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EXPERIMENTAL STUDY ON THE NITROGEN CIRCULATION OF A BIOLOGICAL POND

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The ammonification and nitrification potentials of a biological pond in southern Finland have been experimentally explored at the laboratory scale.

The intention of this study was to explore experimentally at laboratory scale the ammonification (A) and nitrification (B) potentials of a biological pond.



During the impoundment of sewage, nutrient reduction occurs through natural processes by way of incorporation into the microbe and protozoa biomass. Bacterial activity degrades protein through the polypeptide-dipeptide-amino acid chain, releasing ammonia. A part of the amino acids and the released ammonia is taken up by the algal and bacterial biomass of the pond, and a part is oxidized by nitrifying bacteria (*Nitrosomonas*) into nitrites and further into nitrates (*Nitrobacter*). The first phase of nitrification is called nitrification.

The biological pond studied was situated at the Valkeala County College in southern Finland. About 250 persons were living at the college. The average water consumption was 25 m³/day.

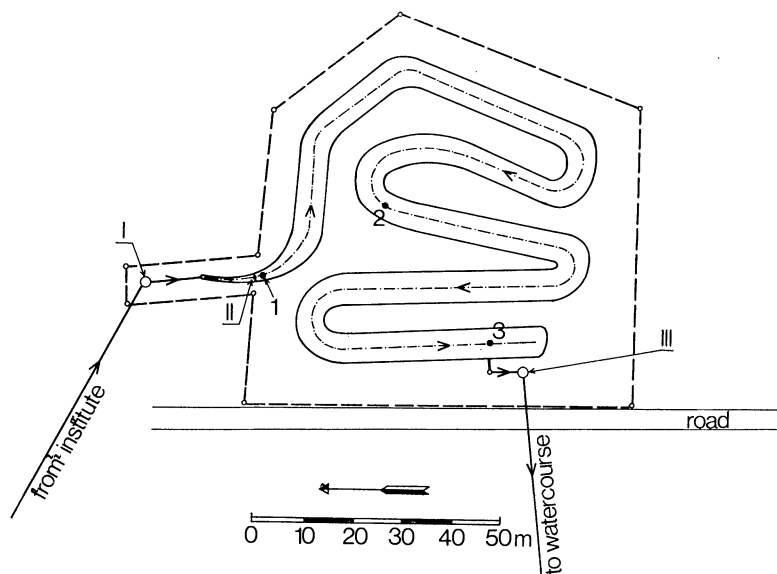


Fig. 1.

General map of the biological pond. I inlet, septic tank, II aeration steps, III outlet. 1, 2, 3 sampling points.

A pre-purification of the college wastewaters took place in a septic tank and a series of aeration steps downstream from it (Fig. 1). The septic tank was constructed of concrete rings 2 m in diameter. The total volume of the tank was 4.7 m³. The average detention time of the wastewater in the tank was from 2 to 4 hr. The construction of the biological pond differed from conventional ponds in that it formed a 300 m long narrow channel. The detention period of the water in the pond was about 2 months.

Table 1 presents the average values of the quality of the pond water at the sampling points in Figure 1 and in raw wastewater. In the summer there has been oxygen in the pond as a result of assimilation activities. In the winter the pond has been oxygen free. The most effective purification has taken place in the aeration steps at the starting end of the treatment plant. Purification in the pond has, however, been less effective. Purification has been more effective in summer than in winter both with regard to BOD load and plant nutrients. The quality and treatment of wastewater at Valkeala County College have been discussed in detail in another connection (Hooli 1973).

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Table 1.

The average quality of wastewater in the biological pond of Valkeala County College. The sampling points are presented in Fig. 1.

	Raw wastewater	Sampling points					
		1		2		3	
		summer	winter	summer	winter	summer	winter
O_2 (mg/l)	0	4,3	0	6,0	0	8,9	0
$KMnO_4$ consumption (mg/l)	275	190	147	173	129	170	145
BOD_7 (mg/l)	370	110	161	63	99	67	110
Tot. P (mg/l)	12,2	6,5	9,0	5,0	10,7	4,3	8,8
Tot. N (mg/l)	42,5	21,4	32,1	11,8	29,3	10,5	28,9

METHODS

Sampling

The samples for the laboratory determinations of potential were taken from different points of the biological pond (Fig. 1). Sample 1 was taken from the influent water, sample 2 from the center of the pond, and sample 3 from the effluent water released into Lake Saanjärvi. The sampling times were June 10th, July 13th, July 30th 1970, and March 17th 1971.

