Photoacoustics: A Potent Tool for the Study of Energy Fluxes in Photosynthesis Research

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1. Introduction

Phytoplankton cells are ideal organisms for the study of various aspects of photosynthesis, since most of their cells are devoted to components related to the harvesting of light energy and its storage as high energy compounds. They lack flowers, roots and all of the many structures and mechanism evolved in the course of the emergence of plants from the primordial oceans and conquering land. The products of photosynthesis are synthesized while carbon from assimilated CO$_2$ is being reduced and oxygen from photolytically split water is evolved. In most open water bodies – freshwater and marine - the energy input of the entire ecosystem depends on microscopic free-floating photosynthetic organisms- the phytoplankton. The determination of phytoplankton biomass and its photosynthesis activity is a great interest to ecologists.

The photoacoustic method allows the direct determination of the biomass of different taxa of phytoplankton and the efficiency of their photosynthesis. The latter is accomplished by relating the energy stored photochemically by photosynthesis to the total light energy absorbed by the plant material.

The method yields rapid, direct results of the efficiency of photosynthesis, compared to standard measurements based on $^{14}$C fixation and oxygen evolution, or compared to indirect results from measurements of variable fluorescence.

We review the history of the application of photoacoustics to photosynthesis research. Our results show that the pulsed photoacoustic technique provides direct information on the biomass and phytoplankton photosynthesis and demonstrate its application in the study of phytoplankton ecology and physiology and in basic research of their photobiology. The photoacoustics has a high potential for following the effects of environmental parameters such as irradiance, nutrient status and pollution on phytoplankton communities and their photosynthetic activity.

2. The history of photoacoustic effect definition

The photoacoustic effect was first investigated in the 1880 by Alexander Graham Bell. During his experiments with the “photophone”, which carried an acoustic signal with a beam of sunlight that was reflected by an acoustic modulated mirror, he noticed that a
rapidly interrupted beam of sunlight focused on a solid substance produces an audible sound. He observed that the resulting acoustic signal is dependent on the composition of the sample and correctly conjectured that the effect was caused by absorption of the incident light.

Recognizing that the photoacoustic effect had applications in spectroscopy, Bell developed the “spectrophone,” essentially an ordinary spectroscope equipped with a hearing tube instead of an eyepiece. (Fig. 1). Samples could then be analyzed by sound when a source of light was applied.

Fig. 1. Historical setup used by Bell (Bell, 1881).

As noted by Bell, “the ear cannot of course compete with the eye for accuracy”, when examining the visible spectrum. Bell published the results in a presentation to the American Association for the Advancement on Science in 1880 (Bell 1880).

In his paper, Bell described for the first time the resonant photoacoustic effect: “When the beam was thrown into a resonator, the interior of which had been smoked over a lamp, most curious alternations of sound were observed. The interrupting disk was set rotating at a high rate of speed and was then allowed to come gradually to rest. An extremely musical tone was at first heard, which gradually fell in pitch as the rate of interruption grew less.” (Fig. 2) The loudness of the sound produced varied in the most interesting manner. Minor

Fig. 2. Schematic setup of “photophone” used by Bell. As light source the sun (or a conventional radiation source) was employed. The acoustic signal was detected with a hearing tube and the ear (Bell, 1881).
reinforcements were constantly occurring, which became more and more marked as the true pitch of the resonator was neared.

As shown in Fig. 3 the light pulse was absorbed by the sample of matter, and then converted to energy equivalents. The resulting energy will be partially radiated as heat (generation of wave), consequently a pressure wave can be detected by an acoustic sensor. The pressure waves are characteristic of the sample and are used to determine composition, concentration, and other thermophysical properties.

![Diagram](image.png)

Fig. 3. The schematic of sound signal detection as used by Bell (Bell, 1881).

“When at last the frequency of interruption corresponded to the fundamental frequency of the resonator, the sound produced was so loud that it might have been heard by an audience of hundreds of people.” (Bell, 1881).

Some time later Viengerov (1938, 1940) used the photoacoustic effect for the measurements of light absorption in gases and obtained quantitative estimates of concentration in gas mixtures based on signal magnitudes. He used blackbody infrared sources, for radiation input and a microphone to detect the acoustic signal. However, his results were affected by the relatively low sensitivity of his microphone as well as undesired photoacoustic effects from the glass chamber, a problem that persists in modern photoacoustic analysis.

