Comparison of two methods for estimating energy storage efficiency in phytoplankton photosynthesis

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The energy storage efficiency of photosynthesis is a sensitive diagnostic tool for probing the physiological status of phytoplankton in the laboratory and in nature. Furthermore, it is also a key factor in determining the economics of photobioreactors and algal mass culture ponds for the production of biodiesel and related applications. Photoacoustics allows direct determination of the energy storage efficiency of photosynthesis as the ratio of energy stored in the photosynthetic process, and not dissipated as heat, to the total light energy absorbed by the photosynthetic apparatus. The aim of the present study was to compare our novel, photoacoustics-based method with an oxygen-evolution-based calculation of photosynthetic energy storage. We also illustrate and discuss the use of photoacoustics for generating photosynthesis versus energy relationship curves. The photoacoustic efficiencies were always higher than the oxygen-based ones, and this difference in results is discussed. Our results show the power of photoacoustics as a tool in the research of various aspects of phytoplankton physiology.

Key words: Efficiency of photosynthesis, photoacoustics, light pipette, phytoplankton.

INTRODUCTION

The estimation of the efficiency of photosynthesis is of great theoretical and practical interest. The efficiency \( \Phi \) by which light energy harvested by photosynthetic pigments is converted into stored products depends on the photosynthetic quantum yield (Dubinsky, 1980) as defined in Equation 1.

\[
\Phi = \frac{\text{moles C assimilated or O}_2 \text{ evolved}}{\text{mole photons absorbed}} \quad (1)
\]

The energy equivalent of the quantum yield, termed energy storage efficiency \( \Phi_E \), can be calculated from the energy content of the enumerator and the spectral distribution of the absorbed light, Equation 2. In these, the enumerator is the caloric value of the stored energy in the process, namely, the difference in energy equivalents between products (photosynthates) and reactants (\( \text{CO}_2 + \text{H}_2\text{O} \)). The denominator is the energy content of the absorbed (not incident!) light.

\[
\Phi_E = \frac{\text{enthalpy (products - reactants)}}{\text{energy absorbed by cells}} \quad (2)
\]

Estimation of the energy storage efficiency of photosynthesis in phytoplankton is difficult (Dubinsky, 1980). From a theoretical point of view, this is true because in the enumerator of the efficiency fraction, usually only tangible products are taken into account. These include either carbon assimilated, determined usually from \(^{14}\text{C} \) assimilation (Dubinsky et al., 1984) or oxygen evolved, measured by electrodes (Dubinsky et al., 1986, 1987; Ben-Zion and Dubinsky, 1988). In any case, all of these ignore the energy stored by...
photosystem I (PSI) activity that does not generate any readily quantifiable product (Herzig and Dubinsky, 1993). A further difficulty lies in defining the fraction’s denominator, namely, the amount of light energy absorbed by the sample. Both of the above-mentioned difficulties are overcome using the photoacoustic method, since 1) in the enumerator, it takes into account all stored energy; and 2) in the denominator, it relates only to the energy absorbed by the sample.

Pump-and-probe and fast-rate–repeated fluorescence methods were also used to estimate photosynthetic rates and quantum yields (Kolber et al., 1998). These methods are fast and automatable but derive the quantum yield from an indirect proxy, namely fluorescence quenching, involving various assumptions (Genty et al., 1989; Kolber et al., 1998). Furthermore, again, energy storage as ATP generated by PSI activity is not accounted for (Herzig and Dubinsky, 1993).

The purpose of this study was to discuss the use of oxygen electrodes, variable fluorescence and photoacoustics, as a tool for studying photosynthesis and in the determination of energy storage efficiency values obtained by means of the photoacoustic method developed by us (Dubinsky et al., 1998; Mauzerall et al., 1998; Pinchasov et al., 2005), with values obtained from oxygen-evolution rates (Dubinsky et al., 1986).

MATERIALS AND METHODS

Sampling

*Nannochloropsis* sp. and *Isochrysis galbana* were grown on Guillard’s F/2 (Guillard, 1975) enriched artificial seawater medium. The samples were then maintained at a constant temperature of 23 ± 0.1 °C. *Peridinium gatunense* (dinoflagellate) was grown in filtered Lake Kinneret water at a constant temperature of 20 ± 0.1 °C. *Chlorella vulgaris* was grown in SCM liquid medium (Moss, 1972) at a constant temperature of 20 ± 0.1 °C.

