A Perfusion Chamber for Electrode Studies on the Physiology of Planktonic Algae[1]

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ABSTRACT

An algal photosynthetic chamber using an oxygen electrode is described. It is unique in that the algae are held in the chamber on a filter. In this manner a high concentration of cells per unit volume of chamber and a high sensitivity to oxygen production is obtained. Other advantages of this system are that the solution around the algae on the filter is changed easily without disturbing the cells and the chamber is easily adapted to continuous closed circulation.

During investigation of photosynthetic processes in marine phytoplankton, there was obvious need for a system in which oxygen evolution could be measured rapidly and continuously and in which the aqueous environment could be changed or modified without addition or subtraction of cells. It seemed likely that algal cells could be concentrated by filtration on membrane or glass-fiber filters for study in a closed system with a Clarke-type oxygen electrode (Carritt and Kanwisher 1959). A system such as this would permit the surrounding medium to be changed without changing cell numbers; the sensitivity would be high, since large numbers of algae could occupy a relatively small volume.

With the above considerations, the lucite chamber shown in Fig. 1 was designed. A central well 40 ml in volume for holding the filter and electrode is surrounded by a cooling jacket containing water. The central well accommodates a 47-mm membrane or glass-fiber filter, supported in the well by a stainless-steel screen. The medium in the well is stirred from the bottom with a $3/4''$ teflon-coated bar. Covering the top of the well is an optically clear disc of lucite sealed to the well by an "O" ring and held in place with three stainless-steel screws. Communication to the central well is by inlet and outlet tubes

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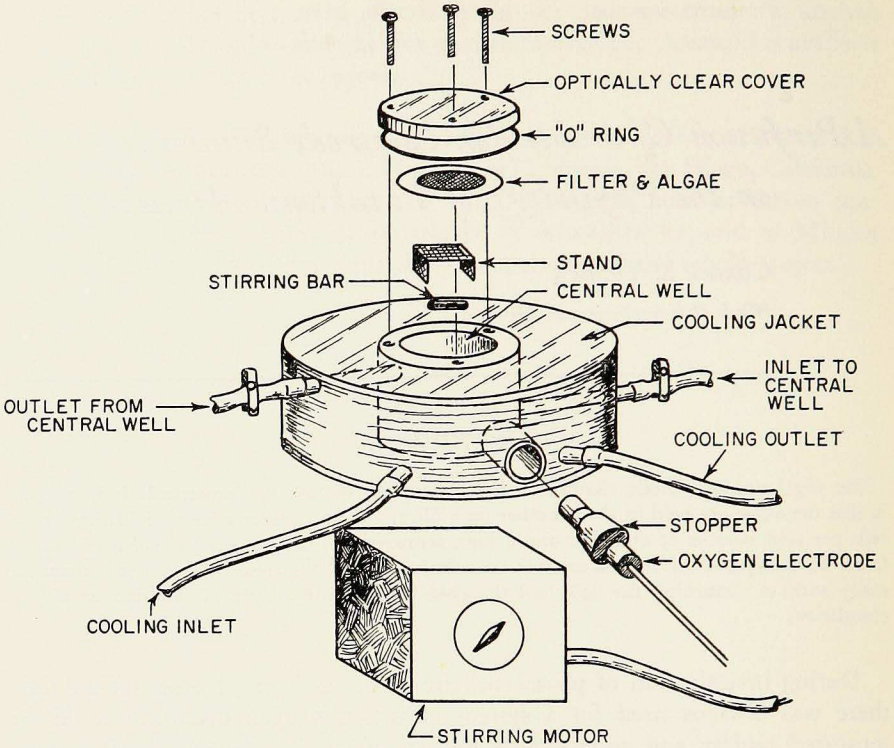

Figure 1. Components of the perfusion chamber.

located at opposite sides and near the bottom and top of the well; these tubes are sealed with short lengths of hose and clamps. The large opening near the bottom of the well houses the oxygen electrode, which is sealed in the chamber with a silicone rubber stopper. Two smaller ports are inlets to the cooling jacket.

