrifying bacteria have been found almost universally in soil and water. Woldendorp [10] has reported about the abundance of sporeforming denitrifying bacteria in arable soils.

Gayon and Dupetit, who identified soil bacteria as the agents responsible for the volatilization of nitrogen, were the first to make a detailed study of the effect of oxygen on this process. Oxygen is generally considered as an inhibitor of the denitrifying process by virtue of its effective competition with nitrate in the oxidation

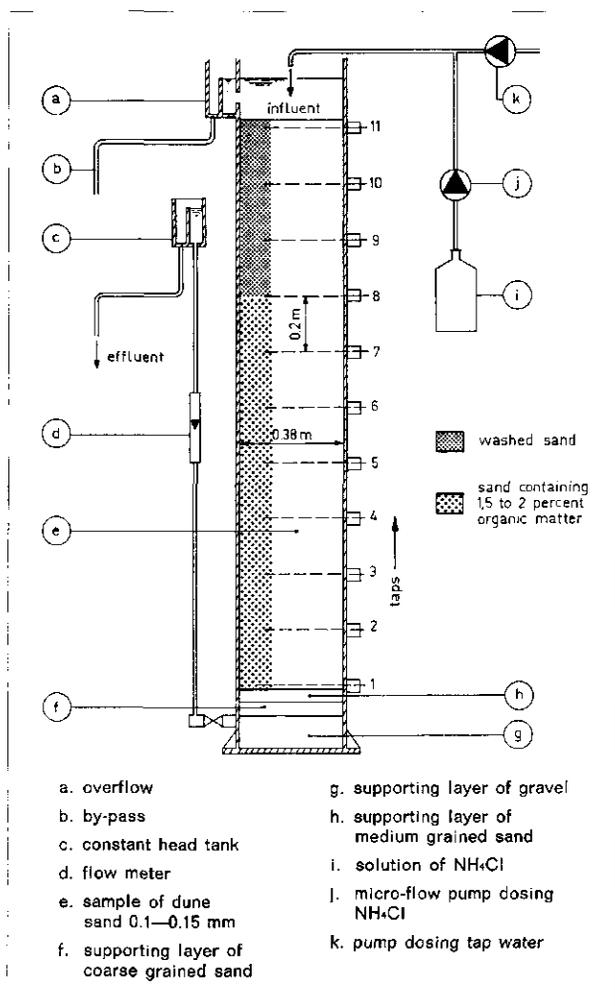


Fig. 1 - Experimental installation.

of organic matter. Wijler and Delwiche [9], studying denitrification in soil systems under simulated field conditions, found that an atmosphere containing 1 per cent of oxygen suppressed denitrification during the early stages of soil incubation. Assuming the presence of an oxygen gradient between the ambient atmosphere and the metabolizing cell and using the soil percolation technique, Greenwood [8] concluded that at about 0.1 p.p.m. of oxygen denitrification took place at about half of its rate under complete anaerobic conditions. It has been found by Verhoeven [17] and Woldendorp [10] that certain aerobic sporeforming nitrate reducing bacteria are capable of reducing nitrate to ammonia. Woldendorp has observed that the release of ammonia by sporeforming nitrate reducing bacteria took place after incubation with glucose, if cells were grown aerobically in the absence of ammonia and were subsequently submitted to anaerobic conditions. Since he could not obtain reduction of nitrate to ammonia in the soil, Woldendorp concluded that soil conditions are unfavourable for the production of ammonia.

Baars [3] has found that in the upper reaches of an aquifer, bacterial numbers per gram of sand rapidly decline with depth. Of these 20 per cent are sporeforming bacteria. Although conditions in the aquifer are unfavourable for growth, it appeared from his study that high bacterial numbers prevail.

3. Experimental

During three years of laboratory investigations the present authors have studied nitrification and denitrification by observing the fate of ammonia, nitrate and dissolved oxygen in tap water, when being percolated through samples of dune sand. Both processes are assumed to occur at the surface of the sand grains. Their rates are partially reflected by changes in the concentration of the above mentioned compounds. This study has been drastically simplified by substituting river water by tap water. Drinking water quality was thought to be of help when unraveling factors governing nitrification and denitrification in dune sand at low percolation rates. In principal the following circumstances were varied:

the rate of percolation;

the ammonia and oxygen contents of the water to be percolated.

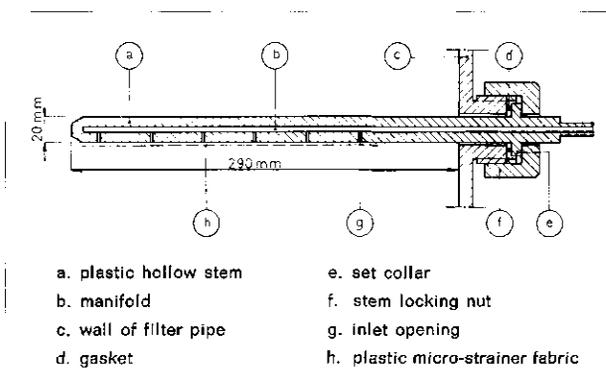
3.1. Installations (see fig. 1 and 4)

Tap water was pumped by pump (k) at a rate of 0.5 to 1.0 $\text{l}\cdot\text{hr}^{-1}$ to the indoor installation represented in fig 1. Ammonium chloride solution was pumped by pump (j) and mixed with tap water. The mixture was percolated through a bed of submerged dune sand of 2m thickness, placed in a plastic cylinder having an internal diameter of 0.38 m. The sand bed (e) was composed of sand, containing from 1.5 to 2.0 per cent organic matter, and washed sand. The layer of washed sand extended from tap no. 8 till 0.05 m above tap no. 11. Before the lower layer was put into the installation, the organic matter content has been determined from the loss of weight on ignition. No attempts have been made to determine the organic matter content of the upper washed sand.

Eleven taps, evenly distributed along the length of the plastic cylinder (see fig. 3), enabled the withdrawal of samples from the sand bed at different levels.

It is assumed that levels chosen 0.2 m apart allow to measure the vertical distribution of inorganic nitrogen and dissolved oxygen in the sand bed. The construction of each tap and its fitting into the wall are indicated in fig. 2. A plastic hollow stem is projecting deeply into the sand bed to collect water in a particular section from different locations. The inlet openings, which are connected to a small diameter manifold, are covered by a plastic micro-strainer fabric to prevent the entry of sand grains into the collecting system. To minimize disturbances of the flow through the sand, samples were drawn off in

Fig. 2 - Construction of tap.



two series. The upper taps were sampled first and the lower ones afterwards, each at a rate of 50 ml.hr⁻¹ (5-10 % of supply).

