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EVALUATION OF THE HETEROTROPHIC ACTIVITY IN WATERS BY MICRO-ANALYTICAL METHODS   .

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ABSTRACT - The radiochemical methods proposed for evaluating heterotrophic activity in waters have been severely criticized in recent years because they are not considered to be sufficiently realistic. Thus, the possibility of directly measuring the heterotrophic consumption of naturally occurring Organic Carbon (OC) in fresh water samples incubated under controlled conditions has been evaluated. The analytical performances of two OC analyzers utilizable for this purpose are discussed here, and some examples of the results obtained by the direct measurement of OC consumption are presented. The consumption rates thus measured range from 11.6 to $50.2 \mu gC/l.h$. Although the technique discussed here is less sensitive than radiochemical methods, the few assumptions needed when making a direct measurement largely offset this loss of sensitivity.

Key words : heterotrophic activity, organic carbon cycle, organic microanalysis.

RÉSUMÉ - Les méthodes consistant à estimer l'activité hétérotrophe à partir de mesures d'incorporation de substrats radioactifs ont été critiquées dans ces dernières années parce que insuffisamment proches de la réalité. Pour obtenir une estimation plus représentative, on a essayé de mesurer directement la consommation hétérotrophe du carbone organique naturellement présent dans les eaux sur des échantillons incubés en conditions contrôlées. Les potentialités analytiques de deux instruments utilisables pour ce but sont ici illustrées et des résultats obtenus par la mesure directe de la consommation hétérotrophe du carbone organique sont présentés. Dans les lacs étudiés on a mesuré des vitesses de consommation du substrat naturel comprises entre 11.6 et 50.2 μg C/l.h. Bien que cette technique soit moins sensible que les méthodes radiochimiques, elle n'implique pas des hypothèses difficiles à vérifier et cet avantage compense largement sa moindre sensibilité.

Mots clés : activité hétérotrophe, cycle du carbone organique, microanalyse organique.

INTRODUCTION

It is essential for those concerned with the heterotrophic processes within the Organic Carbon (OC) cycle to know the total OC flow through the decomposer microheterotrophs. For this purpose the methods which evaluate the microbial heterotrophic activity by tracing the fate of specific labelled substrates are inadequate. The main reason for their inadequacy is due to the fact that they provide an indirect, although very sensitive, measurement. A comprehensive review of those methods and of the problems involved in their use was published by van Es and Meyer-Reil (1982). In order to evaluate directly the flow of naturally occurring OC through the microbial heterotrophic population, the heterotrophic consumption of the natural pool of OC in lake water was followed by microanalytical methods. This approach is based on the assumption that modern OC analyzers are sensitive enough to allow the direct measurement of heterotrophic OC utilization under controlled conditions. As a necessary premise to this approach, the analytical capabilities of the instruments used will be illustrated.

The heterotrophic OC consumption measurements performed up till now have been carried out with a total OC analyzer based on high temperature oxidation of 100 μl of sample (mod. 400/P, Carlo Erba). More information about this instrument and the improvements of it can be found in Bertoni et al. (1982). Mainly because of the extensive routine maintenance required by the above unit, quite recently I started to use a micro-processor controlled total OC analyzer. It is based on U.V. oxidation of a 3 to 8 ml sample carried by an oxygen-sodium persulphate stream (mod. 1850, Astro).

