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Microbial Activities in Undecompressed and Decompressed Deep-Seawater Samples†

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Microbial transformations of ^{14}C-labeled substrates (sodium glutamate, Casamino Acids, glucose, and sodium acetate) were measured in undecompressed seawater samples collected from depths of 1,800 to 6,000 m, during 14- to 21-day incubation periods at in situ temperature (3°C). Each substrate was tested at two concentrations (ca. 0.5 and 5.0 μg/ml) and two in situ pressures. The data were compared to 1-atmosphere (ca. 1.013×10^2 kPa) controls. The rates of ^{14}C incorporation and $^{14}CO_2$ production as well as the amounts of total substrate utilization were generally lower at pressure than in the decompressed controls but were significantly different for each of the four substrates used. The utilization of acetate was the least affected by pressure; rates were similar to those measured at 1 atmosphere in two out of four experiments. In contrast, transformation rates of the amino acids at pressure averaged to only 38% of those in the controls. A single but reproducible "barophilic" response was observed with glucose as a substrate in samples collected from a depth of 4,500 m at a specific area in the northwestern Atlantic Ocean. Except for this latter set of experiments, the transformation of all substrates showed an increased lag period at pressure as compared to the 1-atmosphere controls.

The environmental factors determining microbial activities in the deep sea are probably, in descending order of importance, nutrient concentrations, temperature, and hydrostatic pressure. Oligotrophy, a term nowadays used for waters of low nutrient levels as well as for organisms adapted to live at low nutrient levels (12, 18), puts the basic limits on metabolism. These limits are expressed by threshold concentrations of carbon and energy sources or essential nutrients and may be modified by other environmental factors such as the redox potential in the case of microaerophilic bacteria (6). The uniformly low temperature of 2 to 4°C in the deep sea permits relatively slow growth of low-temperature-adapted "psychrophilic" bacteria (optimal growth rates at 8 to 15°C) but practically eliminates growth of the "mesophilic" microorganisms (optimal growth rates at 25 to 40°C) (1, 17, 27). Between 20 and 25°C temperatures become lethal to psychrophilic bacteria.

The present knowledge of microbial responses toward hydrostatic pressure presents a more complex picture. Although it has never been a technical problem to retrieve deep-sea bacteria at unchanged in situ temperature, pressure-retaining samplers have not been used until recently (9–11, 23, 24). So far the extensive work on pressure effects on growth, metabolism, and enzyme activities of deep-sea bacteria, as reviewed by Morita (16) and Marquis and Matsumura (15), was done with organisms isolated after decompression of the original sample. Since many of such isolates were insensitive to repeated compression and decompression, the general feeling prevailed that, unlike the increase of temperature, decompression may not be lethal or irreversibly damaging to deep-sea bacteria.

This assumption is supported by the fact that "barophilic" bacteria can be isolated under pressure after a brief decompression-recompression cycle (2, 3, 29, 31). These isolates exhibit an apparent pressure adaptation by growing optimally at pressures higher than 1 atmosphere (atm; ca. 1.013×10^2 kPa). The more recently isolated barophilic bacteria were obtained from relatively nutrient-rich deep-sea habitats, namely the intestinal tracts of amphipods and other invertebrates (2, 3, 29; A. Yayanos, A. S. Dietz, and R. Van Boxtel, Proc. Natl. Acad. Sci., in press). Growth of one of these isolates was reduced from its optimum rate at about 500 atm by 90% upon decompression (29), whereas another was found to be "obligately" barophilic, i.e., its growth was entirely inhibited at 1 atm (Yayanos et al., in press).

Barophilic behavior is not readily apparent in deep-sea enrichment studies with natural micro-

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bial populations. Whenever bottles with various kinds and concentrations of growth media were inoculated and incubated on the deep-sea floor, rates of growth and substrate transformations in the control bottles, incubated at 1 atm and in situ temperature, were considerably higher than those in situ (7, 8). It appears that, even if barophilic organisms are present, their growth rate at in situ pressure is still considerably reduced as compared to that of non-barophilic bacteria at 1 atm.

There is, however, no proof so far that some organisms within the natural population are not lost or irreversibly damaged upon decompression. Seki and Robinson (20) demonstrated that growth of microbial populations from depths as shallow as 400 m was adversely affected by a brief decompression-recompression cycle as compared to undecompressed controls. Discussing the large numbers of viable bacteria found in the top sediments of deep-sea cores, ZoBell (30) stated: "Although many bacteria from the deep sea survived, this observation fails to prove that some bacteria, possibly the most sensitive ones, were not destroyed by decompression. Answering this question may require the examination of deep sea bacteria at in situ pressure without subjecting them to decompression." This has now been done in two ways: (i) by the above-mentioned in situ inoculation and incubation studies and (ii) by the use of pressure-retaining samplers and cultivation vessels. Whereas the former is limited to single endpoint measurements, the latter offers the possibility of obtaining data from unlimited time course studies, results of which are reported in the present paper.

