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Problems in Determining Soil ATP

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Adenosine 5' - triphosphate (ATP) measurements are increasingly used to estimate microbial biomass in terrestrial soils, marine and freshwater sediments as well as in activated sludge. The sources of error with current methods may include: ATP adsorption to sediments particularly aluminum and iron oxides (LEE et al. 1971, ionic inhibition of ATP light emission which is dependent on species and concentrations (KARL and LaROCK 1975), ionic enhancement of ATP light emission particularly by Mn^{++} (LUNDIN and THORE 1975), and light emission caused by ADP or other non-adenylates after conversion to ATP by enzymic contaminants in the crude firefly tail preparation (WILDISH et al. 1979).

To estimate the extent of these sources of error an internal standard of ATP is added to the sediment. To estimate the efficiency of an extraction process in releasing ATP from microbial cells in the sample and inactivation of ATP-ases an internal standard consisting of live micro-organisms is added. The purpose of this paper is to draw attention to some inherent difficulties with both types of internal standard.

EXPERIMENTAL

Soil (Finnercy No. 2) was added to conical flasks containing 50 ml of sterile, distilled water. Soil characteristics were as follows: sand 82%, silt 16%, clay 2%, carbon = 1.99%, nitrogen = 0.31%, mg phosphate/ 100g = 288 and pH = 5.5. The following treatments were

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employed: A. distilled water blank, B. gamma irradiated (cobalt 60, 5 Mrads) soil, C. gamma irradiated (Colbalt 60, 5 Mrads), autoclaved soil, D. untreated control soil. In some cases E. coli with an ATP/cell = 3.5×10^{-10} µg and in the senescent phase, as determined by the method of WILDISH et al. (1979), were inoculated into the flasks. Experiments were begun by addition of the sodium salt of ATP, 35 µg/flask, followed by gentle hand swirling. After taking the first sample the flasks were shaken on an orbital shaker (100 orbits/ min). Samples (0.2 ml) were removed at known intervals and injected directly into boiling Tris buffer, pH 7.8, and gently boiled for 1-2 minutes. Extracts were cooled on ice and used directly in the determination of ATP by firefly bioluminescence assay. The following conditions applied: 0.2 ml sample, 0.2 ml Dupont luciferin-luciferase in 0.1 M morpholinopropane sulfonic acid, 0.01 M $MgSO_4$ and pH 7.4. Results were calculated on the basis of the integrated light emission curve for the first one minute after injection corrected for background light and differences in final extract volume.

CHEMICAL ATP KNOWN ADDITION

Results of a typical experiment (Table 1) show

TABLE 1

Percentage recovery of ATP from soil.

	Treatment			
	A	B	C	D
Soil wet weight, g	0	2.7	3.3	6.9
Time, minutes				
1	100	86	84	59
16	100	69	59	35
45	100	53	48	35
60	100	49	50	20

that the interference due to ATP adsorption plus ionic inhibition of light emission is a time-dependent process

reaching a plateau after 45 minutes. In treatment D, further loss of ATP is due to the presence of soil micro-organisms as found also by others (ERNST 1970; CONKLIN and MACGREGOR 1972). HODSON and AZAM (1977) show that with marine bacteria this is due to utilization involving dephosphorylization to adenosine and assimilation of phosphate moieties by the cell.

LIVE MICRO-ORGANISMS KNOWN ADDITION

In a further experiment (Table 2) senescent phase E. coli cells, were added to soil slurries containing ATP. The most marked effect was a time-delayed recoverability of ATP for treatment B2 (~60 min delay) and C2 (~30 min delay) which was relatively shortlived. The most likely explanation for this is that the senescent E. coli cells assimilate ATP degradation products

TABLE 2

Percentage recovery of ATP from soil. E. coli (1=4×10^8 2 = 4×10^9 per flask) added at 60 minutes. Percentages corrected for microbial ATP added assuming ATP/cell remains constant.

	Treatment			
	B1	B2	C1	C2
Soil weight, g	6.4	7.2	6.6	5.9
Time minutes				
1	90	71	83	86
13	44	45	38	34
26	23	23	30	16
55	28	28	23	21
65	25	18	33	20
75	21	11	34	33
90	21	22	31	81
125	17	64	-	24

and change their adenylate pool from AMP to ATP as a result. This explanation requires that the increased level of ATP is intracellular. The total intracellular ATP potentially available: $3.5 \times 10^{-10} \times 4 \times 10^9 \times 30 =$

42 µg is 120% of the added ATP and hence this is a plausible explanation. The value 30 is the experimentally observed range of ATP concentrations in batch cultures from senescent to early stationary phase (WILDISH et al. 1979).

Addition and mixing of micro-organisms to soil, without addition of chemical ATP, as is frequently recommended, or drying of microbial culture solution on glass beads (KARL and LaROCK 1975), would cause considerable stress to the cells. Stress is known to result in a rapid decline in adenylate energy charge (NIVEN et al. 1975) and thus ATP concentration. This may explain why we and others have obtained variable and low recoveries of bacterial ATP after addition to soils.

CONCLUSIONS

Methods for determining extraction efficiency from soils contain sources of error which make such estimates impossible. The method is thus limited to a relative one yielding data which can only be compared when exactly the same technique is followed. Great care must also be taken to avoid microbial dephosphorylation of ATP when assessing the interfering effects involved in the assay by known addition of chemical ATP.

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