All samples were grown in the laboratory under an irradiance level of 30 µmol q m⁻² s⁻¹ in 200 ml Erlenmeyer flasks. At the same time, duplicates were taken from each culture at the same growth phase.

Photoacoustic setup

The experimental setup is shown schematically in Figure 1. A brief, 5 ns, light pulse (Minilite Q-switched Nd:YAG Laser, 532 nm (L), after passing through a pair of 1 mm wide slits (S) to obtain a rectangular beam, is aimed at the suspension of algae whose pigments absorb some of the laser light. The algae (30 ml) are placed into a photoacoustic cell (PAC). A variable fraction of the absorbed light pulse intercepted by the pigments is stored as a product of photosynthesis, whereas the remainder is converted into heat, creating a pressure wave that travels through the water perpendicularly to the laser beam, reaching the hydrophone immersed in the sample. Upon the impact of the heat-generated pressure wave, the ceramic disc in the microphone resonates with decreasing amplitude.

Optimal signal-to-noise ratios were obtained by integrating the root-mean-square (RMS) values over 10 µs. The homemade photoacoustic detector (D) contained a 10-mm–diameter resonating ceramic disc (BM 500, Sensor, Ontario, Canada) as described by Pinchasov et al. (2005). A small portion of the laser pulse was deflected by a beam splitter (BS) and used to trigger the oscilloscope (Tektronix TDS 430A (O)). After low-noise amplification (SRS 560 – low noise amplifier (A)), the signal was processed with the oscilloscope and averaged. The baseline was established and artifacts excluded by alternating a series of 32 shots in which the sample was exposed to the pulse with an identical series in which the laser beam was blocked. The values of the dark series were subtracted from those of the light series. In low-density samples, the signal-to-noise ratio was improved by averaging over 128 pulses. The source of the background light (B) was a quartz-halogen illuminator (Cole-Parmer 4971). The intensity of the background light was adjusted to the desired level by neutral density filters and measured with a LiCor light meter equipped with a cosine quantum sensor.

Efficiency of photosynthesis by photoacoustics

The photoacoustic method is based on the conversion of absorbed light into heat. This heat causes a thermal expansion of the medium, proportional to the absorbed light energy. By increasing the continuous background light intensity from zero to saturation of photosynthesis, the superimposed laser pulse encounters an increasing fraction of the reaction centers, which are closed at any time (Pinchasov et al., 2007). Therefore, a decreasing fraction of the probe laser pulse energy is stored, and corresponding increase in the fraction of the pulse energy is converted to heat, which is sensed by the photoacoustic detector. From these detector responses, the photosynthetic energy storage versus background light-intensity relationship was obtained (Mauzerall et al., 1998).

Thus, the light energy storage efficiency $\Phi_E$ is determined according to Equation 5, and may be expressed as the percentage of light energy absorbed:

$$\Phi_E = \frac{(P_{\text{Alight}} - P_{\text{Adark}})}{P_{\text{Alight}}}$$

where $P_{\text{Adark}}$ is the photoacoustic signal generated by the weak laser pulse in the dark, and $P_{\text{Alight}}$ is the signal produced under the same pulse obtained under the continuous background light ($\sim3000$ µmole quanta m⁻² s⁻¹) (Pinchasov et al., 2007). The difference $P_{\text{Alight}} - P_{\text{Adark}}$ is proportional to the energy stored under a given background irradiance and, as such, provides the enumerator of the efficiency fraction. Since under saturating background light all reaction centers are closed, all of the laser pulse energy is converted to heat. One of the major advantages of the photoacoustic method is the inherent independence of culture density, since in Equation 5, both numerator and denominator of the fraction depend identically on the density of the culture and, thus, on its absorptivity (Dubinsky et al., 1998; Mauzerall et al., 1998).

Light pipette setup

In order to compare the results obtained by photoacoustics (PA) with a more conventional method, we opted for the O₂ polarographic method, using a light pipette device. The light absorbed by the sample from the difference in light transmitted through the medium-filled cuvette with that passing through it when filled with the algal culture being measured, have been estimated The absorbed light was then converted to energy equivalents (E), in watts, through its spectral distribution, integrating the energy equivalents of all wavelengths according to Equation 4:

$$E = h \times \nu$$

Where $h$ is Planck's constant and $\nu$ the frequency at each wavelength.

