JOURNAL OF BACTERIOLOGY, Oct. 1967, p. 1255-1256

Vol. 94, No. 4
Printed in U.S.A.

Use of Glass Fiber Filters for the Rapid Preparation of In Vivo Absorption Spectra of Photosynthetic Bacteria[1]

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Received for publication 13 July 1967

Usually, in vivo light absorption spectra of photosynthetic bacteria are obtained by measuring cell suspensions in glass or quartz cuvettes. In such arrangements, however, the scattering of light by the suspensions as well as sedimentation or even swarming behavior of motile cells (N. Pfennig, Arch. Mikrobiol. **42**:90, 1962) may give rise to various errors. The error caused by light scattering in thin suspensions is partly eliminated by use of an opal glass behind reference and sample cuvettes (K. Shibata, A. A. Benson, and M. Calvin, Biochim. Biophys. Acta **15**:461, 1954).

C. S. Yentsch (Nature **179**:1302, 1957) introduced a new technique for the estimation of chlorophyll concentrations in algal cultures. Algal cells are concentrated on a membrane filter, dried, and cleared with cedar oil. The cleared filter is then mounted in the spectrophotometer housing and absorbancy of the chlorophyll band at 670 mμ is measured. Air is the reference. Modification of this method with a blank filter as reference proved useful in measurements of visible light absorption by particulate matter in the oceans (C. S. Yentsch, Limnol. Oceanog. **7**:207, 1962). Applying the same method for in vivo measurements of enrichment and pure cultures of photosynthetic bacteria, we found it useful for *Chlorobacteriaceae*, i.e., photosynthetic bacteria containing bacteriochlorophylls c or d (A. Jensen, O. Aasmundrud, and K. E. Eimhjellen, Biochim. Biophys. Acta **88**:466, 1964). At wavelengths above 750 mμ, i.e., mainly in the in vivo absorption range of bacteriochlorophylls a and b, however, we were unable to use membrane-type filters because of their high apparent absorbancy in the near infrared region. This difficulty is now overcome by replacing the membrane with glass-fiber filters. By use of the method described below, the preparation of in vivo absorption spectra of photosynthetic bacteria representing all four bacteriochlorophyll types is possible in a time of 3 min per sample. The method proved to be useful in the wavelength range between 350 and 1,200 mμ for all kinds of photosynthetic bacteria enrichment and pure cultures, as well as for the investigation of water samples from different depths of

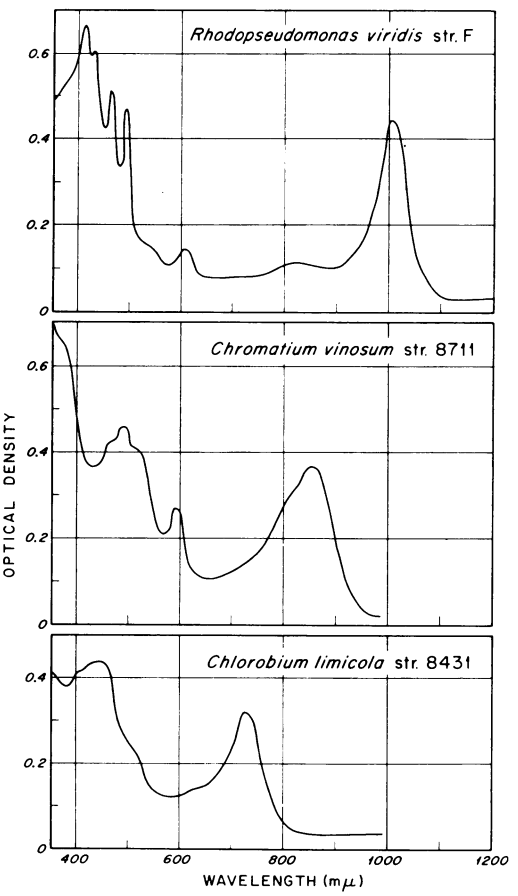

FIG. 1. *In vivo absorption spectra of Rhodopseudomonas viridis strain F (kindly provided by S. W. Watson), Chromatium vinosum strain 8711, and Chlorobium limicola strain 8431.*

[1] Contribution 1949 from the Woods Hole Oceanographic Institution.

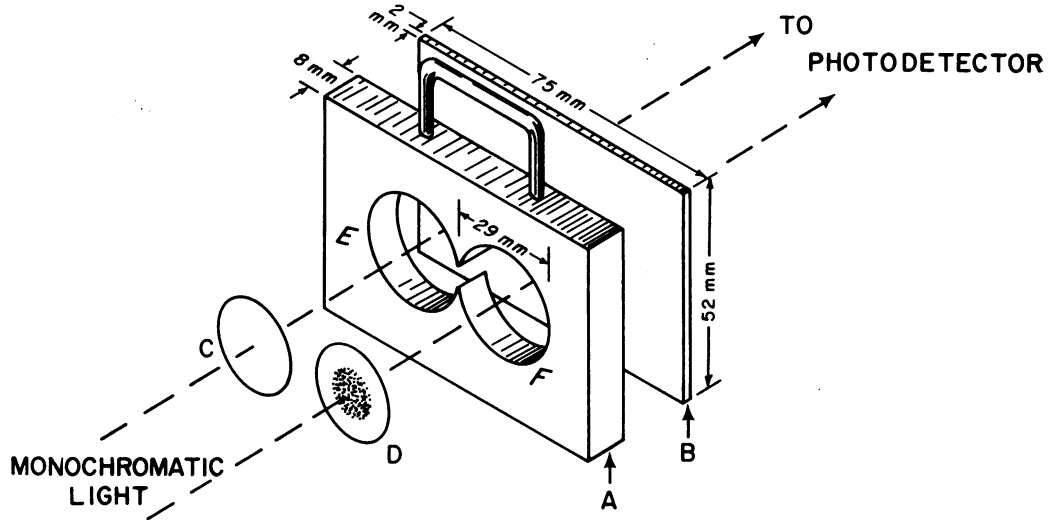

FIG. 2. *Lucite carrier for the mounting of filters in the spectrophotometer. A, lucite block with two wells and handle. B, back plate (optical lucite), to be cemented to the back of block A. C, blank filter (25 mm diameter). D, sample filter. E, well for blank filter. F, well for sample filter.*

meromictic lakes to detect purple and green sulfur bacteria that occur in definite layers between hydrogen sulfide- and oxygen-containing waters. Even with rather small *Chlorobium* and *Rhodopseudomonas* strains, good results are obtained (Fig.1).

Method. Depending on the density of the bacterial suspension, 5 to 50 ml are filtered onto a glass-fiber filter (Gelman GF/C, A, 25 mm diameter). The wet filter is immediately mounted in the special lucite carriage (Fig. 2) which already contains the wetted (water or medium) blank filter. The humidity of the filters is sufficient to attach them to the carriage back. The carriage is positioned into a Beckman DK-1A Recording Spectrophotometer directly in front of the photocell slits. At wavelengths above 750 mμ, the PbS cell is used, below 750 mμ, the photomultiplier (IP28) is used.

This investigation was supported by National Science Foundation grants 861, 5199, and 5456.