The ammonification and nitrification potentials were determined according to the following experimental procedure (Seppänen & Wunderlich 1970, Seppänen 1972).

1. Ammonification Potential

The ammonification study explored the potential capacity of the bacteria in the wastewater to break up proteins and release ammonia (NH_4^+). In the study, 50 ml of nutrient broth (Beef extract 3,0 g, Peptone 5,0 g, Aqua dest. 1 000 ml) was added to the test bottles (vol. 2 500 ml). A daily determination of the NH_4^+ content was made from the bottles by distilling the sample. The ammonification potential was determined from different dilutions of the pond effluent (Figs. 3 and 5) as follows: A \equiv 1:10, B \equiv 1:100, C \equiv 1:1 000, and D \equiv 1:10 000. The water of Lake Saanjärvi was used as diluent.

2. Nitritation Potential

The nitritation study explored the potential of the bacterial population of the wastewater to oxidize ammonia into nitrites. A layer of technical calcium carbonate (CaCO_3) was added to the test bottles (vol. 2 500 ml), and 100 ml of Nitrosomonas base ($(\text{NH}_4)_2 \text{SO}_4$ 2,0 g, K_2HPO_4 1,0 g, NaCl 0,5 g, MgSO_4 0,5 mg, MnSO_4 trace, $\text{Fe}_2 (\text{SO}_4)_3$ trace, distilled water add. 1 000 ml) was added to the sample water (Manual of Microbiological Methods 1957).

The daily NO_2^- content was determined from the bottles (Figs. 2, 4, 6, and 7). The study was used to evaluate the nitritation potential at different points of the pond and in the same dilutions of the effluent as used in the ammonification study.

RESULTS

The most important factor in evaluating the results of both ammonification and nitritation is the length of the lag phase. The more favorable the new conditions are for a certain microbiological process, the shorter the phase. The length of the lag phase depends on the structure of the enzyme system of the microbial population and the density of the microbes active in the process concerned. If the microbial population is adapted to degrading a certain substrate, a new substrate necessitates the development of a new adaptive enzyme system. If only a few microbes are present in the sample bottle, the development of a sufficient population for effective activity takes time.

As the logarithmic growth phase of the bacterial population begins after the lag period, the angle coefficients of the straight parts of the different graphs describing the concentration changes during the growth phase are nearly similar (cf. Figs.).

Fig. 2 (June 10th 1970) indicates clearly that the nitritation potential depends on the degree of dilution in that the potential decreases with a greater dilution. Curves 1, 2, and 3 in Fig. 2 show the nitritation potential of the bottles containing only wastewater from the corresponding sampling points without the addition of carbonate or Nitrosomonas base. A comparison between curves A, B and C and the numbered curves indicates that nitritation can be speeded up by taking into account the environmental requirements of the microbes active in the process, thus obtaining better nitrogen reduction (Seppänen 1972).

Figs. 3 and 4 show the ammonification and nitritation potentials of the pond water on July 13th 1970. A comparison between the two indicates that the lag period for ammonification is considerably shorter than for nitritation (17 days).

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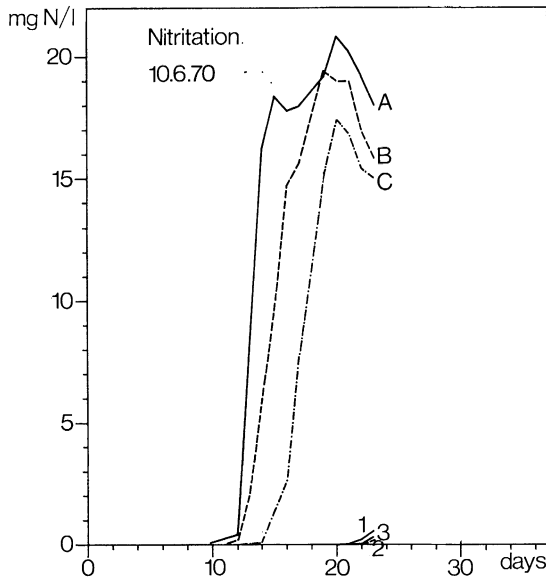


Fig. 2.

Nitritation potential in a biological pond. Curves 1, 2 and 3 show nitritation potential at corresponding sampling points without optimisation (see map, Fig. 1). Graphs A, B and C show the nitritation in diluted and optimised samples taken from point 3. A = dilution 1:10, B = 1:100 and C = 1:1000.

