THE OXYGEN UPTAKE OF MARINE BACTERIA

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In recent studies from the Woods Hole Oceanographic Institution (Waksman and Carey, 1935a), it was shown that stored sea water rapidly undergoes a decrease in oxygen content, with a somewhat parallel increase in numbers of bacteria up to a maximum population level, after which oxygen decrease continues to take place in the water. The rates of bacterial multiplication and oxygen consumption were considered as indices of the availability of the organic substances present in the water, in solution and in suspension. In order to learn whether the marine bacteria of themselves could account completely for these phenomena, a study was undertaken on 25 pure cultures of organisms freshly isolated from the sea,[2] with the object of determining their rates of oxygen consumption in the "resting" condition, and the influence of temperature as well as of added organic matter of both readily and not so readily utilizable nature on these rates. Glucose, C.P., was selected as probably the most readily available substance, and purified alginic acid[3] as one less generally available. The latter compound occurs abundantly in marine algae (Waksman, Carey and Allen, 1934).

[1] Contribution No. 95 of the Woods Hole Oceanographic Institution.

[2] All cultures were isolated and kindly supplied to the writer by Dr. S. A. Waksman. They are being carried in the collection at Woods Hole for future study, but are referred to here by number, awaiting identification or description. The limited objective of this study, as stated above, renders less important the specific name of each organism. Three of the species however, which were recently described (Waksman *et al.*, 1934), are: *Bacterium alginicum* (AA2); alginic acid bacterium No. 8 isolated from diatom tow and related to *Bacterium alginovorum* (AA8); and, *Bacterium fucicola* (AA17).

[3] Prepared and kindly furnished to the writer by Dr. S. A. Waksman.

In order to obtain data that might be compared with previous investigations of both marine (Pütter, 1924, Waksman and Carey, 1935a) and non-marine organisms (Callow, 1924; Cook and Stevenson, 1928; Harvey, 1928; Martin, 1932; etc.), the amount of nitrogen and in most cases the number of cells[4] in the different suspensions were determined. Since the nitrogen content of the dry weight of many bacterial organisms has been found to be approximately 10 per cent (Nicolle and Alilaire, 1909; Hopkins and Peterson, 1929; Hirsch, 1934), one can (by assuming that this value holds approximately for the species in the present study also) readily arrive at a rough figure for oxygen consumption in terms of dry weight of the cells. Dry weight could not be determined in the present study because most of the organisms cytolyzed readily in distilled water, making adequate washing of the cells for weighing purposes almost impossible.

For each species of bacteria, several Petri plates containing sea-water agar (Waksman, *et al.*, 1933) were heavily inoculated and incubated 24 hours at room temperature. The growth was removed from the surface of the agar by gently brushing it into some sterile sea water with the aid of a camel's hair brush. The cells were centrifuged and washed twice with sterile sea water, and finally suspended in sterile M/4 phosphate buffer,[5] at pH 8. The buffered suspension of cells was aerated with a stream of filtered air for ten to fifteen minutes. Two cubic centimeters of the suspension were then transferred to the usual conical-shaped Warburg respirometer vessels in duplicate, each vessel containing 0.2 cc. of 10 per cent KOH solution in the inset, and the oxygen consumption followed manometrically over a period of from two

[4] All the counts, and most of the preparations for the counts, were made by Dr. Margaret Hotchkiss. The writer is also indebted to Mr. Theone Cordon for assistance in the nitrogen determinations.

[5] Sea water itself was not used as the suspension medium in order to avoid the initial rise in pH resulting from the absorption of CO_2 in the respirometer vessels. Preliminary work on one of the organisms indicated no significant difference in the rate of endogenous O_2-uptake of the cells in phosphate buffer, M/2 NaCl-dilute borate buffer, or sea water-dilute borate buffer. Strong borate (M/4) buffer alone abolished O_2 consumption. The oxidation of glucose went on at a much greater rate in phosphate buffer than in either of the other two suspension media tried.

to four hours. The gas phase in the vessels was air, and the speed and amplitude of shaking were, according to earlier experiments with suspensions of various densities of cells and rates of oxygen consumption, more than sufficient to insure full respiration and complete absorption of CO_2. As soon as possible after placing the manometers on the thermostat, the number of cells per cubic centimeter in the respective suspensions was determined by direct microscopic count of a standard calibrated loopful of suspension spread over an area of 4 sq. cm. on a microscope slide and stained with methylene blue. The number of cells was usually near 10^9 per 1 cc. Macro-Kjehldahl determinations of the nitrogen in 50 cc. (or more) of the same suspensions gave, on the average, a value of about 0.06 mgm. nitrogen per 1 cc. of suspension

