Abstract

The microbial mat at the Great Sippewissett Marsh is composed of five layers (in order of increasing depth: gold, green, pink, peach and lower green) of photosynthetic microorganisms. Qualitative and quantitative studies of the photosynthetic pigments of the mat were undertaken to determine which photosynthetic pigments were present in each layer. Samples of each layer were subjected to two extraction procedures: release into aqueuos medium following sonication, and extraction into methanol. The quantity of each pigment in methanol extracts was estimated from known extinction coefficients. In initial analyses, Absorption spectra were determined for each layer that was visually distinguishable along a 1-cm. diameter core. Subsequently, successive discs 1 mm high were separated from each core; each of the resulting samples was 79 mm^3 in volume. This procedure provided samples suitable for quantitative determination of pigment distribution with depth. Chlorophyll a was readily detectable in the gold and gre-n layers, bacteriochlorophyll a (bchl a) in the pink layer, bchlb in the peach layer, and bchl c in the lower green layer. Distribution with depth was as follows: chlorophyll a was most abundant at 2-3 mm, bchl a at 4 mm, bchlb at 5 mm, and bchl c at 6-8 mm. The maximum concentration of chlorophyll a (65-125 ug/ml mat volume) was higher than the maxima of bchl a (35-55 ug/ml) and of bchl c (25-55ug/ml). The concentration of bchl b could not be estimated due to the unavailability as yet of an extinction coefficient for this pigment. Althiugh each pigment was distributed through more than 1-mm region, the order of their maxima relative to depth was the same in each mat core analyzed. Since each of these pigments is characteristic of a particular type of photosythetic microorganism, the vertical succession of the pigments implies that the bacterial community is stratified. An associated microscopical study revealed that the pigment maxima were correlated with the distribution of the several distinguishable microbial types of the mat community (Nicholson and Pierson, these Proceedings).

Introduction

A microbial mat is a complex, miniature ecosystem composed of laminated layers of photosynthetic microorganisms. Mats are a very ancient biological phenomena and there are very few mats which still exist today. The mat studied in this project is located at the Great Sippewissett Marsh and can be considered a living analogue of one of the first forms of cummunity life on earth. The purpose of this study was to determine which photosynthetic pigments were in each layer and what was the vertical distribution of these pigments.

The mat is composed of five layers, gold, green, pink, peach, and lower green in order of decreasing depth. Representative samples of each layer were collected and a 80-100 mm^3 volume of each sample was subjected to 3-5 ml of absolute methanol for 5 min. in the dark to extract the pigments. Methanol extracts from each layer were then filtered through GF/C 2.4 cm Whatman glass microfiber paper. Absorbance spectra of each layer were read on a Cary 14. Core samples of the microbial mat were collected and each core sample was cut (from top to bottom) into 1 mm high disc samples. Each of the resulting disc samples was 79 mm^3 in volume. The disc samples of one core were subjected to methanol extraction, as descibed above, of a fixed dilution. The disc samples of a second core sample were subjected to invivo studies where each disc sample was placed in a constant volume of a Tris buffer (suspending the chlorophylls still in association with their apoproteins). Each suspention was then sonicated for 15-30 second intervals for a total of 2 minutes and then centrifuged for 3 minutes. Absorbance spectra of the supernatant of each suspention was read on the Cary 14. These procedures carried out on the 79 mm^3 disc samples of each core provided suitable for qualitative and quantitative determination of pigment distribution with depth. Light penetration studies using a LICOR meter were also preformed in an attempt to relate amount of pigment in each mm disc to the amount of light lost (absorbed and reflected) in each mm disc.

Results

Absorbance spectra of layers 1-5 (Fig. 1-5) were read from visually distinguishable representative samples of each layer suspended in methanol. The locations of the absorbance peaks of each sample were read in nm and the values were compared to those in Table 1. In the gold and green layers an absorbance peak at 665 nm was

sorbance peak at 773 nm in the pink layer (Fig. 3) revealed the presence of bchl a (Table 1), an absorbance peak at 790 nm in the peach layer (Fig. 4) revealed the presence of bchl b (Table 1), and an absorbance peak at 669 nm in the lower green layer (Fig. 5) revealed the presence of bchl c (Table 1). To verify the change or shift of absorbance peak location of the methanol extractions (from 665 to 669 and from 773 to 790) representative samples were taken as before and subjected to invivo experiments. The samples were suspended in a Tris buffer which pulls the pigments out still attached to their pigment-protein complex. The resulting absorbance spectra give absorbance peaks further spread out and therefore easier to distinguish from the others. By these means a second proof or verification was attained (Fig 6 , Table 1).

