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Quantitative and qualitative evaluation of phytoplankton communities by trichromatic chlorophyll fluorescence excitation with special focus on cyanobacteria

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Abstract

We present results that were obtained with a newly developed fluorometer, the 'PhytoSensor'. They are based on multi-wavelength excitation of chlorophyll fluorescence to detect the phytoplankton biomass and to identify main taxons (among cyanobacteria, green and brown microalgae). A method to evaluate the photosynthetic potential of the phytoplankton was established. Attention was focused on the measurement of the cyanobacterial biomass. A modelling to distinguish between the two spectral groups (blue and red) of cyanobacteria as a function of their pigments and physiological status is proposed. The main innovation of the device results in the recording of the fluorescence induction kinetics of the phytoplankton to confirm and refine the evaluation of the taxonomic composition. The PhytoSensor abilities were compared with pigment analysis, commercial fluorometers, particle and microscopic counting and identification. The PhytoSensor has been used with success to monitor the dynamics of phytoplankton in drinking-water supply reservoirs in Southeast Asia.

Keywords: Bloom monitoring; Chlorophyll fluorescence; Fluorescence induction kinetics; Phytoplankton; Southeast Asia; Water supply

1. Introduction

Chlorophyll *a* (Chl *a*) is an ubiquitous photosynthetic pigment present in all eukaryotic (algae) and prokaryotic (cyanobacteria) phytoplankton organisms. In vivo Chl *a* fluorescence has became an increasingly important tool for the assessment of both biomass and

Abbreviations: Chl, Chlorophyll; FIK, Chl *a* fluorescence induction kinetics; F_0 and F_m, minimal and maximal levels of chlorophyll fluorescence emission, respectively; HPLC, high-pressure liquid chromatography; LED, light-emitting diode; PP, Photosynthetic potential; PS II, Photosystem II

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photosynthetic activity of phytoplankton in situ (Falkowski and Raven, 1997). Very sensitive techniques have been developed (Wilhelm, 2003) among which multi-wavelength fluorometers that allow in vivo and/or in situ taxonomic identification. The current systems are based on the discrimination of 'spectral groups' of phytoplankton (see Beutler et al., 2002). These groups have specific accessory pigments which absorb light efficiently in different ranges of the visible light spectrum (Millie et al., 2002). When they absorb a photon, these pigments become excited and they transfer their excitation energy from pigment to pigment to the Chl *a* molecules, the terminal acceptor of the excitation transfer channel, which emit fluorescence (reviewed in Krause and Weis, 1991). By use of light sources of different excitation wavelengths, an evaluation of the systematic composition of a mixed phytoplanktonic community can be obtained. With the progress in light-emitting diode optic, the feasibility of such an approach has been demonstrated (Schreiber et al., 1993). Two devices, the Phyto-PAM (Heinz Walz, Germany) and the Fluoroprobe (BBE Moldaenke, Germany) (Beutler et al., 2002), are currently available. They have from four to five excitation wavelengths and can detect from three to four spectral groups (green algae, cyanobacteria, 'brown microalgae' (mainly diatoms and dinoflagellates) and a 'mixed group' (cryptophytes)). Recent approaches have been developed to refine the discrimination between the two spectral groups of cyanobacteria (blue and red) and the cryptophytes (Beutler et al., 2003, 2004).

One of the applications of these instruments is the monitoring of the algal and cyanobacterial proliferations (so-called blooms) in water resources used as drinking-water supplies and/or recreational areas (Leboulanger et al., 2002). Blooms are due to eutrophication and more than 40% of worldwide lakes are eutrophic (Briand et al., 2003). Because they can impair the water quality, blooms have been of major concern for the water resource managers (Mouchet and Bonnelye, 1998; Pitois et al., 2000). One of the sources of awareness is the synthesis of toxins by cyanobacteria which are responsible for human health hazards (Haider et al., 2003).

The present work is based on the measurement and interpretation of in vivo Chl *a* fluorescence emission by freshwater phytoplankton. The fluorometer, the 'PhytoSensor', uses the trichromatic excitation of Chl *a* fluorescence to differentiate three spectral groups: green and brown microalgae and cyanobacteria. A mathematical modelling based on pigment content and physiological status differences to discriminate between the blue and red groups of cyanobacteria is tentatively presented. A rough evaluation of the photosynthetic potential (PP) of the phytoplankton was also reached. The main novel aspect proposed by this device is the simultaneous combination of two independent measuring principles, the minimum level of fluorescence and the fluorescence induction kinetics, for a better identification of taxonomic groups. The PhytoSensor has been tested in natural and man-made (sub-) tropical reservoirs in Southeast Asia. Tropical water resources are more sensitive to eutrophication, they show a higher proportion of cyanobacteria and blooms occurring year round are often unpredictable (Lewis, 2000; Oliver and Gant, 2000). The detection of the phytoplanktonic biomass, its proliferation, and the identification of the responsible taxa have been possible.

2. Materials and methods

2.1. Culture of algae and cyanobacteria reference species

The strains of diatoms came from CCMP (Provasoli–Guillard Center for Culture of Marine Phytoplankton, USA), UTEX (University of Texas, USA) and Laboratoire Arago (France) collections, green algae from the University Technology of Malaysia (UTM) collection and cyanobacteria from the UTM and PCC (Pasteur Culture Collection, France) collections. All strains were grown photoautotrophically in continuously sterile air flushed recipients. The following conditions were used. For marine isolates of diatoms: natural seawater F/2 medium, 18 °C, light intensity of $40\,\mu E\,m^{-2}\,s^{-1}$ with a 16 h light/8 h dark cycle. The freshwater isolate of *Phaeodactylum tricornutum* was grown in a Bristol medium. For green algae and the cyanobacteria *Anabaena* sp. and *Pseudoanabaena* sp.: Bold Basal medium, 27 °C, $25\,\mu E\,m^{-2}\,s^{-1}$, 12 h light/12 h dark cycle. For the cyanobacteria *Synechococcus* PCC 7942 and *Synechocystis* PCC 6803, GN medium, 30 °C, $60\,\mu E\,m^{-2}\,s^{-1}$ in continuous light. Cells were harvested during the exponential phase, or when indicated during the stationary phase of growth.

2.2. Cell enumeration, pigment analyses and spectroscopy

Algae and cyanobacteria were identified and counted as units with a Malassez hemocytometer counting chamber as described (APHA et al., 1995). Particle counting was performed with a Particle Counter (PCX Hach) (resolution 1 μm, maximum size 500 μm) on 500 mL at $100\,mL\,min^{-1}$. Chl *a* concentration was measured with a spectrophotometer (Varian DMS 90) after acetone 90% extraction (Jeffrey and Humphrey, 1975) and with a Datalink fluorometer (FL 100, Datalink, France). Pigment analyses were performed by HPLC (Lavaud et al., 2003), and the relative proportions of algae and cyanobacteria were determined as previously described (Wilhelm et al., 1995). Room temperature 680 nm fluorescence excitation spectra were