Two pressure-retaining sampling and cultivating devices have been developed during the past decade (9–11, 23, 24). Working on principles that are slightly different technically, both systems allow studying microbial transformations of radiolabeled substrates in undecompressed deep-seawater samples. Initial results have been published in the above-cited papers. A technical advance toward the acquisition of larger amounts of data with respect to the limited shiptime available has been a "filter sampler" which permits a combination of in situ sample concentration over Nuclepore filters with pressure-retaining retrieval and the cold storage of undecompressed subsamples (9). This system allows the resterilization and multiple use of this sampler at sea and the collection of as many undecompressed samples as storage units were available. The in situ sample concentration was also instrumental in achieving pure culture isolations of deep-sea bacteria in the absence of decompression (H. W. Jannasch, C. O. Wirsen, and C. D. Taylor, manuscript in preparation).

MATERIALS AND METHODS

Sampling, incubation, and subsampling. Undecompressed water samples were retrieved from depths of 1,800 to 6,000 m with pressure-retaining sampler/culture vessels previously described (10, 11). In each sample the pressure was measured initially for the exact determination of the sampling depth and monitored throughout the incubation period. The radiolabeled substrates were added, and the samples were incubated at 3.0 ± 0.5°C in the ship's refrigerated van. The magnetically stirred vessels were then subsampled at certain intervals for measuring the microbial activity. For this purpose 12-ml volumes were removed by sterile transfer units with no loss of pressure while equal volumes of sterile seawater were added back. Thus the samples were diluted 1.1% at each subsampling, but the sample volume as well as the specific activity remained constant throughout the course of the experiment. Details of this procedure have been described earlier (10).

Decompressed control samples were collected with sterile Niskin baggie samplers (General Oceanics Co.) from the same site and depths as the undecompressed samples. These 1-atm controls were transferred to 1-liter flasks, and the radiolabeled substrates were added for incubation at the same temperature as that of the undecompressed samples. Possible temperature increases during sampling (a maximum of 6°C over ambient in the uninsulated pressure-retaining sampler and of 10°C in the Niskin baggie sampler) are not believed to affect psychrophilic organisms irreversibly. This is supported by data of a parallel experiment using an alcohol-rinsed Niskin PVC-bottle sampler (General Oceanics Co.) with a maximum temperature increase of 1°C over ambient. PVC-bottle samplers were not routinely used because of unsterile sampling.

Although most of these experiments were conducted with the sampler/culture vessels aboard the research vessel immediately after sampling, other experiments were done using inocula from the above-mentioned filter sampler (9). The undecompressed and concentrated subsamples were kept in cold storage for inoculation into the prepressurized culture vessels at the laboratory ashore. In these cases the culture vessels, chilled to 3°C, contained filter-sterilized (0.2 μm pore size, Nuclepore) seawater collected at the same site and depth as the undecompressed sample.

The sampling locations are indicated in Fig. 1, and the sampling depths are shown in Table 1.

Substrates. The substrates used in this series of experiments were D-[*U*-^{14}C]glucose, L-[*U*-^{14}C]sodium glutamate, [1,2-^{14}C]sodium acetate, and L-*U*-^{14}C-amino acid mixture from New England Nuclear Corp. and D-[*U*-^{14}C]mannitol and [1-^{14}C]calcium glycolate from International Nuclear and Chemical Corp. Stocks of these radiolaeled substrates were prepared with unlabeled carrier substrates and added to the seawater samples at either of two final concentration levels: approximately 0.5 and 5.0 μg/ml, both having the same activity of approximately 0.006 μCi/ml. Unlabeled Casamino Acids were used as the carrier substrate for the radiolabeled amino acid mixture.