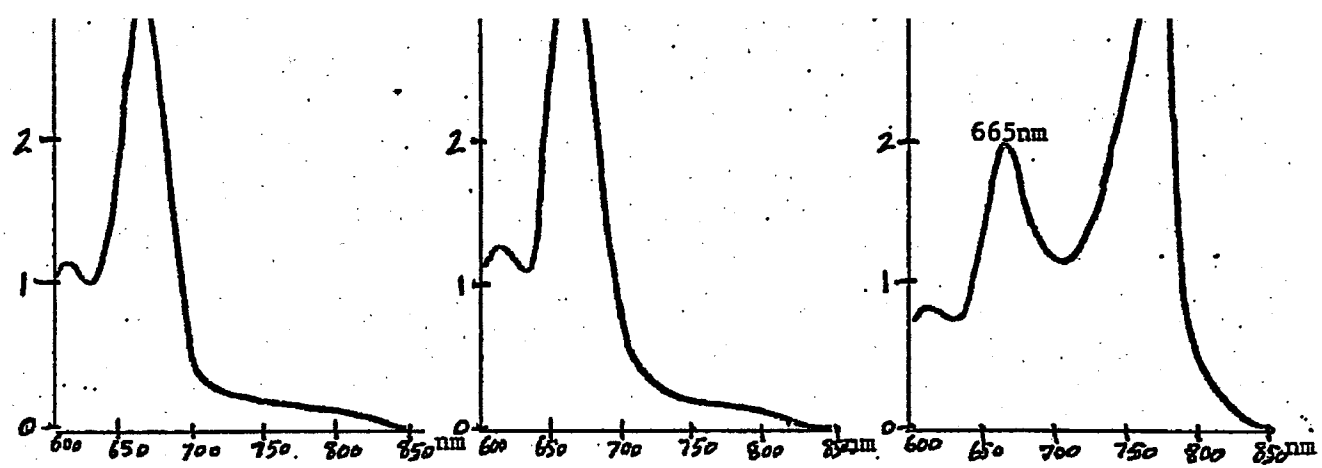
For quantitative studies the absorbance spectra of each 1-mm disc sample of each core(suspended in constant volume of MeOH) was read. Absorbance peaks indicating chlorophyll a, bchl a, bchl b, and bchl c were looked for and measured in absorbance units on each absorbance spectrum of each sample. Using the measured value of each peak (A.U.) and Lambert/Beer's Law the relative concentration of each pigment in each disc sample was calculated in ug/ml in 79 mm^3. Chlorophyll a was apparent 1-4 mm down, being most abundant 2-3 mm down. Bchl a was apparent 2-5 mm down, being most abundant 4 mm down. Bchl b was apparent 4-7 mm down, being most abundant 5 mm down. Bchl c was apparent 5-8 mm down, being most abundant 6-7 mm down.

Maximum concentrations of each pigment were calculated using the highest absorbance peaks and Lambert/Beer's Law. The maximum concentration of chlorophyll a was found to be 65-125 ug/ml mat volume which was higher than the maxima of bchla (35-55 ug/ml) and of bchl c (25-55 ug/ml). The concentration of bchl b

ficient for this pigment.

Using a LICOR meter to measure light intensity, light penetration studies were done on core samples of the mat. These light penetration studies revealed that most light was lost (absorbed and reflected) at the surface or top most part of each layer. The amount of light lost within each layer was very small.

Experiments this summer on the microbial mats included spatial relationships using scanning electron microscopy, photosynthetic activity using ^{14}C uptake, and pigment analysis using absorbance spectra and light penetration studies. With more exact methods of measuring light penetration of each disc sample it could be possible to draw corrilations between light intensity and the amount of a given pigment present. The relative amount of pigments calculated in this project would have had more significance if related to dry mass or protein assays. Unfortunately time did not allow for these further studies. I hope to see further studies on dry mass, protein assays, and oxygen and hydrogen sulfide gradients. These would allow for a more complete profile of the microbial mats at the Great Sippewissett Marsh.



