PROSOPE

H. CLAUSTRE : head of mission and project leader

BACTERIAL PRODUCTION : FRANCE VAN WAMBEKE

Bacterial production during Prosope cruise

METHOD

Bacterial production was estimated from [4,5-^{3}H]-leucine incorporation with the centrifugation method (Smith & Azam, 1992) during PROSOPE cruise.

A mixture of from [4,5-^{3}H]-leucine (Amersham, 155 Ci mmole^{-1}) and cold leucine was added to achieve final concentrations of 16 and 4 nM, respectively in 1.5 ml samples. Two duplicates and one TCA-killed blank were incubated dispended in 2 ml screw cap microcentrifuge tubes in the dark at the in situ temperature, for two hours which was insurred to satisfy linear incorporation with time.

Incubations were stopped by additions of 50 % TCA to reach a final concentration of 5 % TCA. Fifty µl of a Bovine Serum Albumin solution was added as co-precipitant for optimal precipitation of the proteins (final concentration of the BSA 100 mg l^{-1}). Then, a first centrifugation at 16 000 g for 5 min was run in a SIGMA centrifuge refrigerated at 15°C, the supernatent was discarded and 1.5 ml of 5% TCA were added. The samples were vortexed vigorously and centrifuged again. The supernatent was discarded and 1.5 ml of PCS Scintillation cocktail (Amersham) was added. The microcentrifuge tubes were hold in special plastic holders of 20 ml for scintillation counting.

Contrary to what was obtained with the method developped by Smith & Azam (1992) we were unsuccessfull to recover the whole TCA precipitate in the pellets if BSA is not added. With centrifugation technique, bacterial production measured with BSA added were on average 96 ± 13 % (n=21) of those obtained with filtration technique, whereas only 73 ± 9 % were reached without addition of BSA.

Radioactivity was analyzed by a Packard LS 1600 Liquid Scintillation Counter, and corrected with external standard and quench curve. Preliminary experiments of concentration kinetics showed that isotopic dilution was negligible with the 20 nM added. We used conversion factors as stated in Kirchman (1993) with isotopic dilution = 1, i.e. yielding 1.5 kg C per mole leucine incoprporated for both vertical profiles and bioassays.

References

Kirchman, D.L. (1993). Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: P.F. Kemp, B.F. Sherr, E.B. Sherrand J.J. Cole (P.F. Kemp, B.F. Sherr, E.B. Sherrand J.J. Cole(P.F. Kemp, B.F. Sherr, E.B. Sherrand J.J. Coles.), Handbook of methods in aquatic microbial ecology, Boca Raton, Lewis, pp. 509-512.

Smith, D.C., and Azam, F., 1992. A simple, economical method for measuring bacterial protein synthesis rates in sea water using 3H-Leucine. Marine Microbial Food Webs, 6: 107-114.