The water level on top of the sand bed was controlled by an overflow weir (a), discharging part of the influent. The rate of percolation, indicated by flowmeter (d), was checked frequently.

3.2. To carry out experiments under anaerobic conditions an installation was constructed, a diagram of which has been represented in fig. 4. This installation consists of four closed pipes, 0.06 m I.D., each with a length of 1 m, placed in series. The pipes were completely filled with dune sand containing from 1.5 to 2.0 per cent organic

Fig. 3 - Apparatus.

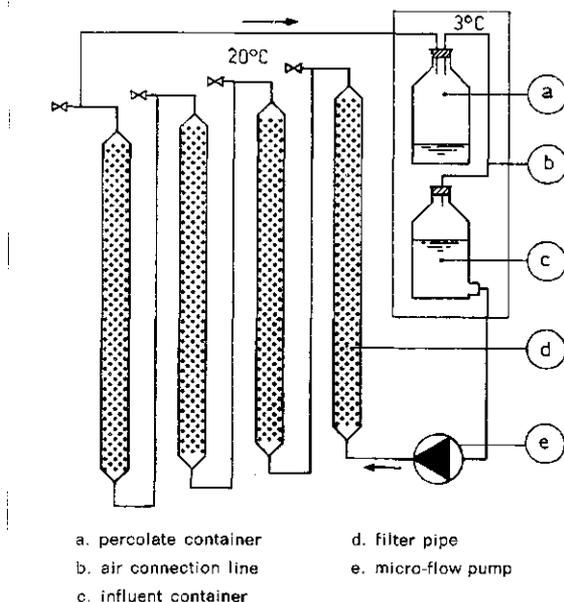
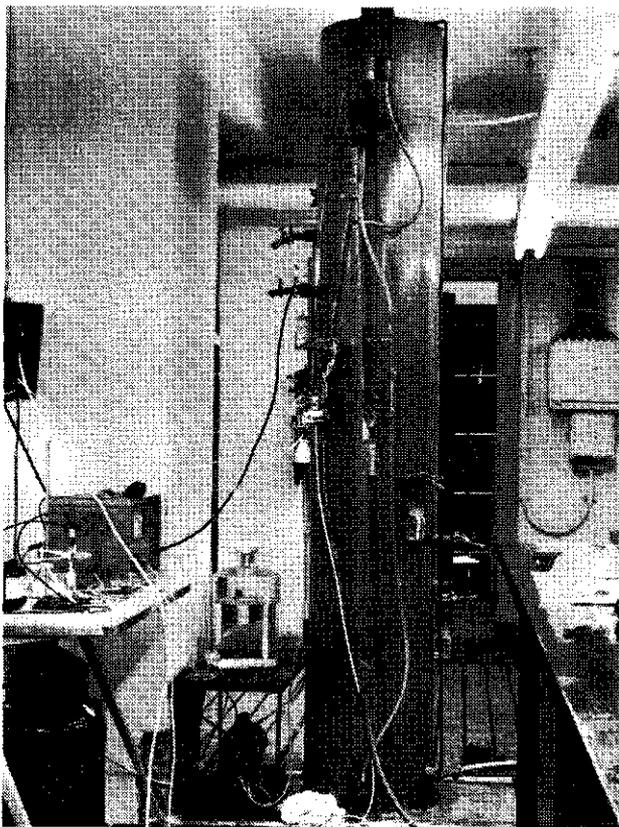


Fig. 4 - Anaerobic installation.

matter. The pores of the sand were saturated with water. Tap water with a temperature of 18° to 20°C was pumped continuously by a micro-flow pump at a rate of 0.43 m.day⁻¹ through four or less of the sand columns. Both the influent and the percolate were stored in containers at 3° C. When filling the influent container pure nitrogen gas was supplied for deoxygenation of the tap water and flushing of the atmosphere in both containers.

3.3. Analyses

The ammonia content was determined colorimetrically with Nessler's reagent. The nitrite content was determined with sulfanilic acid and alphanaphtylamine. The nitrate content was determined by the sodium-salicylate method. The organic nitrogen content was determined from ammonia in the distillate with Nessler's reagent after digestion of the sample. For the determination of oxygen both the Winkler technique on 100 ml samples and an oxygen sensor were used.

TABLE 1

Period	Rate of percolation m.day ⁻¹	Temp. °C	Influent conc. of			pH influent	Remarks
			Ammonia p.p.m. NH ₄ ⁺ -N	Nitrate p.p.m. NO ₃ -N	Dissolved oxygen p.p.m.		
Dec '65 - Febr '66	0.1	17-19	1.1-1.3	2.8-3.1	8.0-8.5	8.0-8.1	Varying the rate of percolation
Febr '66 - Jun '66	0.14	18-20	1.4-1.5	3.0-3.4	7.5-8.0	8.1-8.2	Varying the rate of percolation
Jun '66 - Aug '66	0.2	18-20	0.8-1.1	3.1-3.3	7.0-7.5	8.0-8.2	Varying the rate of percolation
Aug '66 - Nov '66	0.2	19-21	0.7-1.3	2.9-3.3	8.7-9.3	—	Increased aeration
Dec '66 - Apr '67	0.2	17-19	2.5	2.9-3.8	7.0	—	Increased ammonia content
Apr '67 - Jul '67	0.2	19-21	3.0	2.9-3.1	6.0-7.5	—	Dosage of pepton
Jul '67 - Dec '67	0.1	17-21	1.0-1.3	2.5-2.8	7.0-8.0	—	Re-examination

Weekly analyses were made to determine the organic nitrogen content and the distribution of inorganic nitrogen and dissolved oxygen in the profile of the sand bed.

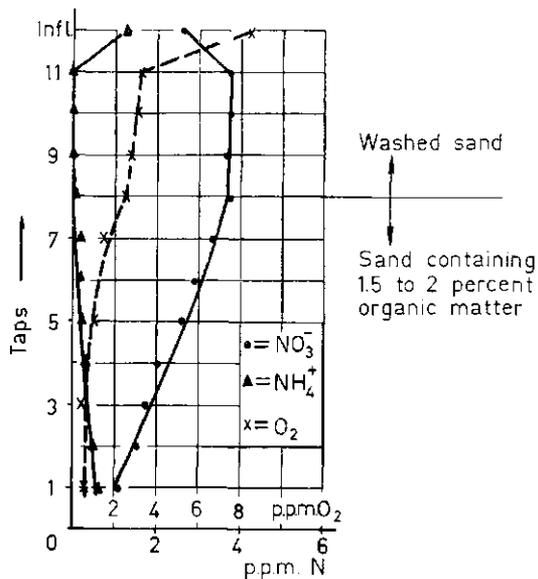


Fig. 5 - Distribution of inorganic nitrogen and dissolved oxygen in filter during 1st period ($v = 0.1 \text{ m.day}^{-1}$).