In practice, an algal suspension is drawn onto the filter using mild suction. The central well, with optical cover removed and intake and outlet ports closed, is filled with the medium. The stirring bar is then placed. The medium should be undersaturated with respect to oxygen so that bubbles resulting from supersaturation are not formed during the photosynthesis experiment. The filter, with algae, is placed on the stainless-steel screen and thoroughly wetted with the medium. After the optical cover is screwed down, the entire unit is tipped to collect the remaining air at the outlet tube. The tube is first opened to permit the air to escape; it is then filled with the medium, using a syringe or a pipette. When the outlet has been closed, stirring is started by centering the central well on a platform magnetic stirrer. To carry off the heat generated

by the stirrer motor, the cooling water should flow through the chamber at a rate of 0.5 l/min.

The culture on the filter is easily illuminated by means of an angled mirror by which light from a suitable source is directed into the central well. If prolonged, intense illumination is used, an infrared filter should be employed. Note that the tip of the electrode should not be exposed directly to intense light.

The sensitivity of a photosynthetic chamber may be expressed as $dM/dt \simeq D(Rp + Rc)/V$, where M is the change in current across the electrode due to a change in oxygen tension in the chamber, Rp is the rate of oxygen production and Rc the rate of oxygen consumption by a single photosynthetic unit, D is the number of photosynthetic units, and V is the volume of the chamber. If the number of photosynthetic units remains constant, then change in oxygen tension will reflect the integrated excess of photosynthesis over respiration, or the reverse. As shown below, details of the shape of curves, relating oxygen tension to time, reflect changes in the rates Rp or Rc.

With an oxygen electrode of the same dimensions and description as that discussed by Kanwisher (1959), and with exposure of 0.5 g of the alga *Dunaliella euchlora* to 800 foot-candles at 20°C, the oxygen tension in a chamber of 20 ml volume is changed the equivalent of 5 mv/hr. (1 mv = 0.7 ml O_2/l). In a chamber of 50 ml volume, with the same amount of these algae, the oxygen tension is changed the equivalent of 3.0 mv/hr. It is emphasized that the sensitivity is greatly altered by varying the thickness of the membrane covering the electrode or by varying other factors controlling the diffusion of oxygen through a membrane. The greatest sensitivity is obtained by using small volumes and large numbers of algae. The size of the chamber is limited by the size of the electrode with respect to the volume of the chamber. According to Kanwisher (1959), the consumption of oxygen by his electrode was 2×10^{-4} ml/hr. with a current flow of one microamp; with a 1-cm platinum electrode, the consumption of oxygen is about 4×10^{-4} ml/hr. Although most investigators may prefer to work with low concentrations of algae, chambers larger than 50 ml may be too insensitive. In the author's experience, small perturbations in either stirring or temperature control are bothersome when working with a full scale of 1–2 mv.

Fig. 1a shows an example of oxygen evolution by *Nannochloris* as a function of light intensity. The data were obtained with a series of Kodak 2″ × 2″ neutral-density filters placed over the optical cover at 5-minute intervals.

The greatest advantage of the system is the ease with which the surrounding medium may be changed. Fig. 2b shows a curve reflecting the change in rate of oxygen evolution by *Dunaliella* after addition of nutrients. For 36 hours, the algae were exposed to 800 foot-candles in a medium containing little or no nutrient nitrogen. At this point the outlet valve to the central well was opened, and a 100-ml syringe containing a new medium with 100 μg-at N/l was attached to the intake inlet. The chamber was completely flushed with

