Limitations of ATP as a measure of microbial biomass

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Estimates of the total living biomass of micro-organisms on decomposing kelp detritus, calculated indirectly from the concentration of ATP, were compared with those obtained directly from cell numbers and volumes. Large overestimates in biomass were obtained from ATP × 250, and C:ATP ratios varied considerably with time. This variation in the C:ATP ratio limits the use of ATP as a measure of microbial biomass.

S. Afr. J. Zool. 1982, 17: 93 – 95

Beraming van die totale lewende biomassa van mikroörganismes op ontbindende seegras detritus, indirek bereken van die ATP-konsentrasie, is vergelyk met dié van gegewens verkry van selgetalle en -volumes. Hoë oorskatting in biomassa is verkry van ATP × 250, en C:ATP-verhoudings het aansienlike variasie getoon afhangende van tyd. Hierdie variasie in C:ATP-verhouding beperk die gebruik van ATP vir die meting van mikrobiale biomassa.

S.-Afr. Tydskr. Dierk. 1982, 17: 93 – 95

Estimates of the total living biomass of micro-organisms are vitally important in many ecological studies, which has consequently lead to the development of numerous techniques for assessing microbial biomass. The presence of non-living detrital material often associated with the micro-organisms complicates the measurement of biomass as it becomes necessary to either separate the two components (which is normally impossible) or to analyse for some constituent that is found in living cells only. Adenosine triphosphate (ATP) is found only in living cells, and providing the level in microbial cells is fairly uniform, the ATP concentration in any sample can be used to calculate total microbial biomass in terms of organic carbon or dry weight (Sorokin & Kadota 1972). A C:ATP ratio of 250:1 has been proposed by Holm-Hansen & Booth (1966) who state that this ratio is constant enough to permit reliable estimates of cell carbon from ATP measurements, and has since been used by numerous authors to calculate microbial biomass without any further investigations into the actual C:ATP ratios of their samples (Hamilton & Holm-Hansen 1967; Hobbie, Holm-Hansen, Packard, Pomeroy, Sheldon, Thomas & Wiebe 1972; Haines & Hansen 1979). Conflicting results have been obtained from ATP-based biomass estimates, with some authors reporting an excellent agreement between results calculated from ATP measurements and those estimated by direct microscopy (Sorokin & Lyutsarev 1978), whereas others eg. Eppley, Harrison, Chisholm & Stewart (1977), obtained significant differences between the two methods. All biomass results estimated from ATP measurements are naturally based upon the assumption that the C:ATP ratio in all micro-organisms remains constant with time, which has recently been queried (see Karl 1980). This paper provides evidence that C:ATP ratios do undergo significant changes, which will subsequently place severe limitations on the use of ATP for estimating microbial biomass.

Materials and Methods

Investigations into the use of ATP for estimating microbial biomass were conducted during a study on the heterotrophic utilization of kelp detritus (see Stuart, Lucas & Newell 1981). In short, incubation experiments were performed in sterilized conical flasks containing 0,5 g powdered kelp debris plus 1,5 l sea water collected from the kelp-bed. Control flasks containing 0,5 g

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Received 25 November 1981; accepted 9 March 1982

freeze-dried, ultraviolet sterilized detritus plus 1,5 l autoclaved sea water were incubated with each experimental flask. Samples of 10 ml each were removed by means of a sterilized syringe on days 0, 1, 2 and then on every fourth day until day 30. ATP was extracted from samples collected from day 2 onwards using the method of Holm-Hansen & Booth (1966). Each sample was filtered through a 0,2-μm nuclepore membrane filter which was immediately immersed in 5 ml boiling TRIS buffer (0,02 M; pH 7,75) for 5 min. All extracts were kept frozen at −20 °C until time of analysis. The ATP content of each extract was assayed by injecting triplicate 0,2-ml aliquots into equal volumes of enzyme preparation (buffered firefly-lantern extract hydrated with TRIS buffer) and after 17 s the peak height of light emitted was measured on a Packard Prias liquid scintillation counter (Barlow 1981). Standard curves covering the range of ATP in the samples were prepared, and blanks consisting of buffer only were run with each batch of samples. Estimates of the total living biomass of micro-organisms (mg C l^{-1}) using the ATP method were compared with those calculated directly from cell numbers obtained by acridine orange direct counts (AODC) (after Hobbie, Daley & Jasper 1977) and cell volumes calculated from scanning electron micrographs (SEM) as described by Linley, Newell & Bosma (1981). Twenty randomly-chosen fields were used to estimate microbial numbers, whereas cell volumes were calculated from at least 50 measurements of bacteria and 10 of protozoa. Formulae appropriate to the various micro-organisms were used to calculate the volumes (eg. formula for a sphere to calculate the volume of cocci and that of a cylinder for rods). Microbial biomass was calculated following Linley *et al.* (1981):

$$\text{Wet biomass (mg } l^{-1}) = \frac{N \times V \times \text{S.G.}}{10^6}$$

where N = number of micro-organisms ml^{-1} ($\times 10^6$); V = mean volume of the cells (μm^3); S.G. = specific gravity of the cells.

The specific gravity of bacteria was taken to be 1,07 (Doetsch & Cook 1973) whilst 1,042 was used for flagellates, 1,039 for ciliates, 1,043 for amoebae and 1,042 for choanoflagellates (Calkins & Summers 1941). Total dry biomass can be calculated using conversion factors of 0,23 for bacteria (Luria 1960), 0,2 for flagellates, ciliates and choanoflagellates and 0,1 for amoebae (A.J. Lastovica, pers. comm.), whereas the carbon equivalent can be estimated assuming a carbon content of 50% of the dry mass (Sorokin & Kadota 1972).

