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A versatile chamber for simultaneous measurements of oxygen exchange and fluorescence in filamentous and thallous algae as well as higher plants

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Abstract

A new chamber was developed for a simultaneous measurement of fluorescence kinetics and oxygen exchange in filamentous and thallous algae as well as in small leaves of water plants. Algal filaments or thalli are kept by a stainless grid close to the bottom window of the chamber in the sample compartment. The grid separates the object from the electrode compartment with the oxygen electrode at the top. This compartment accommodates, in addition, a magnetic stirrer that provides efficient circulation of the medium between the sample and the electrode. This magnetic bar spins on a fixed axis and is driven by an electronically commutated magnetic field produced by four coils which are arranged around the chamber. This design yields a very favourable signal to noise ratio in the oxygen electrode records. Consequently, measurements can be performed even of algae with very low photosynthetic rates such as marine low-light red algae or algae under severe stress. For irradiation of the samples and for fluorescence measurements a fibre optic light guide is used facing the window of the chamber. The four branches of a commercially available light guide serve the following purposes: collection of sample fluorescence and supply of measuring, actinic, and saturating light, respectively.

Additional key words: *Antithamnion*; chlorophyll fluorescence induction; *Ectocarpus*; gas exchange; photosynthetic oxygen release; photosynthesis; submerged aquatic plants.

Introduction

For organisms submersed in water the easiest and rather sensitive method for assessing rates of photosynthesis is the measurement of oxygen exchange. This is usually done polarographically with an oxygen-selective electrode, the most commonly used type of which is the Clark-type electrode (Clark 1956). The most recent developments in the construction of oxygen electrodes focus on single-cell resolution (Land *et al*. 1999, Mancuso *et al.* 2000, Porterfield and Smith 2000) and electrochemical micro-imaging (reviewed by Yasukawa *et al.* 2000). The more conventional macroscopic variants, however, have also been improved and refined several times as manifested in several types of commercially accessible electrodes and measuring chambers. For deeper analysis of the photosynthetic rates, simultaneous measurements of oxygen exchange and chlorophyll (Chl) *a* fluorescence kinetics are very valuable and corresponding models have been also produced commercially (used by Masojídek *et al*. 2000 and others).

Despite all advances, however, to date no devices specifically designed for simultaneously measuring oxygen exchange and fluorescence yield of filamentous algae are commercially available or described in the literature. Measuring oxygen exchange of such organisms under

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Abbreviations: Chl = chlorophyll; F_0 = minimal fluorescence yield of a dark adapted sample, fluorescence in non-actinic measuring radiation; F_M = maximum fluorescence yield of a dark adapted sample; F_V = variable fluorescence, $F_V = F_M - F_0$.
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well defined and reproducible conditions can be very difficult, because they can neither be mounted like a leaf or thallus, nor can they be kept in suspension like unicellular algae. Therefore, in conventional measuring chambers they interfere with the stirrer, which leads to both damage to the measured algae and a high noise of the measurement.

We have constructed a chamber specifically designed for measuring oxygen exchange and Chl fluorescence kinetics of filamentous algae, which we have tested (Küpper *et al.* 2002). Later tests have shown that the chamber is well suited for the measurement on thallus discs of marine macroalgae and of leaf disks or small leaves.

Construction of the chamber

Materials: Fig. 1 shows radial section and horizontal projection of the chamber and its accessory parts. The body of the chamber is made of *Bakelit* or *Vestoran*®. The advantage of *Vestoran* is better mechanical properties, while its disadvantage consists in a slight physical reversible swelling after prolonged use. The grid plate and the axis of the stirring bar were made of stainless steel to provide the necessary rigidity. The windows of the chamber and the temperature control compartment were made of glass.