Although with this latter unit no OC consumption measurements have been made up to now, the first results with lake water samples allow a comparison of the two instruments. In Figure 1, the results are plotted which were obtained by analyzing 20 water samples from Lago Maggiore with a Dissolved Organic Carbon (DOC) concentration ranging from 800 to 2000 μgC/l. Ten samples were analyzed with the 400/P analyzer and the other ten with the 1850 analyzer. On each sample ten analyses were made and the average values were plotted along with their 95% confidence limits. From these plots the latter instrument clearly appears to be more precise. In fact it has a relative standard deviation ranging from 0.6 to 2.2%, while the relative standard deviation of the former one ranges from 3.3 to 3.9%. The minimum measurable OC concentration variation can be calcula-ted from the difference between the closest possible means whose confidence limits do not
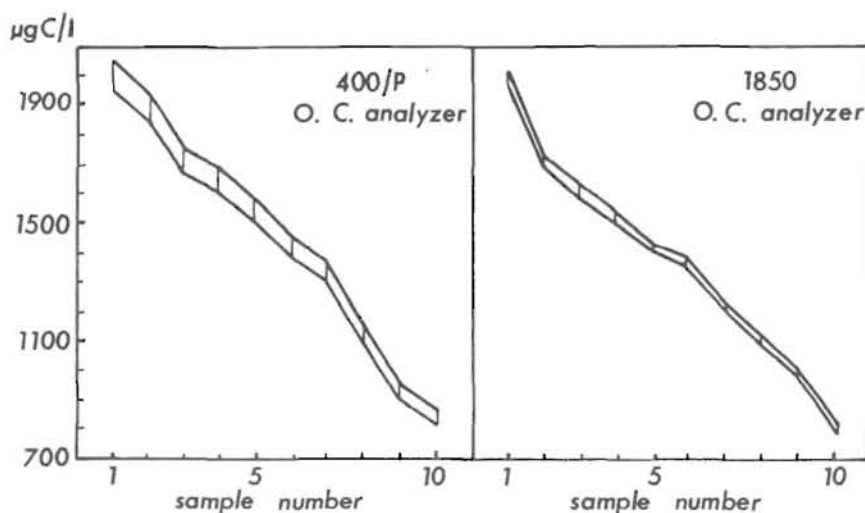

Figure 1 : Comparison between 400/P and 1850 OC analyzer.

overlap. Accepting this it can be concluded that, at the 95% confidence limit, the 400/P allows the measurement of a change in DOC concentration of about 100 μgC/l at the upper limit and of about 45 μgC/l at the lower limit of the concentration range shown in Figure 1. The corresponding quantities are reduced to 65 and 20 μgC/l when using the 1850 analyzer. Moreover the blank values obtained with bidistilled water are different for the two instruments. Based on over 20 analyses, the 400/P analyzer gives an average blank of $409 \pm 16.2 \mu$gC/l and the 1850 analyzer one of $220 \pm 4.6 \mu$gC/l, respectively. This means, accepting the region of quantification for our analyte to start from 10 st.dev. above the blank signal (ACS Committee and Subcommittee, 1980), that the high tempe-rature analyzer can measure OC concentrations above the limit of 569 μgC/l while for the U.V. analyzer this limit is lowered to 266 μugC/l. As for the possibility of working

with sea water, although I have not tested such samples, I believe that it would be impractical to use the high temperature oxidation unit with sea water because the salt content strongly reduces the life span of oxidation catalysts and quickly cloggs the sample injection system. The U.V. oxidation unit, on the other hand, appears to be more useful for salt water, although a lack of precision was observed (Chioetto, pers. comm.).

MEASUREMENT OF HETEROTROPHIC ACTIVITY

The direct measurements of heterotrophic consumption of naturally occurring OC have been performed on samples of 10 l always collected between 9 and 10 a.m. with a plastic Van Dorn type bottle. They were immediately filtered through 10 μm mesh plankton net to remove larger heterotrophs and larger particles and were incubated in the dark at lake temperature in glass bottles under gentle stirring. The OC concentration in the samples was measured continuously, running 10 analyses per hour.

From previous experiments, performed in the above described way on lake water samples both untreated and treated with mercuric chloride, it was possible to conclude that the consumption of naturally occurring organic substrate (particulate OC with size < 10 μm and dissolved OC) can be ascribed exclusively to the metabolic activity of heterotrophs while no measurable physico-chemical losses of organic matter took place (Bertoni et al., 1982). Such experiments lasted for three days or more, to follow the pattern of heterotrophic OC consumption. However, in this paper I will consider only the OC decrease measured over 12 hours of incubation. This period of time is generally long enough to obtain a significant OC decrease and it is short enough not to change natural conditions for microbial life and activity. Thus results of OC consumption may be directly related to naturally occurring events.

