PRODUCTIVITY MEASUREMENTS OF ALGAE USING 14C-TECHNIQUE AND OXYGEN TECHNIQUE UNDER THE INFLUENCE OF INORGANIC NITROGEN SOURCES

BY
LOKMAN SHAMSUDIN

UNIVERSITY OF SOUTHAMPTON
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Lihat sebelah

INTRODUCTION

It is felt that there is an increasing need to have a first hand knowledge on the primary productivity of the ocean as this is related to the abundance of zooplankton and other higher organisms in the marine food chain. In the field of fisheries, such information is of immense importance. The earliest and the most popular method for measuring productivity was to follow the changes in the dissolved oxygen concentration in a water sample using the Winkler titrimetric method. One obvious disadvantage using this conventional oxygen method, which is very insensitive, is that for long incubation period artifacts due to bacterial growth may arise. However, refinement and modification to this method by Bryan et al., (1976) using whole bottle technique, make it possible to get precise and accurate measurements.

Steemann Nielsen (1952) introduced the ^{14}C-technique for the measurement of photosynthesis and this has provided us with a more sensitive and efficient method. Later it was realised however that the interpretation of the ^{14}C-technique itself is a complex matter. For a long period of incubation the ^{14}C-technique gives a significantly lower value for photosynthesis than the oxygen method. This is due to respiration of assilimilated-^{14}C. Consequently, a correction factor was introduced to account for this respiratory loss and Steemann Nielsen (1966) assumed that the obtained result represented gross photosynthesis. Ryther (1969) however argued that the ^{14}C-technique gave a value somewhere between gross and net photosynthesis and that this was the rate of real photosynthesis less the rate of respiration of all organisms in the water under consideration

At present the most popular methods for photosynthetic measurement are those involving parameters such as oxygen production and carbon dioxide fixation derived from algal photosynthesis. The ^{14}C - technique is widely used nowadays especially in oceanic works and if not used correctly, the results obtained can be misleading. As an example when De Vooys (1979) worked on the global oceanic phytoplankton production using ^{14}C - technique he found that his result was approximately 1.5 times greater than that obtained by Platt and Subba Rao (1975) using the same method. Nowadays, to improve the accuracy for productivity measurement, liquid scintillation counters are commonly used for sample counting and also for isotope standardisation. Alternatively, an end window proportional counter may be used if appropriately calibrated. The calibration is done by counting selected number of filters with sample using the end - window counter first and subsequently these same filters are counted again using a liquid scintillation counter.

It had been reported by earlier workers that the productivity measurement by the ^{14}C - technique gives an underestimate as compared to that of the oxygen technique by a factor of approximately two. Many suggestions have been put forward to explain the difference, the principal concepts being related to the nature of nitrogen source available to the phytoplankton cells. The mistake made by many of the earlier workers was in assuming that there is a constant photosynthetic value when determining primary productivity. Another potential error occurs during the

calibration of $NaH^{14}CO_3$ in fluor owing to difficulties associated with aqueous phase from the gel (Inverson et al.,1976). With regards to comparison between the oxygen and the ^{14}C - techniques, Ryther et al., (1971), working on an upwelling region of Peru that there was a two fold difference between the two methods. Similarly, Lande (1973) found there was a poor correlation between the two methods, especially at low photosynthetic rate when the precision of the oxygen method dwindled.

The different usage of terminology can be of great confusion. Strickland (1960) made a clear distinction between net production and net primary production, an appreciation of which is essential when interpreting and dicussing the two methods of measuring plankton production, ie the ^{14}C - technique and the oxygen technique. Gross primary production rate is defined as the gross rate of autosynthesis of the constituents of plant material in water. Actually it is the gross rate of algal photosynthesis including losses due to respiration and organic excretion. Use of the gross rate of photosynthesis is open to criticism because it cannot be measured in practice by both techniques since the dark bottle does not make allowance for the photoinhibition of respiration and this will result in overestimation of oxygen production during algal photosynthesis. It has been recognised that gross production measurement by the oxygen technique may contain error due to photorespiration.

The net primary production rate is defined as the

net rate of autosynthesis of the organic constituents of plant material in water. This does not include respiration of organisms other than the primary producers. The ^{14}C-technique can easily be used to measure net production but this assay is not possible by the oxygen technique because of active heterotrophic organisms are present in the sample. Alternatively, the net production rate can be defined as the net rate of production of plant organisms under the influence of all environment factors. Such a rate can be measured by the oxygen technique but it cannot be measured in natural populations by the conventional ^{14}C-technique. It should be made clear that the oxygen technique and the ^{14}C-technique measure very different overall processes which are inter related. In other words, the ^{14}C-technique measures the carbon flux involved into the system of the living cell while the oxygen technique measures the amount of energy flux into the system. The value of net production using the oxygen method can be negative while that using ^{14}C-technique cannot be negative.

The photosynthetic quotient is taken as the molar ratio of the rate of oxygen production to the rate of carbon dioxide utilisation. Usually the measurement for oxygen production can be carried out by the whole bottle oxygen method while the amount of carbon dioxide utilised is measured as the equivalent amount of carbon fixed. For economic reasons, in the present study the ^{14}C-samples were counted using the thin-window proportional counter which was initially cross-calibrated with a liquid scintillation counter. The liquid scintillation counter was used for the standardisation of $Na^{14}CO_3$ stock. The efficiency of the machine was determined by using ^{14}C-hexadecane of known specific - activity.

Another terminology which is frequently met is the C/N photosynthetic assimilation ratio. This is defined as the ratio of carbon assimilation to that of nitrogen assimilation which comprises of nitrogen nitrate, nitrogen nitrite and nitrogen ammonium. Usually a photosynthetic quotient value of 1.25 is taken for the purpose of calculation as this presumes that the algal population utilises competatively considerable amounts of both nitrate and ammonium. The total photosysthetic nitrogen utilisation is actually the difference between total nitrogen in the dark and total nitrogen in the light, $\Sigma N_D - \Sigma N_L$. The assumption has to be made that nitrification and respiration remain the same in the dark and light bottle.

Many workers have reported major or minor factors which may bring about an effect on the photosynthetic quotient value either increasing or decreasing it. These factors are listed as given below,

a). respiration of assimilated ^{14}C.

b). reassimilation of respired ^{14}C

c). exudation of dissolved organic matter

d). bacterial activities in dark bottle

e). photorespiration

f). type of organic substances synthesised, eg lipid, protein etc

g). nitrogen source either nitrate or ammonium available

Of the factors given above, it is found that the major effect on the photosynthetic quotient value is the source of nitrogen, either nitrate or ammonium available to the phytoplankton cells. The present project was designed to elucidate further the effect of the form of available nitrogen on the photosynthetic quotient.

The planned programme of study in carrying out the project included first and foremost the calibration of both the counters, namely the end window counter and the liquid scintillating counter, and this was followed by the standardisation of $NaH^{14}CO_3$. The other planned programme of study included the following as given below,

a). The change in P.Q. values with time using pure culture grown in f/2 medium.

b). Productivity measurements on pure cultures of algae in media containing various nitrogen sources.

c). Productivity measurements on natural populations of samples taken from Southampton water.

d). Productivity measurements on isolates taken from Southampton Water.

e). Utilisation of carbon and nitrogen by sample taken from Southampton Water.