In 1946 Gorelik suggested the use of the photoacoustic effect for the determination of energy transfer rates between vibrational and translational degrees of freedom of gas molecules. When a sample of gas in a photoacoustic cell is irradiated by photons, which it absorbs, the absorbed energy is used to excite to vibrational or vibrational-rotational energy state in the infrared, visible or ultraviolet ranges of the electromagnetic spectrum. (Rosencwajg, 1980).

Between 1950 and 1970 the photoacoustic gas analyzer employing a conventional light source gave way to the more sensitive gas chromatography technique. Similarly, the spectrophone gave way to the more versatile infrared spectrophotometer.

The development of the laser in the early 1970s had critical implications for photoacoustic spectroscopy. Lasers provided high intensity light at a tunable frequency, which allowed an increase in sound amplitude and sensitivity. In 1968 Kerr and Atwood were the first to apply a CO₂ laser to illuminate a photoacoustic cell (Kerr and Atwood, 1968). More interest in the method was generated when Kreuzer (1971) demonstrated part-per-billion (ppb) detection sensitivities of methane in nitrogen using a helium-neon laser excitation source, and later (Kreuzer 1972) sub-ppb concentrations of ammonia and other gases in mixtures, using infrared CO and CO₂ lasers.

Later, in the 1980s, Patel and Tam (Patel and Tam, 1981, Tam, 1986) have established not only the modern technological basis of the method, by using pulsed lasers as the light source and piezoelectric transducers as the photoacoustic detectors, but also provided the complete theoretical description of the photoacoustic phenomenon, based on the original concepts of Landau and Lifschitz (Landau and Lifschitz, 1959). Since then the photoacoustic method has been adapted and further developed by several groups (Rothberg 1983, Braslavksy 1986).
3. Photoacoustics and photosynthesis

In principle, photosynthesis generates three phenomena which may be detected by photoacoustics, with adequate setups. The thermal expansion of tissue, liquids and gases due to light energy converted to heat is termed as the photothermal signal. That is generated always, when photosynthetic tissue, or cell is exposed to a light pulse, since never is all of the light absorbed by plant tissue stored as products of the process. The unused fraction of the absorbed light energy is converted to heat, resulting in measurable pressure transient. When a leaf is illuminated by a pulse of light, the resulting photosynthetic photolysis of water causes the evolution of a burst of gaseous oxygen. That process leads to an increase in pressure, a change which is readily detected by a microphone as the photobaric signal. For detailed definitions and description see review by Malkin (1996). In addition to these two, absorption of light by components of the photosynthetic apparatus, such as PSI, or PSII, is accompanied by change in its spatial conformation and volume change, or electrostriction.

4. A leaves

Photoacoustic methods provide unique capabilities for photosynthesis research. The pulsed photoacoustic technique gives a direct measurement of the enthalpy change of photosynthetic reactions (Carpentier et al., 1984). A microphone may detect the photoacoustic waves via the thermal expansion in the gas phase. This method allows in vivo measurements of the photosynthetic thermal efficiency, or energy storage, and of the optical cross-section of the light harvesting systems (Carpentier et al., 1985, Buschmann, 1990). Photoacoustic spectroscopy first emerged as a technique for photosynthesis research in the pioneering works of Cahen and Malkin (Malkin and Cahen 1979). Oxygen evolution by leaf tissue can be measured photoacoustically with a time resolution that is difficult to achieve by other methods (Canaani et al. 1988; Malkin 1996).

Photoacoustic measurements can achieve microsecond time resolution and allow determination of fast induction phenomena in isolated reaction centers, photosystems, thylakoid membranes intact cells and leaf tissue (Fig. 4).

![Fig. 4. Setup for photoacoustic photosynthesis measurements in air phase on leaf discs and algae collected on filters (Cha and Mauzarall 1992). Both the sample and reference twin chambers are connected by an air passage to hearing aid microphones.](www.intechopen.com)
These phenomena include states of the oxygen evolving complex in leaf tissue (Canaani et al. 1988) and the earliest steps of photosynthetic electron transport in photosystems (Arata and Parson 1981; Delosme et al. 1994, Edens et al. 2000).