Results

During the first 10 days of incubation, bacteria were the only micro-organisms present in the experimental culture, but these were later succeeded by a large heterotrophic community consisting of flagellates, ciliates, amoebae and choanoflagellates (see Stuart *et al.* 1981). The concentration of ATP (ng ml^{-1}) on each sampling day as well as the C:ATP ratio calculated from direct observations is shown in Table 1. Sterile control samples contained no ATP for the first 10 days, which corresponded to the period that the culture remained entirely free from bacterial contamination, thus indicating that all the ATP present in the experimental flask was derived entirely from the micro-organisms and not from the kelp detritus itself. Between day 2–6 there was a phase of active bacterial growth, which resulted in C:ATP ratios that closely approximated the accepted ratio of 250:1. However, after the sixth day this ratio declined, reaching a value of 49:1 by the last day of the experiment.

Table 1 Concentration of ATP (ng ml^{-1}) ± standard error on each sampling day and C:ATP ratios calculated from direct observations of microbial numbers and volumes, assuming a carbon content of 50% of the dry mass (Sorokin & Kadota 1972)

Day	ng ATP ml^{-1}	± S.E.	C:ATP
2	0,306	0,022	220:1
6	8,810	0,430	215:1
10	10,349	0,254	201:1
14	8,910	0,770	186:1
18	6,838	0,351	132:1
22	4,800	0,285	107:1
26	4,501	0,064	67:1
30	2,723	0,259	49:1

Estimates of the total living biomass of the heterotrophic community obtained directly from cell counts (AODC) and volumes (SEM) were compared with biomass results calculated from ATP measurements (see Figure 1. C:ATP ratios of 250:1 and 100:1 have been used in an attempt to cover the possible range of biomass values (Christian, Bancroft & Wiebe 1975).

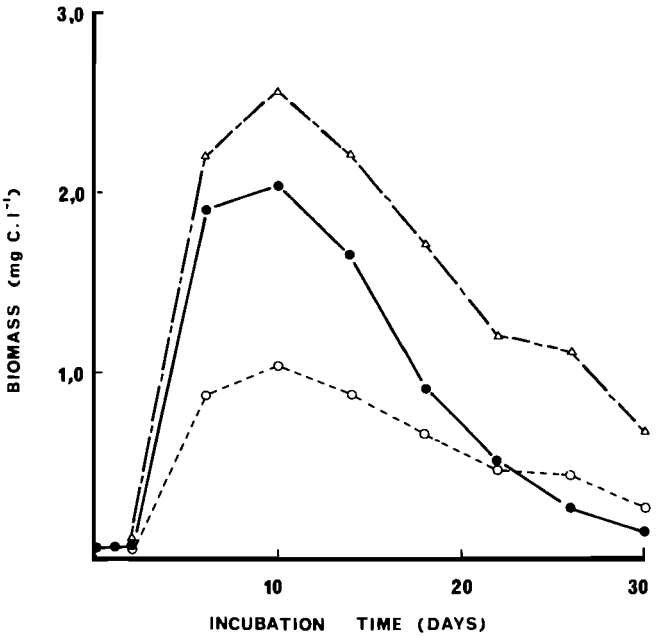

Figure 1 Total microbial biomass (mg C l^{-1}) estimated from direct observations of cell numbers and volumes (● ——— ●), and estimated from ATP concentration × 250 (Δ— ——— – —— Δ) and ATP × 100 (O– – – – O).

Discussion

While it should be noted that changes in the C:ATP ratio could be due to variations in the relationship between

total cell biomass and organic carbon, this is unlikely as it has been found that the carbon content of bacteria determined by CO_2 combustion or isotopically both gave similar results with a mean carbon content of 50 ± 5% of the dry mass (see Luria 1960). It appears that the C:ATP ratio of micro-organisms associated with decomposing kelp detritus varies according to the species composition as well as the age of the culture; the older the heterotrophic community, the lower the C:ATP ratio (see Table 1). Bancroft, Paul & Wiebe (1976) found that in pure cultures of bacteria the C:ATP ratio varied with the growth phase and physiological state of the bacteria, whereas Sakshaug (1977) demonstrated large variations in the C:ATP ratio with different species of diatoms. On the other hand Paerl & Williams (1976) found no such changes in the C:ATP ratio with different species of micro-organisms or under various nutrient conditions. Results obtained in this paper indicate that any biomass estimates based on a single conversion factor would be very inaccurate due to the fact that the C:ATP ratio changes to such an extent during decomposition.

Biomass estimates obtained using the accepted C:ATP ratio of 250:1 gave consistently higher results than those obtained from direct measurements, and from day 20 onwards ATP-based estimates were up to five times higher than those obtained from direct observations. It is possible that during the later stages of decomposition a slight overestimation of ATP could have been obtained owing to the presence of ATP in dead cells as discovered by Chappelle, Picciolo & Deming (1978), although this would not explain the large discrepancy observed during the earlier stages of decomposition. It is obvious from these results that unless one can accurately predict the C:ATP ratio of a sample, one cannot use the ATP content of a sample to obtain the total microbial biomass. Because the C:ATP ratio varies so considerably even within a single species, it is clearly impossible to ever predict the C:ATP ratio with any certainty, suggesting that ATP is an unsuitable measure of living biomass and that this method will probably become totally redundant. However, it is noteworthy that the same trends in biomass are shown by the ATP-based estimates as well as by direct measurements (see Figure 1), suggesting that ATP is nevertheless a convenient measure of changes in the relative abundance of micro-organisms.

Acknowledgements

I would like to thank Ann Gedye for her help and advice in the processing of the ATP samples, and Professor J.G. Field for reading the manuscript. This work was supported by a CSIR post-graduate bursary which is gratefully acknowledged.

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