Initial results on the use of pH-stat titration (AIDA method) for the measurement of algal photosynthesis

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Abstract

The present work deals with the development of a method, named AIDA (algal inhibition detection analysis) for the measurement of algal photosynthesis by pH-stat titration. According to the pH probe signal, a titrator doses a saturated solution of CO_2 to maintain the pH at a set-point value, buffering variations induced by photosynthetic CO_2 consumption. The CO_2 titration curve, after an initial lag Phase, is well interpolated by a straight line, whose slope is proportional to the photosynthetic CO_2 uptake, until photosynthesis becomes limited probably due to nutrients limitation. Preliminary experimental results confirm that, under comparable test conditions, activity estimation was well repeatable. Moreover, the method could sense the photosynthetic response to nutritional changes (N:P ratio) and to the presence of toxicants. Therefore, AIDA shows potential applicability for both limnological studies and biomonitoring.

Keywords: photosynthesis, titration, pH-stat, activity, toxicity

Introduction

There is a growing interest for the assessment of algal photosynthesis for toxicological and for limnological investigations.

With respect to toxicity studies, toxicological analyses of industrial wastewater before discharge in water bodies are more and more frequently required. In Italy, the recent law on water protection (D.Lgs. 152/99) requires that the toxicity of a wastewater is tested by biomonitoring with organisms of various trophic levels, such as *Daphnia magna*, *Ceriodaphnia dubia*, *Selenastrum capricornutum*, bioluminescent bacteria, etc. According to the law, wastewater is acceptable for discharge into surface water when bioassays point out toxicity symptoms in no more than 50 % of the exposed organisms. For public sewers the limit is raised to 80%. Internationally, the most widely adopted algal inhibition test is the OECD (1981a) method, where algal growth is assessed by cell count normally made by a Coulter counter.

With reference to limnological studies, phytoplankton is particularly important since it is responsible for primary production in water and forms the first ring in the trophic chain of aquatic ecosystems. In these investigations, photosynthetic activity could be an important parameter which, however, is often disregarded due the non-availability of simple and reliable determination methods. In general, primary production is evaluated either by the oxygen method of dark and transparent bottles (Gaarder and Gran, 1927), or by the estimation of ^{14}C incorporation (Steemann-Nielsen, 1952). However, both methods involve practical problems due to the need for maintaining bottles submerged in the lake at a fixed depth, for a defined time interval, and results are not always reliable. The second method involves a more scientific approach, but it requires specific skills and equipment (Harris, 1986; Vollenweider, 1974). At present, in studies on eutrophication, primary production and algal blooms are evaluated on the basis of indirect parameters such as water transparency (Secchi disk) or chlorophyll concentration, which is a difficult analysis, the results of which are greatly affected by the extraction procedure and by the algal species.

This paper presents a new method to measure algal activity based on the estimation of CO_2 consumption by pH-stat titration. This is achieved by means of an instrument originally designed to evaluate microbiological activities, such as nitrification (Ramadori et al., 1980, Massone et al., 1998), on the basis of the pH variation they induce.

Materials and methods

AIDA: Principle of the method

The AIDA (algal inhibition detection analysis) method has been developed to measure activity and inhibition of photosynthetic micro-organisms. The principle of operation exploits the ability of these micro-organisms to increase the suspension pH by converting an acid substrate (CO_2) into a neutral product (biomass), according to the gross photosynthesis equation:

$$6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2 \qquad (1)$$

The method requires retaining constant pH by means of an automated titrator. As a matter of fact, in pH-stat conditions and in the absence of other pH-affecting reactions, the amount of CO_2 titrated (as CO_2- saturated solution) used to buffer the pH increase, equals the amount of CO_2 consumed by photosynthesis. Moreover, the rate of titrant addition is proportional to algal activity. Additionally, this method allows the measurement of algal activity at constant substrate (CO_2) concentration.

The procedure is registered at the Patent Office of Politecnico di Milano (*Titrimetric Method to Assess Activity and Inhibition of*

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