

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY, AND FROM THE DIVISION OF PLANT NUTRITION, UNIVERSITY OF CALIFORNIA]

## Bacterial Fractionation of Oxygen Isotopes

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### Introduction

Kamen and Barker<sup>1</sup> have recently discussed the relationship between photosynthesis and the enhanced O<sup>18</sup> content of the atmosphere. It is now fairly clear from the work of Ruben, Randall, Kamen and Hyde,<sup>2</sup> and of Dole and Jenks,<sup>3</sup> assuming that their observations on isotopic fractionation in fresh water are valid for salt water photosynthesis, that the enhanced O<sup>18</sup> content of the atmosphere cannot be explained on the basis of the photosynthesis reaction.

It occurred to one of us (H. A. B.) that the enhanced O<sup>18</sup> content of the atmosphere might result from a relatively more rapid rate of utilization of O<sup>16</sup> than O<sup>18</sup> by bacteria growing in the soil. If photosynthetic oxygen is being continually returned to the atmosphere with an O<sup>18</sup>/O<sup>16</sup> ratio practically that of salt water, then it is necessary for some process to be removing O<sup>16</sup> from the atmosphere at a relatively greater rate than it is removing O<sup>18</sup> if the isotopic composition of atmospheric oxygen is to remain constant. The oxygen dynamics of the atmosphere may be expressed by the equation

$$N_{\text{end}} - N_{\text{start}} = \frac{n}{O_{\text{start}}^{16}} [N_2 - N_1] [1 + N_{\text{end}}] \quad (1)$$

where

$$N_{\text{end}} = [O^{18}]/[O^{16}] \text{ in atmosphere at end of year}$$

$$N_{\text{start}} = [O^{18}]/[O^{16}] \text{ in atmosphere at start of year}$$

$$N_2 = \frac{[O^{18}]}{[O^{18}] + [O^{16}]} \text{ in oxygen put back into the atmosphere through the photosynthesis reaction or other processes}$$

$$N_1 = \frac{[O^{18}]}{[O^{18}] + [O^{16}]} \text{ in oxygen removed from atmosphere through bacterial action or through consumption by other agents}$$

$$n = \text{number of gram atoms of oxygen removed from the atmosphere or returned to it per year, and}$$

$$O_{\text{start}}^{16} = \text{number of gram atoms of oxygen of mass 16 in atmosphere at beginning of year}$$

Thus from equation (1) it is clear that for the oxygen isotopic composition of the atmosphere to remain constant it is necessary that  $N_2 = N_1$ . We estimate the fractionation factor of O<sup>18</sup> for the photosynthesis reaction to be 0.983; hence the fractionation factor of O<sup>18</sup> in the removal of oxygen from the atmosphere should also be 0.983. It is the purpose of this investigation to measure the fractionation factor of O<sup>18</sup> in the removal of oxygen from the atmosphere by growing bacteria.

(1) M. C. Kamen and H. A. Barker, *Proc. Nat. Acad. Sci.*, **31**, 8 (1945).

(2) S. Ruben, M. Randall, M. D. Kamen and J. Hyde, *THIS JOURNAL*, **63**, 877 (1941).

(3) M. Dole and G. Jenks, *Science*, **100**, 409 (1944).

### Theory of the Experiment

It was decided to expose the oxygen to the bacteria in a series of ten culture flasks with oxygen flowing from one flask to the next as fast as needed to maintain a constant pressure throughout the system and with carbon dioxide absorbers in or between the flasks to remove the carbon dioxide formed. As soon as a steady state was reached, it was planned to remove the end or tenth flask and analyze its oxygen isotopically. By making the following assumptions: (1) that all the carbon dioxide is absorbed, (2) that the free volume and the rate of consumption of oxygen in all the flasks are the same, (3) that the oxygen consumption rate is constant with time and (4) that the oxygen isotope fractionation factor for the bacterial consumption of oxygen remains constant with time and composition of the oxygen, it is possible to derive the following equations.