The glucose and alginic acid solutions were made up to 0.2 per cent, sterilized by filtration through a Seitz filter, and stored in sterile containers. One milligram (0.5 cc. solution) of the alginic acid was added to the cells from the side arm of the respirometer vessels, after the rate of oxygen uptake without added substrate had been determined. The glucose was added to duplicate vessels at the start of the experiment, and direct comparison made with the rate of oxygen uptake in check vessels containing corresponding amounts of the same suspension of bacteria, but without the substrate.

In the experiments carried out at lower temperatures, the thermostat was controlled by means of a cooling coil of an electric refrigerating unit,[6] whose relay was connected by a vacuum tube relay with a mercurial thermoregulator in the water bath.

The ratio of oxygen uptake to nitrogen content in milligrams, for 25 species of bacteria at 25°C. over a period of two hours, is shown in figure 1. During this period, the decrease in the rate of respiration with time appears to be in most cases slight. The same results calculated in terms of oxygen consumed per 10^9 bacterial cells are illustrated in figure 2. As one would expect, both methods of calculation show considerable differences among

[6] The writer is indebted to Dr. A. C. Redfield and Mr. Ketchum for the loan of this apparatus, and for the assistance of the latter in assembling it.

the different species in the rates of their endogenous O_2 uptake. The distribution is somewhat more uniform in terms of O_2/N than in terms of O_2/cells, since a larger number of species have a rather slow rate of oxygen consumption expressed in terms of oxygen uptake per cell. It seems evident, from the data at hand,

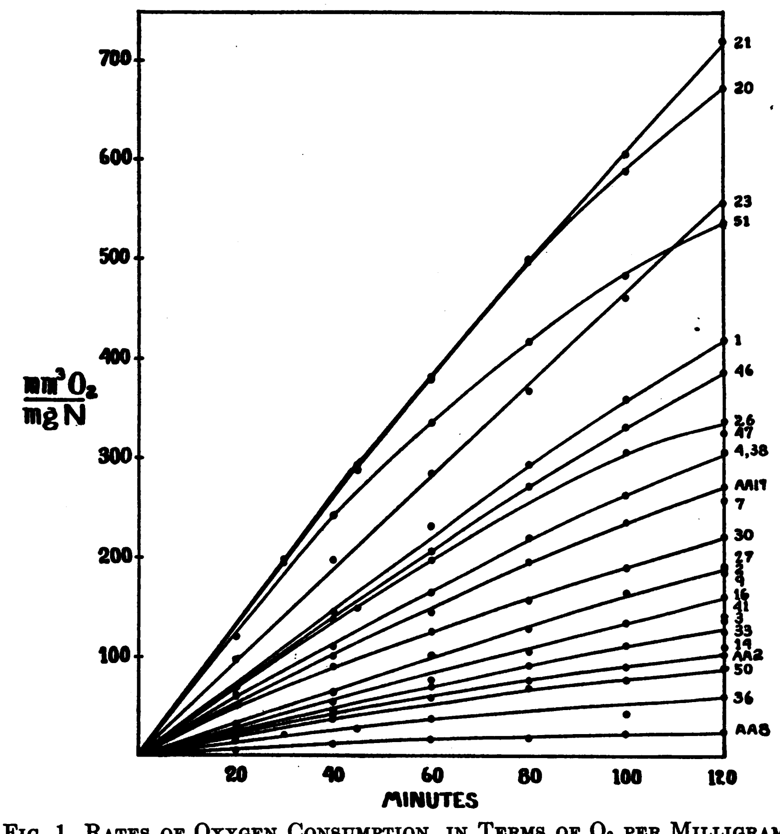

FIG. 1. RATES OF OXYGEN CONSUMPTION, IN TERMS OF O_2 PER MILLIGRAM NITROGEN, BY WASHED CELLS OF 25 SPECIES OF MARINE BACTERIA

that there is no direct relation between the nitrogen content of the cells of different species and their rates of respiration.