3.4. Aerobic experiments

Ammonium chloride diluted with tap water was passed continuously through the sand filter bed represented in fig. 1. Daily analyses were made of the inorganic nitrogen (ammonia, nitrate and nitrite) and dissolved oxygen contents of the influent, the percolate and the samples from taps no. 10 and no. 11. Sampling was started several weeks after the installation had been put into operation. The operating conditions during the test period are summarized in table 1.

Results

The top layer of the sand bed achieved 100 per cent removal of ammonia in the middle of December '65. From then all weekly distributions of inorganic nitrogen and dissolved oxygen in the profile showed a similar pattern as indicated in fig. 5*). It is apparent that in the zone above tap no. 8 mainly oxygen was consumed, while in the zone below this tap mainly nitrate was being reduced. The complete removal of ammonia by nitrifying bacteria was followed by the release of it in the deeper layers of the profile. All samples showed negligible amounts of nitrite indicating that ammonia was completely oxidized to nitrate. The absence of nitrite also indicates that nitrate was being reduced by denitrifying bacteria. The oxygen consumption was governed mainly by the nitrification reaction. Experiments revealed that the uptake of oxygen in the top layer varied from 4.0 to 4.3 mg O₂ per mg NH₄⁺-N supplied to the sand bed. This result is slightly lower than the 4.35 mg O₂ reported by Montgomery and Borne [6]. The weekly average values of the nitrate and ammonia contents of the influent and the percolate obtained from Dec. '65 till Aug. '66 are represented in figs. 6 and 7. When looking at figure 7 it is apparent that ammonia was released continuously by

*) The scale above tap no. 11 has been extended.

the sand bed. The concentrations in the percolate varied from 0.2 to 0.6 p.p.m. NH₄⁺-N.

3.4.1. The rate of percolation

A further inspection of the graphs reveals that there were substantial drops of inorganic nitrogen in the sand bed. Fig. 8, representing the weekly average values, indicates that the biggest drops were obtained during Dec. '65 and Jan. '66 when the rate of percolation was low. It appeared that nitrogen losses, following from the difference between the total inorganic nitrogen contents of the influent and the percolate, were affected by the rate of percolation. From data obtained during the period Dec. '65 - Aug. '66 the authors have computed the average weekly losses of inorganic nitrogen by multiplying the difference between the total inorganic nitrogen contents of the influent and the percolate by the weekly discharge

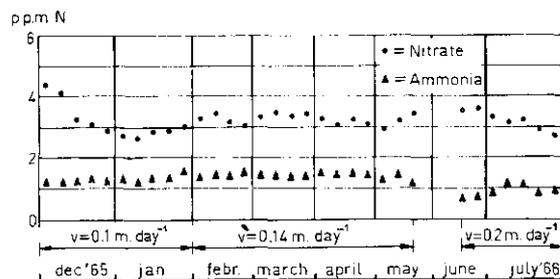


Fig. 6 - Weekly average values of inorganic nitrogen in influent.

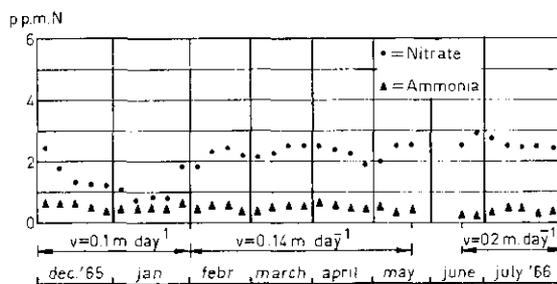


Fig. 7 - Weekly average values of inorganic nitrogen in percolate.

Fig. 8 - Weekly average values of the drop of inorganic nitrogen.

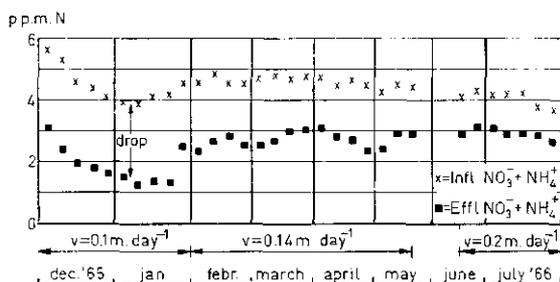


TABLE 2 - Aerobic experiments (Dec. '65 - Aug. '66)

Period	Average dissolved oxygen content in sand bed above tap no. 8	Rate of denitrification (Drop of p.p.m. NO ₃ -N per meter sand bed)		Weekly loss of inorganic nitrogen in total sand bed grams N.week ⁻¹ per m ² of filter area	Weekly quantity of nitrate reduced below tap no. 8 grams NO ₃ -N. week ⁻¹ per m ³ of sand bed	Rate of percolation m.day ⁻¹
	p.p.m. O ₂	above tap no. 8	below tap no. 8			
20—24 Dec. '65	2.5—3.0	none	2.0	1.82	1.40	0.10
27—31 Dec. '65	3.0—3.5	„	1.8	1.72	1.30	
Average weekly value			1.9	1.85	1.35	
3—7 Jan. '66	2.5—3.0	„	1.8	1.72	1.25	0.14
17—21 Jan. '66	2.5—3.0	„	2.0	1.93	1.40	
21—28 Jan. '66	2.5—3.0	„	2.0	1.99	1.40	
Average weekly value			1.9	1.88	1.35	
7—11 Febr.	1.5—2.0	slight	1.5	2.25	1.47	0.14
14—18 Febr.	1.0—1.5	„	1.4	2.20	1.37	
21—25 Febr.	1.5—2.0	0.3	1.1	1.72	1.08	
28—4 March	1.0—1.5	0.6	1.3	2.01	1.27	0.20
Average weekly value			1.3	2.04	1.29	
7—11 March	1.5—2.0	slight	1.3	2.16	1.27	
14—18 March	1.5—2.0	0.5	1.3	2.10	1.27	0.20
21—25 March	1.5—2.0	0.5	1.1	1.72	1.08	
28—1 April	1.5—2.0	0.6	1.3	1.77	1.27	
Average weekly value			1.25	1.94	1.22	
4—8 April	1.0—1.5	0.8	1.3	1.72	1.27	0.20
18—22 April	1.0—1.5	0.5	1.2	1.96	1.18	
Average weekly value			1.25	1.84	1.22	
2—6 May	2.0—2.5	none	1.4	1.81	1.37	0.20
9—13 May	1.5—2.0	slight	1.2	1.57	1.18	
16—20 May	3.0—3.5	none	1.4	1.52	1.37	
Average weekly value			1.3	1.63	1.31	
20—24 June	4.0—4.5	„	0.8	1.68	1.12	0.20
27 June—1 July	3.5—4.0	„	0.8	1.68	1.12	
Average weekly value			0.8	1.68	1.12	
4—8 July	2.5—3.0	„	0.9	1.61	1.26	0.20
11—15 July	2.5—3.0	„	0.8	1.89	1.12	
18—22 July	3.5—4.0	„	0.8	1.89	1.12	
25—29 July	3.5—4.0	„	0.7	1.40	0.98	0.20
Average weekly value			0.8	1.68	1.12	