The number of gram-atoms of oxygen entering any  $i$ th flask per hour is given by the expression

$$2w_B(n + 1 - i) \quad (2)$$

where  $w_B$  is the number of moles of oxygen consumed by the bacteria in one flask per hour,  $n$  is the total number of flasks arranged in series (ten in our experiments) and  $i$  is the number of the flask in line for which the flow datum is desired. Similarly, the number of gram-atoms of oxygen leaving any  $i$ th flask per hour is

$$2w_B(n - i)$$

If  $y_0$  is the atom fraction of O<sup>18</sup> in all flasks at zero time,  $y_1$  the atom fraction of O<sup>18</sup> in the first flask at time  $t$ , and  $y_b$  the atom fraction of O<sup>18</sup> in the oxygen consumed by the bacteria, then

$$\alpha = y_b/y_1 \quad (3)$$

where  $\alpha$  is the single stage fractionation factor for the O<sup>18</sup> isotope in the oxygen consumed by the bacteria. Let  $A$  be the number of moles of oxygen in each flask, and let

$$w = w_B/A$$

then for the first flask

$$y_1 = y_0 + \{wny_0t - \alpha y_1wt - y_1(n - 1)wt\}$$

or

$$\frac{y_1}{y_0} - 1 = w \left[ n - (n - 1) \frac{y_1}{y_0} \right] t \quad (4)$$

where

$$m = n + \alpha$$

Equation (4) is strictly valid only for an infinitesimal increment of time as  $y_1$  is a function of the time. Letting  $x_1$  equal  $y_1/y_0$ , equation (4) may be written

$$dx_1 = w[n - (m - 1)x_1]dt \quad (5)$$

which on integration between  $x$  equal to 1 at zero time and  $x$  equal to  $x$  at time  $t$  becomes

$$x_t = 1 + \left( \frac{n - m + 1}{m - 1} \right) (1 - e^{-w(m-1)t}) \quad (6)$$

A steady state is reached when the exponential term becomes very much smaller than unity; at this point

$$x_{t,\infty} = n/(m - 1) \quad (7)$$

When a steady state has been reached in all ten flasks, it is possible to show by methods similar to the above that the value of  $x_i$  for any  $i$ th flask is given by the equation

$$x_{i,\infty} = \frac{n(n-1)(n-2)\dots(n-i+1)}{(m-1)(m-2)\dots(m-i)} \quad (8)$$

The over-all fractionation factor for the ten flasks would be  $x_{10,\infty}$  while the value of  $\alpha$ , the single stage fractionation factor can be computed from (8) as soon as  $x_{10,\infty}$  has been measured. Equation (8) is illustrated in Fig. 1 where the upper curve gives the over-all fractionation factor in the end flask for lines of flasks beginning with only one flask in the line and going up to 10, the value of  $\alpha$  being arbitrarily taken as 0.996, while the lower curve represents the enhancement of  $O^{18}$  in each flask for the single case of ten flasks in a line. Note that having ten flasks in a line approximately triples the enhancement in the tenth flask in comparison to only one flask in the line, but that the enhancement in the ninth flask in the series of ten is slightly less than twice as good as in the one flask.

Because of the relatively rapid flow of oxygen through the first flask, a steady state condition will be established in it long before a steady state is reached in the end flask. The criterion for the attainment of 99% of the steady state enhancement in the last flask of the line is that the bacteria must have consumed between 4 or 5 times the volume of oxygen originally present in the flask (so that  $\exp(-wat)$  will be reduced to 0.01).

### Experimental Details

Tank oxygen was supplied under 5 cm. pressure through a 48-liter reservoir to the first of ten culture flasks. Each of flasks 1-8 had a total volume of 1100 ml. of which 300 and 40 ml. were taken up by the medium and 50% potassium hydroxide (the latter in a wide side arm blown on to the neck of the flask). The last two flasks had a capacity of 2100 ml. of which 400 and 45 ml. were occupied by the medium and the hydroxide, respectively. Between flasks 5-6 and 9-10 extra carbon dioxide absorbers containing 150 ml. of 50% potassium hydroxide and with a gas space of 350 ml. were inserted.