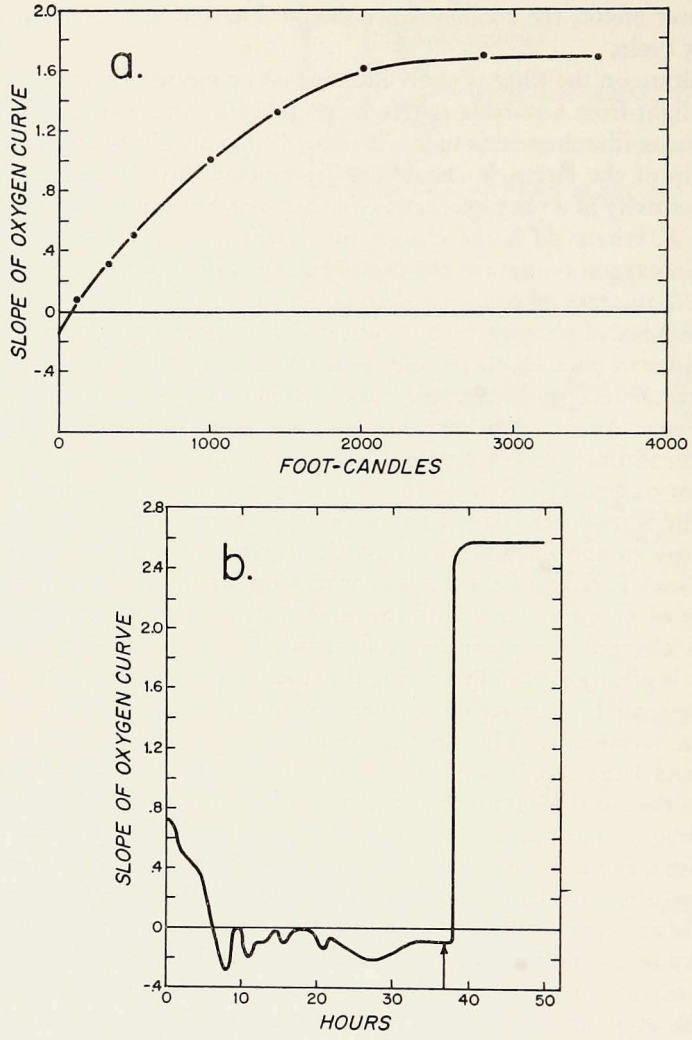

Figure 2. (a) Relationship between oxygen evolution and light intensity; *Nannochloris* sp., 20°C. (b) Change in the rate of oxygen evolution at 800 foot-candles before and after addition (arrow) of nitrate; *Dunaliella euchlora*, 20°C.

the new medium, and both inlets were closed. Once again the algae were exposed to 800 foot-candles. The time-course curve for the next 15 hours shows clearly enhanced oxygen evolution after the medium containing nitrate was supplied.

The chamber may be modified for use in a continuous-flow circulation system, and other parameters may be measured in addition to oxygen evolu-

tion. In one application of this system, the uptake of carbon-14 from the medium was monitored continuously. After the solution was passed through the central well it was forced by a peristaltic pump through an anthracene-scintillation crystal and back into the central well. The beta radiation from C^{14} compounds in solution caused the anthracene crystal to emit light that is proportional to the radioactivity (measured with a photomultiplier tube). Hydrogen-ion concentration and fluorescence of the solution can also be monitored simultaneously in the same system. In some experiments, where stirring is not needed, a photo-detecting device has been placed underneath the central well for viewing the light transmitted through the algae and the support. With a suitable choice of wave lengths (obtained by using optical filters), chlorophyll content as well as cell growth can be measured.

The major disadvantage of the algae-filter system may derive from the tolerance of algae to filtration. Although no systematic survey has been made, it generally appears that most flagellates and diatoms (e.g. *Dunaliella*, *Nannochloris*, *Carteria*, *Isochrysis*, *Skeletonema*, and *Nitzschia*) are very tolerant to filtration. It seems unlikely that many of the coccolithophores and dinoflagellates could stand this treatment.

Depending upon the tolerance and abundance of naturally occurring phytoplankton, the system can be used to study natural populations. The material filtered from 10 to 20 l of coastal water (around Woods Hole) should suffice. However, the sensitivity of the present system is still too low to be used with the sparse populations that can be concentrated from the open ocean.

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