Cultivation biology of *Gracilaria tikvahiae* in the United States

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Introduction

The rhodophyte *Gracilaria tikvahiae* is widely distributed along the eastern coast of the United States and has been considered a prime candidate for phycoculture for purposes including production of agar, bioconversion to methane, fertilizer, and sewage treatment (Ryther, DeBoer & Lapointe 1979; Ryther, Williams, Hanisak, Stenberg & DeBusk 1979; Bird *et al.* 1981; Hanisak 1981a, b). Since 1975, *G. tikvahiae* has been cultivated at the Harbor Branch Institution in Fort Pierce, Florida, as part of a continuing program on seaweed mariculture. One focus of the research in this experimental system has been on manipulating environmental factors that can readily be controlled on a larger, commercial scale (e.g., nutrient enrichments and flow rates of seawater, but not light, temperature, and salinity). This communication will briefly review some of the findings of this research, with an emphasis on how the biology of *G. tikvahiae* interacts with the environmental conditions of the cultivation system.

Yields of *Gracilaria tikvahiae* in outdoor cultivation systems

Gracilaria tikvahiae has been cultivated in many different culture configurations at the Harbor Branch facility, but the highest yields of this species were found in small tanks (55 l) supplied with vigorous aeration and rapid exchange (20–30 times d^{-1}) of enriched seawater (Lapointe & Ryther 1978). Growth of *G. tikvahiae* in this intensively operated system occurred year-round with a mean productivity of 34.8 g dry wt m^{-2} d^{-1} (equivalent to 127.0 t ha^{-1} a^{-1}, about half of which was organic). Growth was maximal (46 g dry wt m^{-2} d^{-1}) at the end of July and minimal (12 g dry wt m^{-2} d^{-1}) in late January (Lapointe & Ryther 1978). Maximal yields occurred at relatively low nutrient enrichments (10–100 μM nitrogen and 1–10 μM phosphorus) and at a stocking density of 2–4 kg wet wt m^{-2}, harvested back to that density at approximately weekly intervals. Yields were found to be directly proportional to the seawater exchange rate, between 1 and 30 culture volumes d^{-1}, for reasons that were not completely understood at the time.

Although the yield of *G. tikvahiae* in these tanks is among the highest for any plant, it must be remembered that these data represent what is possible under rather idealistic conditions; they demonstrate the potential of *G. tikvahiae* to produce biomass, but they are probably not attainable on a commercial level, at least in the near future. It is important to note that the method of cultivation employed on this small scale was very energy-intensive, i.e., large amounts of flowing seawater and aeration were required, and probably could not be practically employed for commercial cultivation, especially for bioconversion purposes, because of the economic and energetic expenses involved.

Such an intensive method of cultivation has been successfully scaled up to larger tanks (i.e., 2.4–29 m^2 in surface area, and 2 400–24 000 l in volume). Over the past five years, the mean productivity of *G. tikvahiae* in such tanks has consistently been in the range of 22–25 g dry wt m^{-2} d^{-1} (80–91 dry t ha^{-1} a^{-1}). Most of the research has involved one particu-

lar clone ('ORCA') of *G. tikvahiae* that was first isolated in December 1977. From an initial weight of a few grams, many tonnes of this clone have been grown at the Harbor Branch facility. During this time, the clone has not reproduced sexually; rather, it propagates vegetatively, reproducing only through fragmentation. Such a sterile clone is useful because, once selected, it can be maintained for long periods of time without changes in its genetic makeup.

