Rate of Oxygen Consumption by Marine Bacteria

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Abstract : To shed more light on O_2 consumption rates of marine bacteria, the procedures which originated with ZoBell, were examined in detail, and the following results were obtained.

Seawater samples seem to be suitable for temporary storage only at low temperatures for short periods of time. It is desirable that each seawater sample treated with care to avoid undue change of O_2 tension before incubation. The incubation of each seawater sample should be designed to terminate just after detecting a discernible amount of O_2 consumption.

Based on the above results a field survey for evaluating the rates of bacterial O_2 consumption in the Hiuchi-Nada area of the Seto Inland Sea was carried out under defined conditions. The rates obtained fell within the range of $3.3\times10^{-11}-1.8\times10^{-10}$ mg O_2/day/cell of bacteria, or $5.8\times10^{-3}-5.4\times10^{-1}$ mg O_2/day/liter of *in situ* water. The implications of the values obtained are discussed.

Introduction

The rate of O_2 consumption by marine bacteria provides useful information not only for understanding the budget of dissolved O_2 but also for studying the progress of mineralization accompanying O_2 consumption.

Many years ago ZoBell[1] proposed certain promising procedures for estimating O_2 consumption rate by marine bacteria. The procedures were modified partially by Tezuka[2], and have received increasing attention during recent years.

Attempts were made to assess more reliable values, and taking the results thus obtaind into account, a field survey for the O_2 consumption rates of bacteria was carried out in the Hiuchi-Nada area of the Seto Inland Sea.

Materials and Methods

Sampling. Seawater samples were taken by non-metallic buckets or van Dorn samplers.

Determination of O_2 consumption rate. The rate of O_2 consumption per unit count of bacteria (Q_{O_2}) was measured according to procedures essentially identical to those described by Tezuka[2]. Except where specially stated, the outline of the procedures is as follows : each water sample was filtered through a sterilized membrane filter of 5 μ porosity to remove organisms other than bacteria. During the filtration treatment, steady attention was devoted to avoid the breakage of cells retaining on filters. The filtrate was poured into two sterilized 300-ml BOD bottles aseptically. One bottle was examined for the initial count of bacteria and for the initial amount of dissolved O_2 immediately. The other bottle was incubated at *in situ* temperature under dark conditions. After a given period of incubation the final count

of bacteria and the amount of dissolved O_2 were determined. The Q_{O_2} values were calculated by the formula described in previous papers[1,2]. The rate of O_2 consumption by bacteria per liter of water was readily obtained by multiplying Q_{O_2} value by the number of bacteria in a liter of water.

Determination of dissolved oxygen. Dissolved O_2 was determined by the Winkler method.

Enumeration of bacteria. In the extinction dilution method, most probable number (MPN) was obtained according to the system of five tubes for each dilution. Direct microscopic counting was done by the procedures described by Lumpkins and Arveson[3]. Pour plate counts were obtained by the usual procedures. The composition of the medium employed for the extinction dilution method was as follows : peptone, 1 g ; yeast extract, 0.1 g ; K_2HPO_4, 0.01 g ; $FeSO_4 \cdot 7H_2O$, 0.005 g ; aged seawater, 1 liter ; pH adjusted to 7.5. A medium having the same composition with 1.5 % agar added was used for the pour plate method.

Percent error was calculated by the equation $S\bar{x}/\bar{x} \cdot 100$ where $\bar{x}$ is the mean and $S\bar{x}$ is the standard deviation of the mean.