The experiments chosen to exemplify this kind of measurement were performed on samples taken from the euphotic layer of Lago Maggiore and of Lago di Mergozzo. General information on these lakes is given by de Bernardi et al. (1984); a study on the OC cycle in Lago Maggiore has been published by Bertoni and Callieri (1982). The results of the experiments are presented in Figures 2 and 3. The average values of 10 analyses for each hour of incubation are plotted with their 95 % confidence limits. The overall decrease of OC is 140 μgC/l in Lago Maggiore and 603 μgC/l in Lago di Mergozzo. As one might
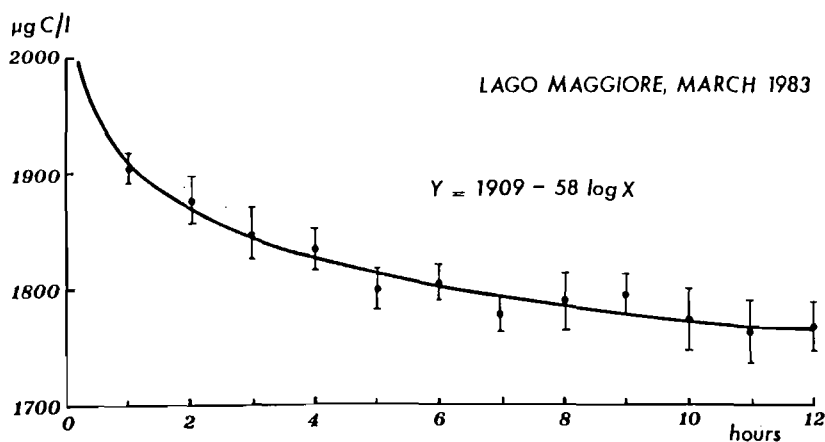
Figure 2 : Decrease in Organic Carbon concentration during the dark incubation of a lake water sample from Lago Maggiore.
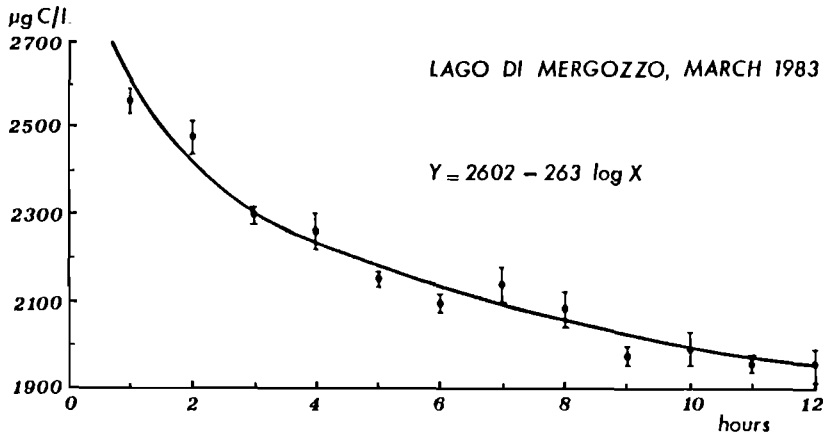
Figure 3 : Decrease in Organic Carbon concentration during the dark incubation of a lake water sample from Lago di Mergozzo.

expect, the OC concentration decreases logarithmically ; thus the significance of the decrease has been evaluated by using the logarithmic regression model. In both lakes the decrease of OC concentration turned out to be statistically highly significant, with $r = 0.978$ for Lago Maggiore and $r = 0.985$ for Lago di Mergozzo.

In order to compare these findings with the results from other methods which express heterotrophic activity in terms of uptake velocity, a rough evaluation of the mean velocity of OC consumption was made. For the two experiments presented here the OC consumption rate turns out to be $11.6\,\mu gC/1.h$ for Lago Maggiore and $50.2\,\mu gC/1.h$ for Lago di Mergozzo. These two values are close to the minimum and maximum rates found in some twenty OC consumption measurements performed from 1981 to 1983. In three cases during this period, working with samples taken in January and February, no significant OC decrease occurred.

It should be noted that the variations in OC concentrations thus measured result not only from heterotrophic breakdown but also from activities such as heterotrophic growth, grazing, autolysis, etc...Nevertheless, since a decrease in OC concentration is generally observed, the catabolic heterotrophic processes turn out to be the prevalent ones.

