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A SIMPLE METHOD FOR THE MASS CULTURE OF MARINE ALGAE

Interest in the mass culture of unicellular algae stems from several sources. Algal culture is being investigated for use in space vehicles as a means of air revitalization, food production, and waste treatment (Benoit 1964). In many overpopulated areas of the world, unicellular algae are considered a potential food supplement for direct human consumption or as food for other animals (Witsch 1960; Spoehr 1953).

Dried algae are now used to supplement livestock feed in China (Choi[1], personal communication; Nakamura 1964). In this laboratory, marine species of unicellular algae are used as foods for oyster and clam larvae in pilot-plant hatchery operations (Loosanoff and Davis 1963). The adoption of hatchery culture of bivalves by industry will bring about an additional demand for a simple inexpensive method of culturing large amounts of algae.

Studies on the mass culture of algae have been largely confined to freshwater species of *Chlorella* and *Scenedesmus* (Burlew 1953; Mayer 1960), probably because of their survival in a variety of environmental conditions and widespread availability. However, these may not necessarily be the most suitable species for some purposes. As was suggested by Mayer (1960), it would be desirable to screen a large number of unicellular algae from the point of view of nutritional composition, toxicity, resistance to contaminants, growth rates in mass culture, and suitability for the particular use intended. The significance of the latter point was demonstrated by observations that larval and juvenile stages of *Crassostrea virginica* and *Mercenaria mercenaria* and larvae of *Ostrea edulis* used certain species of algae as a food source better than others (Davis and Guillard 1958; Walne 1963). This work represents an attempt to determine the feasibility of culturing *Chlorella* and other unicellular marine algae, using a simple method that could be employed in many parts of the world under a variety of conditions. Mass culture can be carried out in the laboratory with good results, but the conditions in some areas are so primitive that glass jars or tubes, artificial illumination, and equipment for sterilizing large volumes of seawater may not be available, and some locations make the large scale culture of marine species impossible. It is important therefore to find unsophisticated methods for the production of large quantities of marine species.

Other investigations into the mass culture of marine algae have depended on natural seawater, both in open tanks and in closed controlled systems (Loosanoff 1951; Raymont and Adams 1958; Wisely and Purday 1961; Davis and Ukeles 1961; Ansell et al. 1963). Although fertilized natural seawater produced a good crop of phytoplankton, the inoculated culture was sometimes replaced rapidly by other organisms introduced with the seawater, such as motile and nonmotile chlorophytes, colorless flagellates, ciliates or other zooplankters (Loosanoff, Hanks, and Ganaros 1957; Ansell et al. 1963). Even under controlled laboratory conditions (Davis and Ukeles 1961), small algal species in seawater occasionally pass through filters and become established in culture carboys. It would be advantageous to use an artificial salt water medium which would

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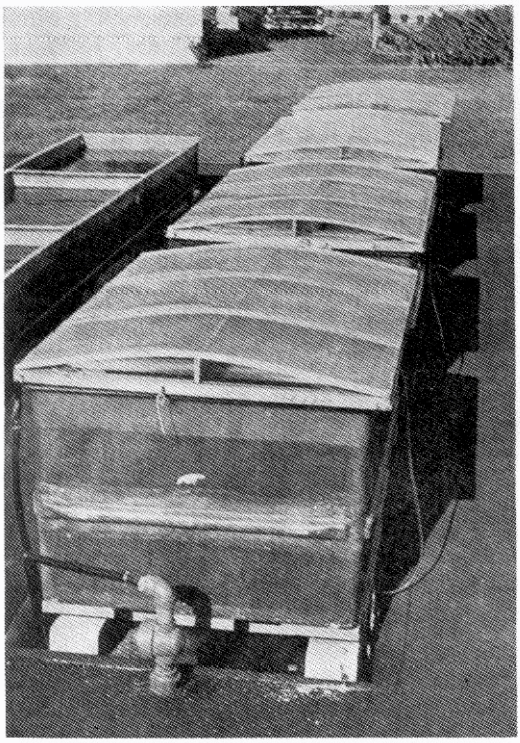

FIG. 1. Outdoor culture tanks, with covers in place, showing valve that regulates the supply of cooling seawater.

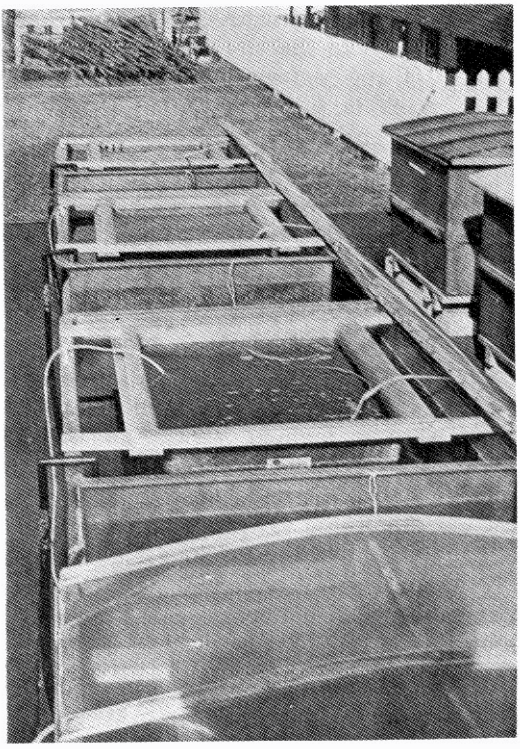

FIG. 2. Culture tanks, with covers removed to show culture vessel inside cooling bath and the aerating and siphoning systems.