from the sand bed. As the average weekly losses were found to be rather constant with time it may be expected that the drop of inorganic nitrogen, after the water had passed the sand bed, was approximately inversely proportional to the rate of percolation. This is demonstrated in fig. 9. This graph suggests that a further lowering of the rate of percolation, viz. below 0.1 m.day⁻¹, would have resulted in appreciable higher nitrogen losses. Were it not

for the release of ammonia the inorganic nitrogen might have been lost completely.

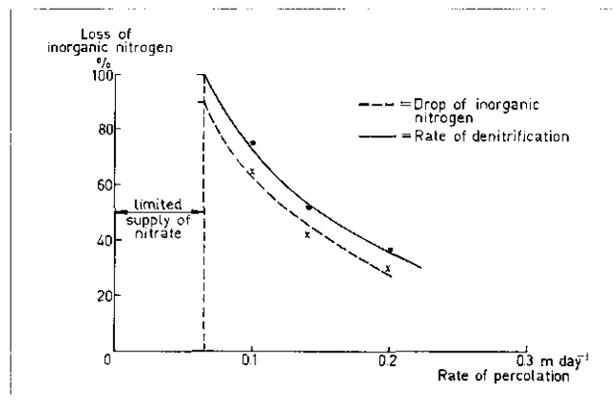
For data regarding the rate of denitrification the reader is referred to table 2. Like the drop of inorganic nitrogen the rate of denitrification below tap no. 8 appeared to be almost inversely proportional to the rate of percolation. This is obvious, since inorganic nitrogen losses result from the denitrification process. Unlike the drop of inorganic nitrogen the removal of ammonia in the top layer of the sand bed was found to be independent upon the rate of percolation. So ammonia was removed completely, irrespective of the rate of ammonia supply.

When conditions are favourable the oxidation of ammonia by nitrifying bacteria depends upon the number of bacteria and the supply of oxygen. The latter being adequate would involve that the top layer of the sand bed allowed enough bacteria to be grown to oxidize ammonia completely.

3.4.2. Dissolved oxygen

The dissolved oxygen content in the zone between tap no. 8 and 11 was governed by the ammonia content of the influent. From the average weekly values, given in table 2, follows that the highest oxygen contents were obtained during the summer when the ammonia content of the influent was low. At that time it became apparent

Fig. 9 - Relationship between the loss of inorganic nitrogen due to denitrification as a percentage of the influent nitrogen content and the rate of percolation.



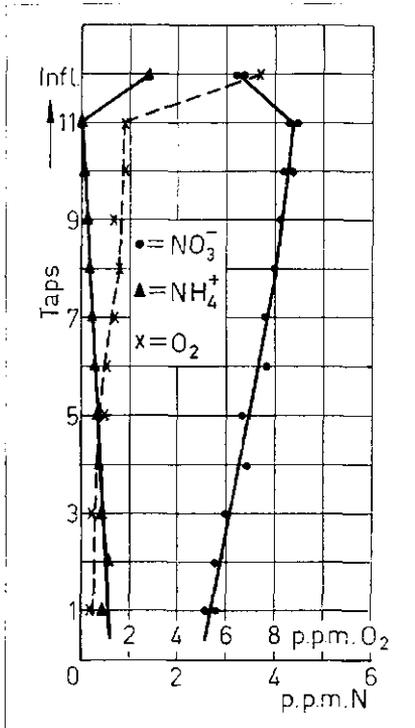


Fig. 10 - Distribution of inorganic nitrogen and dissolved oxygen in filter on 22nd of March.

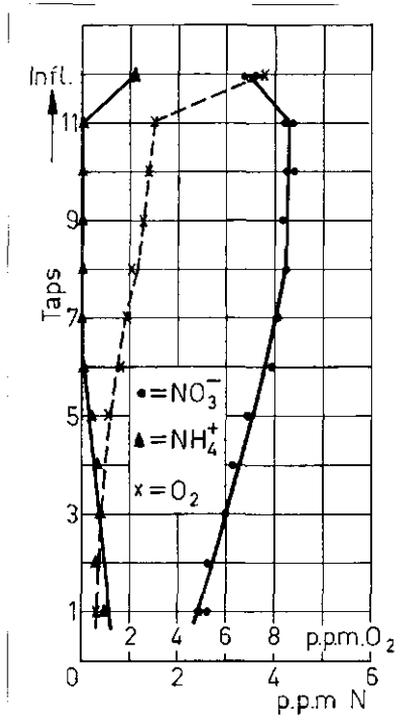


Fig. 11 - Distribution of inorganic nitrogen and dissolved oxygen in filter on 18th of May.

