A novel approach to measuring carbon assimilation and chlorophyll *a* fluorescence in algal suspensions

Performance characteristics of the 6800-18 Aquatic Chamber for measuring CO_2 assimilation by algae in suspension

Established as the industry standard for leaf level photosynthesis measurements, the LI-6800 Portable Photosynthesis System with the NEW 6800-18 Aquatic Chamber makes it possible to measure carbon assimilation and chlorophyll *a* fluorescence from samples in liquid suspension. The system provides a controlled sample environment, with direct control of the headspace CO_2 concentration, light intensity (with independent control of red, blue and far-red light), temperature (through a built-in water jacket), and the O_2 concentration (by connection to tank air).

Built-in automatic controls allow for automated measurements of biological response to CO_2 and light. Chlorophyll *a* fluorescence is measured by a PAM (Pulse Amplitude Modulated) fluorometer, allowing exploration of energy capture at PS_{II} and energy dissipation through various photochemical and non-photochemical processes. The fluorescence measurement is made concurrently with the carbon assimilation measurement, providing a more complete picture of photochemistry of the sample.

Figure 1. The aquatic chamber directly measures CO_2 assimilation by algae in suspension under controlled steady-state conditions. The LI-6800 console (left) provides a conditioned air supply, data file management, and the configuration interface. The LI-6800 sensor head (right) provides flow control valves, gas analyzers, and other sensors. The 6800-18 Aquatic Chamber with fluorometer (far right) holds a 15 mL aquatic sample.

Carbon assimilation in a steady-state system

In contrast with oxygen-based measurements of algal photosynthesis, where O_2 evolution is derived from the change in O_2 concentration over time, the LI-6800 is an open, flow-through, steady-state gas exchange system where CO_2 and O_2 concentrations are constant during the measurement.

In the LI-6800, the carbon assimilation rate is determined by mass balance of an air stream before and after it interacts with a liquid sample. The CO_2 and water vapor concentrations of the air stream are measured by a pair of high-precision infrared gas analyzers (IRGA), that give an effective measurement resolution on the order of 0.1 µmol mol^{-1} for CO_2. The water vapor difference between the incoming and outgoing airstreams is actively minimized by a patent-pending method, and the water vapor concentrations are included in the carbon assimilation calculation to account for volumetric dilution. Flow through the sample cuvette is held constant at a user-determined setpoint and the carbon assimilation rate is calculated from the concentration differences and flow rate:

$$assimilation = flow\left(CO_{2_{in}} - CO_{2_{out}}\frac{1 - H_2O_{in}}{1 - H_2O_{out}}\right)$$

Fundamentally, this mass balance gives the flux of CO_2 between the liquid sample and the cuvette headspace. The flux is coupled to the true biological carbon assimilation rate by mass transfer at the air-liquid interface and the kinetics of the carbonate system for the liquid media being measured.

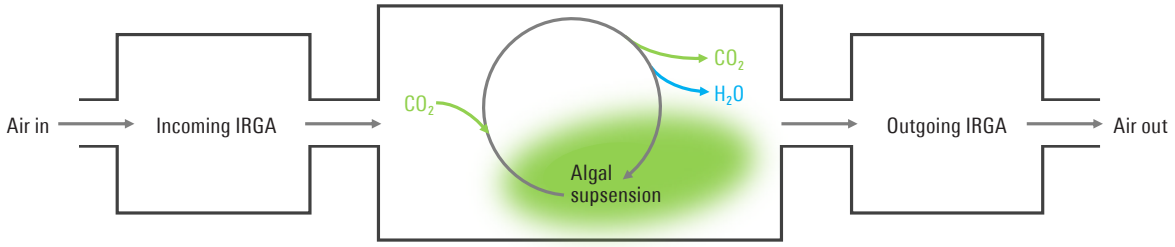

Figure 2. In an open, flow-through system, CO_2 and H_2O in the sample air are measured before interacting with the sample (incoming IRGA) and after (outgoing IRGA). The difference represents biological activity of the sample.

In the 6800-18 Aquatic Chamber, a carefully controlled aeration scheme ensures that under typical measurement conditions, the mass transfer coefficient is not limiting and that the measured flux represents the biological assimilation rate of the sample. To ensure that the carbonate system remains at steady-state during measurements, carbonic anhydrase (CA) is added to the sample media for rapid hydration of CO_2 and interconversion with bicarbonate (HCO_3^-).

Photosynthesis response to irradiance at constant O_2 and CO_2

Figure 3 shows data collected on *Chlorella* at ambient (21%, open symbols) and sub-ambient (0.5%, closed symbols) O_2 concentrations. During measurements, the CO_2 concentration entering the chamber was held constant at 400 µmol mol^{-1} and chamber temperature was held constant at 25 °C using an external water bath. Cells were measured in a saltwater media at 17 ppt salinity.

