

THE OXYGEN CONSUMPTION OF LUMINOUS BACTERIA.

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(Accepted for publication, January 18, 1928.)

In a previous paper (1925) I have published some figures on the total efficiency of luminous bacteria, regarded as machines for light production, which give the ratio of the light emitted to the total energy input, expressed in the same energy units (calories per second). The energy input was calculated by a method of indirect calorimetry, from the oxygen consumption of the bacteria. The oxygen consumed would liberate a definite amount of heat when used to burn the food of the bacteria, a mixture of 40 per cent peptone and 60 per cent glycerol.

Since luminescence of the bacteria is dependent on dissolved oxygen, the oxygen consumption was measured by finding the time necessary for an emulsion of the bacteria in sea water to use up the dissolved oxygen to the point where the luminescence just begins to fade. From the solubility of oxygen in sea water one can then calculate the oxygen consumption, provided the bacteria use equal amounts of oxygen in equal times. Experiment shows that the dimming point represents utilization of at least 99.5 per cent of the dissolved oxygen, a change from the dissolved oxygen in equilibrium with air (21 per cent) to that in equilibrium with < 0.5 per cent oxygen, and that the luminescence intensity of the bacteria is unaffected by different oxygen tensions until the tension falls to < 0.5 per cent oxygen.¹ The oxygen consumption is probably also independent of the oxygen tension until approximately this same value is reached. The time for dimming for an undisturbed tube of bacterial emulsion is proportional to the number of bacteria present, and also approximately proportional to the oxygen tension with which the bacterial emulsion is brought into equi-

¹ Unpublished experiments.

brium, provided of course this is above the critical value (0.5 per cent oxygen). The method would therefore appear to be justified, but requires confirmation, which is the subject of this communication.

The actual figures obtained (Harvey, 1925) for oxygen consumption of the bacteria in seven experiments at temperatures ranging from 18° to 23°C. varied from 6.65 to 17.67×10^{-15} mg. O₂ per bacterium per second, with an average of 11.84×10^{-15} mg. for an average temperature of 21.5°C. These values are 10 per cent too low, since it was believed, at the time the experiments were made, that 2 per cent oxygen was the point at which dimming occurred.

In order to check this method I have recently made determinations of oxygen consumption in the Thunberg-Winterstein respirometer as used by Fenn (1927) for experiments on oxygen consumption by nerve.² The two chambers were of approximately 22 cc. capacity and the capillary held 0.403 gm. of mercury in a length of 153 mm. Since the oxygen consumption in this apparatus is twice the volume movement of the capillary, each mm. movement of the oil drop in the capillary was equivalent to the consumption of 0.000388 cc. O₂. The chambers contained small vessels for the solution of KOH to absorb CO₂. Two cc. of bacterial emulsion in sea water was placed in one chamber and 2 cc. sea water was placed in the other, both in equilibrium with air. The whole apparatus was immersed in a thermostat of running sea water at 21°C. which changed less than 0.01°C. over a period of 2 hours. The readings of the oil droplet movements plotted against time are given in Fig. 1, together with three determinations of the time for another sample of the same emulsion of luminous bacteria to dim, when standing undisturbed at the same temperature.

From Fig. 1 it will be seen that the rate of oxygen consumption is practically independent of rate of shaking and that 2 cc. of bacterial emulsion used 0.0272 cc. O₂ in 1950 seconds or 0.0000139 cc. O₂ per second. One cc. of emulsion would use 0.00000695 cc. per second.

Over the same time interval, at 21°C., it took 12.5 minutes or 750 seconds (time indicated by arrows) for the luminescence of a similar bacterial emulsion to dim. At 21°C. each cc. of sea water will dis-

² I am greatly indebted to Dr. W. O. Fenn for the micro respirometer used in these experiments and to Mr. C. S. Shoup for growing the luminous bacteria and for assistance with the experiments.

solve 0.0052 cc. O_2 (Fox, 1909), at least 99.5 per cent of which is consumed when dimming occurs. Hence the bacteria in 1 cc. sea water emulsion used $0.0052 \div 750$ or 0.00000693 cc. O_2 per second, a value in perfect agreement with the micro respirometer. I conclude that the dimming method gives a very good index of the oxygen consumption of the bacteria.