Results and Discussion

The reliability of Q_{O_2} values was examined by parallel determinations for the replicate samples. As shown in Table 1, bacterial counts, O_2 consumptions, and Q_{O_2} values converged to similar levels, and the percent error of Q_{O_2} values is about 27 %. The fact shows

Table 1. *Variation between the rates of O_2 consumption per unit count of bacteria (Q_{O_2}) in the parallel determination for the replicate samples*

		O_2 consumption (mg/20 h/L)	Bacterial counts (MPN/L)		Q_{O_2} value (mg O_2/day/MPN)
			Initial	Final	
Run	1	0.170	3.5×10^7	5.4×10^8	1.1×10^{-9}
	2	0.150	2.7×10^7	3.5×10^8	1.4×10^{-9}
	3	0.145	2.4×10^7	2.8×10^8	1.7×10^{-9}
	4	0.170	5.4×10^7	5.4×10^8	9.7×10^{-10}
	5	0.178	7.0×10^7	5.4×10^8	9.3×10^{-10}
Mean		0.163	4.2×10^7	4.5×10^8	1.2×10^{-9}
Percent error		8.7	46.5	27.9	26.7

Note: The water sample was collected from the surface of Tosa Bay. Incubation temperature, 25.0°C ; incubation period, 20 hours.

that Q_{O_2} values are highly reproducible under given conditions.

In a strict sense, the formula involved in the determination of Q_{O_2} value can apply only when the number of bacteria varies exponentially during each incubation period. The influence of incubation period on apparent Q_{O_2} values was examined at several incubation temperatures by using the water samples collected during both summer and winter. As shown in Table 2 A, irrespective of *in situ* temperature, incubation period suitable to the above-described requisite was distinctly shortened by increasing the incubation temperature

Table 2A. *Variation of the apparent Q_{O_2} values as functions of incubation temperatures and periods*

In situ temp. (°C)	Incubation temp. (°C)	Incubation period (days)	O_2 consumption (mg/L)	Counts of heterotrophs (MPN/L) Initial	Counts of heterotrophs (MPN/L) Final	Q_{O_2} value (mg O_2/day/MPN)
28.1*	10	0 — 0.5	0.007	1.3×10^{6}	3.3×10^{6}	6.5×10^{-9}
		0 — 1.0	0.012	〃	2.3×10^{6}	6.9×10^{-9}
		0 — 2.0	0.023	〃	2.3×10^{7}	1.5×10^{-9}
		0 — 3.0	0.043	〃	3.3×10^{7}	1.5×10^{-9}
	15	0 — 0.5	0.011	〃	5.4×10^{6}	7.5×10^{-9}
		0 — 1.0	0.039	〃	2.4×10^{7}	5.0×10^{-9}
		0 — 2.0	0.130	〃	3.5×10^{8}	1.0×10^{-9}
		0 — 3.0	0.201	〃	4.9×10^{8}	8.1×10^{-10}
	20	0 — 0.5	0.019	〃	3.5×10^{7}	3.7×10^{-9}
		0 — 1.0	0.098	〃	2.2×10^{8}	2.3×10^{-9}
		0 — 2.0	0.209	〃	4.9×10^{8}	1.3×10^{-9}
		0 — 3.0	0.252	〃	1.3×10^{8}	3.0×10^{-9}
	25	0 — 0.5	0.039	〃	5.4×10^{7}	5.5×10^{-9}
		0 — 1.0	0.153	〃	3.5×10^{8}	2.5×10^{-9}
		0 — 2.0	0.291	〃	1.1×10^{8}	5.9×10^{-9}
		0 — 3.0	0.354	〃	4.9×10^{7}	9.0×10^{-9}
	30	0 — 0.5	0.090	〃	5.4×10^{8}	2.0×10^{-9}
		0 — 1.0	0.232	〃	9.2×10^{8}	1.7×10^{-9}
		0 — 2.0	0.315	〃	7.0×10^{7}	9.1×10^{-9}
		0 — 3.0	0.410	〃	2.2×10^{7}	1.9×10^{-8}
12.0**	10	0 — 0.5	0.004	1.4×10^{7}	2.2×10^{7}	4.5×10^{-10}
		0 — 1.0	0.015	〃	7.9×10^{7}	4.0×10^{-10}
		0 — 2.0	0.104	〃	7.0×10^{8}	3.0×10^{-10}
		0 — 3.0	0.200	〃	9.2×10^{8}	3.1×10^{-10}
	15	0 — 0.5	0.007	〃	7.0×10^{7}	4.0×10^{-10}
		0 — 1.0	0.035	〃	4.6×10^{8}	2.7×10^{-10}
		0 — 2.0	0.123	〃	3.5×10^{8}	5.9×10^{-10}
		0 — 3.0	0.255	〃	3.5×10^{8}	8.1×10^{-10}
	20	0 — 0.5	0.011	〃	3.5×10^{8}	2.1×10^{-10}
		0 — 1.0	0.046	〃	5.4×10^{8}	3.2×10^{-10}
		0 — 2.0	0.132	〃	1.7×10^{8}	1.1×10^{-9}
		0 — 3.0	0.295	〃	1.0×10^{8}	2.3×10^{-9}
	25	0 — 0.5	0.023	〃	5.4×10^{8}	3.2×10^{-10}
		0 — 1.0	0.065	〃	3.5×10^{8}	6.2×10^{-10}
		0 — 2.0	0.142	〃	3.3×10^{7}	3.2×10^{-9}
		0 — 3.0	0.310	〃	1.1×10^{7}	3.5×10^{-8}
	30	0 — 0.5	0.032	〃	3.5×10^{8}	6.1×10^{-10}
		0 — 1.0	0.074	〃	3.5×10^{8}	7.1×10^{-10}
		0 — 2.0	0.156	〃	7.0×10^{7}	2.2×10^{-9}
		0 — 3.0	0.340	〃	2.4×10^{7}	6.1×10^{-8}