A further example of what can be obtained by microanalytical methods is presented in Figure 4.
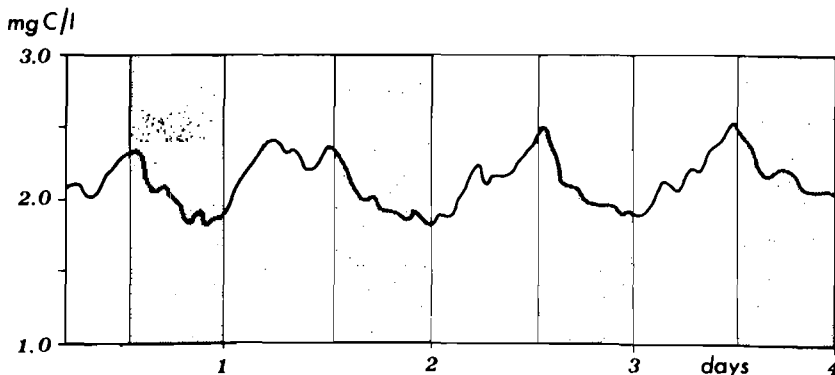

Figure 4 : Variations in Dissolved Organic Carbon concentration during the light-dark cycle in a lake water sample from Lago di Mergozzo (dark periods are shaded).

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The results are plotted from an experiment performed in March 1983 on Lago di Mergozzo waters to evaluate the possibility of directly measuring the variations in DOC concentration caused by the autotrophic - heterotrophic coupling. A 20 l lake water sample was taken in the morning (10 a.m.) at a depth of 2 m and immediately filtered through a 126 μm mesh plankton net to remove larger filter feeders whilst not noticeably altering the majority of the algal population. The sample was then incubated at lake temperature in a 12 hours light-dark cycle. An on-line filter (Whatman GF/C precombusted) placed between the incubation bottle and the analytical instrument made it possible to continuously measure the DOC concentration in the sample during the incubation time, running 10 analyses per hour. Although only the results from the first 4 days of incubation are shown here, the experiment lasted for 7 days. The microcosm collapsed on the 6th day and no significant variations in DOC concentration occurred after this time. It is not the purpose of this paper to undertake a detailed discussion about autotroph-heterotroph coupling. Here I want only to stress that, at least in a mesotrophic water body, the influence of such coupling on DOC concentration is directly measurable.

CONCLUSIONS AND OUTLOOK

The OC consumption rates estimated from the direct measurements which were performed in Lago Maggiore turned out to be higher by a factor of 10 compared to the rates measured in the same water body with labelled organic substrate uptake method (Melchiorri *et al.*, 1975; Bertoni, 1983). The rates obtained by the direct measurements are of the same order as those measured in German lakes with the dark assimilation method (Overbeck, 1981) and from sedimentation trap data (Ohle, 1976). Furthermore a direct estimation of heterotrophic uptake of natural DOC at three stations in the North Atlantic revealed an average rate of 9.8 μgC/l.h (Sieburth *et al.*, 1977). These findings would support the consideration (Overbeck, 1983) that the heterotrophic activity evaluated by labelled organic substrate uptake method is an underestimation of the whole heterotrophic activity in an ecosystem. If this is true, the heterotrophic utilization of the pool of natural organic substrate may be large enough to produce, in a few hours, variations of the substrate concentration which are large enough to be measured by the more recent analytical instrument both in lake and sea water.

The direct measurement of the heterotrophic activity by microanalytical methods, of course, also suffers from some drawbacks. In particular I would like to mention the longer incubation time required compared to the radiochemical methods. This can be overcome by avoiding the incubation itself by using an automatic sampler that, during a preset period of time, takes samples at hourly intervals during the night, fixing them immediately (Bertoni, 1985). If previous knowledge of the hydrodynamics or the use of a free floating buoy guarantees that always the same water mass is sampled, the series of samples collected by such an instrument can permit the construction of an OC concentration variation curve from which the heterotrophic activity in the water mass can be estimated. If that instrument is suspended in the euphotic layer to take samples during a night-day cycle, the variations in OC concentration owing to the autotroph-heterotroph coupling can also be studied. Sampling an open system by using the above mentioned instrument presents obvious advantages compared to the closed incubation systems.

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