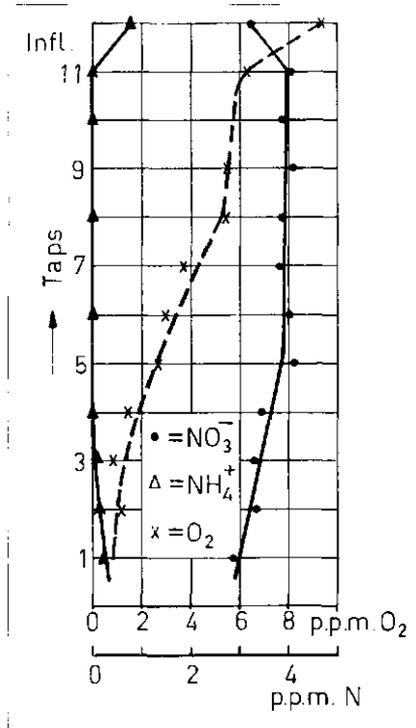


Fig. 13 - Distribution of inorganic nitrogen and dissolved oxygen in filter at a high oxygen level.

that ammonia was influenced by nitrifying bacteria present in the supply tubing. Their activity could be minimized by a frequent renewal of the tubing. Experiments revealed that denitrification and the release of ammonia were influenced by the dissolved oxygen content of the interstitial water. Data in table 2 indicate that denitrification occurred at 2.0 p.p.m. of oxygen. It is shown in fig. 10 that ammonia was released in all sections of the sand bed, including the layer of washed sand, if the oxygen content below tap no. 11 fell below 1.5 to 2.0 p.p.m.

When oxygen exceeded 3.0 p.p.m., however, no reduction of nitrate could be perceived whilst under this circumstance the release of ammonia was confined to the zone below tap no. 8 (see fig. 11).

It is striking that the oxygen content below tap no. 8 did not fall below 0.5 p.p.m. The incapability of the denitrifying microorganisms to exhaust the oxygen supplied to the lower section of the profile is shown in fig. 12, indicating the frequency distribution of dissolved oxygen in the percolate obtained during the first quarter of 1966.

From August till November '66 the influent dissolved oxygen content was raised by dispersing air into the water on top of the sand bed. As measures to obtain a as constant composition of the inflowing water as possible were not always successful, unavoidable variations of the ammonia content took place. It appeared that aeration and low ammonia contents resulted in a downward shift of the level below which denitrification did start and

Fig. 12 - Frequency distribution of dissolved oxygen in percolate during the first quarter of 1966.

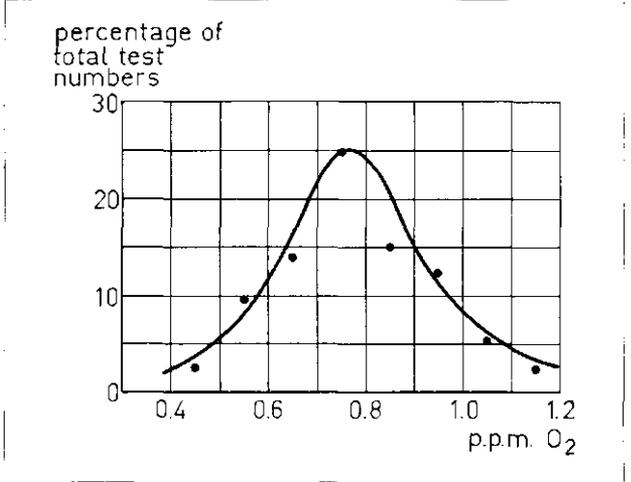
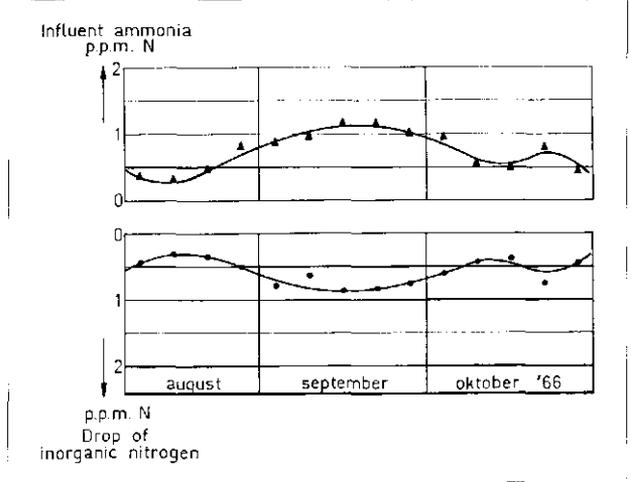


Fig. 14 - Relationship between influent ammonia and drop of inorganic nitrogen.



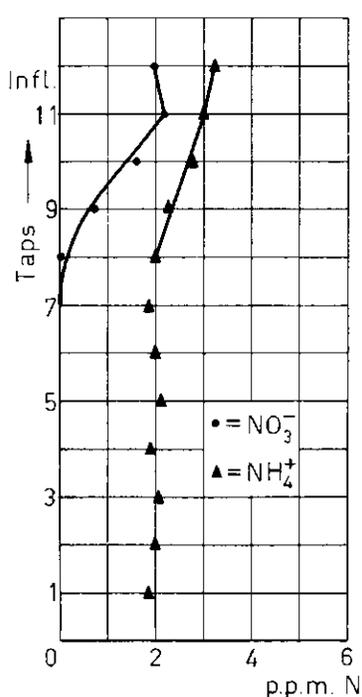
release of ammonia was to be perceived, as illustrated in fig. 13. It may thus be expected that from August till November '66 ammonia had affected the drop of inorganic nitrogen in the sand bed. This is shown in fig. 14, indicating the relationship between the weekly average values of the influent ammonia content and the resulting drops of inorganic nitrogen. The influence results from the pronounced effect of ammonia on the dissolved oxygen content in the zone of denitrification.

Evidence was obtained that denitrification ceased if the oxygen level in the main body of the interstitial water did exceed 3.0 p.p.m. It was found that release of ammonia was confined to the lowest section of the profile in which the oxygen level had fallen below 1.5 to 2.0 p.p.m. Till November '66 there was a gradual drop of the ammonia content of the percolate. Release of ammonia, however, did not cease.

3.4.3. High ammonia content

During Dec. '66 and the first quarter of '67 the ammonia content of the influent was increased to about 2.5 p.p.m. $\text{NH}_4^+\text{-N}$, while the oxygen content was lowered to about 7.0 p.p.m. By these measures the oxygen demand of the influent was raised, in fact, so as to deplete the dissolved oxygen content of the interstitial water by nitrifying bacteria. Under these circumstances, however, the dissolved oxygen contents of samples withdrawn from taps no. 10 and 11 were found to vary from 1.0 to 1.5 p.p.m. Since it was found by Greenwood [8] that nitrification in soil is inhibited at appreciable lower oxygen levels, it is probable that nitrifying bacteria, thriving on the surface of the sand grains, were not able to exhaust the oxygen in the interstitial water. This finding is supported by the presence of small concentrations of oxygen in the percolate. It must be noted that

Fig. 15 - Distribution of inorganic nitrogen in filter during dosage of peptone.



the top layer in which nitrification was apparent had a depth of no more than 0.05 m. Like in the former periods, when complete removal of ammonia was attained, the rate of oxygen uptake in the top layer varied from 4.0 to 4.3 mg O_2 per mg $\text{NH}_4^+\text{-N}$ removed in that layer. It was found that increased ammonia resulted in the presence of ammonia in the whole profile of the sand bed. Slight variations of the ammonia content, however, do not permit to draw the conclusion whether release or adsorption of ammonia had occurred in the lower section of the sand bed.

