1. Extraction

Mats developing in a sand substrate or gelatinous mats can be extracted with methanol (100% minus the water in the mat), acetone, or a maxture: acetone/methanol (7/2). Distinct layers recognizable by differences in color can be carefully separated from each other and then extracted with the solvent. The addition of small amounts of $CaCO_3$ or $MgCO_3$ to the solvent will prevent acidification and subsequent conversion of chlorophylls to phaeophytins. It is important to mix the samples thoroughly with the solvent, and with tough mats it may be necessary to use tissue grinders or sonication to achieve adequate mixing for complete extraction. Once pigments are in methanol, they are extremely sensitive to degradation and photooxidation. The extracts should be kept cold and dark (work in dim light). Extraction will take a few minutes to a few hours depending on how well the sample is macerated. The extract can be clarified by settling, low speed centrifugation, or pressure filtration through glass-fiber filters. The absorption spectrum of the extract should be recorded as soon as possible.

It may be necessary to re-extract the sample to remove all pigments. Methanol (95%) and acetone will extract all chlorophylls and carotenoids. However, the water-soluble phycobilins will not be extracted with organic solvents.

2. In vivo spectra

Pigments may be examined directly in living material by doing absorption spectra on mat layers that are evenly suspended in an appropriate buffer. Since suspensions of cells are turbid, it is necessary to compensate for this by suspending cells in sucrose or BSA or by disrupting the cells. Cells are most easily and quickly broken by sonication. After sonication, spectra must be recorded immediately. Although the cells are destroyed, the pigments remain

with those of intact cells and considerably different from the spectra obtained from extracted pigments no longer oriented in the native pigment/protein complex.

3. Quantifying Pigments

The best quantitative data are obtained by using extinction coefficients for the purified pigments in the same organic solvent used for extracting the mat samples. However, since there are several different pigments present in the extract, the relative heights of peaks are altered by interactions in closely located bands. Quantitative data are only meaningful if related to some other parameter such as dry mass or protein present in the mat sample.

It is very difficult to obtain quantitative data from the complex spectra of disrupted cells. These spectra are immensely useful, however, for determining exactly which wavelengths of light are absorbed by a particular mat layer. They may also reveal significant variations in absorption maxima that permit a closer identification of the actual organisms present.