There have also been attempts to grow *G. tikvahiae* in what may be considered as a nonintensive type of culture, earthen ponds with polyvinyl chloride liners. These ponds varied in size from 10–20 m^2, in depth from 0.4–0.8 m, and had volumes up to 25 000 l. In most cases, *G. tikvahiae* was scattered, unattached, along the bottom of the ponds. Initially, no aeration was employed, and the exchange of seawater was relatively low (2 volume turnovers d^{-1}). Yields in this type of 'bottom culture' were significiantly lower than in the energy-intensive method and usually ranged from 5–8 g dry wt m^{-2} d^{-1} (18–29 dry t ha^{-1} a^{-1}). Besides this considerable reduction in yield compared to the more intensive cultivation, other problems were encountered that were primarily associated with a reduction in water motion. The most serious of these was the growth of diatom mats on the sides of the ponds. These mats eventually detached from the liners, became entrapped in and floated the seaweed to the surface, where, without agitation, surface temperatures reached 40 °C in the summer. Such a high temperature is lethal to *G. tikvahiae*. In addition, once the seaweed was floating, it was quite susceptible to being blown to one end of the pond by even moderate winds. The resulting heterogenous distribution of seaweed further reduced yields – during the day, part of the pond was overcrowded with seaweed, causing much self-shading, while the rest of the pond was empty; thus, light energy, the ultimate limiting factor for seaweed growth, was squandered in the system. Also, at night, the dense patches of seaweeds became anaerobic. These problems could be alleviated by applying gentle aeration to the ponds, enough to circulate water around the seaweed without moving the plants themselves. In so doing, thermal stratification was eliminated, diatoms and other potential fouling organisms were more readily washed out of the pond, the distribution of *G. tikvahiae* within the pond was fairly homogenous, and anaerobic conditions were avoided. Consequently, yields in such ponds were approximately twice that of completely unaerated ponds, but still only about half that of the energy-intensive mode of cultivation.

'Bottom culture' was also studied, on what may be considered a pilot scale, in a 0.1-ha (0.25-acre) pond. This pond was stocked twice with several tonnes of *G. tikvahiae*. Each time cultures were sustained for only 6 months, during the cooler months of the year. Mean productivity was ca 7 g dry wt m^{-2} d^{-1}. In both years, growth stopped with the advent of warm summer temperatures. No aeration was employed in this pond. Serious problems were also encountered with grazing by amphipods. At present, no further work with this pond is planned. Instead, research has concentrated on minimizing the amount of aeration and seawater flow required to grow *G. tikvahiae*, and is being conducted in tanks.

Seawater requirement and carbon dioxide

As noted earlier, large amounts of seawater are required for maximal growth of *G. tikvahiae*. The relationship of yield to seawater flow can be described by a hyperbolic curve (e.g., Ryther, *et al* 1983). Several experiments over the years have indicated that this requirement for seawater is not related to mineral nutrients (i.e., nitrogen, phosphorus, or trace metals). Rather, it could be due to one or more other factors, including wider fluctuations in pH and temperature or a build-up of some toxic substance under conditions of low flow.

There is now considerable evidence that growth of *G. tikvahiae* is limited by the availability of CO_2 under low flow rates; thus, enhanced yields at higher flow rates are due to an increased CO_2 supply. Early evidence for such a carbon limitation was provided by Lapointe & Ryther (1979) who noted that the pH in culture tanks of *G. tikvahiae* increased to ca. 9.0 as the rate of water exchange was reduced, and suggested that the elevated pH may have been responsible for the reduced yields observed at low seawater exchanges. At such a high pH, free CO_2 is almost completely unavailable and substantial decreases in photosynthesis of *Gracilaria* occur (Blinks, 1963; Ryther & DeBusk 1982).

Longer-term studies have focussed on the influ-

ence of pH and inorganic carbon levels on yields of *G. tikvahiae*. These studies have demonstrated that *G. tikvahiae* grows better on CO_2 than on bicarbonate and that the pH effect on growth is due to the influence of pH on the relative availability of CO_2 and bicarbonate (Ryther *et al.* 1983). Interestingly, little CO_2 becomes available to the cultivation tanks via aeration. Preliminary experiments found that total inorganic carbon in a culture tank previously stripped of inorganic carbon increased from 0.4 to 1.1 mM after 9 days of aeration (typical total inorganic carbon values in seawater are ca 2.4 mM). In nonaerated tanks previously stripped of inorganic carbon, there was little increase in inorganic carbon demonstrating that the diffusion of CO_2 into seawater from the atmosphere is relatively slow and not sufficient to support the high rates of growth of which *G. tikvahiae* is capable.

