

NOTES AND COMMENT

DIFFERENTIAL TITRATION WITH STRONG ACIDS OR BASES VS. CO₂ WATER FOR PRODUCTIVITY STUDIES

In the October 1959 issue of *Limnology and Oceanography* Beyers and Odum present curves contrasting differential titrations of natural waters using H₂SO₄ and H₂CO₃ solutions. They show a marked divergence of the curves so obtained and suggest that CO₂ changes computed from differential titrations with strong acids or bases are too low. They have, however, made an error which invalidates their argument. They failed to take account of the fact that a molecule of H₂SO₄ reacts with bicarbonate to produce 2 molecules of H₂CO₃, hence the addition of one mole of H₂SO₄ is equivalent to the addition of 2 moles of H₂CO₃. If one takes account of this discrepancy, then their curves coincide in the early part of their range. Usually, in natural waters, one is interested in a relatively small portion of the curve (pH 8-9, or 7-8) and such a portion of the curve can be examined conveniently, and accurately, by simply bubbling respired air through the natural water to bring its pH to the desired starting point, then performing the differential titration with 0.02N NaOH. Each ml of this solution will absorb 20 micromoles of H₂CO₃ from solution, or, at higher pH, will convert 20 micromoles of bicarbonate to carbonate, in close analogy to the changes produced by photosynthesis. The addition of NaOH will, of course, alter the total alkalinity slightly, but this influence is small when examining the limited region of the pH curve usually required in a study under natural conditions. The titrations with CO₂ water, described by Beyers and Odum, provide reliable curves, but the titration with 0.02N NaOH is much more simple and convenient, and is equally reliable in every natural water that I have encountered. Beyers and Odum do not refer to the work of Moberg *et al.* (1934) in which they show that differential titration curves in sea water agreed closely with total CO₂ determinations via the Van Slyke apparatus. Neither did they understand my (1956) comparison of differential titration curves

with the curves computed from Dye's nomograms or they would have noted that my differential titration curves yield higher CO₂ changes than are computed from Dye's nomograms. Frey and Stahl (1958) also observed a discrepancy in this direction. Beyers and Odum leave the erroneous impression that Dye's nomograms are more reliable than differential titrations with NaOH in fresh water. This is not the case. On the contrary, differential titration with NaOH is the method of choice for relating pH change to CO₂ change in productivity studies because it avoids the problems associated with the handling of H₂CO₃ solutions.

In the graph presented by Beyers and Odum below, the HCl curve agrees closely with the CO₂ curve between 8.7-7.5 which is in contrast to their previously published curve (Beyers and Odum 1959). This is the point I wish to make. When a differential titration with HCl or NaOH is extended over more than one pH unit, slope deviations become important.

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Verduin (1960) believes that adding one molecule of NaOH has the same effect on the bicarbonate equilibrium as withdrawing one molecule of H₂CO₃ (as by a plant in

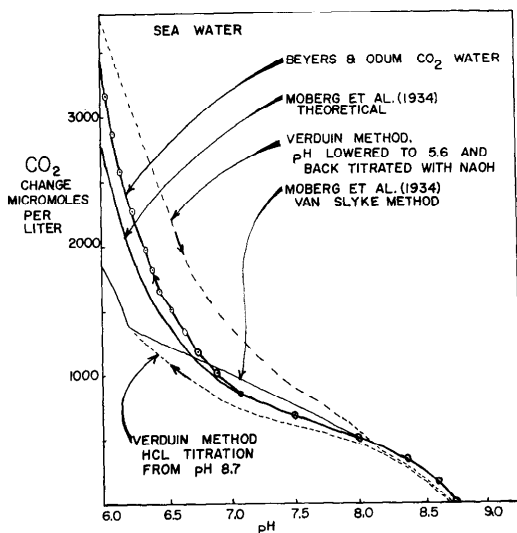


FIG. 1

photosynthesis). His contention is wrong theoretically and experimentally. To convert the previously published graphs (Beyers and Odum 1959) into the form recommended by Verduin, one simply multiplies the H_2SO_4 molarity by 2. The H_2SO_4 and H_2CO_3 curves still do not agree.

The effects are different since the equilibria are shifted in a different manner. The error of his method of computation is not a constant factor but varies markedly with pH. The error is small at pH 8.5 and very large at pH 7.5. For computing metabolism from observed changes in pH it is the slope that is important.

Verduin writes a stoichiometric formula with exactly one mole of H_2CO_3 equivalent to a mole of HCl or NaOH step by step in the titration. One cannot do this. The only way to convert a titration with a strong acid or base into a curve of total CO_2 and pH is to titrate to completion to get alkalinity and then calculate the total CO_2 for each state of equilibrium as conveniently described in textbooks by Sverdrup and Harvey.