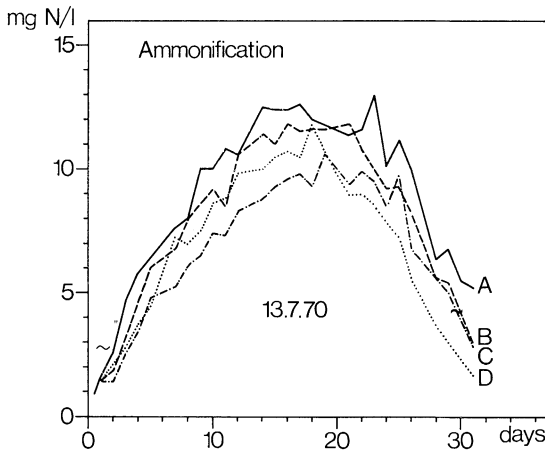


Fig. 3.

Ammonification potential in a biological pond. Curves show ammonification in different dilutions of the sample taken from point 3. Nutrient broth was added to the bottles. A = dilution 1:10, B = 1:100, C = 1:1000 and D = 1:10000.

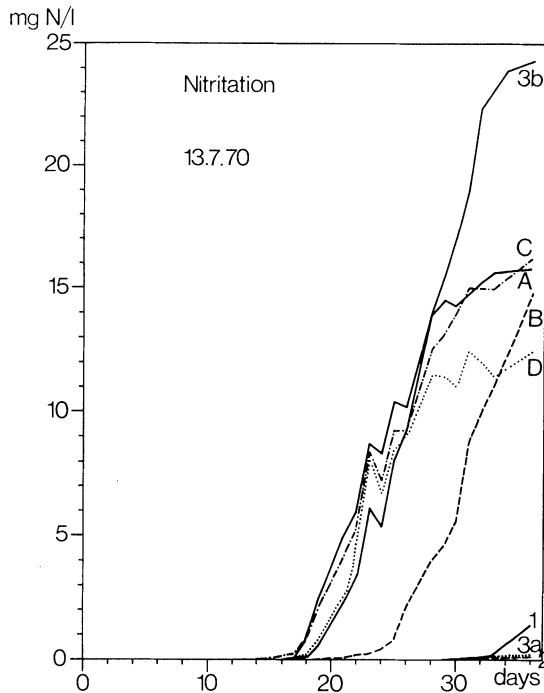


Fig. 4.

Nitritation potential in a biological pond. Curves 1, 2 and 3a show nitritation at corresponding sampling points without optimization (see map, Fig. 1). Graphs A, B, C and D show nitritation in diluted and optimized samples from point 3. Graph 3b is an undiluted optimized sample. A = dilution 1:10, B = 1:100, C = 1:1000, and D = 1:10000.

As ammonification is one of the central microbiological processes in nature and numerous different bacteria are ammonifying in type (Kusnezow 1959), it is natural that the ammonification potential is relatively high in domestic wastewater containing a large number of both nutrients and types of bacteria.

The long lag phase for nitritation indicates that either the number of nitrifying bacteria (*Nitrosomonas*) in the wastewater is small or conditions are not favorable for effective nitrification.

Figs. 5 and 6 show the results for the samples taken on July 30th 1970. The dilutions were the same as with the previous samples. Fig. 5 shows that the ammonification potential in the greatest dilution (1:10 000) was smaller than in the lesser dilutions. On the other hand, the nitritation potential was highest in

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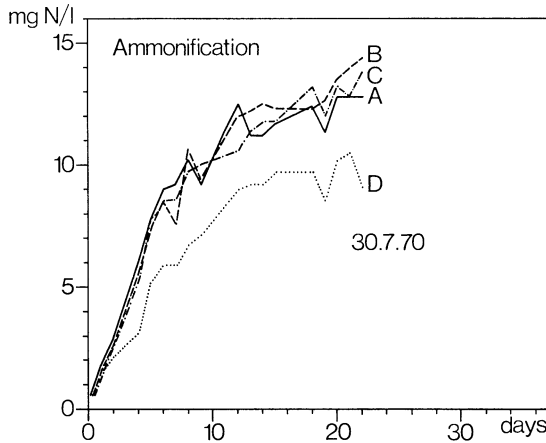


Fig. 5.

Ammonification potential in a biological pond. Curves show ammonification in different dilutions of the sample taken from point 3. Nutrient broth was added to the bottles. A = dilution 1:10, B = 1:100, C = 1:1000, and D = 1:10000.

the greatest dilution. The reason for this may be that some component of the wastewater inhibits nitrification in a strong concentration and thus nitrification becomes more effective in greater dilutions.

