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ATP AS A BIOMASS INDICATOR FOR CLOSED ECOSYSTEMS

CATHY T. TAKANO, CLAIR E. FOLSOME and DAVID M. KARL[a]

Exobiology Laboratory, Department of Microbiology, and [a]Department of Oceanography, University of Hawaii at Manoa, Honolulu, Hawaii 96822, U.S.A.

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Nucleotide measurements have been used as biomass indicators in microbial ecology. We report the use of this metric in materially closed, energetically open microbial ecosystems. Our results indicate that: (i) both ATP concentrations and pO_2 show an increase from time of closure; (ii) ATP concentrations (0.2 1.2 ng/ml) are approximately 2 3 times higher than open ocean water; (iii) there is an apparent oscillation of ATP concentrations on the order of 50 days; and (iv) within experimental limitations the ratios of ATP concentrations to observed total cell counts is constant.

Introduction

Previous studies (Kearns and Folsome, 1981) have shown that gaseous oxygen concentrations can be used as an indicator of internal stability and biological activity. Little experimental research has been done to determine actual biological activity or biomass of these systems. A relatively simple and reliable technique was needed to make such determinations. Nucleotide measurements have been widely used to determine total biomass in a variety of marine, freshwater and terrestrial environments (Karl, 1980), and are easily adapted to the study of closed microbial ecosystems.

ATP is attractive as an estimate of total biomass since: (i) it is distributed ubiquitously in all living cells; (ii) it is easily extracted from microbial assemblages and can be precisely measured; (iii) it is not associated with dead cells nor absorbed onto detrital material; and (iv) there exists a fairly constant ratio of ATP to total cell carbon for all microbial taxa independent of metabolic activity or environmental conditions (Karl, 1980). The properties and reliability of this technique are well established and ATP concentrations are measurable in small samples, which is essential in the study of closed microbial systems.

Materials and methods

Small closed marine microbial ecosystems were constructed as previously described (Kearns and Folsome, 1981). In addition to the 2-liter flasks, 1- and 5-liter flasks were also prepared with comparable amounts of sand, water and sediment. Oxygen measurements were determined by quantitative gas solid chromatography at 1- 2-day intervals, using 25-μl (STP) gas samples.

Sampling and nucleotide extraction

Water samples (2 ml) were collected from a sideport using a glass syringe. Immediately following collection, samples were filtered as 1-ml replicates, through a 25-mm 0.2-μm Nuclepore membrane. Particulate material retained by the filter was extracted for adenine nucleotides in 5 ml boiling 60 mM potassium phosphate buffer (pH 7.4) for approximately 5 min. All extracts were stored frozen (−20°C) prior to analysis.

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ATP assay

Lyophilized firefly lantern extracts (Sigma FLE-50) were stored frozen prior to use. Each vial was rehydrated with 5 ml dH_2O and allowed to stand at 25°C for approximately 10 h. The enzyme was then further diluted with equal volumes (approx. 15 ml each) of $MgSO_4$ (0.04 M) and $KHAsO_4$ buffer (0.1 M, pH 7.4). Since ATP concentrations were low in these samples, DL-Luciferin was added to a final concentration of 10 μg/ml, and the enzyme solution was allowed to stand for 10 h before use. When used under these conditions, the limit of detection was approximately 0.01 ng ATP/ml extract. ATP assays were performed and data were reduced as previously described (Karl and Holm-Hansen, 1978).

Results

All four experimental flasks showed a pO_2 increase from time of closure. The kinetics of pO_2 change for several ecosystems are shown in Fig. 1. A, B and C show flasks which were monitored at regular intervals for 6 months, while D was monitored for 5 months from time of closure. All show an overall increase in pO_2: A increased only slightly from 20.6% to 21.7% by day 100 and has remained fairly constant; B has increased from 20.5% to 22.22%; C from 20.5% to 23.5% by day 130 and D increased from 20.4% to 23.0% by day 140.

