

## REVIEW OF THE MAIN AVAILABLE METHODS FOR ESTIMATING THE MICROBIAL BIOMASS AND ITS ACTIVITIES

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In many fields of Soil Science, there is a need to have a simple and accurate method for estimating soil microbial biomass. Soil microflora which represents between 2 and 4 % of carbon and 4-8 % of soil nitrogen, takes a big place in nutrients cycles of agronomical importance (C, N, P, S) : The pool of mineral nutrients stocked in this alive compartment of organic matter changes with the fluctuation of microbial populations led by farmer or environment actions. Its role in degradation of organic matter or biocides are related to the problems of soil or environment preservation. The authors make a critical review of the main methods available for the microbial biomass estimation.

— Measurements by direct observation (7, 8, 11, 12) with optical or electron microscopy are often considered as references methods. Continual improvements of these techniques have significantly increased the accuracy of measurements. Besides the fact that direct observation of microorganisms suppose too long and tedious handlings, calculation of biovolume and biomass include coefficients which are marred with mistakes because characteristics of microflora in situ is unknown.

— Counting methods (M.P.N.), obtained by plate or liquid cultivation after inoculation with suspension-dilution of soil, are probably the more usual methods. Often, these techniques which have significant improvements, are not very precise and reproductive ; they give a number of viable propagules, and so underestimate the number of bacteria. They are partly inadapted to fungi counting.

— Quantitative analysis of the compounds specific of living organic matter suppose that the compounds are specific of microbial cells and their extraction is quantitative. Analysis of cells walls and membranes constituents (5, 6) (muramic acid, hexosamines, lipopolysaccharides) raises the question of the extraction and purification, because similar compounds exist in non-living organic matter. Also analysis of D.N.A. depends on these factors, but the study of specific nucleic bases can be precise its origin (microbes or plants). Numerous methods have been proposed to measure ATP (1, 3, 4, 14, 15) which is characteristic of living cells. Except the questions of extraction and interferences with soil constituents, ATP can't be expressed sometimes in terms of biomass, because the value of the yield coefficient between ATP and biomass for microbes in situ is unknown.

— Microbial activities measurements (10) have been often used as indexes to study dynamic of microbial populations. Global or specific, microbial activity is not inevitably related to a size of population. Most experiment on enzymatic activities show that measurements, performed in artificial conditions, generally express a potentiality which depends on experimental conditions. Promising way is the use of more global tests as short-term glucose respiration (13) which are correlated with biomass, but which have the disadvantage of the discrimination to a few microbial populations.

— Biocidal methods (2, 9) consist to kill soil microflora with biocides (generally  $\text{CHCl}_3$ ) and to incubate fumigated soil after inoculation. Mineral carbon and nutrients released by dead cells mineralization are proportional to soil biomass. Except methodological questions, this method is very easy, precise and can be use with isotopic tracers. On the other hand this approach is not adapted with the measurements of short-term fluctuations.

It is difficult to compare the approaches described in this review. They answer more and less directly to a precise aim and the results obtained for a question with different methods are rarely the same. The expression in term of biomass is sometimes hazardous for certain methods.

In reality, they can be classified in two groups :

— The first where methods can be expressed directly or not to biomass : direct observation, biocidal methods.

— The second, where methods measure the activities of the total or a part of the microflora.

Endly, for the questions wich are us concerning at the moment (study of nutrients cycles), the incubation-fumigation method will prove for the future an interessant tool.

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