Method for In Situ Measurement of Nitrification in a Stream

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A method is described in which the oxidation of NH$_3$-N to NO$_2$-N and NO$_3$-N in a stream was measured in situ by use of an equilibration chamber. The conversion was stoichiometric.

Studies of a New York tributary of the Allegheny River consisting essentially of coolant water from a fertilizer manufacturing complex revealed what appeared to be nitrification. This stream receives approximately 11.5 million gallons of heated water per day, averaging 36.8 ± 1.1°C and carrying about 1 ton of nitrogenous compounds per day. These compounds exist primarily in the form of ammonia and nitrate. The quantity of both nitrite and nitrate increased 140 and 120 kg/day, respectively, with downstream progression. A corresponding reduction in the quantity of ammonia was detected, although the conversion of NH$_3$-N to NO$_2$-N and NO$_3$-N was not stoichiometric. Increases in nitrite and nitrate in natural systems with concomitant, though not necessarily stoichiometric, decreases in reduced nitrogen forms is usually attributed to nitrification (2, 3). Such indirect evidence, however, does not preclude the possibility that extraneous additions of either nitrite or nitrate or the reduction of nitrate to nitrite may have occurred (5). The method described here permits direct measurement of the nitrification process.

With an equilibration chamber, designed for use in situ, we have shown that measurable nitrification takes place over a 4-h period. This retention period was equivalent to the time required for water to traverse the 2.6-mile (ca. 4.18 km) length of the stream. Furthermore, the rate of oxidation seen in the chamber was equivalent to the rate of oxidation occurring in the stream itself, as calculated with upstream-downstream measurements, and required the presence of stream-bottom mud.

The equilibration chamber (Fig. 1) was constructed of marine plywood (0.75 inch [ca. 1.90 cm]) and was divided into three equal compartments with 0.75-inch holes drilled at each end of each compartment to allow water to flow through. Stream-bottom mud was placed in two of three compartments to a depth of 3 to 4 inches (ca. 7.62 to 10.16 cm) to simulate stream conditions, and one compartment was kept free of mud as a control. The chamber was securely anchored in the stream, and aluminum screens were fitted over the holes to filter debris from the compartment.

Stream water was permitted to flow through the chamber for at least 24 h before each experiment. A portion of the stream was trapped over the stream bottom by closing the holes with rubber stoppers. After allowing the system to equilibrate for 2 h, water samples were taken for 0-h analyses and again at 2 and 4 h. Ammonia, nitrite, and nitrate were measured according to Standard Methods (1).

Combined results representing five separate tests are shown in Table 1. The mean concentration of nitrite and nitrate increased from 1.5 to 2.1 mg of N/liter and from 3.5 to 4.8 mg of N/liter, respectively, an increase of 1.9 mg of N/liter over the 4-h period. The mean concentration of ammonia, on the other hand, decreased 1.8 mg of N/liter (from 5.7 to 3.9 mg of N/liter) during the same period.

![Equilibration Chamber](http://aem.asm.org/Downloaded from http://aem.asm.org on May 5, 2020 by guest)
TABLE 1. Changes in the concentration of ammonia, nitrite, and nitrate in the equilibration chamber over a 4-h period

| Time (h) | N-form | Concen (mean ± SEM [mg/liter]) | $\Delta NO_2-N$/$\Delta NH_4-N$ |
|----------|--------|--------------------------------|-------------------------------|
| 0        | NH$_4$-N | 5.7 ± 0.2                      | 1.0/0.9                       |
|          | NO$_2$-N | 1.5 ± 0.0                      |                               |
|          | NO$_3$-N | 3.5 ± 0.1                      |                               |
| 2        | NH$_4$-N | 4.8 ± 0.2                      | 1.9/1.8                       |
|          | NO$_2$-N | 1.9 ± 0.0                      |                               |
|          | NO$_3$-N | 4.1 ± 0.3                      |                               |
| 4        | NH$_4$-N | 3.9 ± 0.2                      |                               |
|          | NO$_2$-N | 2.1 ± 0.0                      |                               |
|          | NO$_3$-N | 4.8 ± 0.2                      |                               |

*All assays were performed in duplicate. No changes occurred in the concentrations of NH$_4$-N, NO$_2$-N, or NO$_3$-N in the open compartment containing stream-bottom mud or the closed compartment with no mud. Significance levels between means of NO$_2$-N + NO$_3$-N and NH$_4$-N at various time intervals were obtained by analysis of variance ($P < .01$). Concentrations given are means ± standard error of mean (SEM) of five separate determinations.

![Graph](http://aem.asm.org/)

**Time, hrs**

**Fig. 2. Effect of KClO$_3$ ($5 \times 10^{-3} \text{M}$) on nitrification in the equilibration chamber over a 5-h period.**

Symbols: O, Chamber compartment with KClO$_3$ added; ●, control compartment.

N/liter over the same time period. The differences between means was assessed by analysis of variance ($P < .01$), and the results clearly show that nitrite and nitrate were formed at the expense of NH$_4$-N.

Added confirmation that nitrification was responsible for conversion of nitrogen compounds in the chamber was obtained with a nitrification inhibitor (4). The inhibitor, KClO$_3$, was added to a final concentration of $5 \times 10^{-3} \text{M}$ to one chamber compartment while the other compartment was used as a control. Equilibration and sampling were performed essentially as before. The results (Fig. 2) show a 70% reduction in nitrification in the presence of the inhibitor, KClO$_3$.

The presence of a relatively large population of chemoautotrophic nitrifying bacteria, particularly in the bottom mud of both the chamber and the stream, was subsequently confirmed (J. Pierce and J. P. White, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, G130, p. 47).

Use of the equilibration chamber appears particularly advantageous in studying a number of processes where the concentration of substrate or product varies in a moving boundary of water. With proper adaptation, the chamber can also be designed to control such environmental variables as temperature, light, and substrate concentration.

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