A question naturally arises concerning the accuracy with which the number of cells was determined. The results of both the plate counts of viable cells and the direct counts of total cells, just before and following a four-hour period in the respirometers, are reported in table 1. The plate counts are in fairly good agreement, indicating a slight increase (except for species 1) in the number of cells where glucose was added and a slight decrease without glucose. The direct counts show greater variability.

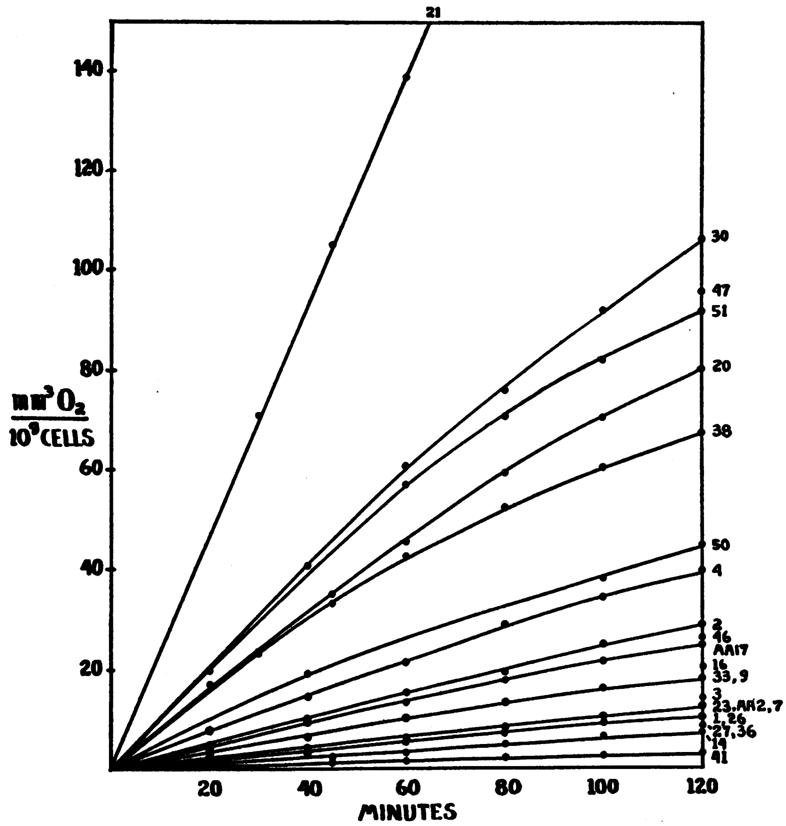

FIG. 2. RATES OF OXYGEN CONSUMPTION, EXPRESSED IN TERMS OF O_2 PER CELL, BY 24 SPECIES OF MARINE BACTERIA

A comparison of the rates of endogenous oxygen uptake by two bacteria, at 5°, 15° and 25°C., is given in figure 3, A and B. Successive experiments, using the same species, were plotted, to show the degree of variation at the different temperatures. Out of a total of 16 experiments, the extreme variation in oxygen uptake per milligram nitrogen amounted to 88 per cent, which apparently

TABLE 1

Comparison of plate counts and direct counts of the cells in three suspensions previous to and following a period of four hours in Warburg respirometer vessels

SUSPENSION OF SPECIES NUMBER	PLATE COUNT: CELLS × 10^9/CC.			DIRECT COUNT: CELLS × 10^9/CC.		
	Before	After 4 hours		Before	After 4 hours	
		No glucose	+ 1 mgm. glucose		No glucose	+ 1 mgm. glucose
AA17	0.65	0.60	0.75	1.05	0.55	0.60
1	1.35	1.20	0.85	0.65	0.70	0.30
21	1.05	1.00	1.30	1.25	0.62	0.40