* The water sample was collected from the surface of Tosa Bay.
** The water sample was collected from the surfaee of Uranouchi Bay.

from 10° to 30°C ; and the apparent Q_{O_2} values which obtained in prolonged incubation at higher temperatures, decreased abnormally due to underestimation of the integrated sizes of bacteria.

Therefore, the data described in Table 2 A were recalculated for the following short intervals : 0.5—1.0 day ; 1.0—2.0 days ; 2—3 days. The newly obtained Q_{O_2} values (Table 2 B) decreased streadily with time, so long as the bacterial populations increased. Accordingly

Table 2 B. *Recalculated Q_{O_2} values as functions of incubation temperatures and shorter incubation periods*

In situ temp. (°C)	Incubation Temp. (°C)	Incubation period (days)	O_2 consumption (mg/L)	Q_{O_2} value (mg O_2/day/MPN)
28.1	10	0.5 — 1.0	0.005	3.6×10^{-9}
		1.0 — 2.0	0.011	1.2×10^{-9}
		2.0 — 3.0	0.020	7.2×10^{-10}
	15	0.5 — 1.0	0.028	2.2×10^{-9}
		1.0 — 2.0	0.091	7.5×10^{-10}
		2.0 — 3.0	0.071	1.7×10^{-10}
	20	0.5 — 1.0	0.079	1.6×10^{-9}
		1.0 — 2.0	0.111	3.3×10^{-10}
		2.0 — 3.0	0.043	1.4×10^{-10}
	25	0.5 — 1.0	0.114	1.4×10^{-9}
		1.0 — 2.0	0.138	6.7×10^{-10}
		2.0 — 3.0	0.063	8.4×10^{-10}
	30	0.5 — 1.0	0.142	4.0×10^{-10}
		1.0 — 2.0	0.083	2.5×10^{-10}
		2.0 — 3.0	0.095	2.3×10^{-9}
12.0	10	0.5 — 1.0	0.011	3.0×10^{-10}
		1.0 — 2.0	0.089	3.1×10^{-10}
		2.0 — 3.0	0.096	1.2×10^{-10}
	15	0.5 — 1.0	0.030	2.9×10^{-10}
		1.0 — 2.0	0.088	2.2×10^{-10}
		2.0 — 3.0	0.132	3.8×10^{-10}
	20	0.5 — 1.0	0.035	1.6×10^{-10}
		1.0 — 2.0	0.086	2.7×10^{-10}
		2.0 — 3.0	0.163	2.7×10^{-9}
	25	0.5 — 1.0	0.042	1.9×10^{-10}
		1.0 — 2.0	0.077	5.7×10^{-10}
		2.0 — 3.0	0.168	8.4×10^{-9}
	30	0.5 — 1.0	0.042	2.4×10^{-10}
		1.0 — 2.0	0.082	4.7×10^{-10}
		2.0 — 3.0	0.184	4.3×10^{-9}