As the ammonia content below tap no. 11 appeared to be almost constant with depth, it may be concluded, that when once it has penetrated the lower layers, ammonia is hardly removed.

In contrast with the foregoing picture, ammonia appeared to be removed in the lower sand layer if a readily degradable organic substance, like peptone, was present. During the second quarter of 1967 incremental amounts of peptone were dosed continuously in addition to the supply of ammonia, given in table 1. The maximum concentration amounted to 40 p.p.m. Experiments revealed that during this period the activity of nitrifying bacteria gradually decreased to a small percentage of the values obtained in the former periods, whilst most of the oxygen remained to be consumed in the top layer. It is likely that under these circumstances heterotrophic bacteria had developed in the top layer. By virtue of their faster growth rates these would have been more successful in competing for the available oxygen and thus have detained nitrification.

The simultaneous dosage of peptone and ammonia resulted in higher rates of denitrification below tap no. 11. Nitrite was absent. Both the rate of denitrification and removal of ammonia depended upon the amount of peptone supplied to the sand bed. The maximum concentration of peptone caused a complete exhaustion of the available nitrate, as shown in figure 15.

In this connection it is of interest to mention that Marshall et al. [14] and Woldendorp [10] have found that certain actively nitrate reducing bacteria utilize ammonia for cell synthesis. Judging from the close relationship in our experiment between the utilization of ammonia nitrogen and the reduction of nitrate nitrogen (the ratio appeared to be about 25 per cent) a similar observation seems evident, so that it may be concluded that the removal of ammonia was effected by denitrifying bacteria.

3.4.4. Resumption of the release of ammonia

During the last two quarters of 1967 one of the former process conditions was re-installed. After the addition of peptone had been stopped nitrification recovered soon and, like in the former periods, the removal of ammonia was fully completed in the top layer of the sand bed. It was found that the release of ammonia had been resumed.

3.5. Anaerobic experiments

With the aid of tracer techniques Nommik [18] has demonstrated that a mixed soil flora is capable of reducing nitrate to ammonia under strictly anaerobic conditions and at a neutral to alkaline pH. Woldendorp [10] has found that this is also true of pure cultures of

TABLE 3 - Anaerobic experiments in 1m sand columns (Aug. - Nov. '67)

Period	Rate of percolation: 0.43 m.day ⁻¹ Oxygen content: less than 0.1 p.p.m.		
	Reduction of nitrate Total drop in p.p.m. NO ₃ -N	Release of ammonia Increase in p.p.m. NH ₄ ⁺ -N	Weekly quantity of nitrate reduced grams NO ₃ -N, week ⁻¹ per m ³ of sand bed
31— 2 Aug. '67	1.94	0.85	1.47
2—15 Aug. '67	2.20	1.18	1.68
15—25 Aug. '67	1.45	1.13	1.12
Average weekly value			1.42
13—20 Sept.	1.33	0.70	1.34
20—26 Sept.	1.13	0.46	1.12
26— 3 Oct.	1.08	0.54	1.05
Average weekly value			1.17
3—10 Oct.	0.97	0.30	1.47
10—21 Oct.	0.48	0.38	0.70
21—31 Oct.	0.69	0.32	1.05
Average weekly value			1.07

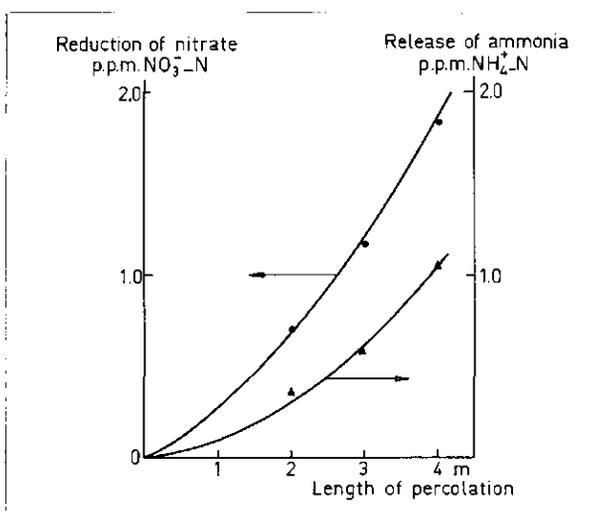
certain sporeforming nitrate reducing bacteria. These results suggest that the formation of ammonia in dune sand may be affected by anaerobiosis.

In order to verify this question experiments were carried out in a sample of dune sand taken from the same store from which the sand for the aerobic experiments had been taken. The installation is represented in fig. 4. Tap water was pumped continuously through sand columns of 4, 3, 2 and 1 m length. Sampling was started two weeks after the installation had been put into operation. Frequent analyses were made of the ammonia, nitrite, nitrate and dissolved oxygen of the influent and the percolate.

The dissolved oxygen as well as the nitrite content appeared to be less than 0.1 p.p.m. Considerable amounts of ammonia were released by the sand under this condition. This appears from table 3.

It is fairly probable that the rate of denitrification and release of ammonia were directly proportional to the length of percolation, since the sand was homogeneous.

Fig. 16 - Influence of the length of percolation on the rate of nitrate reduction and the release of ammonia at an oxygen level below 0.1 p.p.m.



That the facts show differently, as is shown in fig. 16, may be due to the sequence of the tests.

In subsequent anaerobic experiments denitrification was found to be stimulated by percolation of minor quantities of glucose, sodium acetate and glutamic acid, in addition to tap water. The substrates were tested separately in concentrations of less than 10 p.p.m. During several months of investigation in four sand columns (see fig. 4) it appeared, however, that ammonia in the percolate had dropped to a negligible concentration. It is fairly probable, that glucose, etc. had been degraded by actively denitrifying bacteria which utilized ammonia. In the absence of a readily degradable organic substance the ratio of ammonia nitrogen released to the nitrate nitrogen reduced was about 0.5. Even higher values were obtained during the period following after one month of stopping the installation. When, however, aerated tap water without any addition of substrates was percolated through the sand bed ammonia in the percolate dropped rather rapidly. Whether the formation of ammonia was affected by oxygen or ammonia had been oxidized to nitrate is a question which has to be solved in a further study.