In the work of da Silva (da Silva et al, 1995) the photoacoustic method has been demonstrated to be suitable, efficient and reliable technique to measure photosynthetic O₂ evolution in leaves.

The O₂ evolution in intact undetached leaves of dark adapted seedlings was measured during photosynthesis with the objective to detect genetic differences (da Silva et al., 1995).

Photoacoustic method can also measure state photosynthesis in intact cells and leaf tissue if the measuring pulses are given in combination with continuous background light (Kolbowski et al. 1990).

5. Phytoplankton

A simple technique based on photoacoustic measurements allowed us to determine the biomass, as well as the efficiency of photosynthesis, for different taxa of phytoplankton in situ (Dubinsky et al., 1998).

The experimental system is shown schematically in Figure 5 and 6 (Dubinsky et al. (1998), Mauzerall et al. (1998), and Pinchasov et al. (2005)).

Fig. 5. Photoacoustic phytoplankton cell (Dubinsky et al., 1998). The reddish algal culture is of Porphyridium cruentum and the laser pulse at 560nm.

The laser pulse is incident upon the suspension of algae, whose pigments absorb part of the laser beam. A variable fraction of the absorbed light pulse is stored in the photochemical products of photosynthesis. The remainder of the absorbed light is converted to heat, producing an acoustic wave that is intercepted by a detector (for details see Pinchasov et al. 2005). The signal contains a noisy background and later reflections from the walls of the vessel as well as from impedance mismatch within the detector.
The use of piezoelectric films acoustically coupled to a liquid sample and a pulsed laser light source increased the time resolution of the photoacoustic technique to the microsecond scale (Nitsch et al., 1988; Mauzerall et al., 1995). Photoacoustic thermodynamic studies have been carried out on isolated photosynthetic reaction centers from bacteria \textit{Rb. sphaeroides} (Arata & Parson, 1981), on PS I from cyanobacteria (Delosme et al., 1994), and on PS II from \textit{Chlamydomonas reinhardtii} (Delosme et al., 1994).

The resulting electric signal PA, is stored and subsequently analyzed on a computer. Thus, the light energy storage efficiency $f$ is determined following Eq. 1.

$$f = \frac{(PA_{\text{light}} - PA_{\text{dark}})}{PA_{\text{light}}}$$  \hspace{1cm} (1)

$PA_{\text{dark}}$ is the photoacoustic signal generated by the weak laser pulse in the dark and $PA_{\text{light}}$ is the signal produced under the same pulse obtained under saturating (~3000 µmole photons m$^{-2}$ s$^{-1}$) continuous white light from a quartz-halogen illuminator (Pinchasov 2006).

We illustrate the application of the method by determining the effects of photoacclimation, nutrient limitation and lead poisoning on phytoplankton cultures from different taxa.

6. The effect of nutrient limitation on photosynthesis

We were able to follow the effects of the key environmental parameter, nutrient status, on the photosynthetic activity of phytoplankton. The nutrients examined were nitrogen, phosphorus and iron (Pinchasov et al., 2005).

The algae for these cultures were harvested by centrifugation from the nutrient-replete media in which they were grown, and resuspended in media from which N or P was omitted. Cultures were followed over two weeks and compared for their photosynthetic energy storage efficiency.
As seen in Fig. 7, all three algal species showed a sharp decrease in efficiency; by ~50±5% in the P limited, and ~60±5% in the N limited cultures, as compared to the nutrient replete controls (=100%). Fig. 8 shows the light energy storage efficiency under different ambient irradiance levels, resulting in an energy-storage curve for *Isochrysis galbana*.

Fig. 7. The effect of nutrient limitation on relative photosynthetic efficiency. For each species photosynthetic energy efficiency of the nutrient replete control was taken as 100%. Controls (clear columns) were grown in nutrient replete media, whereas in the -P (gray) and -N (horizontal hatch) cultures, phosphorus and nitrogen were omitted from the medium. (Pinchasov et al., 2005).
Fig. 8. The effect of nutrient limitation on the photosynthesis – irradiance relationship of *Isochrysis galbana*. Nutrient replete control (−Δ−), phosphorus limited (−◊−), and nitrogen limited (−□−).

The maximal storage in the nutrient replete control was taken as 100% (Pinchasov et al., 2006).