Optical path: Light is delivered to the chamber by a commercially available (*Walz*, Germany) light guide with one corporate/joint 10 mm diameter output/input branch leading to the chamber and four thinner output/input branches with homogeneously mixed fibers. Of the four branches, the two larger ones were used to detect the fluorescence signal and to supply the "actinic light", while the smaller two branches were used for supplying the modulated measuring radiation and saturating flashes. "White actinic light" was provided by a 100 W (24 V) halogen lamp with neutral density filters and heat filter, focussed into the light guide by two lenses. "White" saturating flashes were provided by a 100 W halogen bulb inside a *KL1500* lamp from *Schott* (Mainz, Germany), complemented in the lab with a mechanical shutter. Measuring radiation was produced by a red LED, as implemented in the *Walz 101* system.

The joint arm of the light guide was inserted through an adapter from the bottom to get into contact with the glass window of the constant temperature compartment. The divergence of the beam from this light guide is large enough to produce a homogeneous irradiation on the 25 mm diameter sample area 25 mm above the end of the light guide. The radiation passes through the water-filled cooling compartment of the chamber, so that much of the heat is absorbed.

Sample and electrode compartments, stirring and filling: The sample compartment of the chamber is designed to provide a large-area, but thin space for the sample in order to minimise effects of shading in case of filamentous algae. The sample is separated from the electrode compartment by a stainless steel grid, the distance of which from the window is adjusted with spacers. An axis for a magnetic stirring bar is mounted on the grid. This construction prevents the sample from getting damaged by the stirring bar and provides an efficient mixing that brings about a fast medium exchange between the sample and the electrode compartment. The fixation of the stirring bar on an axis prevents its jerky lateral movements which occur when it flows freely.

The stirring bar is driven by an electronically commutated rotating magnetic field which is produced by four coils controlled by a laboratory-assembled electronics with the push-pull driver *L293D* as the core component. This design of the magnetic drive makes the centre of the circular chamber accessible from both sides. On one side is the window for irradiation and fluorescence measurements, on the other side the oxygen electrode.

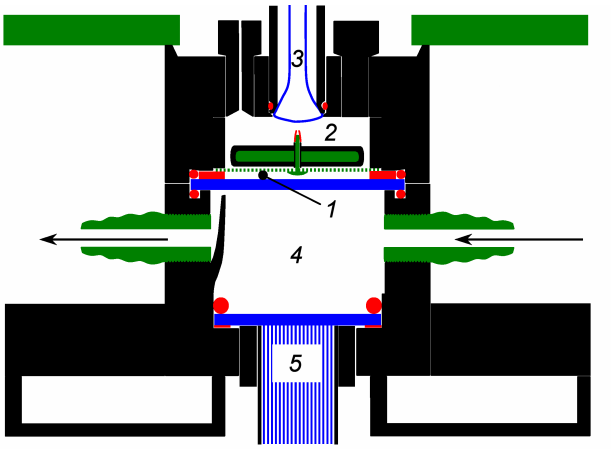
The sample and electrode compartment are filled with the medium after its assembly with the sample inserted. Two stoppers in the top wall of the chamber (next to electrode insertion) serve this purpose. One of them is provided with a capillary opening allowing for escape of surplus medium.

Temperature control in the chamber is made by the flow of temperature controlled water from a thermostat through the temperature control (TC) compartment below the window of the chamber. The window of the sample compartment was made of optical glass rather than plastic in order to improve heat conductivity. Since the thickness of the measuring compartment is small as compared to the window surface, the rate of heat flow through the latter is sufficient for reliable TC. To keep bubbles from accumulating at the surface of the glass plate separating the TC and sample compartments, a baffle was inserted in front of the exit from the TC-compartment, with a narrow (about 1 mm) gap between its upper edge and the glass plate.

Detectors/measuring system: Kinetics of fluorescence induction are measured by using a PAM modulated fluorimeter (*Walz*, Effeltrich, Germany) with a PIN diode as a detector. The fluorimeter is connected to the measuring chamber as described above.