Analyses. The incorporation of labeled carbon from the different substrates into cell material and the respired $^{14}CO_2$ were measured following the procedures described earlier (26, 27). Samples of 10 ml were

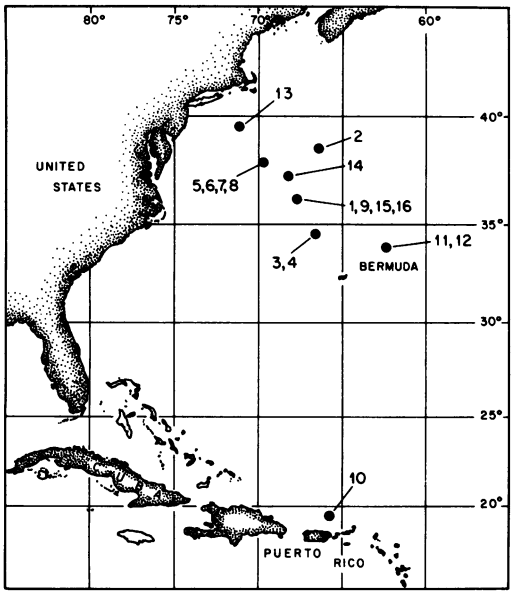

FIG. 1. Area of sampling and sample numbers.

filtered (0.2 μm pore size, Amicon Corp.) and washed twice, first with 10 ml and then with 5 ml of chilled seawater. Data of background filter controls run with sterile seawater containing the labeled substrates were subtracted from the incorporation values. Background volatilization controls were likewise subtracted from the respiration values. Samples were counted with an Intertechnique SL-20 liquid scintillation counter. Correction for quenching was done by the channels ratio method.

RESULTS

The experiments with four of the six substrates (glutamate, Casamino Acids, glucose, and acetate) resulted in measurable microbial activities at in situ pressures and temperature. The incorporation and respiration of the labeled carbon from each of these substrates at two concentration levels in water samples from two depths and incubated at the two in situ pressures are presented in Fig. 2 to 5. Each data set (with the exception of Fig. 3B, 170 atm) includes the 1-atm controls. The actual initial substrate concentrations in the undecompressed samples varied and were therefore individually determined (Table 1).

The generally negative effect of pressure on microbial activity appeared strongest in the glutamate-enriched samples. The rates of substrate utilization, as calculated from the combined data of incorporation and respiration, were two to five times lower at pressure than in the 1-atm controls (Table 1). The total utilization of glutamate within the given time periods, averaged for the four experiments, was 27% at pressure and 56% at 1 atm. There was no significant difference of total substrate utilization at the two pressures and substrate levels used in these experiments. The insignificant decrease of pH and dissolved oxygen measured at the end of the 3-week incubation period did not indicate a limitation of substrate utilization by these factors.

When a Casamino Acids mixture was used as a medium, noticeable effects of pressure on

TABLE 1. Supplementary data on the effect of in situ pressure on the utilization of four substrates by undecompressed and decompressed natural microbial populations from the deep sea

Substrate	Sample site no. (see Fig. 1)	Pressure at sampling depth and of incubation (atm)	Initial concn (μg/ml)	Ratio of utilization rates (in situ pressure/1 atm)
Sodium glutamate	1	183	0.50	0.37
	2	306	0.615	0.31
	3	180	5.58	0.19
	4	300	5.78	0.50
Casamino Acids	5	183	0.426	0.41
	6	350	0.286	0.48
	7	170	4.04	Not done
	8	313	1.40	0.41
Glucose	9	183	0.350	0.96
	10	600	0.189	0.45
	11	185	6.09	0.75
	12	450	5.46	2.65
Sodium acetate	13	177	0.483	0.59
	14	385	0.452	0.99
	15	175	5.86	1.00
	16	462	4.25	0.69

substrate utilization were observed. The rates of incorporation and respiration are about two times lower at pressure than at 1 atm. The total substrate utilization estimated as above was 41% at pressure and 56% in the controls.

The microbial utilization of glucose appeared to be much less affected by pressure than that of the amino acids. As compared to the 1-atm controls, the rates were somewhat reduced in two experiments, almost equal in one experiment (Fig. 4A, 183 atm), and considerably higher in another (Fig. 4B, 450 atm). Since, in this latter case, the data were quite unexpected, a chance for the repetition of this particular experiment at the same site and depth (Fig. 1, number 12) was sought and obtained. The results were basically confirmed: the ratio between the utilization rate at pressure and at 1 atm was 2.65 in the first and 1.85 in the repeated experiment (second set of data not presented). The total utilization of glucose averaged 62% at pressure and 64% in the 1-atm controls.

