

STUDIES ON THE CHLOROPHYLLS AND PHOTOSYNTHESIS OF
THERMAL ALGAE FROM YELLOWSTONE NATIONAL
PARK, CALIFORNIA, AND NEVADA

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Much work has been done on the geological history of the hot springs and geysers, and several taxonomic studies of the flora and fauna of thermal water have also been made. The works of Weed (1), Setchell (2), Vouk (3), Brues (4), Copeland (5), and Howard (6), and references they cite, give a reasonably good survey of the findings in this field. The author has found but little physiological data on the chlorophyll-containing plants of thermal waters.

The main purpose of this study was to investigate the chlorophylls of algae growing in the hot springs and geyser pools of Yellowstone National Park and compare them with the chlorophylls found in other green plants growing at much lower temperatures. Studies of algae from hot springs in California and Nevada were also undertaken.

On March 6, 1937, through the courtesy of Mr. Joseph Joffe, Assistant Superintendent of Yellowstone National Park, the author received five samples of plants collected in the park on March 1 and shipped to Yellow Springs, Ohio, in glass bottles in the water in which they were found growing.

Taking a typical case the following data will indicate the nature of the study of each sample: Sample *Phormidium laminosum* (Ag.) Gom., collected from Mammoth area, temperature of water 65°C. Phase test and basicity test normal. Absorption spectra of algae in water:

I	II	III	IV	End abs.	Order of intensity: I, IV, II, III.
676	620	585	497	457	

Acetone extract after transference to ether gave the following absorption bands expressed in Ångstroms:¹

I	Shadow	II	III	Shadow	IV	V	VI	End abs.	Order of
662.8	642.8	613.2	577.6	533.5	482.7	453.6	431.8	423.5	

intensity: IV, VI, I, V, III, II.

¹ None of the plant pigments were separated or purified due to the small quantities available.

The ether extract was saponified with 40 per cent methyl alcoholic potash. The absorption bands in the ether layer above the potassium chlorophyllin were:

I	II	End abs.	Order of intensity: I, II.
482.7	452.1	425.0	

Spectrum of saponified chlorophyll or potassium chlorophyllin in the methyl alcoholic KOH mixture:

I	II	III	End abs.	Order of intensity: III, I, II.
642.2	509.8	481.0	453.5	

Absorption bands of acidified ether extract or pheophytin:

I	II	III	IV	V	End abs.	Order of intensity: IV, I, V, III, II.
666.6	606.3	534.0	480.7	450.5	431.8	

Two other samples contained *Phormidium laminosum* (Ag.) Gom. and in one case *Spirulina labyrinthiformis* (Menegh.) Gom. mixed with *Phormidium*. All the above data apply to these samples. The temperature where these algae grew ranged from 48.5–65°C. One sample from the spring called Cleopatra (70°C.) consisted of sulfur bacteria only, although other samples contained Myxophyceae.

The most important features of these data were the abnormal ratio of chlorophyll *a* to *b* and the evolution of oxygen which took place when the plants were irradiated while at 20°C. This latter reaction would tend to indicate that such Myxophyceae normally growing at 65°C. could carry on the process of photosynthesis at normal temperatures. This would also show that light energy need not necessarily be supplemented by temperatures above 20°C. in order for these algae to carry on the process of photosynthesis for several days at least. No attempt was made to grow the algae at 20°C. or at higher temperatures.

After obtaining the above data, a collecting trip was made in Yellowstone National Park during July, 1937. Samples were taken from the Mammoth area, Madison Junction, Lower Geyser Basin, and Upper Geyser Basin. Care was exercised to collect from pools where the plants were continuously exposed to the hot waters for days at a time and not merely bathed intermittently with the hot water. Thirty-three samples were dried immediately (40–50°C.) and stored. In many cases some samples were dried and others preserved in formaldehyde. Some of these were examined on August 8 and 9, 1937, and repeated examinations made in October, 1938. Collections consisted largely of the calcareous or siliceous deposits to which the algae adhered.