It is interesting to compare the oxygen consumption of luminous bacteria with that of other bacteria. Of the numerous papers on

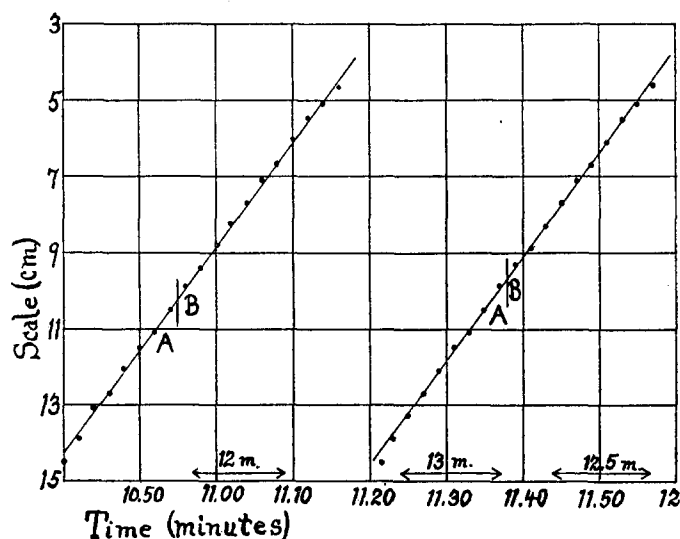


Fig. 1. Readings of index drop scale of micro respirometer in cm. plotted against time in minutes. Below the mark, (A), the respirometer was shaken 98 times per minute; above, (B), at 120 times per minute. The horizontal arrow lines indicate the times for bacterial emulsions to use dissolved oxygen, as determined by the dimming method.

respiration of bacteria (*cf.* Stephenson and Whetham (1924), Novy and Soule (1925), Brooks (1918-1922), Callow (1924) and Pütter (1924)), only Callow and Pütter give values which can be properly compared, namely, oxygen consumption per weight or volume of bacteria. The luminous bacterium used by me in efficiency studies was a cylinder averaging 2.2μ long by 1.1μ in diameter with rounded ends. Its surface area was $7.6\mu^2$ and its volume $1.695\mu^3$. When

TABLE I.

	Organism	Observer	Volume in μ^3	Surface in μ^2	Temperature	Oxygen consumption in mg. per hr.		
						Per individual	Per kg.	Per sq. m.
Aerobic	<i>Photobacterium phosphorescens</i> ..	Harvey, 1925	1.7	7.6	21.5°	4.26×10^{-11}	$2.5 \times 10^{4*}$	5.6
	<i>Bacillus fluorescens non-liquifaciens</i>	Pütter, 1924	0.06	1.26	15°	1.8×10^{-10}	3×10^6	143
	<i>Bacillus fluorescens liquifaciens</i>	Müller, 1912	0.19	1.90	21°	3.56×10^{-10}	5.9×10^6	280
	Various bacilli.....	Callow, 1924			22°	7.64×10^{-10}	4.01×10^6	400
							1 to $5.4 \times 10^{3\dagger}$	
Facultative anaerobic	<i>Bacillus coli</i>	Müller, 1912	0.47	3.9	22°	1.28×10^{-10}	0.271×10^6	33
	Yeast.....	?	180	150	26°	27.3×10^{-10}	0.0168×10^6	20
	<i>Colpidium</i>	Pütter, 1905	153×10^3	16.9×10^3	17°	0.368×10^{-6}	2250	23

* On the assumption of a density = 1 for *Photobacterium phosphorescens*.

† On the assumption of 85 per cent water in bacteria, since Callow's results are based on dry weight of bacteria.

oxygen consumption per gm. of luminous bacterium is calculated (assuming a density of 1) we obtain an average value of 0.291 cc. O₂ per gm. per minute or 17.46 cc. O₂ per hour. Callow finds 5 to 25 cc., O₂ per gm. dry weight per hour, for several types of aerobes. Assuming 85 per cent water in the bacterium, these figures become 0.75 to 3.75 cc. O₂ per gm. moist bacterium per hour.

Calculating the average oxygen consumption in terms of a single bacterium, per kilo of weight and per sq. m. of surface, we obtain the values given in the accompanying table. We see that the oxygen used per surface area and per kilo is very much less than the values found by Pütter, although greater than those recorded by Callow.