The photosynthetic response to irradiance is characterized by two general regions. At low irradiance, light is limiting to photosynthesis and carbon assimilation increases linearly with increasing irradiance. The initial slope of this region of the curve gives the apparent quantum yield; photons required per carbon assimilated. As irradiance increases, the rate of rise in photosynthesis decreases such that the photosynthetic rate ultimately approaches some asymptotic maximum. At this point light is no longer limiting, and maximum photosynthetic rate is constrained by other processes.

When O_2 concentrations are reduced in solution, the likelihood of oxygenation of RuBP by RuBisCO — the first step of photorespiration — is reduced and the overall efficiency of carbon assimilation relative to energy capture increases. Under these conditions, a steeper initial slope to the photosynthesis-irradiance response and higher maximum photosynthetic rate are expected, as is a reduction in the electron transfer rate per carbon assimilated. This increase in efficiency is driven by the relative abundance of CO_2 and O_2 and the selectivity of RuBisCO between the two. It is expected to have little to no impact on the efficiency of PS_{II}; as shown here by the near identical behavior of Φ_{PSII} between the cells at ambient and sub-ambient O_2. Photorespiration impacts electron sink strength, such that 1-qL may be expected to decrease some with an increase

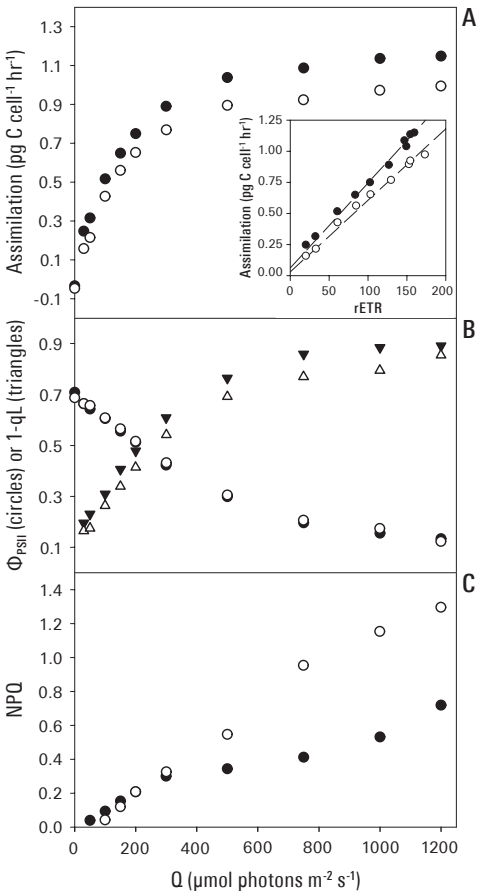

Figure 3. Panel A shows the net carbon assimilation rate as a function of light intensity (Q) and relative Electron Transfer Rate (rETR). Panels B and C show measurements derived from chlorophyll *a* fluorescence as a function of light intensity. The quantum efficiency of PS_{II} (Φ_{PSII} when Q>0 or F_v/F_m when Q=0) is shown in the middle panel (circles) along with the fraction of closed reaction centers (1-qL, triangles). The bottom panel reflects the captured energy being dissipated through non-photochemical processes (NPQ).

in photorespiration. Active regulation of a portion of NPQ related to cyclic electron flow can serve to balance the energetic products of photochemistry, ATP and NADPH, such that as photorespiration is suppressed down regulation of NPQ is expected.

Photosynthesis response to CO_2 at constant O_2 and light intensity

Figure 4 shows an example CO_2 response curve collected on *Monoraphidium* at ambient O_2 concentration and 700 µmol photons m^{-2} s^{-1} light intensity. The CO_2 concentration entering the chamber was varied during these measurements and chamber temperature was held constant at 25 °C using an external water bath. Cells were measured in a freshwater media buffered to pH 7.0 with a TRIS buffer.

The photosynthetic response to CO_2 is non-linear in nature. At low concentrations, CO_2 is limiting and assimilation changes proportionally to the concentration. Under these conditions, the portion of energy ultimately used in carbon assimilation decreases, leading to a rise in non-photochemical quenching and a rise in 1-qL, indicative of a decreasing electron sink strength. This leads to a reduction in the quantum efficiency of PS_{II} (Φ_{PSII}) as the system becomes progressively limited by energy dissipation.

Photorespiration is likely to increase as the ratio of O_2 to CO_2 increases. Low CO_2 concentrations may also lead to engagement of any active carbon concentrating mechanisms (CCM), increasing the number of electrons required to support assimilation. At high CO_2, regeneration of RuBP becomes limiting as electron transport reaches its maximum capacity. Above this point, only modest increases in the actual assimilation rate are expected, driven by suppression of photorespiration and disengagement of any CCM as the concentration increases.

