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Measurement of photosynthetic oxygen production

1 Introduction

All material methods for measuring primary production are based on the stoichometry of photosynthesis. They measure rates of CO_2 utilization, changes in the concentration of organic products or, in the case of oxygen techniques, rates of oxygen evolution during photosynthesis. The observed changes in oxygen concentration of the water result from photosynthetic release as well as respiratory consumption of oxygen. Changes in the light are defined as net oxygen production, oxygen decline in the dark is named community dark respiration.

Basically the oxygen method does not require expensive equipment or hazardous chemicals. It was used by GAARDER & GRAN (1927) to carry out the earliest measurements of in situ productivity and is still the most common method for monitoring metabolism of aquatic ecosystems. The development of oxygen-sensitive electrodes enabled continuous and short-time measurements in enclosures as well as in surface waters. Production and respiration of oxygen can be measured in enclosed samples of pelagic (GAARDER & GRAN 1927) or benthic communities (POMEROY 1959) or estimated from the oxygen budget of whole river stretches (ODUM 1956) or of epilimnion or hypolimnion of stratified lakes or sea basins (e.g., WELCH 1968). The latter calculations have to take atmospheric exchange into account.

2 Measured processes

The observed change in concentration of dissolved oxygen results from a variety of physiological processes (see, e.g., HARRIS 1978, FALKOWSKI & RAVEN 1997). During illumination oxygen is photosynthetically produced but also consumed by mitochondrial

respiration and perhaps by light respiration and the Mehler reaction. Mitochondrial respiration occurs by algae as well as by heterotrophic organisms (bacteria, fungi, animals) which (except for axenic cultures) jointly act in the water sample.

Photosynthesis: Cyanobacteria, prochlorophytes, eukaryotic algae and higher plants (but not anaerobic photosynthetic bacteria) use light energy to oxidize water. They yield molecular oxygen during this so-called light reaction of photosynthesis:

$$2\ H_2O + \text{light} \text{----->} 4\ H^+ + 4\ e^- + O_2$$

Light reaction occurs in photosystem II which is located in thylakoid membranes.

Dark respiration: „Dark" or mitochondrial respiration occurs in the light as well as in darkness. Its pathways include glycolysis, the oxidative pentose phosphate pathway, the tricarboxylic or Krebs cycle, and the mitochondrial electron transport chain. Dark respiration serves to form ATP and reductants and to produce carbon skeletons from complex organic compounds. Respiration is therefore essential for maintenance as well as growth of cells. Maintenance respiration rates are variable between species but fairly constant for a given population. The component of respiration associated with cell synthesis is proportional to the growth rate (see GEIDER 1992). Its rate increases with temperature (Q_{10} ca. 1.7-2.0). Mitochondrial respiration might be inhibited as well as stimulated in the light. GRANDE et al. (1989), e.g., found ratios between mitochondrial respiration in the light and in the dark ranging from 0.2 - 12.5.

Photorespiration: The light-dependent oxidation instead of carboxylation of ribulose bisphosphate is named photorespiration. It produces glycolate which is subsequently exudated, oxidised or used for synthesis of amino acids. Intensity of photorespiration increases with increasing ratios of oxygen to carbon dioxide. Photorespiration is an energy-dissipating process which reduces both the initial slope and the light-saturated rate of photosynthesis. It was reviewed, e.g., in a special issue of Aquatic Botany (1989, volume 34).

Mehler reaction: This is a light-dependent redox reaction, in which photosynthetically produced oxygen is reduced to H_2O_2 in photosystem I. There is no net oxygen consumption.

3 Common applications

Bottle method: Water samples from the various depths are enclosed in both duplicate transparent (light) and in dark bottles. Bottles must be filled immediately and carefully (to avoid exchange of oxygen or trapping of gas bubbles). The bottles are either incubated at the sampling depth or are moved vertically through the euphotic layer. The lenght of incubation must allow measurable changes in oxygen concentration but avoid supersaturated conditions in the light bottles. It ranges from less than one hour under conditions of very high algal biomass to about 6 hours in less productive waters. Simultaneous incubation of light and dark bottles enables the measurement of both net oxygen production and dark respiration. If the rate of respiratory oxygen consumption is the same in the dark as in the light then the difference between the oxygen values of the light and the dark bottle is a measure of gross photosynthesis. The method was described in more detail by, e.g., WETZEL & LIKENS (1991).