Pütter has pointed out that when oxygen consumption per sq. m. of respiring surface is compared, different groups of organisms whose size varies greatly give similar values. I am inclined to question the validity of this conclusion except as a very general statement. However, much of the data which has accumulated on unicellular forms and single cells would have far greater value had the experimenters taken the trouble to determine the number, surface and volume (or weight) of the cells studied.

The most important point in comparing the oxygen consumption of cells is to have sufficient oxygen available so that the center of the cell will receive diffusing oxygen more rapidly than it can be used. Only if this is true will oxygen consumption be independent of oxygen tension. Too great oxygen tensions, as pure oxygen, should also be avoided, since there is evidence, at least in the case of these luminous bacteria, that oxygen consumption may be slowed under these conditions.¹

Gerard (1927) and Fenn (1927) have both published the equations, $C_0 = \frac{Ar^2}{4D}$, for calculating the oxygen pressure, C_0 , in atmospheres at

the surface of a long cylinder respiring at a definite rate which allows all parts to receive adequate oxygen. In this equation, A is the oxygen consumption of the tissue in cc. oxygen per gm. per minute, r is the radius of cylinder and D is the diffusion coefficient of oxygen for the tissue in cc. oxygen diffusing per sq. cm. for a pressure gradient of

one atmosphere per cm. The equation becomes $C_0 = \frac{Ar^2}{6D}$ for a sphere

and approximately $C_0 = \frac{Ar^2}{5D}$ for a short cylinder such as the luminous bacterium.³

Using Krogh's (1919) diffusion coefficient of oxygen for connective tissue, 1.15×10^{-5} , and an oxygen consumption of 0.29 cc. per gm. per minute, with $r = 0.000055$ cm., C_0 turns out to be 1.53×10^{-5} atmospheres. Remembering that the bacteria are surrounded by sea water and not air, we must allow for the solubility of oxygen in sea water. One cc. sea water will dissolve 0.0255 cc. oxygen at N. T. P. from one atmosphere of pure oxygen at 20°C. Hence $1.53 \times 10^{-5} \div 2.55 \times 10^{-2} = 0.6 \times 10^{-3}$ or 0.0006 atmosphere oxygen in equilibrium with sea water should maintain an adequate respiration of the bacteria. Let us assume that the point of inadequate oxygen supply is the point where luminescence begins to dim.⁴ As this value (0.0006 oxygen) is about 4 times smaller than the value (approximately 0.0026) at which we observe the luminescence to begin to dim, I am led to believe that the diffusion coefficient, the only value in the equation not directly measured, is too high. It seems very likely, then, that oxygen penetrates far less readily into a luminous bacterium than into gelatin gel or connective tissue. It is very possible that these cells are surrounded by a chitin-like envelope as some text-books of bacteriology assert. Krogh (1919) found the diffusion of oxygen through chitin to be about 9 times as slow as through connective tissue.

SUMMARY.

Oxygen consumption of luminous bacteria determined by the Thunberg micro respirometer and by the time which elapses before the luminescence of an emulsion of luminous bacteria in sea water begins to dim, when over 99 per cent of the dissolved oxygen has been consumed, agree exactly.

Average values for oxygen consumption at an average temperature

³ This equation was derived for me by my colleague, Dr. J. W. Alexander. Inasmuch as the assumption is made in the derivation that oxygen consumption is independent of oxygen concentration for *all* oxygen concentrations, it is questionable if this equation can be applied to an object as small as a bacterium.

⁴ This question is under investigation at present.

of 21.5°C. are 4.26×10^{-11} mg. O₂ per bacterium; 2.5×10^4 mg. per kilo and 5.6 mg. O₂ per sq. m. of bacterial surface.

The only correct comparison of the oxygen consumption of different organisms or tissues is in terms of oxygen used per unit weight with a sufficient oxygen tension so that oxygen consumption is independent of oxygen tension.

Measurement of the oxygen concentration which just allows full luminescence, compared with a calculation of the oxygen concentration at the surface of a bacterial cell just necessary to allow the observed respiration throughout all parts of the cell, indicates that oxygen must diffuse into the bacterium much more slowly than through gelatin or connective tissue but not as slowly as through chitin.

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