(A) An analysis of the purple bacterial layers in the microbial mats at Sippewissett Marsh.

Background:

Preliminary work on the mats has revealed an interesting variation to the usual pattern of layering found in other similar intertidal systems. The usual pattern found in mats is a surface layer of cyanobacteria beneath which is found a pink/red layer of purple sulfur bacteria. In Sippewissett mats, beneath this pink/red layer, we have found a third distinct layer of peach-colored purple sulfur bacteria. Initial analysis of the pigmentation patterns for the three layers revealed the presence of chlorophyll a in the cyanobacterial layer, bacteriochlorophyll a (Bchl a) in the pink layer and Bchl b in the peach layer. The presence of two distinct purple photosynthetic bacterial layers in laminated mats has not been previously reported. The presence of a distinct layer of Bchl b containing purple bacteria in intertidal mats has not been previously reported. The mats at Sippewissett therefore reveal an interesting diversity in their bacterial components.

The objective of the project is to determine which physiological parameters (sensitivity to light, sensitivity to O_2, need for H_2S) and which pigment/protein molecular complexes form the basis of the diversity in purple sulfur bacteria observed in the Sippewissett mats.

the 3 mat layers.

b) To isolate in pure culture so as to identify to genus and species the major organisms present in the Bchl a--containing and Bchl b--containing layers.

c) To determine which of the following environmental parameters show variations that correlate strongly with the distribution of the two different photosynthetic bacterial layers: light intensity, O_2 levels, H_2S levels.

d) To compare the pigmentation patterns of the pure cultures isolated from the mats with the patterns in the actual mat layers to confirm the identity of the sources of all the pigment--protein complexes.

e) To vary the environmental parameters of light intensity, O_2 and H_2S levels with the pure cultures to see if their responses to these factors in a controlled laboratory setting are similar to their natural distribution in the field.

(B) An analysis of the pigment/protein complexes found in the filamentous, gliding marine green photosynthetic bacteria.

Background:

An organism appearing similar to the filamentous, gliding, thermophilic bacterium Chloroflexus aurantiacus was observed independently by Jane Gibson and R. W. Castenholz in marine mat material at Woods Hole in 1983. Both investigators are currently trying to isolate the organism. Using the large masses of this organism observed during the summer 1983, our initial observations

mesophilic photosynthetic gliding bacteria previously, none is currently available in pure culture. The objective of this project will be to isolate the organism in pure culture and to analyze the pigment/protein complexes for comparison with those of other photosynthetic green bacteria and in particular for comparison with the thermophilic strains. There are no other known examples of photosynthetic bacteria that have thermophilic and mesophilic strains from which one can compare the properties of the integral membrane pigment/protein complexes.

Specific objectives for summer, 1984:

a) To isolate this organism in pure culture.

b) To isolate in spectrally pure form the photochemical reaction center pigment/protein complex.

c) To compare the isolated reaction center complex with the one previously characterized from the thermophilic Chloroflexus aurantiacus in terms of its optical absorption characteristics, molecular weight, polypeptide composition, and thermal stability.

d) To determine the distribution of the filamentous photosynthetic bacteria in the Sippewisset Marsh.

(C) The search for a missing link

Background:

After a few beers it is at least fun, if not instructive, to imaginatively explore the process of evolution among the early phototrophs on earth. Many microbiologists have done this (even without the beer) and many have conjured

evolution of photosynthesis there was a time (perhaps when H_2S was no longer so readily available as a reductant and probably just prior to the evolution of the water splitting photosystem II reaction center), when a phototrophic organism existed that took advantage of the abundant Fe^{2+} on earth and used it as a source of electrons. It is certainly possible energetically. Such an organism would have had an abundant source of electrons and might have been very close to, if not the immediate ancestor of, the O_2-evolving cyanobacteria. The reaction center of this creature would have been very similar to the PSII reaction center of cyanobacteria although it would have had a slightly more negative redox potential. Of course, there is no reason why such an organism could not have persisted to modern times. It would grow in an anaerobic environment, rich in Fe^{2+}, exposed to light and would produce copious amounts of Fe^{3+} as a metabolic waste product. It would be most interesting to find and study this organism. It would be a very significant organism in putting together a story about life on earth from 2 to 3 BYA. The existence of such a creature would have a significant impact on our interpretations of the origins of the banded iron formations. I confess I have looked for it before (at MBL, summer 1976). I did not find it. Searching will not be easy.