Measurements of net photosynthetic oxygen release and respiratory oxygen uptake were carried out using a custom made Clark-type electrode (*Theta'99*, Praha, Czech Republic). The electrode was inserted from the top centre of the chamber (Fig. 1). Both the *Walz* PAM and the oxygen electrode were connected to a 16 bit A/D converter *OxyCorder* (*Photon Systems Instruments*, Brno,

Czech Republic), providing a simultaneous digital recording of both parameters in a computer.



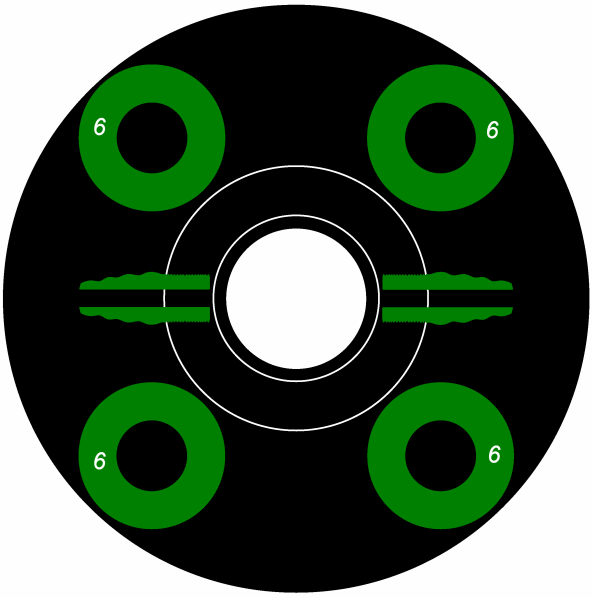


Fig. 1. Schematic drawing of the measuring chamber. *Top:* vertical section. *The arrows* indicate the flow direction of the cooling water. *Bottom:* horizontal projection, showing the position of the magnetic coils in relation to the base plate, body of the chamber, and the water inlet/outlet of the temperature control compartment. Materials used are indicated by colours: *black:* hard plastic (*Vestoran*®, epoxy resin); *red:* rubber or silicone; *green:* metal (stainless steel, ferrit, copper); *blue:* glass. Descriptions of parts: *1:* sample compartment; *2:* electrode compartment with stirring bar on axis; *3:* Clark-type electrode; *4:* temperature control compartment, which serves also as a heat filter for incoming radiation; *5*: light guide; *6*: magnetic coils.

Plants for testing: *Ectocarpus siliculosus* (Dillwyn) Lyngbye (strain Port Aransas) and *Antithamnion plumula* (Ellis) Tharet in LeJolis were kindly provided by Prof. D.G. Müller and Dr. I. Maier (Universität Konstanz).

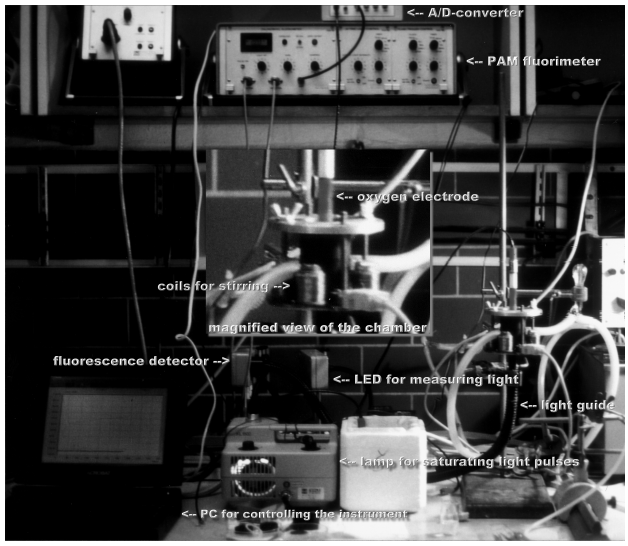


Fig. 2. Photograph of the complete system, the inset picture in the middle shows a magnified view of the chamber itself. The figure shows the system without insulation in order to make the magnetic coils visible.

