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Author(s) K. Roch & A. Kaffka

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DETERMINATION OF THE ACTIVITY OF NITRIFYING BACTERIA IN SURFACE WATERS BY A MODIFIED BOD-TEST.

by K. Roch and A. Kaffka

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Abstract

A modified method of the BOD-test was applied in order to find out the seasonal changing activity of the nitrifying bacteria in surface waters.

In studies of the river ELBE the samples collected at TEUFELSBRÜCK (km. 630.1) were diluted 1:10 with aerated tap water and mixed with 5.2 mg/l. NH_4^+-nitrogen (0.37×10^{-3} Mol/l). As the result of this admixture a seasonal changing increase of BOD was found. This increase was caused by the activity and number of the $NH4^+$ - oxidising bacteria and depended on temperature. Furthermore these investigations indicated that the low $NH4^+$ -content functioning in summer as a limiting factor does not allow a higher influence of nitrification on the BOD and oxygen balance in the receiving water. With regard to water hygiene the nitrifying flora can affect the oxygen balance significantly, particularly in rivers of long retention time or in estuaries loaded by nitrogenous waste waters during the summer time or if the river water is artificially heated by cooling water discharge.

Surface water receives a considerable amount of organic and inorganic nitrogen compounds through industrial and domestic waste, as well as from the drain-off of agricultural areas, which are often spread with fertiliser several times while under cultivation. The organic substances are utilised by a large number of heterotrophic bacteria as source of energy and carbon, and are reduced to low-molecule combinations. CO2 and ammonia are finally liberated as end products by aerobic reduction. Further oxidative mineralisation of ammonia nitrogen is mainly limited to a small group of bacteria of the genera <u>Nitrosomonas</u> and <u>Nitrobacter</u>.

Their maximum possible oxygen consumption on the complete oxidation of $NH4^+$ to $NO3^-$ can be calculated stoichiometrically according to the equations:

		consumption mg O2 per mg N
Nitrosomonas:	$2\ NH_3 + 3\ O_2 \rightleftharpoons 2\ NO_2^- + 2\ H^+ + 2\ H_2O$	3.43
Nitrobacter:	$2\ NO2^- + O_2 = 2\ NO_3^-$	1.14
	$2\ NH_3 + 4\ O_2 \rightleftharpoons 2\ NO_3^- + 2\ H_2O + 2\ H^+$	4.57

As the nitrifying bacteria however utilise NH4+ and NO2- nitrogen as a source of energy as well as a source of nitrogen for cell construction and are reduced on assimilating CO2, the actual value lies at 4.33 mg. O2 (WOLF 1971). Nitrosomonas requires from 1 mg. NH4+ -N 3.22 mg. O2 for oxidation, Nitrobacter from 1 mg. $NO2^-$ N 1.11 mg. O2 (ANONYM 1971).

For a long time the oxygen requirements of the nitrifying bacteria were thought to be unimportant in influencing the purity of the water, if one disregarded occasional contrary reports (HURWITZ et al. 1947, BUSWELL et al. 1953).

Only in recent years have writers repeatedly referred to the loading of the oxygen balance in surface waters through nitrification (SIDDIQI et al. 1967, COURCHAINE 1968, ROCH & KAFFKA 1971, ANONYM 1971 etc.). This loading emerged particularly from the results of experiments in which the amount of nitrification on BOD was tested. HURWITZ et al. 1947, MONTGOMERY & BORNE 1966, WOOD & MORRIS 1966, SIDDIQI et al. 1967, COURCHAINE 1968).

According to some experiments on water from the ELBE, published in 1971, the amount of nitrification determined by BOD_5 lay between 8.8% and 81.5%. The highest values were measured downstream of the KÖHLBRANDHÖFT purifying plant. COURCHAINE obtained results similar to those of 1968 in experiments on the GRAND RIVER. According to these, the BOD of surface water below the outlets of large purifying plants can be greatly increased through nitrification at favourable water temperatures.

According to BRAUNE & UHLEMANN (1968), nitrification in river water only begins to be measurable above c. 15°C. In the course of a year, therefore, temperatures permitting only limited nitrification are present naturally over a long period. According to COURCHAINE, however, favourable or almost optimum temperatures for nitrifying bacteria can be obtained locally through the entry of warm cooling-water, so that temperature is also ruled out as a limiting factor during the cold season. Also the fact that the BOD can be altered through the influence of nitrification in the above-mentioned degree in the laboratory at 20°C, gives one to expect that in water even nitrification makes stronger claims on the oxygen balance than is generally accepted, since higher temperatures occasionally occur here.