4. Discussion

From the aerobic experiments it has appeared that the percolation of tap water, enriched with ammonia, through a fully saturated sample of dune sand at a rate of 0.1 to 0.2 m.day⁻¹ resulted in the consumption of oxygen in the upper reaches of the sand bed and the reduction of nitrate in the lower ones. It is shown that the oxygen consumption was governed mainly by the nitrification reaction, giving rise to an oxygen uptake of 4.0 to 4.3 mg O₂ per mg NH₄⁺-N supplied to the sand bed. Denitrification was apparent in the upper reaches of the sand bed at low dissolved oxygen contents. Under this condition the rate of denitrification was not uniformly distributed throughout the sand bed at the start of the experiment. The biggest rates were obtained in the layer below tap no. 8 (see fig. 1) consisting of sand with an initial concentration of 1.5 to 2.0 per cent of organic matter. The layer above tap no. 8 consisted of washed sand. As it may be safely assumed that the organic matter content in washed sand was inferior to that of

the lower layer it is probable that denitrification was associated with organic matter in the sand. Organic matter was being mineralized by bacteria inoculated with the sand. Verhoeven and Goos [16] have demonstrated that a number of organic substances are converted into CO_2 and water in the denitrification process. It is interesting to note that not all organic matter can be oxidized. It is mentioned by Nason and Takahashi [11] that denitrifying bacteria are not capable of oxidizing acids and hydrocarbons anaerobically.

It is probable that the bacteria were rather inactive since they were deprived of an external food supply. Our data revealed that less than 0.2 mg of $\text{NO}_3^- \text{N}$ were reduced per month and per gram of organic matter initially present in the sand. So the endogenous phase is evident and it may be expected that the rate of mineralisation, i.e. the average weekly quantities of nitrate reduced had decreased slowly. This is clearly shown in table 2 and 3. From the divergence between the average weekly quantities of nitrate reduced below tap 8 and the average weekly losses of inorganic nitrogen in the total sand bed (see table 2) it can be deduced that the rate of mineralization in the layer of washed sand had gradually increased due to adsorption of organic matter and invasion of dead cells from the top layer. No bacteriological examination has been undertaken to verify this conception. It is true that in our investigation never any organic nitrogen has been found in the interstitial water, but this determination is too coarse a measure to draw a conclusion upon.

The nitrification process was confined to the top layer of the sand bed. Our results indicate that nitrifying bacteria were capable of removing from 1.5 to 1.8 p.p.m. of $\text{NH}_4^+ \text{N}$ at an influent dissolved oxygen content of 8.0 to 8.5 p.p.m. Higher ammonia contents resulted in the penetration of it to the deeper layers. Under this circumstance dissolved oxygen was low (1.0 to 1.5 p.p.m.) whilst ammonia was hardly removed herein. Removal of ammonia did take place in the presence of readily degradable organic matter. In a sandy soil Woldendorp [10] has found that after a dressing of ammonia part of it was converted into soil organic matter, whereas in the case of a nitrate dressing less nitrogen was recovered. It is fairly probable that ammonia was utilized for growth by actively denitrifying bacteria. From the above mentioned picture it would follow that the removal of ammonia in dune sand is a pure biological phenomenon. Organic matter gave rise to the development of heterotrophic bacteria, however, which were competing for the available oxygen. These results suggest that in the practice of infiltration removal of ammonia will be affected by the organic constituents in river water.

An inspection of figs. 5, 10, 11 reveals that the oxygen content approached asymptotically to zero, which is indicative of an oxygen consumption being dependent upon the rate of oxygen transfer. Following Skerman and Mc Rae [20] it is assumed that in the percolation of water through sand the mass transfer of oxygen and nitrate to the biosphere at the surface of the sand grains is governed by molecular diffusion and the movement of water particles. The fact that the rate of denitrification, expressed as the drop of nitrate per meter sand bed, was found to be almost inversely proportional to the rate of percolation indicates, that the rate of nitrate transfer was

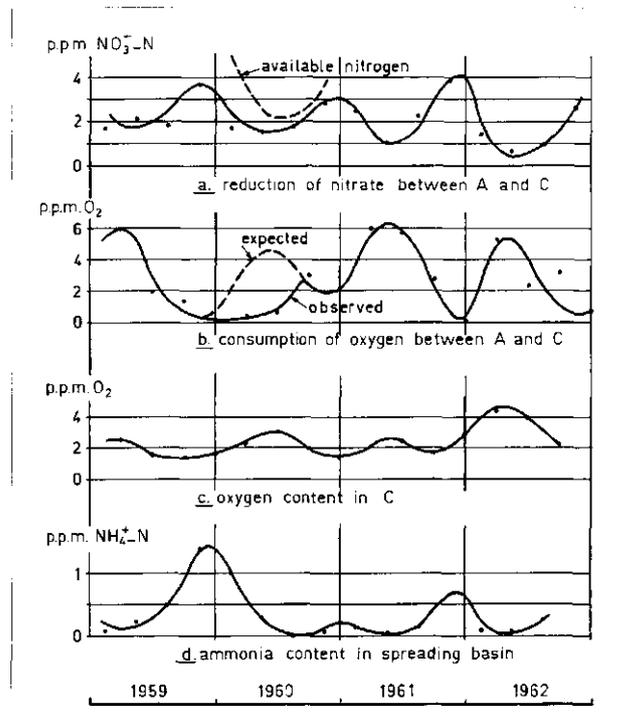


Fig. 17 - Field experiments by Leeftang.

adequate due to its concentration and its oxidative power. This finding is supported by the distribution of nitrate obtained in the sand bed (see fig. 5). It further appears that the consumption of oxygen continued while nitrate was being reduced. In laminar flow the rate of mass transfer will be low so that the rate of mineralisation may easily surpass the rate of oxygen transfer. As a consequence the oxygen content in the biosphere is lowered and nitrate will be reduced subsequently. It may thus be concluded that an oxygen gradient had been present between the main body of the interstitial water and the biosphere. Denitrification was found to start at an oxygen level of about 3.0 p.p.m. In view of the above mentioned picture it is clear that this value should not be regarded as an absolute one but to be dependent upon the rate of oxygen transfer in the boundary layer and the rate of mineralisation.

In this connection it is of interest to point out that Meiklejohn [19] has reported about denitrification in aerated cultures.