The culture medium contained 4% yellow corn meal, 1% Difco yeast extract, 1%  $CaCO_3$ , 0.5%  $K_2HPO_4$ , 0.05%  $MgSO_4 \cdot 7H_2O$ , 0.005%  $FeSO_4 \cdot 7H_2O$ , a trace of  $MnSO_4$  and  $Na_2MoO_4$ . The inoculum was 10% by volume of a similar medium originally inoculated with soil. The reaction flasks were shaken on a horizontally rotating shaker in order to maintain an adequate oxygen supply, to prevent the development of anaerobic bacteria which produce hydrogen and to facilitate the absorption of carbon dioxide (which turned out to be the most troublesome part of the research). The temperature was 28°.

Volume of oxygen consumed during the first twenty-

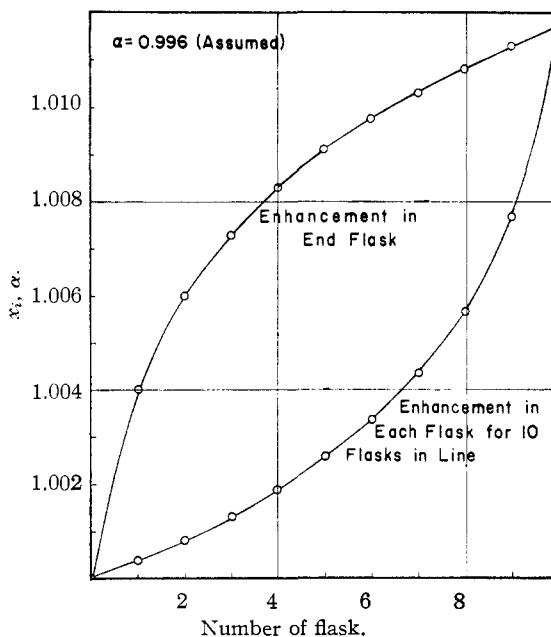


Fig. 1.

eight hours of incubation amounted to 30-40 liters. At the end of this period the alkali in the side arm of flask ten was dumped into the medium to absorb carbon dioxide remaining in the gas which may have been as much as 50% of the total. After shaking flask ten for fifteen minutes gas was allowed to enter it first from flask nine and then from the rest of the system. This procedure probably resulted in the oxygen in ten having an  $O^{18}$  composition equal to the average of the steady state value of flasks nine and ten. Flask 10(1) was then removed and replaced by a similar flask 10(2) which had been flushed with oxygen and evacuated. Gas was introduced into it first from flask 9, then from the rest of the system and incubation continued for another 28 hours after which flask 10(2) was handled like 10(1). Liter samples of oxygen for isotopic analysis were taken from flasks 10(1), 10(2), from the oxygen tank and from the reservoir. Aliquot samples were analyzed as follows:

TABLE I

COMPOSITION OF DIFFERENT OXYGEN SAMPLES IN VOLUME PER CENT.

	Tank	Reservoir	Flask 10(1)	Flask 10(2)
$O_2$	99.5	98.5	81	77.3
$CO_2$	0	0	2.5	8.3
$N_2$	0.5	1.5	16.5	14.4

The oxygen taken for isotopic analysis was pumped out of each flask over copper in a furnace at 300° and the oxide formed later reduced to water also at 300° with tank hydrogen. The same hydrogen was used in all oxygen reductions.

The technique of purifying the water and measuring its density was essentially that of Dole and Jenks.<sup>3</sup> A modified density technique was tried in which the water whose density was to be measured was transferred to one chamber of a double chambered float and the density of the whole measured by suspension in normal water. This method which was similar to that described by Gilfillan and Polanyi<sup>4</sup> turned out to be extremely time consuming and was abandoned.

(4) E. S. Gilfillan and M. Polanyi, *Z. Physik, Chem.*, **166A**, 254 (1933).