The increased activity of the nitrifying bacteria during the summer months leads to a decrease in concentration of NH_4^+ and to peaks of the NO_2^- and NO_3^- values. This is caused by the rapid multiplication of the nitrifying bacteria at summer temperatures (RHEINHEIMER 1959, 1964).

That nitrification on the BOD test is small in spite of increased activity and a higher number of cells at temperature peaks (ROCH & KAFFKA 1971) can be attributed to the very low amount of NH_4^+ nitrogen in the water during this period. With sufficient NH_4^+ content, however, the number of nitrifiers is significant for the degree of BOD. Their multiplication hardly mattered on account of their long generation period of 24 hrs. at 20°C during the 5-day duration of the BOD experiment.

No conclusions can be made on the oxidation potential of the nitrifying bacteria through the usual BOD-test. A direct count offers great difficulties and the determination of their activity, according to RHEINHEIMER (1959), is very time-consuming. In our experiments, therefore, we determined the seasonally varying activity of the nitrifying bacteria according to a modified method of the BOD-test, eliminating the limiting factor of the concentration of NH_4^+.

In addition the water from the ELBE was diluted with aerated mains water in the proportion 1:10 and mixed with 5.2 mg/l. NH_4^+ -N (0.37×10^{-3} Mol/l.). In the individual experiments there existed approximately equal concentrations of NH_4^+ (5.3-5.6 mg/l. NH_4^+ -N).

Method

The samples were removed from April to December 1969 once or twice monthly at TEUFELSBRÜCK (Elbe kilometers 630.1), about 5 km. below the outflow of the KÖHLBRANDHÖFT purifying plant[1]. Work was begun on the samples in the laboratory 2 hours at the latest after removal. The following procedure was taken for the BOD-test:

[1] (about 370,000 m^3/day)

1. 1:5 diluted ELBE water (800 ml. ELBE water + 3200 ml. of aerated mains water).
2. 1:5 diluted ELBE water with 0.1 Mol/1. NH_4Cl for suppressing nitrification, after SIDDIQI et al. (1967) (800 ml. of ELBE water + 3200 ml. water for dilution with 0.1 Mol/1. NH_4Cl and 80 ml. of a 0.067 molar phosphate buffer after SORENSEN, pH 6.9).
3. 1:10 diluted ELBE water with 0.37×10^{-3} Mol/1. NH_4Cl (400 ml. ELBE water + 3600 water for dilution + 5.2 mg/1. NH_4^+ -nitrogen).

300-ml. Winkler bottles were filled with these solutions and incubated at 20°C for 5 to 10 days. The oxygen content was determined by the Winkler method, that of the BOD after the standard German procedure. For the determination of nitrite and ammonium nitrogen, we employed the methods modified for the auto-analyser by O'BRIEN et al. (1962). The nitrate was determined with sodium salycilate.

Results and Discussion

The addition of 5.2 mg/1. of ammonium nitrogen to ELBE water which had been diluted tenfold had the effect of clearly increasing the BOD. This clearly depended on promotion of nitrification (Tab. 1, Figs. 1 & 2).* It was so high, particularly in the summer months, that the oxygen was exhausted after only 7 days. The increase in oxygen consumption was connected with a decrease in the concentration of ammonium and a corresponding rise in that of the nitrite and nitrate. The oxygen consumption for the nitrifying bacteria (3rd series of experiments, Tab. 1) can be calculated by the above-mentioned equation from the formation of nitrite and nitrate. For instance, it amounted to 3.98 mg/1. O_2 after 10 days' heating:

NH_4^+ -N decrease:	1.11 mg/1.	
NO_2^- -N increase:	0.99 mg/1.	= 3.20 mg/1. O_2
NO_3^- -N increase:	0.18 mg/1.	= 0.78 mg/1. O_2
		= 3.98 mg/1. O_2

With regard to the tenfold dilution of water from the ELBE, a BOD of 40 mg/1. O_2 resulted for the nitrifying bacteria. The values for BOD_5 turned out to be similarly high for the warm season. Prolonging the time of heating to 10 days clearly shows that with the duration of heating and favourable concentration of ammonium the relative proportion of the nitrifying bacteria shows a strong increase in BOD. The ammonium

* at the end of this translation.

nitrogen consumed during flow-off is almost equivalent to that of the products of oxidation. It should be taken that in the method which employed river water diluted tenfold, the processes of assimilation and autolysis are extremely small for the nitrifying bacteria and heterotrophic organisms.