The evolved oxygen was also converted to the same energy equivalents, assuming all photosynthates to be glucose. Thus, the efficiency of photosynthetic light energy storage, or $\Phi_E$, the energy equivalent of the quantum yield, was calculated as the ratio of energy stored as photosynthates (Equation 5) of the total light energy absorbed:

$$\Phi_E[\%] = \frac{\text{energy stored as photosynthesize}}{\text{light energy absorbed}}$$

The light pipette (Illuminova, Upsala, Sweden) device (Figure 2) consists of a quartz halogen light source, a computer-controlled photon flux dispenser that illuminates a cuvette equipped with a stirrer, and a micro-oxygen electrode (MI-730, Microelectrodes, Inc., Londonderry, Northern Ireland). The object unit contains the cuvette into which the 5 ml sample is injected by a small syringe. The computer-controlled light source delivers precise photon fluxes of 0 to 3500 µmole quanta m$^{-2}$ s$^{-1}$ (photosynthetically active radiation). Light sensors before and after the cuvette provide information on the average light in the cuvette and the light absorbed by the sample. A magnetic stirrer in the cuvette keeps the algae in suspension, ensuring that they are uniformly illuminated over time, and provides the water movement needed for the polarographic electrode.

The temperature of the sample is controlled by a circulating refrigerated-water bath. The command unit connects the other subunits to the computer and is fitted with several controlling devices, e.g., for regulation of the stirrer's velocity, to change...
between manual or computer-controlled measurements, and for calibration procedures, e.g., for adjusting zero and 100% oxygen saturation, as well as several vacant spaces for optional external UV light and a pH electrode. The data were processed and stored in a computer, and analyzed by specifically developed software. The rate of oxygen evolution was calculated on the basis of the ambient O$_2$ concentration (Walker, 1987; Wolfstein and Hartig, 1998; Danilov and Ekelund, 2001). Efficiency was estimated as follows: changes in oxygen concentration were converted to oxygen-evolution rates according to temperature and salinity saturation tables. The conversion to energy was done assuming all photosynthates to be glucose. The energy in the light absorbed by the sample at each irradiance level was calculated from the difference in intensity between light before and after the cuvette and the cuvette’s area. The maximal efficiency values obtained from the oxygen-evolution rates measured compares with the light pipette to those derived from the photoacoustic measurements (Figure 3). The data points were chosen from the two photosynthesis-energy curves for each species, one generated from the oxygen-evolution rates and the other (Figure 4a) from the photoacoustic signal (both were obtained from the same sample and time). Standard deviations for three replicate measurements and regression analysis of the data were done and values are presented in the graphs.

The photosynthesis versus energy curve was generated as described in Pinchasov et al. (2005) and Pinchasov et al. (2007) by changing the intensity of the continuous background light, and replacing the photoacoustic signal obtained in the darkness, PAdark in Equation 5, by that obtained under the different background intensities, as described above. This curve (Figure 4a) is compared to analogous P vs E (formerly P vs I) generated from oxygen electrode (Figure 4b) (Berner et al., 1986) and variable fluorescence (Falkowski et al., 1986) (Figure 4c).

RESULTS AND DISCUSSION

As can be seen in Figure 3, the results obtained by photoacoustics correlate well with the oxygen-based results. When the application of photoacoustics to the generation of photosynthesis-versus-energy curves is compared to other methods, a few differences are notable. It is important to point out that both photoacoustics (Figure 4a; Berner et al., 1986) and variable fluorescence (Figure 4c; Falkowski et al., 1986) increase as more reaction centers (traps) are progressively closed, hence thermal dissipation and fluorescence losses increase. Actually, both are constituents of the “non-photochemical quenching” (NPQ) losses of light energy absorbed by cells and not stored as products of photosynthesis. Therefore, these methods cannot reveal decreases in photosynthetic rates due to photoinhibition and they both reach a maximal signal level as all centers are closed. These differ from curves based on either oxygen evolution (Figure 4b, Berner et al., 1986) or $^{14}$C (Figure 4d) assimilation, as these reveal
Figure 4. Photosynthetic efficiency for algae from different taxonomic groups by: (a) photoacoustics light pipette (b) oxygen evolution (Berner et al., 1986) (c) fluorescence (Falkowski et al., 1986), and (d) $^{14}$C assimilation.