For the iron limitation experiments the algae were cultured in iron-replete media, under the same conditions as in the nitrogen and phosphorus depletion experiments. The photoacoustic experiments were conducted after two weeks in these media. As the iron is progressively depleted, the ability of the three species to store energy decreases Fig. 9.
Fig. 9. The effect of iron concentration on photosynthetic efficiency. Controls (clear columns) were grown in iron replete media containing 0.6 mg L\(^{-1}\). The iron concentration in the iron-limited cultures was (hatched columns, from left to right), 0 mg L\(^{-1}\), 0.03 mg L\(^{-1}\), 0.09 mg L\(^{-1}\), and 0.18 mg L\(^{-1}\), respectively (Pinchasov et al., 2005).

In our experiments, the exposure of the Cyanobacteria \textit{S. leopoliensis} to different concentrations of lead resulted in some major changes in chlorophyll concentration and photosynthesis Fig. 10 (Pinchasov et al., 2006).

Figure 11 shows the changes in photosynthetic efficiency following lead application. The reduction of photosynthesis reached ~50% and ~80% with 25 ppm and 200 ppm correspondingly. It is important to emphasize that these results are similar in trend to the
decrease in chlorophyll concentration. Most of the decrease seen after the first 24 hours already took place in the first 40 min, and probably even earlier.

With increasing lead concentration and duration of exposure, inhibition of photosynthesis increases. Since the photoacoustic method yields photosynthetic energy storage efficiency results that are independent of chlorophyll concentration, it means that the observed decrease in efficiency is not due to the death of a fraction of the population, but rather due to the impairment of photosynthetic function in all cells, possibly due to inactivation of increasing fractions of the photosynthetic units.

Fig. 10. Relative photosynthetic efficiency of *Synechococcus leopoliensis* (Cyanobacteria) versus irradiance after 7 days of exposure to lead. (■) MFM medium and 0 ppm, (∆) phosphorus free medium (MFM-P) (●) MFM-P and 25 ppm, (□) MFM-P and 50 ppm, (○) MFM-P and 100 ppm, (○) MFM-P and 200 ppm (Pinchasov et al., 2006).
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Fig. 11. The effect of lead on relative photosynthetic efficiency of *Synechococcus leopoliensis* (Pinchasov et al., 2006).

Fig. 12. An experimental photoacoustic setup, where the pulses previously obtained by a laser were produced by red light emitting diodes. The cuvette with the algal culture faces the LED array, whereas the microphone, visible on the left, is horizontal, placed at the rear window of the cuvette.
Recently Gorbunov et al., (submitted) were able to conduct photoacoustic measurements on *Chlamydomonas reinhardtii* and to determine the allocation of energy to either photosystem by using PSI or PSII deficient mutants. In these experiments the brief exciting pulses hitherto produced by lasers were generated by red light emitting diodes (Fig. 12), and the saturating, continuous light was provided by blue LEDs (Fig. 13).

![Fig. 13. The same setup as in fig 12. The blue LEDs provide the saturating, continuous light.](image)

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In these experiments the authors also estimated the contribution of electrostriction to the photoacoustic signal by comparing results at room temperature with ones measured at 4 °C, the temperature at which the photobaric signal is eliminated, and electrostriction is singled out.

### 7. Conclusions

1. Photoacoustics can be used to reliably estimate the concentration of photosynthetic pigments in phytoplankton cultures or assemblages.
2. The efficiency of energy storage by phytoplankton photosynthesis can be estimated directly, easily, rapidly and reliably by photoacoustics.
3. The effects of any environmental stressor, such as temperature, nutrient limitation, high/dim light and pollutants on the photosynthetic capacity of phytoplankton can be detected rapidly by photoacoustics.

4. Future work is likely to seek the replacement of lasers by LED sources, allowing the development of portable systems suited for field work, including submersible profilers.

8. References


Photosynthesis is one of the most important reactions on Earth, and it is a scientific field that is intrinsically interdisciplinary, with many research groups examining it. We could learn many strategies from photosynthesis and can apply these strategies in artificial photosynthesis. Artificial photosynthesis is a research field that attempts to replicate the natural process of photosynthesis. The goal of artificial photosynthesis is to use the energy of the sun to make different useful material or high-energy chemicals for energy production. This book is aimed at providing fundamental and applied aspects of artificial photosynthesis. In each section, important topics in the subject are discussed and reviewed by experts.

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