In the truly siliceous areas of the park, nine samples were collected and examined. The absorption spectra, phase tests, and basicity tests of the extracted chlorophylls were normal. The ratio of chlorophyll *a* to *b* varied. Chlorophyll *b* was never absent but did not reach more than 15 per cent of the total chlorophyll present. The plants found were *Phormidium Treleasei* (Ag.) Gom. and *Synechococcus lividus* Copeland. The temperatures from which collections were made varied from 54.4–72.2°C. In one case sulfur bacteria were collected from waters 82.2°C. The elevation varied from about 7,000 to 7,500 ft.

A typical examination of these chlorophylls is given below: Sample No. 13A was collected near Green Pool, Upper Geyser Basin, July 15, 1937. The temperature of the water was 72.2°C. The collection consisted of *Phormidium Treleasei* (Ag.) Gom. and *Synechococcus lividus* Copeland. The phase test was normal with typical potassium chlorophyllin with an absorption band at 642 $m\mu$. The basicity test was good. An acetone extract with transference of the chlorophyll to ether by means of water gave the following absorption bands:

I	II	III	IV	V	VI	VII	VIII	End abs.	Order of in-
663.4	644.5	613.0	578.0	535.5	483.0	456.0	433.0	410.0	

tensity: VIII, I, VI, VII, IV, III, V, II.

Pheophytin produced by adding 10 per cent HCl to the ether solution in the cold:

I	II	III	IV	V	VI	VII	End abs.	Order of intensity:
667.2	605.7	562.5	534.3	505.7	480.0	452.1	421.0	

I, VII, VI, IV, V, II, III.

Chlorophyll *b* was about 15 per cent of the total chlorophyll present. The examination of this material, which had remained in a dried condition in a glass jar, gave essentially the same chemical tests and the same absorption spectra when examined September 20, 1938.

From the calcareous Mammoth Hot Springs (elevation 6,500 ft.) area 20 samples were taken. The temperatures, where algae were found growing and collections made, ranged from 37–72°C. The pH of the waters varied from 6.5–6.7 in this region according to Copeland (5). The species included: (1) *Phormidium Treleasei* (Ag.) Gom.; (2) *Synechococcus lividus* Copeland; (3) *Spirulina labyrinthiformis* (Meneg.) Gom.; (4) *Phormidium laminosum* (Ag.) Gom.; (5) *Synechococcus elongatus* Nag. *Phormidium Treleasei* and *Synechococcus lividus* were often found together.

At Mound Terrace (Mammoth area) six collections were made from

waters with the following temperatures: 67°C.; 60°C.; 56.8°C.; 43°C.; 40°C.; 36.6°C. *Phormidium Treleasei* (Ag.) Gom. was a constant and the most abundant form present. In the three higher temperatures some *Synechococcus lividus* Copeland was found. It was quite clear that the temperature range of growth of these two species was not too restricted and examination of the chlorophylls gave normal chlorophyll and pheophytin absorption bands. Collections 11A, 7C, 4B, 4C, growing at temperatures from 59–67°C. and composed of the same genera and species as the samples immediately above, gave the following absorption bands when an acetone extract was transferred to ether: Sample 7C is representative of this group:

I	II	III	IV	V	VI	VII	VIII	IX	X	End abs.
664.0	644.0	610.9	576.5	548.8	532.5	501.0	481.2	455.5	432.5	420.0

Order of intensity: X, I, VIII, IX, VII, III, V, IV, VI, II.

The pheophytin absorption spectrum of 7C in ether was:

I	II	III	IV	V	VI	End abs.	Order of intensity: I, VI, V,
667.0	606.8	548.5	534.5	509.5	472.0	453.5	

III, II, IV.

In the samples 11A, 7C, 4B, and 4C there was an absorption band at about 548 $m\mu$ which is not present in any other known chlorophyll extract from plants. This also remained in the pheophytin spectrum. Whether this is some unusual accompanying pigment or a modified chlorophyll cannot be settled at the present time. It does not seem to be a matter of species or temperature at which the plants were growing, but it is worth observing that there was no such finding in the strictly siliceous sinter region of the Upper and Lower Geyser Basin. These modified chlorophylls appeared only in the calcareous waters. Such absorption spectra indicate another probable modification of the chlorophylls now known.