As pumping seawater is probably the single most expensive operating cost for any land-based seaweed cultivation system (Huguenin 1976), it may prove to be more cost-effective to develop suitable methods of pH control (i.e., additions of CO_2 or waste acid). If *G. tikvahiae* is to be cultivated for bioconversion purposes, a ready remedy to this problem may exist if the CO_2 that normally composes 40% of the biogas could be recycled back to cultivation sites. In this regard, carbon nutrition should be considered as a necessary part of nutrient management strategies, as are nitrogen, phosphorus, and trace metals.

Aeration requirements

As shown earlier, high yields of *G. tikvahiae* require relatively large amounts of aeration. Recent experiments (Ryther, *et al* 1983) demonstrated that periodic aeration (i.e., 15 min · h^{-1}, for a total of 6 h d^{-1}) was as efficient as continuous aeration (24 h d^{-1}). Even without further refinements, these results indicate that aeration requirements can be greatly reduced.

The beneficial effects of aeration on seaweeds can be attributed to the following: (1) it increases photosynthetic efficiency, by rotating the seaweeds in such a way that they are able to maximize the absorbance of light rather than having a high degree of self-shading; (2) it increases nutrient uptake rates, by reducing diffusion boundary layers; (3) it increases the availability of metabolic gases (carbon dioxide, oxygen), both by reducing diffusion boundary layers and by direct enhancement from the airline; and (4) it flushes out competing algal cells and spores, thereby also reducing the epiphyte problem. While aeration is clearly beneficial to seaweeds being cultivated in land-based systems, it appears that the requirement for aeration is partly an artifact of the culture configuration; seaweeds in situ derive the same benefits as above from water movement (i.e., currents). Other methods of providing circulation in seaweed culture tanks (e.g., paddle wheels; Neish *et al.* 1977) may be just as effective as aeration in maintaining high yields.

Nutrient management

Maximal yields are possible only when nutrient conditions are not limiting to growth. Thus, it would at first seem that continuous addition of nutrients would maximize growth, but in fact such a practice is wasteful and can actually result in a reduction in yield due to its enhancement of the growth of epiphytes, which are often considered to be the most serious threat to maintaining seaweed cultures (Hanisak 1978). Fortunately, seaweeds have a considerable capacity to store nutrients when external supplies are available and then draw upon these reserves when external nutrients are depleted (Chapman & Craigie 1977; Hanisak 1979). This phenomenon has been dramatically demonstrated for *G. tikvahiae,* which, after soaking in seawater enriched with high levels of nutrients for only 6 h, will have taken up and assimilated enough nutrients to grow at non-nutrient-limited rates for 7–14 days (Ryther, *et al* 1981).

As with CO_2, there are inexpensive means of providing nutrients for the cultivation of *G. tikvahiae,* including wastes such as sewage and fermentation residues from anaerobic digesters. For example, nutrients from digester residues can be recycled through the cultivation system with an efficiency of 73%, a value high enough to eliminate the requirement for applying conventional fertilizer (Hanisak 1981b).

Conclusions

The successful cultivation of *Gracilaria tikvahiae* in this experimental system is due to its rapid growth rate over a wide range of environmental

conditions, its ease of propagation by purely vegetative means, the favorable year-round growing season in Florida, and the development of successful management strategies. While each species is unique, the experiences at Harbor Branch should be helpful to others attempting to cultivate seaweeds. It is evident that the design of any commercial seaweed mariculture operation needs to consider carefully the biology of the species of interest. After determining that the alga will be able to grow fairly well under the prevailing light, temperature, and salinity conditions, management strategies need to be developed based on biological information such as nutrient uptake ability, inorganic carbon preferences, and seawater circulation.