the incubation of a water sample should be designed to terminate just after detecting a discernible amount of O_2 consumption. Based on the above facts, reasonable criteria for incubation period were proposed as a function of incubation temperature : incubation should be carried out for 2 days or more at temperatures lower than 10°C ; for 1 or 2 days at temperaturers ranging 10° to 20°C ; for 1 day or less at temperatures higher than 20°C. These criteria were employed for the later experiments, and the criteria appear to be valid also for water samples collected from other parts of the coastal sea.

In most water samples O_2 tensions are apt to vary, especially during filtration treatments. Thus the effect of lower O_2 tension on Q_{O_2} value was examined. The depletion of O_2 tension was accomplished by evacuating the water sample. As shown in Table 3, the apparent Q_{O_2} values decreased somewhat by lowering the concentration of dissolved O_2. The results were confirmed by two separate runs. ZoBell[1] described the rate of O_2 consumption by marine bacteria as independent of O_2 tension within a relatively broad range. The dis-

Table 3. *Effect of O_2 tension on the apparent rate of bacterial O_2 consumption*

Dissolved O_2 (%)	Counts of heterotrophs (MPN/L)		O_2 consumption (mg/day/L)	Q_{O_2} value (mg O_2/day/MPN)
	Initial	Final		
118	5.4×10^7	2.1×10^8	0.059	5.1×10^{-10}
70	1.3×10^7	4.9×10^8	0.048	3.6×10^{-10}
47	5.4×10^7	2.3×10^8	0.027	2.1×10^{-10}

Note: The water sample was collected from the surface of Tosa Bay. Incubation temperature, 19.5°C; incubation period, 2 days.

crepancy may be ascribed to the difference in bacterial flora. Therefore, it is desirable that water samples be treated with care to avoid undue change of O_2 tension before incubations.

The effect of temporary storage of water samples on Q_{O_2} values was examined by using water samples collected from: Tosa Bay which receives clear, low-nutrient-content, oceanic water; and Uranouchi Bay which has turbid, nutrient-rich water. As shown in Table 4, there was no significant difference between the original samples and the stored samples

Table 4. *Effect of the temporary storages of water samples on the apparent rate of bacterial O_2 consumption*

Sampling area	Storage temp. (°C)	Storage period (day)	O_2 consumption (mg/L/day)	Counts of heterotrophs (MPN/L)		Q_{O_2} value (mg O_2/day/MPN)
				Initial	Final	
Uranouchi Bay	5	0	0.285	2.3×10^7	3.3×10^8	2.2×10^{-9}
		0.5	0.399	1.3×10^7	7.9×10^8	2.1×10^{-9}
		1.0	0.367	4.5×10^5	1.3×10^9	2.3×10^{-9}
	20	0	0.285	2.3×10^7	3.3×10^8	2.2×10^{-9}
		0.5	0.261	7.9×10^6	4.6×10^8	2.4×10^{-9}
		1.0	0.285	3.3×10^6	1.3×10^9	1.3×10^{-9}
Tosa Bay	5	0	0.090	1.1×10^6	2.4×10^8	2.0×10^{-9}
		0.5	0.098	4.5×10^5	2.8×10^8	2.3×10^{-9}
		1.0	0.082	2.0×10^5	1.1×10^8	4.7×10^{-9}
	20	0	0.090	1.1×10^6	2.4×10^8	2.0×10^{-9}
		0.5	0.073	3.3×10^6	7.9×10^7	3.1×10^{-9}
		1.0	0.081	1.4×10^6	7.9×10^7	4.2×10^{-9}

Note: Incubation temperature, 21°C; incubation period, 1 day.

from the viewpoint of the quantitative aspect of Q_{O_2} values. But if examined in detail, the Q_{O_2} values obtained for the water samples kept at 20°C for 1 day appeared to change slightly. Therefore, most water samples seem to be suitable for temporary storage only at low temperatures for short periods of time.