### Discussion of the Experimental Results

The experimental data for the density measurements are collected in Table II. Results are expressed in p. p. m.,  $\gamma$ , in excess of the density of the standard which was taken as that of the tank sample. The first number in each case is the  $\gamma$ -value with reference to the immediately preceding density measurement of the standard and the second number, the  $\gamma$ -value calculated using the immediately succeeding density measurement of the standard.

TABLE II

DENSITY DIFFERENCES OF WATER SAMPLES				
Purification No.	Tank	Reservoir	Flask 10(1)	Flask 10(2)
1	Standard	4.0 5.9	5.2 7.2	4.6 6.6
2	Standard	0.3 0.3	2.4 2.0	2.2 1.8
3	Standard	0.3 2.0	-0.1 1.6	0 1.8
4	Standard	Not analyzed	2.04	Not analyzed
5	Standard	1.1 1.0	1.4 1.3	2.1 2.1
6	Standard	Lost	1.6 1.7	1.4 1.5
Av. of purifications 2, 5 and 6	0	0.8	1.7	1.8

In computing the averages we have rejected purifications 1, 3 and 4 because of the discrepancy in the density values between those calculated using the preceding and succeeding standard densities. Averaging the tank and reservoir density values, which should be the same (there were twice as many density measurements of the tank sample than of the reservoir sample hence the tank average value was given double weight) and subtracting this weighted average from the average of the two flask samples, we arrived at the final excess density of the bacterial oxygen water equal to  $1.5 \pm 0.5 \gamma$ .

The over-all fractionation factor,  $x$ , is related to  $\gamma$  by the equation

$$x = 1 + (\gamma/222)$$

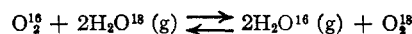
which for  $\gamma$  equal to 1.5 yields 1.0068 for  $x$ . The single stage fractionation factor,  $\alpha$ , can now be calculated from  $x$  using equation (8) taking the average of the ninth and tenth flasks as follows

$$x = \frac{10!}{\alpha(\alpha+1)(\alpha+2) \dots (\alpha+9)} \left\{ \frac{1+\alpha}{2} \right\}$$

This equation could be solved for  $\alpha$  using gamma

functions, but in the narrow range where we are working it is easier to compute  $x$  for a few values of  $\alpha$  and interpolate graphically. We obtain  $\alpha = 0.997$  for  $x$  equal to 1.0068. As the fractionation factor required to explain the enhanced  $O^{18}$  content of the atmosphere is 0.983 as remarked above, it is clear that bacterial concentration of  $O^{18}$  in the atmosphere is almost negligible. If, using the figure 0.983, we compute the  $\gamma$  value we would have observed if the bacteria concentrated  $O^{18}$  to this extent, we obtain 10.8  $\gamma$  which is six times greater than the average computed from the data of Table II.

We conclude that the postulate originally used by one of us in 1936,<sup>5</sup> namely, that the establishment of the following equilibrium in the stratosphere at  $-55^\circ$



is responsible for the atmospheric concentration of  $O^{18}$ , still appears to be the most reasonable explanation of the excess abundance of  $O^{18}$  in the atmosphere. Even this hypothesis fails by about 1  $\gamma$  to reproduce quantitatively the experimental observations.

In conclusion one of us (M. D.) gratefully acknowledges a grant from the Graduate School Fund of Northwestern University in support of this research.

### Summary

Bacterial concentration of  $O^{18}$  in the atmosphere by preferential removal of  $O^{16}$  has been studied by passing oxygen through a series of 10 culture flasks inoculated with soil bacteria and analyzing isotopically the residual oxygen accumulated in the tenth flask. The single stage fractionation factor is found to be 0.997, which falls considerably short of the value required to explain the enhanced  $O^{18}$  content of the atmosphere, 0.983.

A mathematical theory of the experiment which served as a guide in planning the work and from which the single stage factor was calculated from the over-all fractionation factor is given.

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(5) M. Dole, *J. Chem. Phys.*, **4**, 268 (1936).