Verduin apparently misunderstood Moberg, Greenberg, Revelle, and Allen (1934), believing that these authors had reported stepwise titrations with strong acid. Moberg *et al.*, changed the total CO_2 content stepwise by adding or withdrawing CO_2 by

passing CO_2 or CO_2 free air through sea water. Then they plotted points on a graph of total CO_2 and pH (colorimetrically) with points determined in three ways, with only fair agreement:

- A. Total CO_2 was determined with the Van Slyke method (See Fig. 1).
- B. Total CO_2 was computed from equilibrium constants (See Fig. 1).
- C. Total CO_2 was computed from the sum of methyl orange alkalinity and phenolphthalein alkalinity. This line was similar to the Van Slyke method.

They did not, however, report a stepwise titration curve of pH versus strong acid or base.

In the Figure 1 we have titrated sea water with CO_2 water as previously described and plotted moles as a function of pH observed.

For comparison, the theoretical curve from equilibrium constants by Moberg *et al.* is plotted. The agreement with the curve from CO_2 water is better than with these authors' own experimental data with the Van Slyke method and colorimetric pH methods. Also in the Figure 1 we have plotted a titration of pH with HCl on the same water and have computed according to Verduin's instructions (one mole CO_2 as equivalent to one mole HCl). In both graphs correction is made for dilution so that the molar concentrations refer to the actual concentration in the titration vessel at all times. The curves are different in slope.

Verduin indicates that sometimes he lowers the pH with CO_2 and back titrates with NaOH. This procedure we have tried on the same water. It gives still another curve not consistent in slope with Verduin's acid curve (See Fig. 1).

Beyers has improved the CO_2 titration method for routine use with a special burette with a magnetic stirring capsule for equilibrating CO_2 over a flat gas-water interface in a swollen upper portion. He has over a hundred 24-hour recordings of balanced aquarium metabolism with a Beckman zromatic pH meter and Honeywell recorder.

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LIGHT AND DARK BOTTLE EXPERIMENTS IN ALASKA

In the course of an investigation, during the summer of 1958, into the effect of the Katmai ashfall on the trophic status of two lakes in the Kodiak region, Alaska, measurements of primary production were made.¹ Both lakes have surface areas of about 45 ha, maximum depths of about 29 m, and are located within 2 miles of each other on Afognak Island.

The "light and dark" bottle technique of Gaarder and Gran (1927) was used although the low sensitivity of the method renders it marginal for use on oligotrophic lakes. Frey and Stahl (1958) were able to use it successfully, however, on several arctic lakes. Dissolved oxygen concentrations were determined by the un-modified Winkler method. The length of exposure was usually 24 hours, rarely extended to 48 hours. We first made certain that it was possible to fill three BOD bottles from the same sample of lake water contained in a Kemmerer bottle, fix them and, upon subsequent titration, obtain identical results for each (within the limits of the experimental error). The Kemmerer bottle was not coated with plastic or wax, but had been in use for a number of years. The use of the same kind of sampler did not appear to affect the results obtained by Frey and Stahl (*ibid.*).

Satisfactory results were obtained from our early experiments, *e.g.* in mid-July gross photosynthetic rates of 25-50 $\mu\text{g C/L/day}$ were measured. From then on, a situation

developed in which the bottle exposed to light contained less oxygen than the dark bottle at the end of the experiment. The difficulty appeared first in Upper Jennifer Lake in the deeper regions and spread toward the surface. In Little Kitoi Lake the phenomenon appeared about two weeks later near the surface and spread downwards. It became nearly impossible to obtain positive indications of carbon fixation in either lake for the remainder of the summer.

Some representative data are given in Table 1. In practically every case, the dark bottle apparently contained more dissolved oxygen than the light bottle at the end of the experiment. In Little Kitoi Lake, the blank (sample taken from the same Kemmerer bottle, fixed and titrated at the beginning of the experiment) consistently showed an oxygen concentration higher than either the light or the dark bottle, while in Upper Jennifer Lake the blank ordinarily contained less than either light or dark bottle. The August 25-26 data for Upper Jennifer Lake are plotted in Figure 1.

A possible explanation for the higher oxygen concentrations in dark than in light bottles might be found in the recently discovered dependence of respiration rates of some photosynthetic organisms upon oxygen concentration (Gessner and Pannier 1958). These authors indicate that respiration rates in darkened bottles will be too low due to the lack of the locally high oxygen tensions present in light bottles. Thus it is entirely possible for calculated gross photosynthetic rates to take on negative

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