Judging from the foregoing tables, rapid growth of bacteria occurred during incubations. The phenomenon which has been recognized as "bottle effect", occurs under a closed system of relatively small volume, and may cause the variation of Q_{O_2} values. Therefore a trial was made to prevent bottle effect by increasing the incubation volume from 100 ml up to

Table 5. *Effect of volume of incubation bottle on the rate of bacterial O_2 consumption*

Volume of bottle (ml)	Counts of heterotrophs (MPN/L) Initial	Final	O_2 consumption (mg/day/L)	Q_{O_2} value (mg O_2/day/MPN)
100	4.5×10^5	1.7×10^8	0.058	2.0×10^{-9}
	〃	1.1×10^8	0.060	3.0×10^{-9}
300	4.5×10^5	1.1×10^8	0.051	2.6×10^{-9}
	〃	2.4×10^8	0.047	1.2×10^{-9}
500	4.5×10^5	1.4×10^8	0.051	2.1×10^{-9}

Note: The water sample was collected from the surface of Tosa Bay.
Incubation temperature, 19.5°C; incubation period, 2 days.

500 ml. As shown in Table 5, rapid growth of bacteria during incubation was not prevented effectively; and, as was expected, the mean generation times were far shorter as compared with those obtained under the open system of chemostats[4]. The Q_{O_2} values obtained in the present experiments should be interpreted as potentialities rather than naturally occurring rates.

There are much quantitative data showing the fact that divergent counts of bacteria are obtained by employing different methods. Therefore, Q_{O_2} values were calculated using the following different counts for the same sample: direct microscopic counts, extinction dilution counts, and pour plate counts. As typical data are shown in Table 6, apparent discrepancies are noted in the in the Q_{O_2} values obtained, but the overall rates of O_2 consumption by

Table 6. *Oxygen consumption rates based on the bacterial counts obtaining by different enumeration methods*

Method	Bacterial counts (MPN/L) Initial	Final	O_2 consumption (mg/day/L)	O_2 consumption rates (mg O_2/day/ bact. count)	(mg O_2/day/ L of water)
Direct counting	2.2×10^6	7.6×10^9	0.312	3.4×10^{-10}	7.4×10^{-4}
Extinction dilution counting	2.6×10^4	8.2×10^7	0.312	3.1×10^{-8}	8.0×10^{-4}
Plate counting	1.4×10^4	4.6×10^7	0.312	5.5×10^{-8}	7.7×10^{-4}

Note: The water sample was taken from the surface of Tosa Bay.
Incubation temperature, 26°C; incubation period, 1 day.

bacteria in a liter of water converged to a similar level.

The field survey for estimating the rates of bacterial O_2 consumption was carried out at 8 stations in the Hiuchi-Nada area, Seto Inland Sea (Fig. 1). For estimating Q_{O_2} values incubations were conducted under the following set of minimally disturbed conditions:

Table 7. *Oxygen consumption rates by bacteria in the Hiuchi-Nada area of the Seto Inland Sea*