Acetate proved to be the most preferable substrate of the four tested, having the highest

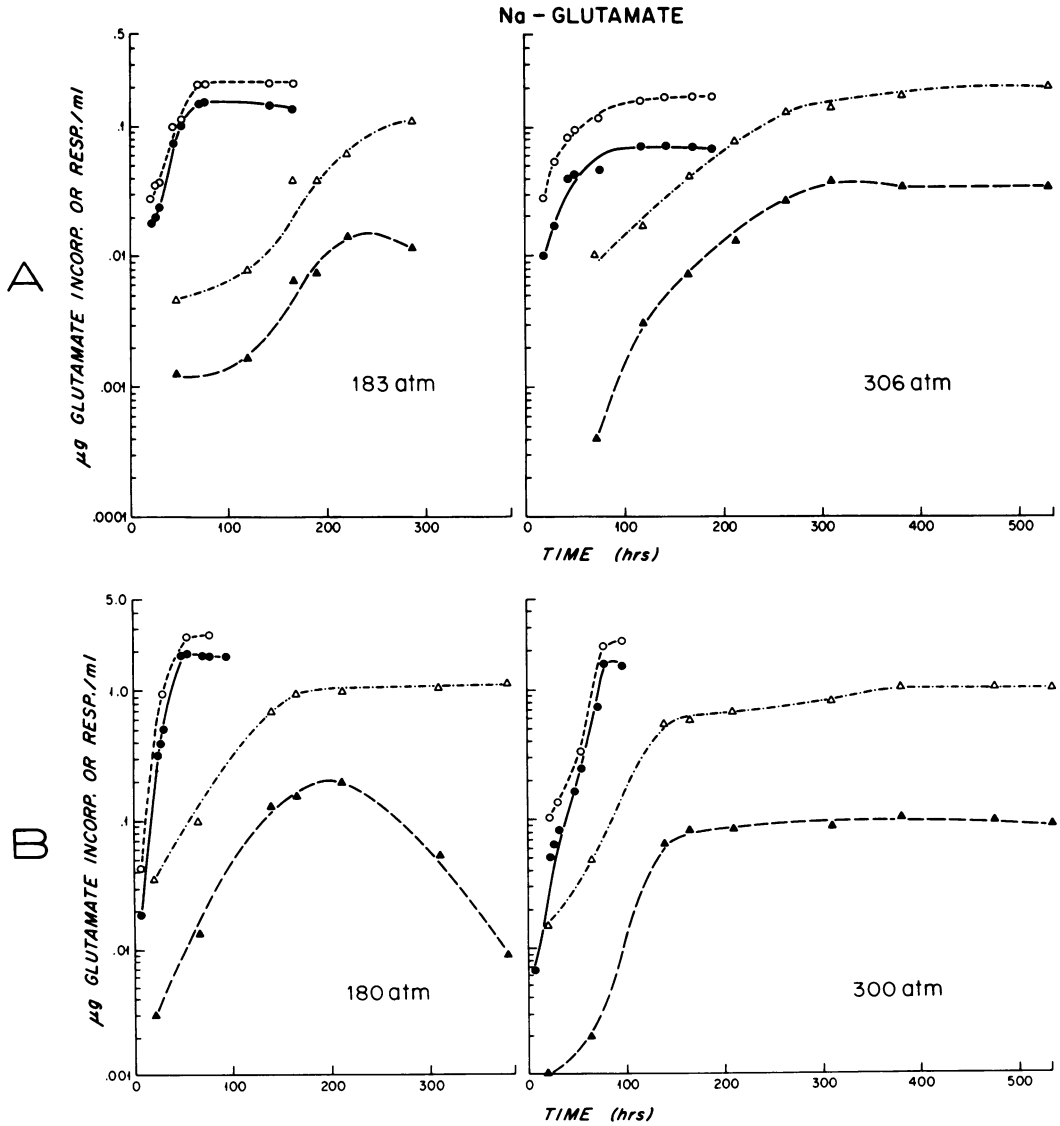

FIG. 2. Incorporation (filled symbols) and respiration (unfilled symbols) of radiolabeled carbon from sodium glutamate at two levels of concentration (A, ca. 0.5 μg/ml; B, ca. 5.0 μg/ml; see Table 1) in seawater sampled from two depths and incubated at in situ pressure (△, ▲, undecompressed samples) and at 1 atm (○, ●, decompressed controls).

rates of utilization. In two of the four experiments the rates measured at pressure appeared equal to those of the 1-atm controls (Fig. 5, Table 1). The average percentages of total substrate utilization at pressure and in the controls were practically indistinguishable (88.7 and 88.0) and considerably higher than for the other substrates.

Mannitol and glycolic acid, when incubated at the same two concentration levels used throughout these experiments and at in situ pressures (180 and 480 atm) and temperature (3°C), did not give rise to any noticeable activity of [^{14}C]carbon incorporation or respiration within 3 weeks. Upon release of pressure to 1 atm, glycolic acid started to be utilized after a brief lag but did not reach a total substrate utilization of more than 6%.