There is no apparent similarity in pO_2 kinetics for these flasks except for B and C which are 2- and 5-liter systems, which were closed at the same time and which

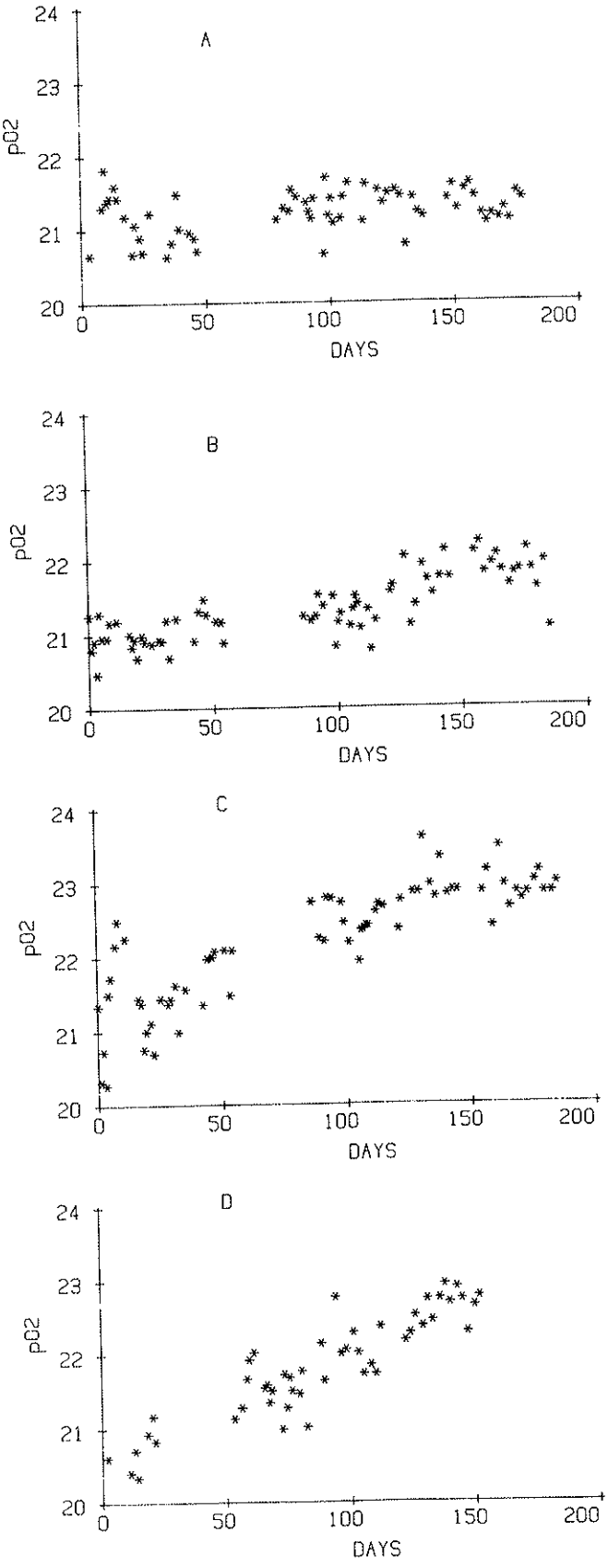


Fig. 1. The kinetics of oxygen concentration change for four microbial ecosystems. (A) a closed 1-liter flask. (B) closed 2-liter flask, taken from same site as A. (C) closed 5-liter flask, taken from same site as A. (D) closed 5-liter flask, taken from same site as A, but containing coral fragments instead of sand and sediment. All measurements were performed from the day of closure.

used the same initial materials. These have reached a plateau after an initial increase in pO_2, but it is uncertain whether they have reached steady state. A, which was closed approximately 2 weeks later, has not shown a significant increase in pO_2 over 6 months. D is the only flask in which pO_2 is still increasing, although this flask has been closed for only 5 months. This flask differs from the others in that it contains coral fragments instead of sand and sediment.

ATP measurements were performed on surface water samples from all systems, but only B contained detectable concentrations of ATP. This flask did not show any detectable concentrations of ATP (ATP $<$ 50 ng/l) before day 50. After 50 days ATP concentrations increased, as shown in Fig. 2. There appears to be a cycling of ATP concentrations on the order of 50 days with the peak ATP concentration increasing with each cycle. A comparison of the graphs of O_2 and ATP for flask B shows that the cycling in ATP concentrations is independent of O_2 concentrations. Model linear regression analysis failed to reveal any correlation ($P < 0.01$) between pO_2 and ATP concentrations. There is a correlation between ATP concentrations and observed bacterial counts for similar systems.