The difference which occurred between the BOD-values of the 2nd series after heating for 5 days (+ 5.2 mg/l. NH_4^+ -N) and the 3rd series (nitrification completely suppressed through the addition of 0.1 Mol/l. NH_4Cl) (Tab. 1. Fig. 1) was set as a relative amount for the activity of the nitrifying bacteria. This amount varied seasonally, its maximum occurring at the same time as that of the water temperature (Fig. 3). Since the experiments were performed under constant laboratory conditions (in darkness at 20°C), this is evidence for the fact that the number of nitrifying bacteria in the ELBE greatly increases in the summer.

Certainly in all experiments during heating, the oxygen was consumed mostly by the nitrite-formers. The reason for the low nitratation is probably due to the fact that the concentration of nitrite when heating begins was too low, and that at the temperature for heating, the activity of the nitrite-formers is greater than that of the nitrate-formers. For the same reason there should also occur summer nitrite peaks in the ELBE. Thus the seasonally changing activity of the nitrite-formers and their numbers was comprised primarily by this method. A corresponding method is conceivable for determining the activity of the nitrate-formers. In place of the existing NH_4Cl a few milligrams of nitrite can be added, and the oxidation of the NH_4^+ -N contained in the surface water by <u>Nitrosomonas</u> can be neutralised by the addition of thio-urea or allyl thio-urea (WOOD & MORRIS 1966, MONTGOMERY & BORNE 1966).

The dependence of the nitrification on the ammonium nitrogen content of water from the ELBE, which was shown in experiments with undiluted samples of water from the same place (ROCH & KAFFKA 1971) did not occur in these experiments. The other variations in ammonium concentration in ELBE water thus had no further influence because of the addition of 5.2 mg/l. NH_4^+ -N to water which had been diluted tenfold.

The experiments have shown that the number of nitrifying bacteria which occur in the ELBE in summer is sufficient, when there is sufficient NH_4^+ content, to exceed the oxygen requirements for the microbial oxidation of organic compounds several times. Thus the maximum of the BOD_5 of the nitrifying bacteria determined by the modified method lay at 42 mg/l. O2 in the lukewarm water of September 1969. According to these findings the NH4+ content was the limiting factor for nitrification in water from the ELBE during the warm season. It was further shown what significance nitrification can have for the BOD-test and the oxygen balance in surface water at summer temperatures or with thermic loading by warm cooling-water, if waste water with large volumes of NH4+ is introduced or if agricultural surfaces which have been spread with manure are flooded by high water in summer. While in fast-flowing streams the oxygen requirements of the nitrifying bacteria can have no substantial influence on the quality of the water on account of their long generation period, the oxygen balance can be considerably charged in slow-flowing waters and also in tidal estuaries; through this there can result abuses of water hygiene.

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Dipl. Biol. K. ROCH und Priv. Doz. Dr. Dr. A. KAFFKA, Hygienisches Institut, D-2 Hamburg 36, Gorch-Fock-Wall 15-17

Table 1. The influence of 5, mg/l NH_4^+-nitrogen on the biochemical oxygen demand (all values expressed as mg/l)

Datum	6.5.	11.5.	13.5.	16.5.
Versuchsreihe I				
fünffach verdünntes Elbewasser				
NH_4^+-N	0,32	0,32	0,32	0,32
NO_2^--N	0,01	0,01	0,02	0,03
NO_3^--N	0,51	0,51	0,52	0,54
BSB, O_2		4,1		6,0
Versuchsreihe II				
fünffach verdünntes Elbewasser + 0,1 Mol NH_4Cl/l				
NO_2^--N	0,01	0,01	0,01	0,01
NO_3^--N	0,50	0,49	0,49	0,49
BSB, O_2		4,0		5,0
Versuchsreihe III				
zehnfach verdünntes Elbewasser + 5,2 mg NH_4^+-N/l				
NH_4^+-N	5,35	5,14	4,95	4,24
NO_2^--N	0,01	0,03	0,22	1,0
NO_3^--N	0,51	0,62	0,65	0,69
BSB, O_2		4,8	12,0	43,0