Although an economic analysis of the cultivation of *G. tikvahiae* in the United States is not yet complete, the fact that seawater, aeration, and nutrient inputs into the system can be substantially reduced without adversely affecting yield is promising, particular for applications having high economic value (i.e., agar). However, prospects for the cultivation of *G. tikvahiae* in a land-based system for bioconversion purposes are less promising due to high cultivation costs relative to the low economic value of methane. Any commercial marine biomass farm will probably need to take advantage of natural water circulation patterns in open waters.

Besides continuing to investigate means of reducing seawater and aeration inputs for the cultivation of *G. tikvahiae,* present work at Harbor Branch involves isolating fast-growing or agar-rich strains of *Gracilaria.* Significant improvements in both biomass yields and chemical composition are considered possible both with traditional genetic techniques as well as with more recent genetic engeneering techniques that are presently being developed for other types of organisms.

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References

Bird, K. T., M. D. Hanisak & J. H. Ryther, 1981. Changes in agar and other chemical constituents of the seaweed, *Gracilaria tikvahiae,* when used as a substrate in methane digesters. Resour. Conserv. 6: 321–327.

Blinks, L. R., 1963. The effect of pH upon the photosynthesis of littoral marine algae. Protoplasma 57: 126–136.

Chapman, A. R. O. & J. S. Craigie, 1977. Seasonal growth by *Laminaria longicruris:* relations with dissolved inorganic nutrients and internal reserves of nitrogen. Mar. Biol. 40: 197–205.

Hanisak, M. D., 1978. Cultivation and bioenergetics of the agarophyte *Gracilaria tikvahiae.* Int. Coun. Explor. Sea, Code Number C.M. 1979/F:24: 8 pp.

Hanisak, M. D., 1979. Nitrogen limitation of *Codium fragile* ssp. *tomentosoides* as determined by tissue analysis. Mar. Biol. 50: 333–337.

Hanisak, M. D., 1981a. Methane production from the red seaweed *Gracilaria tikvahiae.* Proc. int. Seaweed Symp. 10: 681–686.

Hanisak, M. D., 1981b. Recycling the residues from anaerobic digesters as a nutrient source for seaweed growth. Bot. mar. 24: 57–61.

Huguenin, J. E., 1976. An examination of problems and potentials for future large-scale intensive seaweed culture systems. Aquaculture 9: 313–342.

Lapointe, B. E. & J. H. Ryther, 1978. Some aspects of the growth and yield of *Gracilaria tikvahiae* in culture. Aquaculture 15: 185–193.

Lapointe, B. E. & J. H. Ryther, 1979. The effects of nitrogen and seawater flow rate on the growth and biochemical composition of *Gracilaria foliifera* var. *angustissima* in mass outdoor culture. Bot. mar. 22: 529–537.

Neish, A. C., P. F. Shacklock, C. H. Fox & F. J. Simpson, 1977. The cultivation of *Chondrus crispus:* Factors affecting growth under greenhouse conditions. Can. J. Bot. 55: 2263–2271.

Ryther, J. H., N. Corwin, T. A. DeBusk & L. D. Williams, 1981. Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae* (McLachlan, 1979). Aquaculture 26: 107–115.

Ryther, J. H., J. A. DeBoer & B. E. Lapointe, 1979. Cultivation of seaweeds for hydrocolloids, waste treatment, and biomass for energy conservation. Proc. int. Seaweed Symp. 9: 1–17.

Ryther, J. H. & T. A. DeBusk, 1982. Significance of carbon dioxide and bicarbonate-carbon uptake in marine biomass production. Presented at the Symposium 'Energy from Biomass and Waste VI'. January 25–29, 1982. Lake Buena Vista, Florida.

Ryther, J. H., T. A. DeBusk, D. A. Andrews & C. Habig, 1983. Cultivation of *Gracilaria* as a biomass source for energy. Proc. World Mariculture Soc. (In press).

Ryther, J. H., L. D. Williams, M. D. Hanisak, R. W. Stenberg & T. A. DeBusk, 1979. Biomass production by marine and freshwater plants. Third Annual Biomass Energy Systems Conf. Proc. Solar Energy Res. Inst., Golden, Colorado: 13–23.