

Kratz W A & Myers J. Nutrition and growth of several blue-green algae. *Amer. J. Bot.* 42:282-7. 1955. [Department of Zoology, University of Texas, Austin, TX]

Media and methods were developed that supported maximum growth rates of three blue-green algae (cyanobacteria). *Anacystis nidulans*, a new species brought into culture, had a temperature optimum of 41 °C and the highest specific growth rate of any autotrophic organism [The *SCI*<sup>®</sup> indicates that this paper has been cited in over 635 publications since 1955, making it one of the most-cited papers ever published in this journal.]

Jack Myers  
Department of Zoology  
University of Texas Austin,  
TX 78712-1064

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"This paper was part of the dissertation of my student, Bill Kratz, since deceased. He extended a line of work begun with the green alga, *Chlorella*. The rationale for the study was that the microalgae are microbes, rich in protein, with minimal skeletal crud; that such cell material is the end product of their photosynthetic metabolism; and that the specific growth rate, the first order rate constant for growth, measures performance of cellular machinery.

"Supporting methods had already been developed. Aeration with CO<sub>2</sub>-enriched air is a requirement because of the high C content (50 percent) of the cells produced. Light must be provided to thin layers of culture, which minimize self-shading. For these requirements, we had developed two culture systems. One was an elegant steady-state culture device. The other was the ultimate in simplicity: aerated test-tube cultures in which growth was estimated by periodic readings of absorbance.

"Extending such rationale and methods to the blue-green algae seemed a formidable task. These organisms were billed as sluggish growers, mostly limited to lower temperatures (<25°C). This reputation came from a microbiological study<sup>1</sup> and from a botanical

study directed toward ecology.<sup>2</sup> Growth had been estimated in these studies after 7 to 14 days incubation. One important feature of these cells was a high pH (>7) requirement. This made the media problem more formidable because of the difficulty in avoiding precipitation of HPO<sub>4</sub> = , Mg<sup>++</sup>, Ca<sup>++</sup>, Fe<sup>+ + +</sup>, and trace elements. Further, the classical 5-percent CO<sub>2</sub>-in-air mixture was not compatible in practice with a high pH.

"With four species assembled from other laboratories, Bill began an attack on the problem of media composition. Meanwhile, he searched for other species by the simple procedure of selection culture: collect natural waters, judiciously spike them with media components, and incubate in test-tube cultures. Because Texas waters are often warm, they were incubated at various temperatures up to 40°C.

"The technical media problems proved to be solvable. With the species already at hand, Bill could achieve cell concentrations and specific growth rates (5 to 8 percent per hour) comparable to those of many green algae. Meanwhile, there was an exciting development in one of the selection cultures. An alga sampled from a campus creek grew rapidly (30 percent per hour) at up to 40°C. Getting the unialgal culture from it was easy. Getting a bacteria-free culture was more difficult and resisted all Bill's attempts. Fortunately, he had sent a culture to M.B. Allen. When she graciously sent back an axenic culture, he quickly completed the paper.

"Bill went on to further study of his blue-greens, but his greatest impact had been made. He had brought the blue-green algae from the realm of exotic curiosities to experimentally usable microbes. More practically, viewed in terms of its numerous citations, the paper presented a widely useful organism and media for its culture. Although *Anacystis nidulans* probably was misnamed,<sup>3,4</sup> it came to be studied more than any other blue-green alga (or cyanobacterium) in captivity."

1. Allen M B. The cultivation of Myxophyceae. *Arch. Mikrobiol.* 17:34-53. 1952. (Cited 115 times since 1955.)

2. Gerloff G C, Fitzgerald G P & Skoog F. The mineral nutrition of *Coccochloris pentocystis*. *Amer. J. Bot.* 37:835-40, 1950.

3. Stanler R Y, Kunisawa R, Mandel M & Cohen-Bazire G. Purification and properties of unicellular blue-green algae. *Bacteriol. Rev.* 35:171-205, 1971. (Cited 395 times.)

4. Rippka R, Deruelles J, Waterbury J B, Herdman M & Stanier R Y. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* 111:1-61, 1979. (Cited 215 times.)