Date (1972)	Station	Depth (m)	O_2 consumption (mg O_2/L/day)	Bacterial counts (cells/L)			Q_{O_2} value (mg O_2/cell/day)	Rate of bacterial O_2 consumption in original water (mg O_2/day/L of water)
				Original water	Filtered water			
					Initial	Final		
26 July	4	0	0.222	5.0×10^8	5.0×10^8	2.9×10^9	1.6×10^{-10}	8.1×10^{-2}
		10	0.052	1.4×10^8	2.3×10^7	2.1×10^9	1.1×10^{-10}	1.6×10^{-2}
	6	0	0.046	3.6×10^9	4.5×10^8	2.0×10^9	4.5×10^{-11}	1.2×10^{-1}
		10	0.037	4.5×10^8	2.3×10^8	2.9×10^9	3.5×10^{-11}	1.6×10^{-2}
	12	0	0.174	1.4×10^9	1.8×10^8	4.1×10^9	1.4×10^{-10}	1.9×10^{-1}
		10	0.089	1.2×10^8	1.0×10^8	3.4×10^9	9.6×10^{-11}	1.1×10^{-2}
27 July	17	0	0.200	1.8×10^9	4.5×10^8	5.7×10^9	9.7×10^{-11}	1.8×10^{-1}
		10	0.088	3.9×10^7	6.8×10^7	2.7×10^9	1.2×10^{-10}	5.8×10^{-3}
	21	0	0.435	4.5×10^9	2.5×10^9	2.7×10^{10}	4.2×10^{-11}	1.9×10^{-1}
		10	0.219	5.4×10^7	2.3×10^7	4.5×10^9	1.8×10^{-10}	9.7×10^{-3}
	23	0	0.210	1.8×10^9	1.8×10^8	3.1×10^{10}	3.5×10^{-11}	6.4×10^{-2}
		10	0.091	1.4×10^8	1.9×10^7	7.1×10^9	7.6×10^{-11}	1.1×10^{-2}
28 July	19	0	0.258	2.5×10^9	9.0×10^8	2.1×10^{10}	4.1×10^{-11}	1.0×10^{-1}
		10	0.096	2.1×10^8	6.8×10^7	3.3×10^9	8.4×10^{-11}	1.8×10^{-2}
29 July	9	0	0.352	1.7×10^{10}	4.0×10^8	5.3×10^{10}	3.3×10^{-11}	5.4×10^{-1}
		10	0.264	2.5×10^8	1.3×10^8	2.3×10^{10}	5.5×10^{-11}	1.4×10^{-2}

Note: Bacterial counting, direct microscopic counting; Incubation period, 1 day; Incubation temp., 21°−24°C.

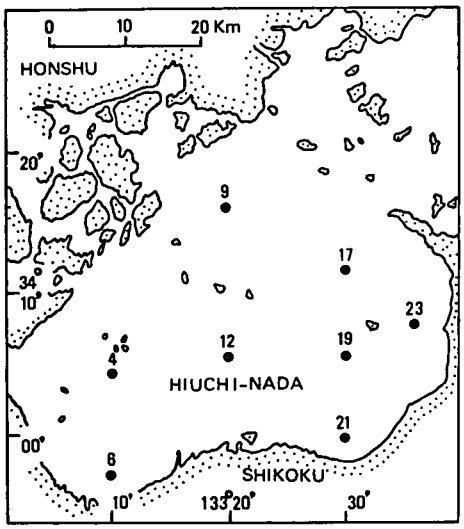

Fig. 1 Hiuchi-Nada area in the Seto Inland Sea, showing locations of the stations examined.

that is, incubations of seawater were carried out under careful precautions just after sampling at *in situ* temperatures, under O_2 tensions similar to *in situ* levels, for periods suitable to the above-described criteria, in 100-ml BOD bottles. As shown in Table 7, the Q_{0_2} values fell within the range of 3.3×10^{-11}—1.8×10^{-10} mg O_2/cell/day with a mean of 8.4×10^{-11} mg O_2/cell/day.

A definite pattern of the Q_{0_2} variation is not confirmed statistically. The values do not coincide exactly with those reported by Johnson[5] and ZoBell[1]. The discrepancies are due mainly to the difference in methods of bacterial counting. The rates of O_2 consumption by bacteria per liter of water were within the range of between 5.8×10^{-3}—5.4×10^{-1} mg O_2/liter/day. The rates were relatively high at surface layers of water owing to the localization of bacteria. But extreme deficiencies of dissolved O_2 do not occur due to bacterial removal of O_2 in the area of the sea. The bulk of O_2 consumed by bacteria is recognized as being used in the mineralization process. Thus assuming the respiration quotient of bacteria is 1.0, bacterial mineralization rate of carbon compounds (mg C/day/cell) can be estimated roughly by multiplying the O_2 consumption rate by 0.375.

Laevastu *et al.*[6] suggested that O_2 consumption, determined under defined conditions, can serve as an index of water type. Informations about O_2 consumption rates of bacteria are useful for elucidating the characteristics of bacterial ecosystems as well as water types-if the determinations are carried out under clearly defined, comparable conditions.

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