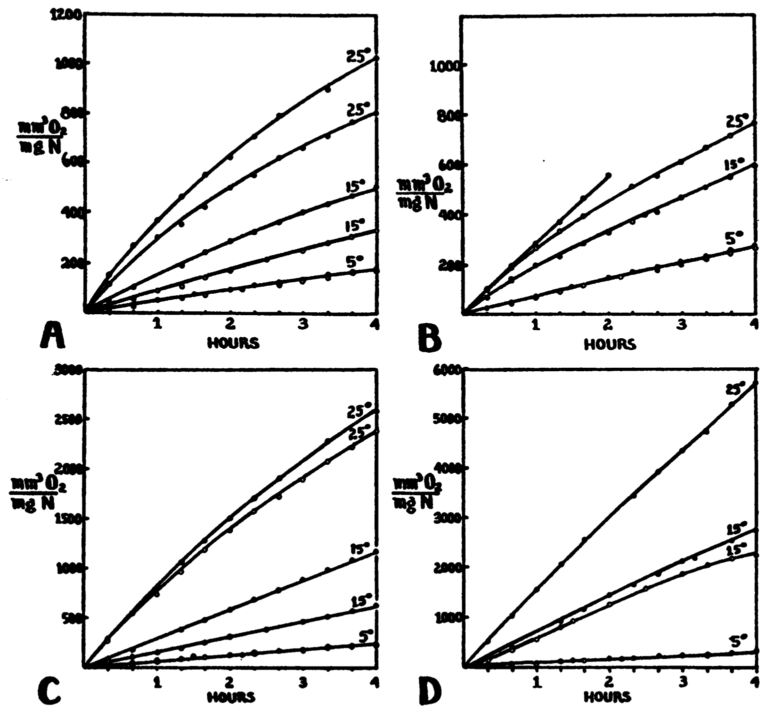

FIG. 3. OXYGEN CONSUMPTION OF WASHED CELLS OF 2 SPECIES OF MARINE BACTERIA, IN TERMS OF O_2 PER MILLIGRAM N, WITH AND WITHOUT ADDED GLUCOSE, AT DIFFERENT TEMPERATURES

A and B, without glucose; C and D, with glucose. Solid and hollow circles represent values obtained in repeated experiments.

is not excessive for bacteria. Callow (1924), for example, found that the variation may amount to as much as 250 per cent, in terms of O_2/milligrams dry weight. In the present study, the extreme variation did not exceed 200 per cent in terms of O_2/number of cells. The rate of oxygen consumption in the presence of 1 mgm. glucose is illustrated in C and D, figure 3. These may be compared directly with A and B, for they were made on the same suspensions of bacteria. It was found that, as was the case with luminous bacteria (Johnson, 1935), the increase in oxygen consumption due to addition of glucose varied considerably in

TABLE 2

Oxygen consumption of washed cells of marine bacteria with and without added glucose or alginic acid

SPECIES NUMBER	CUBIC MILLIMETERS O_2 PER MILLIGRAM N PER 2 HOURS											
	5°C.			15°C.			25°C.					
	En-dogenous	With 1 mgm. glucose	Increase	En-dogenous	With 1 mgm. glucose	Increase	En-dogenous	With 1 mgm. glucose	Increase	En-dogenous	With 1 mgm. alginic acid	Increase
			per cent			*per cent*			*per cent*			*per cent*
23	142	156	10	331	1,417	328	503	3,631	621	557	847	52
21	149	167	12	382	561	46	659	1,546	134	721	778	7.9
AA17	92	126	36	232	446	92	464	1,156	149	271	333	22
1	82	397	378	143	1,141	691	421	2,022	380			
2	39	131	235	164	1,221	644	243	2,864	1,078	190	321	68
4	54	129	138	84	492	485	314	1,830	482	306	350	14
7	85	274	222	164	1,212	639	334	2,637	689	258	405	56
14	89	231	159							112	126	12
41				52	1,733	3,232	74	1,546	1,989	141	182	29
33										136	148	8.8
AA2										104	219	110
AA8										26	31	19
Average..	91	201	148	194	1,028	769	378	2,154	690	256	340	36.2

successive experiments, as well as with different species of organisms. The oxygen consumption of all the bacteria studied, using glucose and sodium alginate as substrates, is summarized in table 2. Only those organisms which gave an increase of more than 5 per cent oxygen uptake over a period of two hours, in the presence of alginic acid, were listed. The great abundance of marine bacteria capable of oxidizing alginate (Waksman, Carey and Allen, 1934) is very obvious. The temperature coefficients for the endogenous uptake as well as for the oxidation of the glucose are reported in table 3. The unusually high Q_{10} of 9.1, between 5° and 15°C., for the oxidation of glucose by species number 23, seems to suggest the possibility that, for some bacteria, there may be a critical temperature between 5 and 15°, below which glucose is oxidized only very slowly.