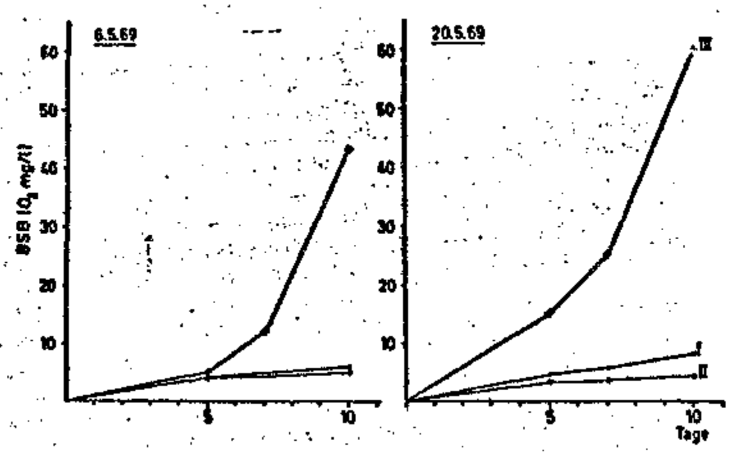

Fig. 1. The influence of admixture of 5,2 mg/l NH_4^+-N and 0,1 Mol/l NH_4^+-N on BOD. A: Experiment 6.5.1969, B: Experiment 20.5.1969.

I •——• Elbewater diluted with tap water 1:5

II o——o Elbewater diluted with tap water 1:5 + 0,1 Mol/l NH_4Cl + buffer pH 6,9

III +——+ Elbewater diluted with tap water 1:10 + 5,2 mg/l NH_4^+-N.

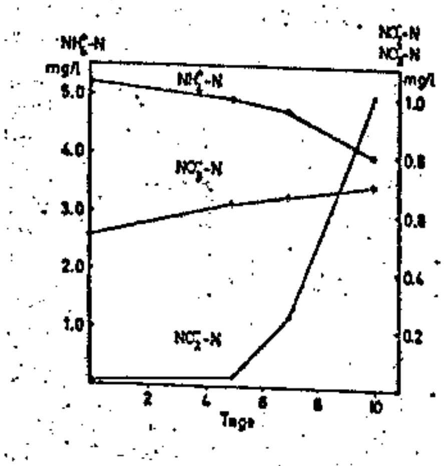

Fig. 2. Alterations of NH_4^+-, NO_3^-- and NO_2^--concentrations during 10 day incubation as a consequence of admixture of 5,2 mg/l NH_4^+-N to Elbewater diluted with tap water 1:10 (6.5.1969).

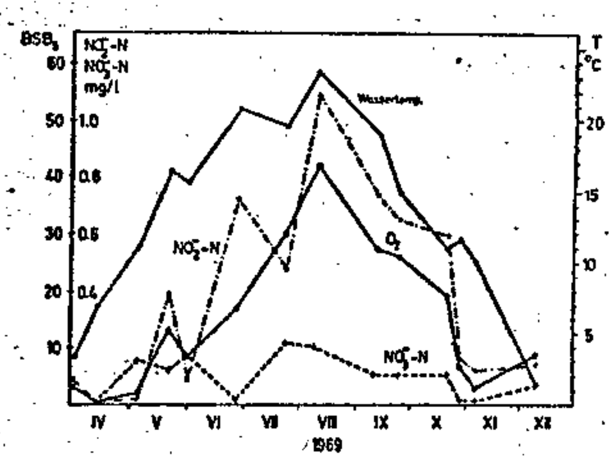

Fig. 3. The seasonal temperature fluctuation and alteration of NH_4^+-oxidation and BOD due to nitrifying bacteria as consequence of admixture of 5,2 mg/l NH_4^+-nitrogen during 5-day incubation (samples collected at Teufelsbrück)

- ●——● Water temperature
- ○——○ BOD_5 of nitrifying bacteria
- ·–·–·–· increase of NO_3^- after 5-day incubation
- + – – – + increase of NO_2^- after 5-day incubation.

Notice

Please note that these translations were produced to assist the scientific staff of the FBA (Freshwater Biological Association) in their research. These translations were done by scientific staff with relevant language skills and not by professional translators.