DISCUSSION

The experimental approach to study heterotrophic microbial activity in a natural habitat by following the conversion of a tracer substrate

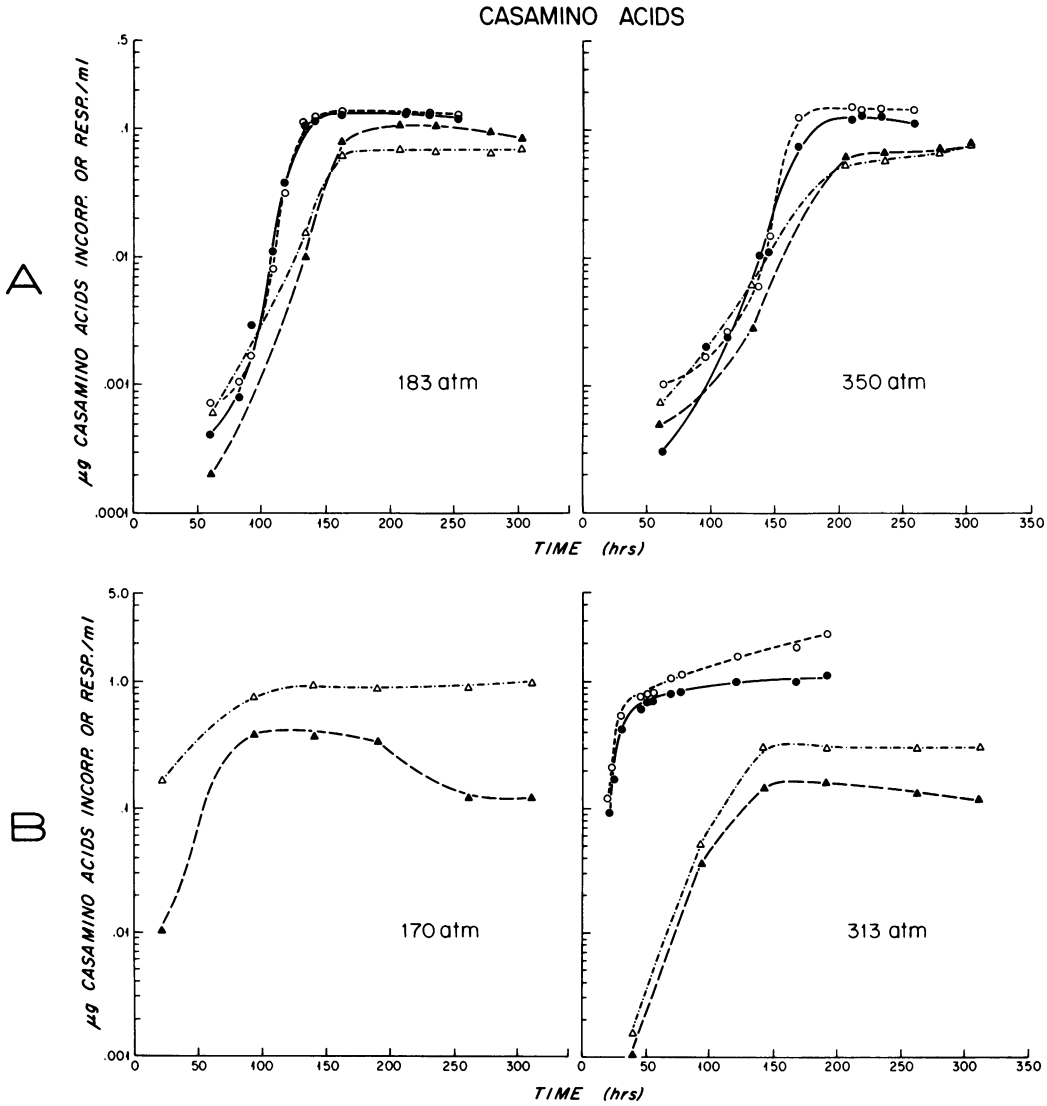

FIG. 3. Incorporation (filled symbols) and respiration (unfilled symbols) of radiolabeled carbon from Casamino Acids at two levels of concentration (A, ca. 0.5 μg/ml; B, ca. 5.0 μg/ml; see Table 1) in seawater sampled from two depths and incubated at in situ pressure (△, ▲, undecompressed samples) and at 1 atm (○, ●, decompressed controls).

appears generally limited by the necessary choice of a substrate and of its concentration. It is commonly assumed that this choice can be based on analytical data. However, the finding of metabolically utilizable compounds in seawater may indicate either (i) that their concentrations represent steady-state levels of a continuous turnover process, or (ii) that these substrates are, for a number of reasons, not utilized below a particular threshold concentration.