Specific objectives for summer, 1984:

a) To discuss this organism and create a description of it*.

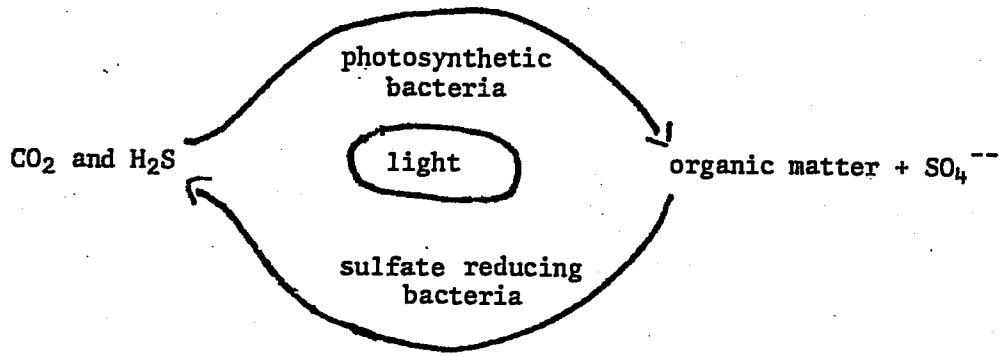
b) To design and construct a suitable enrichment medium for it.

c) To locate likely habitats, obtain samples and attempt to enrich for and isolate it.

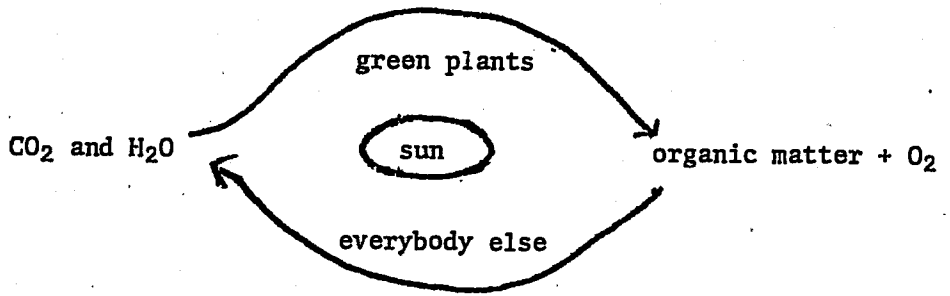
*Part (a) is best carried out in the evenings at the Captain Kidd.

cosms: Winogradsky, Pfennig, and Hot Spring Columns.

These devices are in essence micro-worlds with simplified balanced nutrient cycling system that looks like this:



which may be compared to the well-known global ecosystem:



1. Winogradsky column.

A large glass cylinder is firmly packed part way with anoxic and other muds from shallow marine, estuarine or freshwater environments and filled almost to the top with natural waters from the habitat. Organic detritus in the mud will provide substrates for anaerobic respiration and fermentation. The mud may be supplemented at the bottom of the cylinder with large or small amounts of insoluble organic matter (e.g., paper toweling) and 1:1 amounts

H_2S-producing bacteria. The packed mud should be free of trapped bubbles. The cylinder is covered loosely and exposed to a tungsten lamp (not close enough to heat). Illumination is from one side only. Near infrared filters can be used to select particular populations of photosynthetic bacteria.

A diagram of such a column is shown. For more details see Aaronson, S. 1970, Experimental Microbial Ecology, Academic Press, pp. 6-11.