Afterwards Skerman and Mc Rae [20] have shown that reduction of nitrate under this circumstance started as soon as the oxygen supply became limited. Denitrification ceased, however, under the condition of an adequate supply of oxygen. Since they applied a vigorous agitation a turbulent mixing prevailed, so that a minute oxygen gradient may be expected. They found that denitrification started at an oxygen level of about 0.2 p.p.m.

The reduction of nitrate in the deeper layers of an aquifer has been observed by Leeftang [2]. The present authors have replotted some of the field data regarding the reduction of nitrate and the consumption of oxygen between bore hole A, at a depth of 1 to 2 m below the bottom of the spreading basin and bore hole C some 50 m downstream (see fig. 17). River water was infiltrated

at a rate of about 0.4 m day⁻¹. Although not strictly comparable on account of the widely differing rates of mineralisation and the lower temperatures in the soil (8 to 15° C), it appears that the field data agree with the above mentioned results. Like in the present investigation there was a simultaneous consumption of oxygen and reduction of nitrate. Data reveal that both parameters are negatively correlated. The oxygen level at which the reduction of nitrate did cease, however, is surprisingly high (about 4 p.p.m.).

Surveyed at large the oxygen contents in bore hole C were rather high. In 1960 the oxygen consumption was low and one might wonder whether the nitrate during this year was on the verge of exhausting.

Leeflang has found that the ammonia content of the recovered groundwater varied from 0.2 to 0.5 p.p.m. Our results indicate that minor quantities of ammonia may be released at low dissolved oxygen contents. Whether the formation of ammonia was affected by dissolved oxygen in the interstitial water is a question which is difficult to answer, since ammonia may be oxidized by nitrifying bacteria. At an oxygen level of about 2.0 p.p.m., in fact, no ammonia was released. Under anaerobic conditions, however, the release of ammonia was increased considerably.

5. Conclusions

For the practice of infiltration of river water the following interpretations can be put upon the above mentioned results:

5.1. Since it is fairly probable that the nitrification process is confined to the bottom of the spreading basin, i.e. the top few cm's of the aquifer, the disturbance of it may result in the penetration of ammonia to the deeper layers where it is hardly removed.

5.2. The rate at which ammonia is removed by nitrifying bacteria will, to a certain extent, be affected by the concentration and the degradability of the organic constituents of river water. The adsorption of ammonia in the deeper layers is promoted by the removal of easily degradable organic matter by denitrifying bacteria.

5.3. At dissolved oxygen contents below 3.0 p.p.m. the mineralisation of adsorbed organic matter may result in the reduction of nitrate. That oxygen is consumed simultaneously may be explained from the fact that the supply of oxygen cannot keep pace with the consumption of it by bacteria, so that nitrate is reduced subsequently.

5.4. At dissolved oxygen contents below 1.5 p.p.m. ammonia may be released. The release of ammonia is strongly favoured by anaerobiosis.

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References

- Huisman, L. and van Haaren, F. W. J. (1966), JWSA Congress, Barcelona, Special Subject no. 3, 1-43.
- Leeflang, K. W. H. (1965), *Kwaliteitsveranderingen door infiltratie - Zeventiende Vakantiecursus in Drinkwatervoorziening*, Delft, 55-81.
- Baars, J. K. (1960), *Principles and applications in aquatic microbiology*, Ed. Heukelekian and Dondero, Publ. John Wiley & Sons, Inc, 344-365.
- Maschhaupt, J. G. (1938, 1941), *Lysimeteronderzoekingen aan het Rijkslandbouwproefstation te Groningen en elders*. Versl. Landbk. Onderz. 44 (1) A (1938), 184 p.p. Versl. Landbk. Onderz. 47 (4) A (1941), 524 p.p.
- Kolenbrander, G. J. (1969), *Nitrate content and nitrogen loss in drainwater*, Netherl. J. Agriculture (in press).
- Montgomery, H. A. C. and Borne, B. J., *The inhibition of nitrification in the BOD-test*, WPRL Stevenage, reprint 488, 1966, 14 p.p.
- Downing, M. A., Painter, H. A., Knowles, G. (1963), *Nitrification in the activated sludge process*, WPRL Stevenage, reprint 422, 1963, 25 p.p.
- Greenwood, D. J. (1962), *Nitrification and nitrate dissimilation in soil*, Plant and Soil, 17, 3, 378-391.
- Wijler, J. and Delwiche, C. C. (1954), *Investigations on the denitrifying process in soil*, Plant and Soil, 5, 2, 155-169.
- Woldendorp, J. W. (1963), *The influence of living plants on denitrification*, Thesis Wageningen, Netherlands.
- Nason, A. and Takahashi, H. (1958), *Inorganic nitrogen metabolism*, Annual Review of microbiology, 12, 203.
- Delwiche, C. C. (1956), *A symposium on inorganic nitrogen metabolism*. Eds. Mc. Elroy, W. D. and Glass, B. H., 233-256, John Hopkins Press, Baltimore.
- Wheatland, A. B., Barrett, M. J., Bruce, A. M. (1959), *Some observations on denitrification in rivers and estuaries*, Journal and Proceedings Institute of Sewage purification, 149-162.
- Marshall, R. O., Dishburger, H. J., Mc Vicar, R. and Hallmark, G. D. (1953), *Studies on the effect of aeration on nitrate reduction by Pseudomonas species using N¹⁵*, J. Bact., 66, 254-258.
- Pfaff, C. (1963), *Das Verhalten des Stickstoffs im Boden nach langjährigen Lysimeterversuchen*, Zeitschrift für Acker- und Pflanzenbau, 117, 77-99.
- Verhoeven, W. and Goos, J. J. C. (1954), *Studies on true dissimilatory nitrate reduction*, Antonie van Leeuwenhoek Journal of Microbiology and Serology, 20, 93-101.
- Verhoeven, W. (1952), *Aerobic sporeforming nitrate reducing bacteria*, Thesis Delft, Netherlands.
- Nommik, H. (1956), *Investigations on denitrification in soil*, Acta Agriculturae Scandinavica, 6, 195-228.
- Meiklejohn, J. (1940), *Aerobic denitrification*, Ann. Applied Biol., 27, 558-573.
- Skerman, V. B. D. and Mac Rae, J. C. (1957), *The influence of oxygen availability on the degree of nitrate reduction by Pseudomonas Denitrificans*, Canadian Journal of Microbiology, 3, 505-530.
- Sacks, L. E. (1948), *Metabolic studies on denitrification*, Thesis University of California.