The former may be true for productive surface waters where a more-or-less constant input of organic carbon and energy sources does exist at a level that is able to sustain a continuously growing microbial population. In such a situation, the measurable concentration of a growth-limiting substrate, similar to the steady-state concentration in a chemostat, indicates the efficiency of utilization, but it is independent of and not indicative for the actual amount of substrate consumed (4).

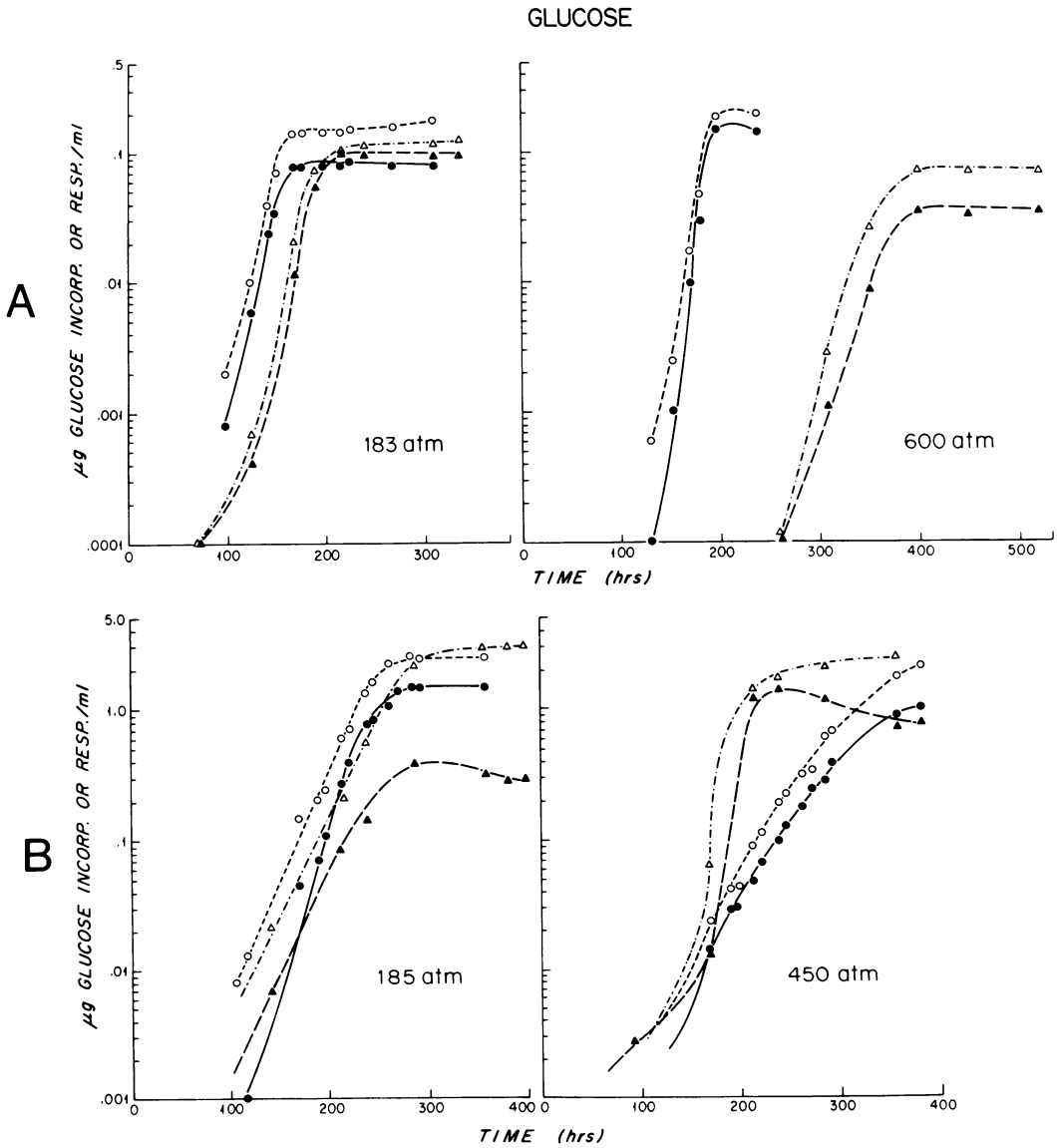

FIG. 4. Incorporation (filled symbols) and respiration (unfilled symbols) of radiolabeled carbon from glucose at two levels of concentration (A, ca. 0.5 μg/ml; B, ca. 5.0 μg/ml; see Table 1) in seawater sampled from two depths and incubated at in situ pressure (△, ▲, undecompressed samples) and at 1 atm (○, ●, decompressed controls).