advantage is the fast repetition of the laser pulse, which, at 10 Hz, allowed us to acquire 32 to 128 signals in a few seconds and obtain very good signal-to-noise ratios. This compares most favorably with the difficulty inherent in the estimation of energy absorbed in the oxygen method. By that method, light absorbed by the sample has to be estimated from the difference between light entering the sample and that leaving it. Such calculations involve assumptions regarding reflection by cuvette surfaces and light scattered, not absorbed by the cells. In addition, measurements of oxygen-evolution rates are slow and as such, restrict the number of replicate measurements (Dubinsky et al., 1986; Dubinsky, 1980). The higher values obtained by photoacoustics may be due to a combination of two factors:

a) The inclusion of energy stored by PSI activity as ATP generation (Herzig and Dubinsky, 1993), which is not...
accounted for in the enumerator of the fraction with the oxygen method.

b) Additional energy dissipation takes place between the thermal dissipation detected by the hydrophone (~10 µs) and the evolution of oxygen (1.5 to 10 ms), resulting in less energy being finally stored as products of photosynthesis. The difference between methods may eventually be developed to study and estimate the energy losses between energy absorbed and that finally stored in the biomass increment. In recent studies, the applicability and power of photoacoustics in studying the effects of nutrient limitation (Pinchasov et al., 2005), lead toxicity (Pinchasov et al., 2006), and trophic status of water reservoirs (Pinchasov et al., 2007), were demonstrated. However, the method is restricted to relatively high chlorophyll concentrations since, until now, we were only able to receive good results at chlorophyll concentrations above 1.5 ± 0.1 µg/ml, and this requires relatively expensive equipment, including the laser itself, a digital oscilloscope, and a low-noise amplifier. It should be pointed out that, the efficiency and quantum yield of photosynthesis were also estimated based on ¹⁴C assimilation (Dubinsky and Berman, 1976). In that method, besides the problem of determining the light absorbed in samples, which was solved by these authors by using in situ water columns, there is major uncertainty regarding the calibration of the radiocarbon assimilation results as representing net or gross photosynthesis, and their dependence on sample size "bottle effects" (Gieskes et al., 1979) and inclusion/disregard of excreted products of photosynthesis (Tilzer and Dubinsky, 1987).

Estimates of photosynthetic energy storage efficiency are becoming of great importance, since the conversion of spectral reflectance of oceans into estimates of productivity depends on the correct evaluation of quantum yields (Maritorena et al., 2000). In turn, photosynthetic efficiency is most sensitive to a variety of environmental factors, including light inhibition and nutrient limitation (Maritorena et al., 2000; Repka et al., 2004; Pinchasov et al., 2007). The xanthophyll cycle is activated upon exposure to supraoptimal light intensities, enhancing thermal dissipation of excess light intensity, thereby reducing the risk of photodynamic damage to the photosynthetic apparatus (Niyogi et al., 1997; Yokthongwattana et al., 2001; Jin et al., 2003).

Thus, photoacoustics is an additional tool for probing the effects of various environmental and physiological factors on the partitioning of absorbed light energy between photochemical storage of products and its degradation to heat. It is the only direct method for measuring – for instance – the effect and efficiency of activation of the xanthophyll cycle to dissipate potentially harmful excess light energy. In general, it can determine any environmentally induced changes in NPQ of fluorescence. While different algae are all likely to show higher energy storage efficiency when measured by photoacoustics than by other methods, they differ considerably since some taxa (e.g., Rhodophyta and several cyanophytes) lack the xanthophyll cycle while others, such as diatoms and chlorophytes, have a highly developed one (Rmiki et al., 1996; Niyogi et al., 1997; Masojidek et al., 1999; Sagert and Schubert, 2000). The different O₂ or ¹⁴C-based and the photoacoustic methods are complementary; since, when applied concomitantly, they allow the estimation of energy dissipation stages taking place in the time interval τ between the absorption of light and the assimilation of carbon.

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