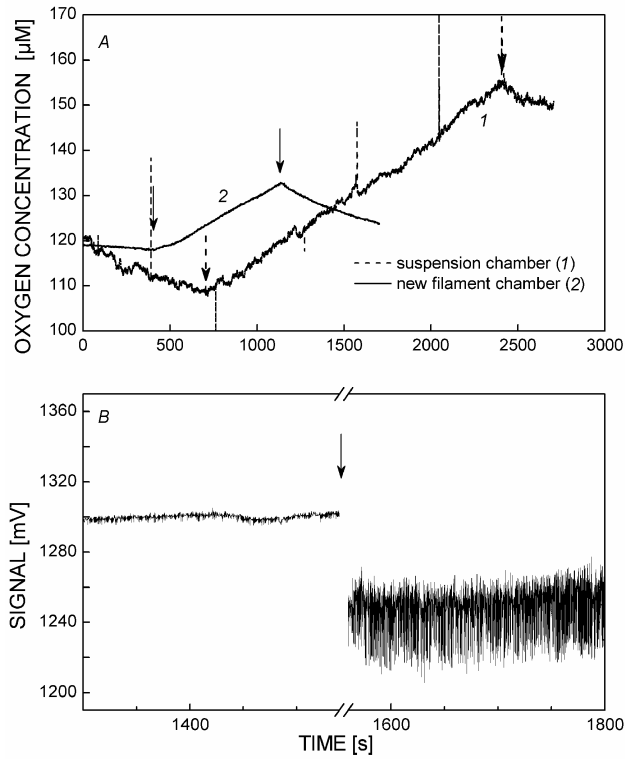


Fig. 3. Performance of the measuring chamber: (*A*) Signal/noise ratio in the old and new measuring chamber, 3 mg *Antithamnion plumula* filaments per cm^3 of chamber volume, 18 °C. *The arrows* (*solid* for new, *dashes* for old chamber) indicate the start and end of the actinic irradiation. (*B*) Oxygen measurement in the new measuring chamber: stirring bar with and without axis. *The arrow* indicates the time point of changing over from the measurement with axis to the one without axis.

Ectocarpus and *Antithamnion* were grown in *ASM-1* medium (Maier and Calenberg 1994) at a photon flux density of 8 µmol m^{-2} s^{-1} with a 14 : 10 h light : dark cycle.