For most oligotrophic waters the existing levels of utilizable substrates are too low to sustain continuous microbial growth and often represent threshold concentrations (5, 6). Moreover, the input of organic materials reaching the deep sea in an undecomposed state depends, the deeper the more so, on the sinking rate of larger particles from fecal pellets to fish carcasses. Therefore, the distribution of organic carbon and energy sources will be heterogeneous, and their local concentrations will be relatively high. This would lead to patchy growth of microorganisms. When, in such microhabitats, the substrate levels are reduced to near-threshold concentrations, turnover rates approach zero.

It has been argued (24, 25, 28) that the more similar the concentrations of test substrates are to the concentrations actually found in seawater, the more realistic will be the conversion rates measured. On the basis of the above discussion, this may not be so. By adding the test substrate at conveniently high concentrations to the natural microbial population, the resulting "potential" rate of conversion may actually be more,

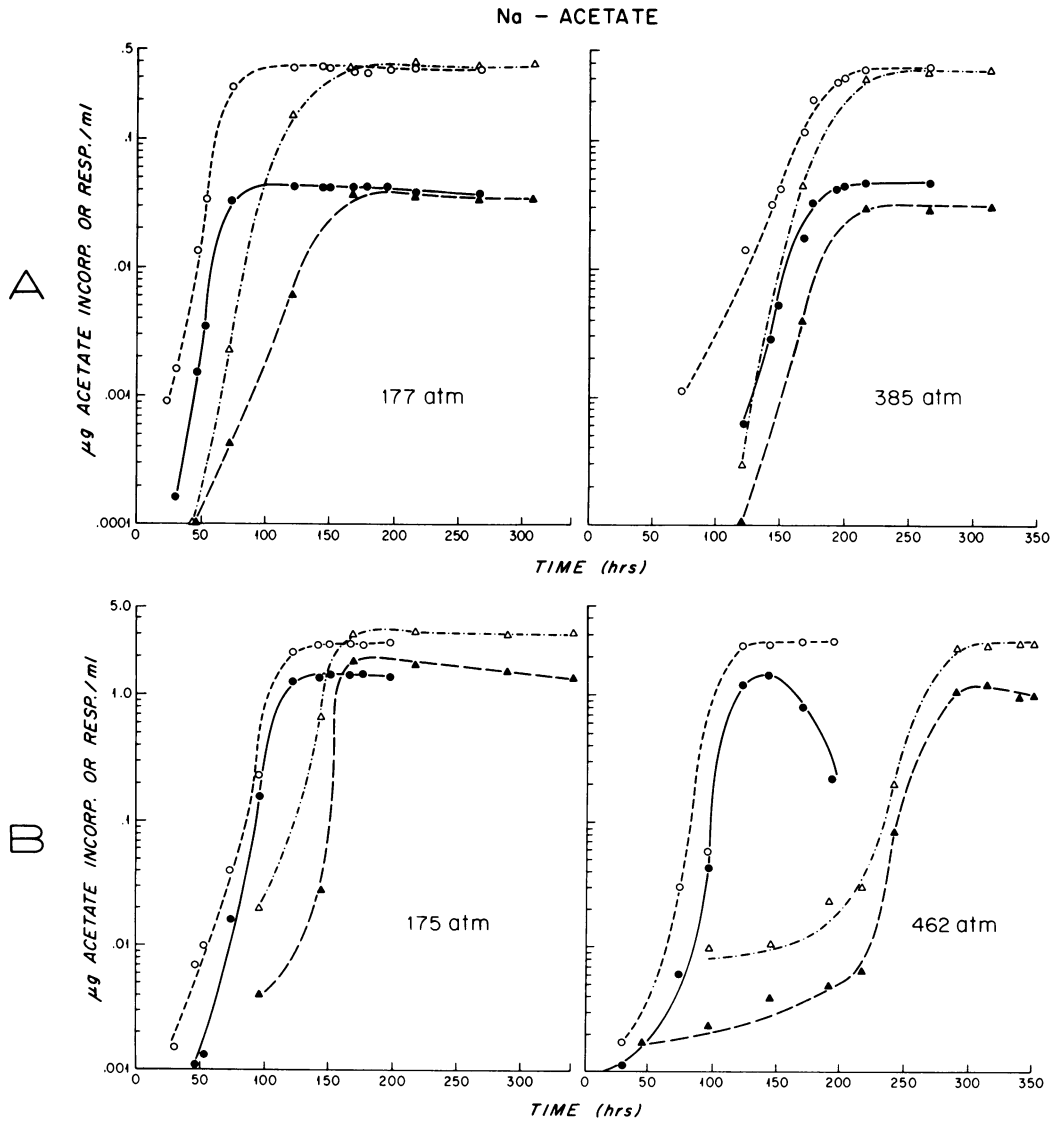

FIG. 5. Incorporation (filled symbols) and respiration (unfilled symbols) of radiolabeled carbon from sodium acetate at two levels of concentration (A, ca. 0.5 μg/ml; B, ca. 5.0 μg/ml; see Table 1) in seawater sampled from two depths and incubated at in situ pressure (△, ▲, undecompressed samples) and at 1 atm (○, ●, decompressed controls).

certainly no less, realistic for deep-sea conditions than by using near-in-situ concentrations, assuming a continuous supply of the substrate at a constant level.