Laboratory incubations: A variety of incubators was used for laboratory or shipboard measurement of oxygen production. Samples are usually exposed to an artificial light gradient. Light sources should resemble the spectral composition of sunlight as close as possible but the vertical changes of the light spectrum in the water column were rarely simulated. An assemblage of microelectrode and light source of variable intensity but constant spectral composition („light pipette") enables measurements of oxygen evolution of the same concentrated algal sample at a range of light intensities within an hour or so. Laboratory measurements should take the temperature-dependence of the involved processes into account.

4 Priniciples of oxygen measurement

The Winkler method: This method is based on the oxidation of manganous hydroxide by the oxygen dissolved in the sample. The product is transformed to manganic sulfate which liberates iodine from previously added potassium iodide. The quantity of free iodine is

equivalent to the amount of dissolved oxygen. It is determined by titration with a standard solution of sodium thiosulfate. Errors may occur in waters containing suspended organic solids, dissolved organic compounds, nitrite, or iron salts. The precision of the Winkler method is improvable by microtitration with automatic detection of the endpoint. The method has been described in full detail in APHA 1989, and WETZEL & LIKENS (1991).

***Oxygen electrodes*:** Oxygen electrodes consist of a cathode, usually made of platinum or gold, and a silver / silver chloride anode. A constant potential is maintained between both poles to electrochemically reduce oxygen at the cathode to water and hydroxide ions. At the same time, silver of the anode is oxidized. The silver is regenerated by the surrounding hydrous solution of KCl. The electric signal of this polarographic method is proportional to the oxygen flux. It depends on the surface area of the cathode. The mostly used Clarke-electrodes separate sample and cathode by a thin (10-30 µm) membrane of Teflon or polyethylene. The diffusion of oxygen through membrane and electrolyte is often speeded up by stirring. Rate of diffusion and thus signal of the electrode depends on temperature and exchange rate of the sample. The output also depends on salinity and air pressure. Macroelectrodes require a rapid flow of sample across the membrane. Their output is usually compensated for temperature. Most of them are unreliable at oxygen concentrations below 1 mg l^{-1} and they are poisoned by hydrogen sulfide. Microelectrodes provide faster response and are less sensitive for flow of water but are more fragile. Their output is usually not corrected for temperature and is less constant compared with macroelectrodes. Oxygen electrodes require repeated calibration by chemical methods.

References

AMERICAN PUBLIC HEALTH ASSOCIATION (APHA) (1989): Standard methods for the examination of water, sewage, and wastewater.- 17 th ed. American Public Health Association, New York. 1550 pp.

FALKOWSKI, P.G. & RAVEN, J.A. (1997): Aquatic photosynthesis.- Blackwell Science, Oxford. 375 pp.

GAARDER, T. & GRAN, H.H. (1927): Investigation on the production of plankton in the Oslo-Fjord.- Rapp. Cons. int. Explor. Mer 42, 1-48

GEIDER, R.J. (1992): Respiration: taxation without representation ?- In: Primary productivity and biogeochemical cycles in the sea.- (Eds.: FALKOWSKI, P.G. & WOODHEAD, A.D.) Plenum Press, New York, London, 333-360

GRANDE, K.D.; MARRA, J.; LANGDON, C.; HEINEMANN, C. & BENDER, M.L. (1989): Rates of respiration in the light measured in marine phytoplankton using an ^{18}O isotope-labelling technique.- J. Exp. Mar. Biol. Ecol. 129, 95-120

HARRIS, G.P. (1978): Photosynthesis, productivity and growth: The physiological ecology of phytoplankton.- Arch. Hydrobiol. Beih. Ergebn. Limnol. 10, 1-171

ODUM, H.T. (1956): Primary Production in Flowing Waters.- Limnol. Oceanogr. 1, 102-117

POMEROY, L.R. (1959): Algal productivity in salt marshes of Georgia.- Limnol. Oceanogr. 4, 386-397

WELCH, H.E. (1968): Use of modified diurnal curves for the measurement of metabolism in standing water.- Limnol. Oceanogr. 13, 679-687

WETZEL, R.G. & LIKENS, G.E. (1991): Limnological analysis.- 2 nd ed. Springer, New York. 391 pp.

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