2. Pfennig column

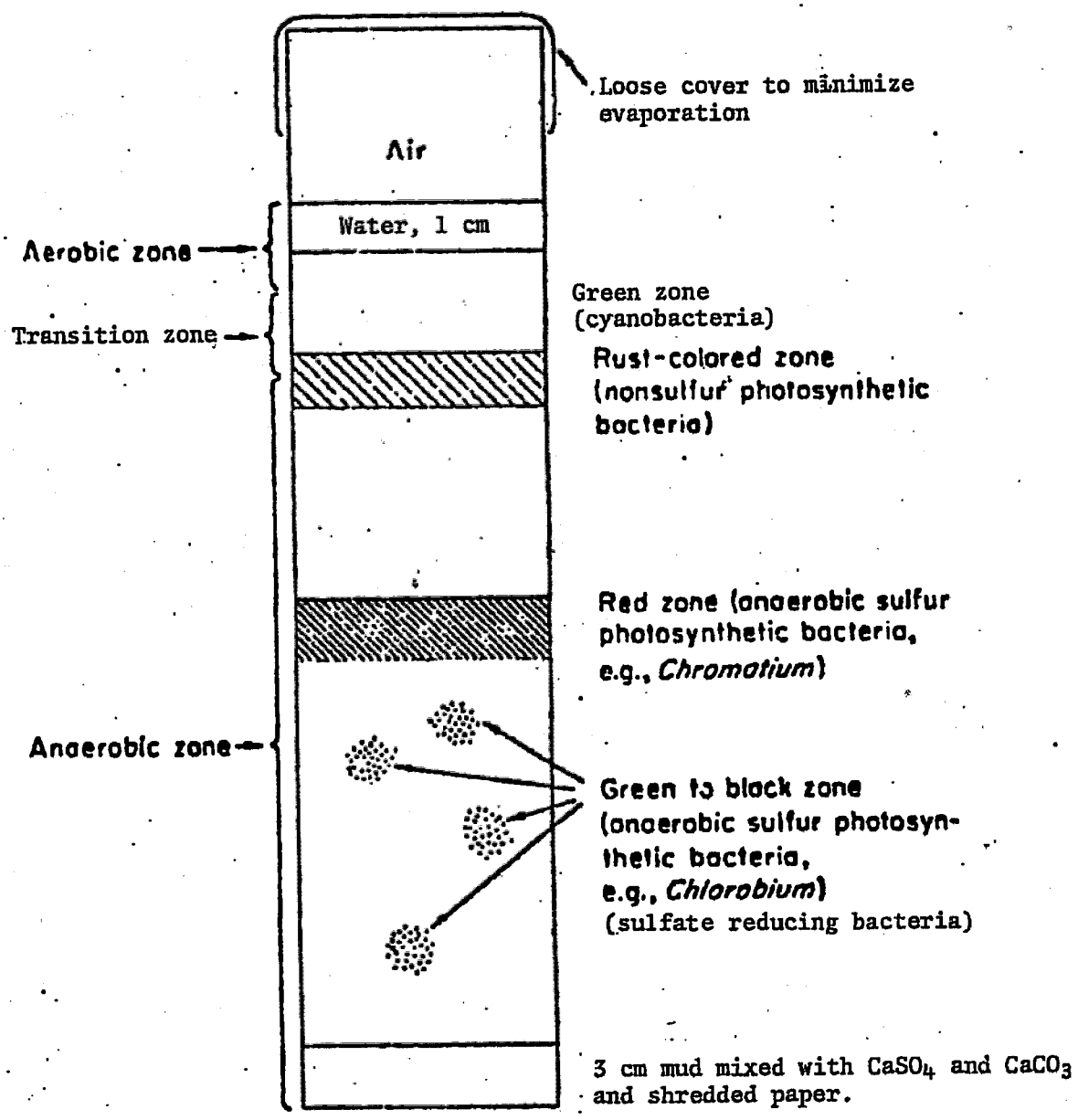
The Pfennig column is similar to the Winogradsky column. The total amoung of organic matter is less in a Pfennig column, hence the rate of sulfide production is lower. One part (by volume) of sludge from the primary settling basin of a sewage plant is mixed with 1 part garden soil and ½ part $CaSO_4$. A layer of this mixture about 5-8 cm high is placed in the bottom of a cylinder which is filled with water. The cylinder is kept dark for two weeks at room temperature before beginning illumination.

The Pfennig columns can be illuminated with either white light or light filtered through a near infrared filter. The upper aerobic zone may contain colorless sulfur bacteria such as species of Beggiatoa.

3. Hot Spring Column

The hot spring column is a modification of the Winogradsky column in which hot spring sediment and inoculum is used to recreate the sequence of thermophilic microbial communities which develop in the normal thermal gradient of a hot spring. This may be done by packing the mixed sediment and inoculum into glass cylinders (2/3 full) adding spring water to the top and applying a spot source of heat and light on the sediment with an incandescent flood lamp or infrared

be established along the sediment-glass interface. The gradient may be regulated by the lamp wattage or its distance from the column. Thermophilic cyanobacteria and photosynthetic bacteria will develop in their normal range of temperatures, usually in conspicuous bands which are largely unispecific. These columns may be maintained for several months. $CaSO_4$ and paper toweling may be included in the bottom of the column to promote the growth of SO_4-reducing bacteria resulting in sulfide-rich conditions.



Winogradsky column.

Revised from Aaronson, 1970