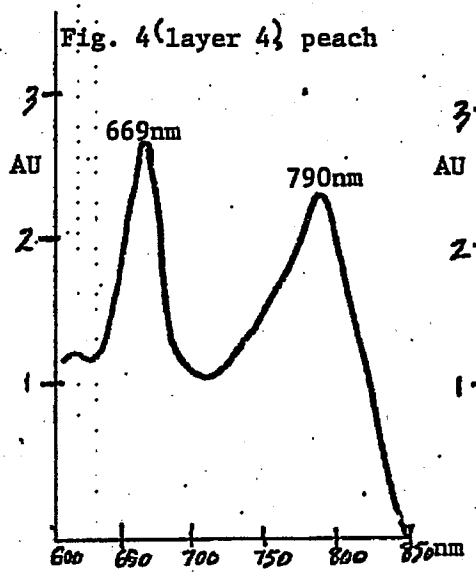


Fig. 4(layer 4) peach

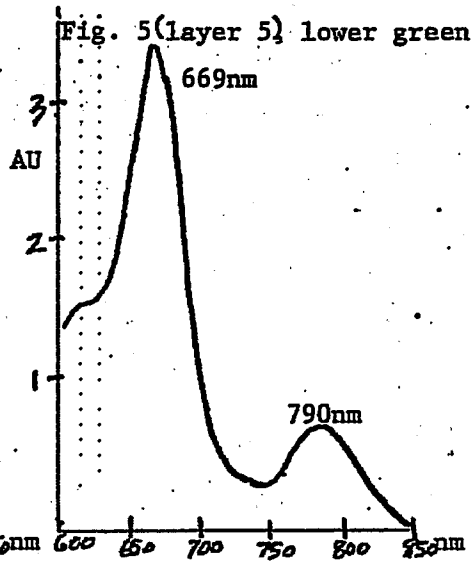


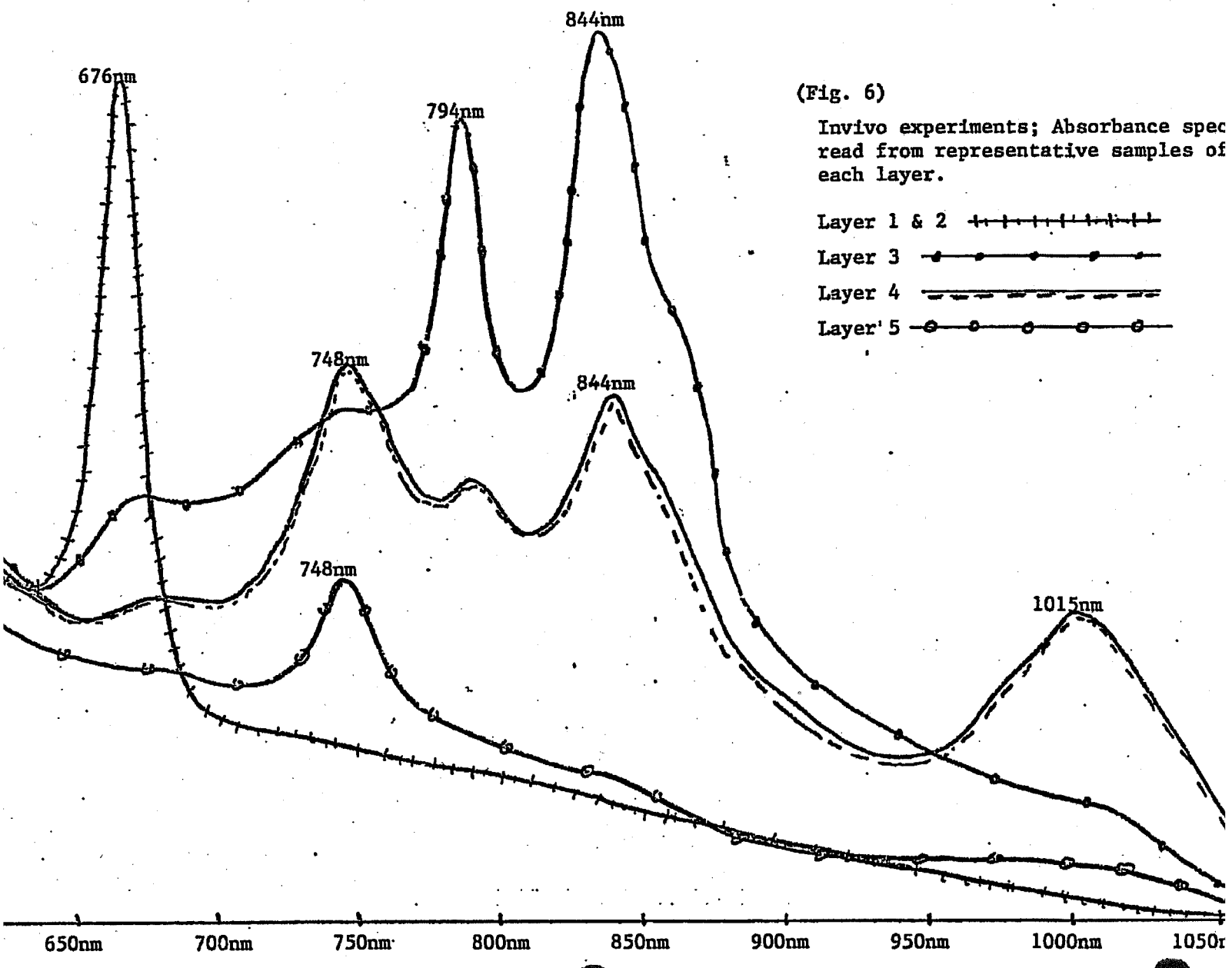
Fig. 5(layer 5) lower green

(Fig. 1-5) nm is measured on the x axis, absorbance units is measured on the y axis. MeOH extractions.

* Table 1.

Pigment	Invivo	MeOH
Chlorophyll a	676nm, 621nm	664nm, 614nm
bchl a	805nm, 830-890nm	771nm
bchl b	835-850nm, 1020-1040nm	794nm
bchl c	745-755nm	660-669nm
bchl c_s	740nm	667nm
bchldd	705-740nm	654nm
bchl e	719-726nm	646nm

*from 4th ed. Biologt of Microorganisms , T.P. Brock, B.W. Smith, and M.T. Matigan.



(Fig. 6)

Invivo experiments; Absorbance spec
read from representative samples of
each layer.