In the summer of 1939, studies of algae from "The Geysers" in Sonoma county, California, and at "Beowawe," Nevada, were made.

Material from The Geysers in California was collected from waters ranging in temperature from 49–66°C. All specimens showed the presence of a unicellular green alga which is probably *Chlorella sp.* The chlorophylls from these specimens were preserved in 5 per cent formaldehyde and upon examination gave normal pheophytin absorption spectra. This loss of Mg by the chlorophyll molecule during the storage of algae in 5 per cent formaldehyde is normal. It seems reasonable to assume that the chlorophylls of these green algae are also normal in respect to ordinary properties. To find no blue-green algae in the six specimens collected was rather unexpected.

The collections from Beowawe, Nevada, were taken from water of temperatures from 60–71°C. The elevation was about 4700 ft. The species represented were *Phormidium Treleasei* (Ag.) Gom. and *Phormidium laminosum* (Ag.) Gom. The chlorophylls were preserved in 5 per cent formaldehyde and spectroscopic examination showed a normal pheophytin spectrum in each case. Thus there appears to be no reason to assume that these chlorophylls were not essentially normal. It is interesting to note that the species here were also common in the Yellowstone material.

In all the habitats investigated, it was evident that the algae grew more abundantly in waters ranging in temperature from 37–50°C. This was also true of a single species such as *Phormidium Treleasei* (Ag.) Gom. In Yellowstone Park, where the altitude was from 6000 to 7500 ft., there would be a white to rose-colored thin mineral crust on many pools. Under this one often found considerable algae. This probably indicated that the radiation at the higher altitudes must have been too intense for these plants and they grew better where there was some shading. Nothing like this was found in the Geysers in California or at Beowawe, Nevada.

CONCLUSIONS

From the data obtained it seems most reasonable to conclude that the thermal Myxophyceae studied have, for the most part, normal chlorophylls with chlorophyll *b* constituting about half of the usual amount present in most green plants. Evolution of oxygen upon irradiation at normal room temperature was positive. These algae undoubtedly carry on photosynthesis in their natural habitat at the higher temperatures. What possible changes in the mechanism of the photosynthetic process might be found here must remain for future investigation to decide. Whether these species exist in thermal waters through adaptation or are relics of past ages cannot be definitely stated; but the fact that, for the most part, normal chlorophylls are present may add more credence to Vouk's contention that the flora of thermal waters is chiefly due to adaptation of these forms to high temperatures. After finding algae growing in intense radiation at high temperatures with the water often charged with hydrogen sulfide or containing arsenic, one feels that these plants are well worth further physiological study. An examination of the nature of the proteinaceous enzymes and the properties of many of the other chemical compounds associated with cell metabolism and photosynthetic reactions should be of much value.²

² All identifications of algae were made by Dr. Francis Drouet of the Field Museum, Chicago, Illinois.

SUMMARY

1. Myxophyceae normally growing at 65°C. evolved oxygen upon irradiation and showed evidence of retaining the power to carry on the process of photosynthesis at 20°C. This indicates that extra thermal energy is not essential for photosynthesis at least over a short period of time.

2. Chlorophyll *a* and *b* found in several species of Myxophyceae growing in waters ranging in temperature from 37–72°C. are essentially the same as found in plants growing all over the world. Certain standard chemical tests and spectroscopic examination of the chlorophylls were used as the criteria for these comparisons. The ratio of chlorophyll *a* to *b* often varied considerably but in general chlorophyll *a* showed an increase over the percentage found in most plants.

3. Green algae (*Chlorella sp.?*) were the only forms found at The Geysers, California. The temperature of the waters from which collections were made varied from 49–66°C.

4. Collections from Beowawe, Nevada were from waters ranging from 60–71°C. The algae belonged to the Myxophyceae and the species were like some of those found in Yellowstone National Park.

5. In some of the calcareous regions of Yellowstone National Park spectroscopic study of the chlorophylls revealed an unidentified absorption band at 548 m μ .

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