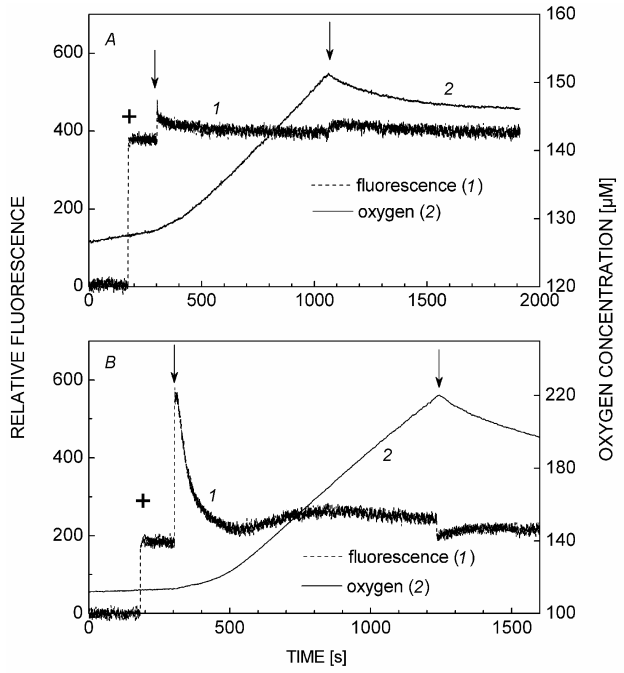


Fig. 4. Test measurements with diverse organisms and measuring regimes. *The arrows* indicate the start and end of the actinic irradiation, *the + signs* indicate the start of the fluorescence measuring pulses in *A* and *B*. For all measurements, 3 mg fresh mass of algae per cm^3 of chamber volume were used. The positive slope before switching on the "actinic light" was caused by the temperature change of the medium after filling it into the chamber, because these test measurements were started briefly afterwards. This effect is not observed when the medium is pre-conditioned to the correct temperature before filling it into the chamber, as shown in Fig. 3. (*A*) Oxygen and fluorescence measurement of *Antithamnion plumula* at 18 °C and an actinic irradiance of 65 µmol m^{-2} s^{-1}. (*B*) Measurement of oxygen and fluorescence of *Ectocarpus siliculosus* at 18 °C and an actinic irradiance of 65 µmol m^{-2} s^{-1}.

Performance of the system: For measurements of filamentous algae and similar objects the new chamber yields a much better signal/noise ratio compared to measuring chambers designed for unicellular algae (Fig. 3). This was achieved by the following design characteristics (Figs. 1 and 2):

(*a*) The sample compartment and the electrode compartment comprising the magnetic stirring bar are separated (Fig. 1). In measuring chambers designed for suspensions, interference of the filaments with the stirring bar causes irregularities of the stirring. Another detrimental consequence of this interference is that the filaments become fragmented or aggregate. In the new system the filaments are fixed in the shallow sample compartment and in this way separated from the stirrer by the stainless steel grid (Fig. 3*A*). Since the filaments do not move even their aggregation is prevented. The circulation of the medium between the sample and electrode compartments is fast enough to provide a prompt response of the electrode.

(*b*) The stirring bar of the new chamber is fixed on an axis. This prevents it from jerky lateral movements, which generate strong noise (Fig. 3*B*).

(*c*) The stirring bar is moved by a rotating magnetic field generated by four coils controlled electronically with a four channel push-pull-driver. This drive yields a much (> 10 times) lower noise compared to a mechanically commutated motor, because of the complete absence of sparking.

In addition this construction has further advantages contributing to the reproducibility of the measurements: Algal filaments are uniformly spread in the sample compartment in a thin layer (usually < 1 mm) and, consequently, irradiation of the sample is very homogeneous. The geometry of the sample compartment and the inserted water filter provide a good temperature homogeneity and stability in the sample even in case of strong irradiation; less than 1 °C temperature rise was measured when irradiating the chamber with about 1 000 µmol "white actinic light".

Examples of measurements

The primary stimulus for the design and construction of the chamber came from the need to perform measurements with marine filamentous brown and red algae. At their optimum growth temperature (around 18 °C) the species used to display a relatively feeble photosynthetic activity and in our studies their photosynthetic rate was further reduced by heavy metal toxicity (Küpper *et al.* 2002). As shown in Figs. 4*A* and 4*B*, the instrument presented here meets these demands very well.

Fig. 4*A* shows a measurement of the red filamentous alga *Antithamnion plumula*. The fluorescence kinetic trace reveals the typical features of Chl fluorescence induction in organisms with phycobilisomes (Campbell *et al*. 1998): a high F_0 and correspondingly small F_V/F_M of about 0.3. The onset of photosynthetic oxygen evolution is very slow, the rate of oxygen release reaches its maximum only after about 200 s. The reaction to switching off the "actinic light" demonstrates that this slow response is a property of the alga, not of the measuring system: The transition from light to dark causes an immediate stop of oxygen release and onset of oxygen uptake caused by the respiration.

The filaments of *Ectocarpus siliculosus* are similar to *Antithamnion plumula* only in the sense that the photosynthetic rate is very low. The fluorescence kinetic trace is typical for brown algae (Dring *et al.* 1996, Pearson *et al.* 2000), with an F_V/F_M of about 0.6 and a very slow relaxation to the steady state of fluorescence as late as

500 s after the onset of actinic irradiation. The oxygen release, in contrast, starts and stabilises faster than in *Antithamnion*, the maximum rate is reached after less than 100 s. The bend between oxygen release and oxygen uptake is not as sharp as in *Antithamnion*.

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