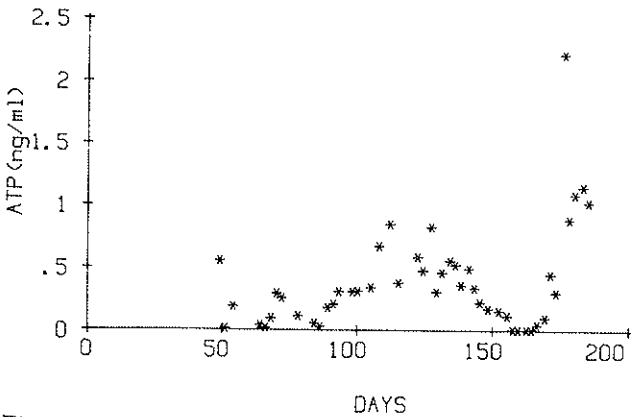


Fig. 2. ATP concentrations measured at 1—2-day intervals plotted against time, in days. The data show that after an initial period of undetectable ATP concentrations a significant increase occurs, marked by an apparent oscillation of total ATP concentrations on the order of 50 days.

TABLE 1

Station	Depth (m)	ATP (ng/ml)	Carbon (μg/l)[a]	Ref.
N. Pacific Gyre	0—100	0.02—0.12	5.0—28.7	8
S. Calif. Bight	0—100	0.13—0.35	31.2—87.5	2
S. Calif. Bight (1—10 km offshore)	0—100	0.5 —1.5	125—375	1
Kaneohe Bay	6—10	0.20	50	7
Closed Ecosystem		0.20—1.20	50—300	

[a] A C/ATP Ratio of 250 was used (Karl, 1980).

Table 1 shows a comparison of ATP and carbon concentrations for the closed system analyzed in this study and several ocean environments. Kaneohe Bay represents the location where our initial inoculum was collected, North Pacific Gyre is an oligotrophic region and the Southern California Bight an area of upwelling. Biomass estimates derived from the ATP data for our closed system, 50—300 μg C/liter, are comparable to the values measured for eutrophic coastal waters and areas of intense upwelling. When the values for a closed are compared to the average values measured for the Kaneohe Bay region, it is evident that ATP and carbon concentrations are generally greater than the steady state. Our results also show at least an order of magnitude greater ATP concentration than measured previously in oligotrophic ocean regions.

Discussion

Measurements of ATP concentrations indicate the total metabolically active biomass present. pO_2 measures the difference between primary production and consumption. Hence these two metrics do not have to be correlated. Our experimental results do

show an observed correlation of ATP with cell counts and no correlation with pO_2.

These flasks do show the same pO_2 response as has been demonstrated previously (Kearns and Folsome, 1981): they also show a slower increase in pO_2, this is not totally unexpected since there was no attempt to duplicate those initial inocula.

We have shown that a modification of the boiling phosphate buffer technique (Karl and Craven, 1980) can be used to measure ATP concentrations of closed ecosystems. Our results indicate that materially closed ecosystems manifest at least a 2--3-fold increase in ATP concentrations over open ocean water. We also observed an apparent oscillation in the total ATP concentration which occurs on the order of 50 days. Three such cycles have been recorded: the peak ATP concentration increases with each successive cycle. The oscillations of ATP concentrations appear to be independent of pO_2 concentrations. There is a correlation between experimentally determined ATP concentrations and observed cell counts for similar systems. This suggests that ATP measurements may be used to estimate total biomass in closed systems, though further research is required to determine the accuracy of these estimates.

Our results show that closed ecosystems exhibit positive increases of pO_2, although much slower than previously described, and do not reach a steady state value even after 5--6 months. ATP concentrations are significantly higher than the steady state levels observed in open ocean water and appear to be cycling on the order of 50 days. This cycling appears to be independent of pO_2 concentrations, but a correlation does exist between ATP concentrations and observed bacterial counts for similar systems.

Acknowledgements

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