The amount of dissolved organic matter in sea water is small, amounting to only around 2.3 grams of carbon in 1 cubic meter (Krogh, 1934), equivalent to 0.00023 per cent carbon, as compared with 0.016 per cent carbon in most of the experiments in the present study. Since glucose appears to be the most readily

TABLE 3

Temperature coefficients for oxygen consumption per milligram N per two hours of 8 species with and without added glucose

SPECIES NUMBER	Q_{10} (ENDOGENOUS)		Q_{10} (+ 1 MGM. GLUCOSE)	
	5° to 15°C.	15° to 25°C.	5° to 15°C.	15° to 25°C.
AA17*	2.5	2.0	3.5	2.5
21*	2.5	1.7	3.3	2.1
23*	2.3	1.5	9.1	2.5
1	1.7	2.9	2.8	1.7
2	4.2	1.4	1.1	2.3
4	1.5	3.7	3.8	3.7
7	1.9	2.0	3.2	2.1
Average......	2.3	2.18	3.8	2.4

* Average values obtained from 2 or 3 repeated experiments.

utilizable sugar (Kendall and Gross, 1930), it would hardly seem likely that the rate of endogenous oxygen uptake by bacteria in sea water would be increased to any considerable extent by the dissolved organic matter, above the values given in table 2 for the respective species. During active bacterial reproduction, the rates of oxygen consumption might be expected to be higher than during the "resting" periods. In this connection, it is interesting to compare these results with the data of Waksman and Carey (1935b, table VI), on the rate of oxygen consumption and bacterial multiplication in stored sea water. They found that at 22°C., the addition of 10 mgm. glucose per liter (0.001 per cent) caused an increase in total oxygen consumed over a period of two days (between the first and third) of 197 per cent. The average per cent increase in O_2 uptake by the "resting" cells of the 8 species studied here, at 25°C., was found to be 690 per cent. However, the concentration of glucose was 40 times that used by Waksman and the per cent increase was found to be considerably less at lower concentrations of glucose. Waksman's data show that, between the first and third day, when the bacterial population remained practically stationary, the oxygen consumption per cell (taking the average number of cells over the two day period) per hour amounted to 16.5×10^{-9} mm.,^{3} without glucose, and to 18.4×10^{-9} in the sample containing an initial concentration of 0.001 per cent glucose. In comparison with these values, the average value for the endogenous oxygen uptake per cell per hour, over a period of two hours, of 24 species taken at random, was 21.3×10^{-9} mm.,^{3} at 25°C.; this is an extraordinarily close agreement, considering that the figures were arrived at by two such widely different methods. Furthermore it is a definite indication that the bacteria alone could easily account for the oxygen consumption in stored sea water, as described by Waksman and Carey (1935a and b).

In the present investigation, no organism was found capable of consuming as much oxygen as 534×10^{-9} mm.^{3} per cell per hour, as reported by Pütter (1924) for *Bacterium fluorescens-liquefaciens* in sea water. It is necessary, however, in making comparisons with the rates of O_2 uptake for various bacteria reported by different workers to emphasize that they have not all been obtained by the same procedure; it has also been frequently found that organisms of the same species may vary widely at different times in their rates of respiration.

SUMMARY

The rates of oxygen uptake by washed cells of 25 species of bacteria freshly isolated from the sea were found to vary between 2×10^{-9} and 132×10^{-9} mm.^{3} per cell per hours, with an average for all species of 21.3×10^{-9} mm.^{3} In terms of milligrams of nitrogen the variation was between 15 and 360 mm.^{3} O_2 per hour, with an average of 140, for all species.

The increase in the rate of O_2 uptake in a 0.04 per cent glucose

solution varied for 8 species between 10 and 378 per cent, with an average of 148 per cent, at 5°C.; between 46 and 3232 per cent, with an average of 769 per cent, at 15°C.; and between 134 and 1989 per cent, with an average of 690 per cent, at 25°C. The average temperature coefficients for the endogenous O_2 uptake of 8 species were Q_{10} 2.3, between 5° and 15°C., and 2.18, between 15° and 25°C. For the oxidation of glucose, the average Q_{10} was 3.8, at 5° to 15°C., and 2.4 at 15° to 25°C.

Sodium alginate, in concentration of 0.04 per cent increased the rate of oxygen consumption by the washed cells of 11 out of the 25 species tested, the per cent increase ranging from 7.9 to 110 per cent, with an average of 36.2 per cent.

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