The LI-6800 Portable Photosynthesis System with the 6800-18 Aquatic Chamber provides a novel approach to measuring CO_2 exchange from aquatic samples. With support for controlled sample environment, advanced programming options for automated control over sample conditions, and built-in data file management, the aquatic chamber presents novel research opportunities for aquatic biologists.

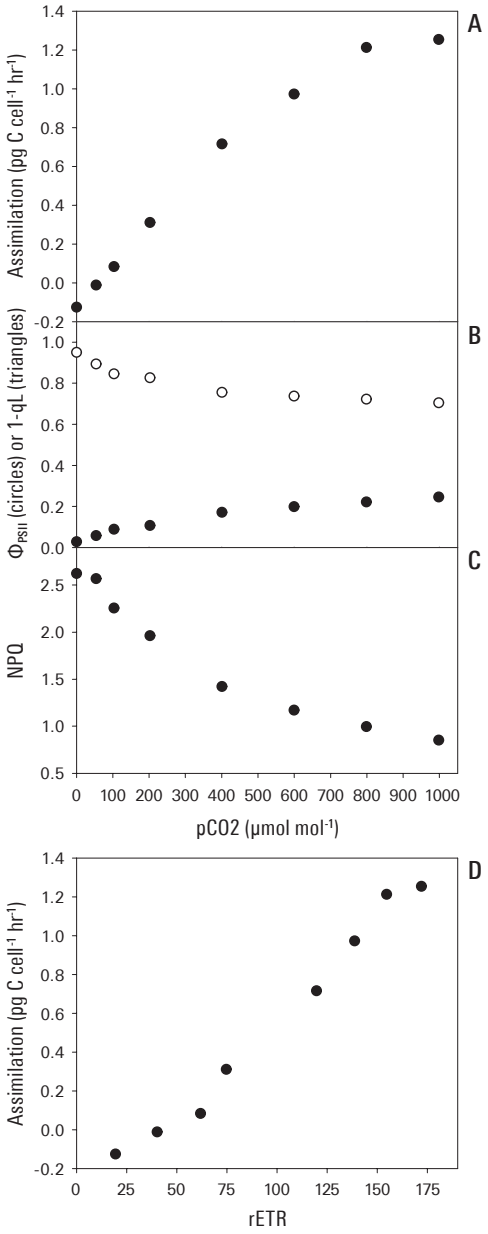

Figure 4. Panel A shows the net carbon assimilation rate. Panels B and C show measurements derived from chlorophyll *a* fluorescence as a function of the equilibrium CO_2 concentration (pCO_2) in the sample cuvette. The quantum efficiency of PS_{II} is shown in the panel B (Φ_{PSII}, closed symbols) along with the fraction of closed reaction centers (1-qL, open symbols). Panel C shows the degree of captured energy being dissipated through non-photochemical processes (NPQ). Net carbon assimilation as a function of relative Electron Transfer Rate (rETR) is plotted separately in panel D.

Specifications

Sample Cuvette

Wetted materials: 316 stainless, float glass, Viton, PTFE, silicone, acetal

Cuvette working volume: 0 – 20 mL, 15 mL recommended sample volume

CO_2 Gas Analyzer

Operating Principle: Non-dispersive Infrared (NDIR)

Measurement Range: 0 – 3100 µmol mol^{-1}

Precision (1-sigma) @ 4 second averaging @ 400 µmol mol^{-1}: < 0.1 µmol mol^{-1}

Accuracy: 1% of reading at > 200 µmol mol^{-1}, +/- 2 µmol mol^{-1} at < 200 µmol mol^{-1}

CO_2 Control

Range: 0 – 2,000 µmol mol^{-1}

Fluorometer

Part Number: 6800-01A

Red/Blue actinic light output: 0-3000 µmol m^{-2} s^{-1}

Far-red light output: 0 – 20 µmol m^{-2} s^{-1}

Saturation Flash Intensity: 0 – 16,000 µmol m^{-2} s^{-1}

Red actinic peak wavelength: 625 nm

Blue actinic peak wavelength: 475 nm

Far-red peak wavelength: 735 nm

Temperature

Operating temperature: 0 to 50 °C with no solar load (non-freezing)

Storage temperature: -20 to 60 °C with chamber clean and dry

Temperature control: User provided water bath. #10-32 threaded connections to chamber.

Operating fluid environment

Temperature: non-freezing to 50 °C

Salinity: 0 – 35 %

Auxiliary Ports

pH (probe not included): 12 mm diameter O-ring sealed port and integrated amplifier. Passive glass-electrode based pH probe, with BNC connector (nominal -59 mV/pH slope, user calibrated).

Septa: Silicone-PTFE septa

Specifications subject to change without notice

LI-COR® Biosciences

Global Headquarters

Lincoln, Nebraska, USA
Intl.: +1-402-467-3576
envsales@licor.com

979-19316 8/20

Outside the United States – Regional Offices and Distributors

www.licor.com/env/contact

ISO 9001:2015 certified