eliminate the immediate introduction of undesirable organisms. Preliminary laboratory experiments in which growth of axenic cultures was measured under controlled conditions indicated that the commercially prepared salt mixture, Rila Marine Mix[2], might be used. The salt was dissolved in distilled, demineralized, or tap water, and supplemented with a nitrogen source and a vitamin mix of thiamine and B_{12}. This type of culture medium was used in laboratory mass cultures in place of the natural seawater previously employed (Davis and Ukeles 1961). Excellent growth of *Monochrysis lutheri*, *Isochrysis galbana*, *Phaeodactylum tricornutum*, and *Dicrateria* sp. was obtained during a period of 6 months in continuous culture.

A similar basal medium made up with Rila salts was used in cultures maintained outdoors in 280-liter fiber glass tanks. Each culture tank was placed inside a larger fiber glass tank and secured on a frame so that the water level in the inner tank was slightly higher than that in the outer tank. Seawater pumped from the nearby harbor was continuously circulated in the outer tank which thereby served as a cooling bath for the culture. Several aquarium-type stone aerators were placed in each culture tank and connected to a Marco®[3] air pump to provide aeration and stirring in the bottom of the culture. Each pair of nested tanks was covered with heavy-duty polyoxyethylene sheeting stretched over a wooden frame (Figs. 1 and 2).

The tanks were filled with tap water and Rila Marine Mix (2.0%w/v) was added, supplemented with urea (0.02%w/

[2] Rila Products, Teaneck, New Jersey.

[3] Registered trademark, J. B. Maris Co., Bloomfield, New Jersey.

v), tris (hydroxymethyl) aminomethane (0.2%w/v) and the vitamin mixture (thiamine 0.02 mg and vitamin B_{12} 0.2 μg/100 ml). Each tank was inoculated with 10 liters of a culture taken from the unialgal mass culture apparatus maintained in the laboratory. The growth response of the following four species was followed during the summer: *Dunaliella euchlora*[4], *Chlorella* sp. (580), *Dicrateria* sp., and *Monochrysis lutheri.*

The temperature of the cultures ranged from 19.5 to 23.0C, even during an extremely warm period when air temperatures were as high as 38.0C. The *p*H of the cultures varied from 7.9 to 9.1 and was relatively high in the afternoon and low in the early morning. During the first month of growth, only an occasional ciliate and a few diatoms were observed as gross contaminants. Cell counts revealed an initial increase in cell populations. The population in each tank was 0.3×10^6 cells/ml with the exception of *Chlorella* sp., where the initial population was about 10 times as high. Within 10 days there was an increase to 1.9×10^6 cells/ml for *M. lutheri*, 1.4×10^6 cells/ml for *Dicrateria* sp., 0.7×10^6 cells/ml for *D. euchlora*, and 8.7×10^6 cells/ml for *Chlorella* sp. (580). The initial increase was followed by a period of decline when cultures were being harvested (for use as foods in pilot-plant hatchery studies) at a rate of 60–100 liters a day from each tank. All cultures were used as foods by juvenile stages of oysters and clams with good results.

The most consistent maintenance of population levels and the least amount of invasion by other species occurred in the *Chlorella* culture. In contrast, there was little growth of *D. euchlora*, and the culture was eventually overgrown with diatoms. The observation that growth of *D. euchlora* was poor in this medium was confirmed in laboratory mass cultures. The addition of phosphate increased the populations of *D. euchlora* to levels equivalent to those obtained when enriched natural seawater was used as the basal medium. Obvious benefit to other species in culture was also observed. All species that were maintained in natural seawater in the mass culture apparatus previously described (Davis and Ukeles 1961) are now being cultured in this enriched artificial seawater medium. The chemical composition of Rila salts is given by the manufacturer so that appropriate modifications can be made for particular species as nutritional information becomes available. An increased yield in outdoor tanks can be anticipated in future work.

While investigating the mass culture of *Phaeodactylum tricornutum* in 1,000-liter outdoor concrete tanks, Ansell et al. (1963) encountered difficulty in controlling environmental conditions, as well as in maintaining such large cultures. In subsequent work, it was found more convenient to use 15-liter carboys (Ansell, Raymont, and Lander 1963). Tanks of an intermediate size make it possible to maintain relatively large volumes of a variety of species, and the cultures can be harvested or discarded without great loss. Fiber glass is a satisfactory material for the construction of culture tanks because it can be handled and shaped easily, it is sturdy enough for repeated outdoor use, and it is relatively nontoxic. Although plastics are generally not inhibitory, it is necessary to test each formulation (Dyer and Richardson 1962). It is a good practice to fill each tank with water and treat with successive washes before use. Enrichment conditions and a heavy inoculum of one species should establish conditions that suppress the growth of other organisms in the culture tank. A species can be cultured until undesirable contamination appears, then harvested, and the cells stored under refrigeration, dried or lyophilized.

This work has demonstrated that a variety of unicellular marine algae can be cultured on a large scale in artificial seawater with a minimum expenditure. Improvements will ultimately depend upon data

[4] This strain may be identical with *D. tertiolecta* Butcher (McLachlan 1960).

obtained in the laboratory on optimal conditions for algal growth.

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