The two substrate levels used in this study, in the approximate proportion of 1:10, resulted in data that showed no statistically significant difference for the rates of substrate conversion. This may indicate that these two substrate concentrations were within the range of substrate saturation, i.e., distinctly above the range where conversion or growth rates are almost proportional to substrate concentrations.

The data of this study also showed that the two in situ pressures used for each pair of the experiments under otherwise equal conditions did not result in significantly different rates of metabolism. An increasing lag with increasing pressure, however, as observed in an earlier study (10), was definitely discernible.

One major result of this study confirmed earlier observations (14, 15) concerning the dependence of pressure effects on the type of substrate used in the growth or utilization experiments. There is a reproducible order of substrate "preference" in terms of conversion rates and total utilization. Possible physiological-biochemical reasons for this variability or loss of barotolerance and barophilism in pure cultures of microorganisms are discussed in reviews by Marquis and Matsumura (15) and by Landau and Pope (13). In principle, pressure has less of an effect on catabolic processes and ATP generation than on biosynthesis and ATP utilization. This pressure-dependent, variable utilization of substrates will affect the categorization of bacterial isolates with respect to their barotolerant or barophilic behavior. An organism clearly demonstrated to be barophilic when growing on one particular substrate may not necessarily be barophilic under natural conditions of growth.

Studies with natural populations cannot be expected to reveal this behavior of individual species, with one possible exception in the data presented here. The barophilic response observed in the experiment with sample 12 (Table 1; Fig. 4B, 450 atm) appeared to be worthy of reporting because a repetition of the experiment with a sample collected from the same area and depth 3 months later resulted in very similar data. This "Bermuda Rise" site was originally selected for microbiological transformation studies in the particularly organic-rich sediments of this area at a depth of 4,550 m. Whether the microbial population in the water column 50 m above this sediment contains a particular barophilic component or single species remains to be studied.

As in earlier preliminary decompression studies (10), the conversion of radiolabeled carbon from glutamate and Casamino Acids under pressure diminished at some level of incomplete substrate utilization but resumed activity when decompressed to 1 atm at unchanged in situ temperature or when the temperature was increased from 3 to 20°C at unchanged in situ pressure (data not presented). Tabor et al. (24) reported similar observations with respect to decompression of samples incubated with glutamate and acetate. The earlier observed increase of the ratio of respiration to incorporation with increasing pressure (10) was, in the present study, only noticeable with glutamate as a substrate.

If the effect of pressure on the total microbial turnover of organic matter in the oceans is considered, the depths of sampling and the corresponding pressures of incubation used in this study cover the ecologically significant range. According to Sverdrup et al. (22), about 24.2% of the oceans' total volume comprises the 0- to 1,000-m top layer, 73% comprises the 1,000- to 5,000-m layer, 2.7% comprises the 5,000- to 6,000-m layer, and the remaining 0.1% comprises the deep trenches below 6,000 m to the maximum depth of about 11,000 m. Although measured only for the sediment-water interface on the ocean floor (21), the oxygen uptake per area in the shelf zone (0- to 200-m depth) was estimated (B. B. Jorgensen, *in* B. Bolin and R. B. Cook (ed.), Proceedings of the SCOPE Workshop on Interactions of Biogeochemical Cycles, in press) to be 83% of the total, 15% in the continental slope zone (200- to 4,000-m depth), and 2% on the deep-sea bottom below 4,000 m. The generally observed decline of biomass with increasing depth (19) corroborate these estimates which are interpreted as indicating the diminishing food supply with depth. At this time, both sets of data—(i) those of pressure effects on microbial activity and (ii) those of increasing oligotrophy with increasing depth—are still insufficient for a comparative assessment of their particular roles in the biological activities of the deep sea.

Measuring microbial transformation activities in undecompressed and decompressed samples of deep-seawater is still complicated by the unknown proportion between the two components of the natural microbial population: the surface-derived, more or less barotolerant, and the pressure-adapted barophilic bacteria. The next step in this work will be studies with pure cultures isolated before decompression. Such a technique has now been developed, and the first set of data is being prepared for publication.

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