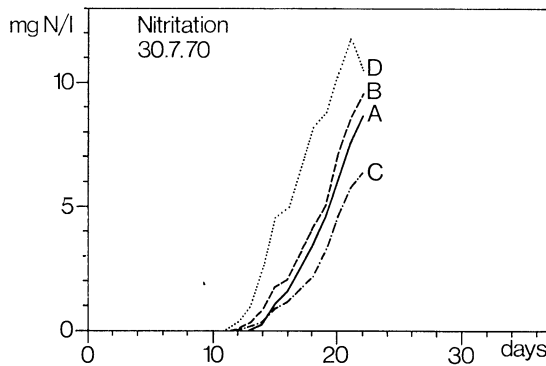


Fig. 6.

Nitritation potential in a biological pond. Curves A, B, C and D show the nitritation in diluted and optimized samples taken from point 3 (see map, Fig. 1) A = dilution 1:10, B = 1:100, C = 1:1000, and D = 1:10000.

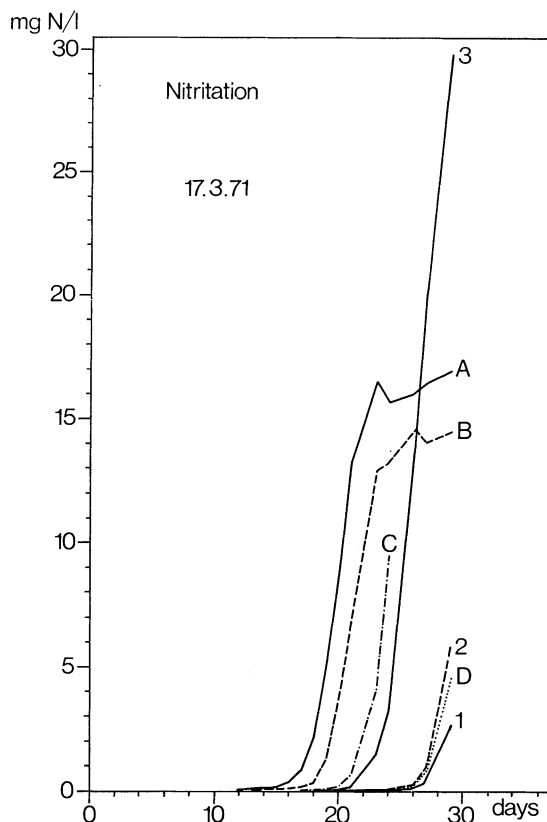


Fig. 7.

Nitritation potential in a biological pond. All curves show nitritation in optimized samples. Curves 1, 2 and 3 are samples from corresponding sampling points (see map, Fig. 1). Curves A, B, C and D show the nitritation potential in diluted samples taken from point 3. A = dilution 1:10, B = 1:100, C = 1:1000, and D = 1:10000.

Nitritation potential in the winter was studied from samples taken on March 17th 1971 (Fig. 7). The test procedure differed slightly from the previous times. No *Nitrosomonas* base or CaCO_3 grains had been added to the summer samples from points 1 and 2. Now both were added to all sample bottles. The high NO_2 value in bottle 3, as compared with the other bottles, was caused by the oxidation into nitrites of the ammonia released in the ammonification of nitrogen compounds present in the wastewater. By the time the wastewater reached the outflow of the pond (sample 3), the proteins had become degraded so far during the long retention period, that effective ammonification released ammonia.

CONCLUSIONS

An effective treatment of wastewater implies a knowledge of the biological processes of the elements that are to be removed. At the present moment nitrogen and phosphorus are regarded as the nutrients most detrimental to water-courses (Ryhänen 1968, P. Seppänen 1970). Chemical treatment is increasingly being used to remove phosphorus. Nitrogen is still for the most part removed biologically through denitrification. In order to be able to remove the nitrogen through denitrification, the dissolved ammonia has to be oxidized by way of nitrites into nitrates. However, a part of the nitrogen compounds bonded with the proteins is already removed with the sludge during activated sludge treatment. A method that can be used to regulate the operation of a biological pond is to keep the wastewater concentration on an optimal level for bacterial activity.

Nitrogen reduction in biological ponds can be improved by determining the most favorable wastewater concentrations for the biological circulation of nitrogen components through experimental potential determinations. The results of this study indicated that both the ammonification and nitrification potentials depended on wastewater concentration. No special interest is attached at the moment to the operational control of biological sewage treatment facilities. By studying the potential of the different bioprocesses for each individual pond, the reducing effect of the ponds can be improved with fairly simple methods, as, for example, by simply regulating wastewater concentration.

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