CULTIVATION OF MACROSCOPIC MARINE ALGAE AND FRESHWATER AQUATIC WEEDS

Progress Report

For the Period May 1, 1979 - December 15, 1979

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I. Summary

A. Seaweed culture:

The "ORCA" clone of the red seaweed Gracilaria tikvakiae, isolated in December 1977, has now been grown continuously in 2600-1 aluminum tank culture for two years. Yields of the seaweed over the past twelve months, in cultures receiving four exchanges of water per day enriched with 50-100 μmoles/1 NO_3-N, 5-10 μmoles/1 PO_4-P and trace metals (concentrations of enrichment depending upon the nutrient content of the seawater) and in vigorous aeration maintaining the plants in suspension, have averaged 12 g ash-free dry weight/m^2.day, equivalent to approximately 44 t afdw/hectare.year (17.5 t/acre.year). These yields are almost exactly the same as those obtained using similar methods over the previous year.

During the past year a new nutrient enrichment procedure was initiated in which the commercial inorganic trace metal mixture, used alone up to that time, was supplemented with a chelated iron source (Fe-EDTA). Following that change in enrichment procedure, new experiments on the effect of reducing the flow of water through the cultures proved highly successful, growth at one volume exchange/day being equal to that obtained with four exchanges/day. It was concluded that, with the earlier inorganic trace element enrichment procedure, one or more essential elements precipitated in the seawater or were otherwise made unavailable to the plants, and that the increased growth with increasing rates of water exchange had resulted from an

enhanced supply of the limiting trace element(s) in the seawater. For reasons not understood, that effect was not apparent when it was looked for in the smaller (50 l), screening tanks, perhaps because it was obscured by other growth-limiting factors associated with slow exchange rates in the smaller cultures (i.e., large fluctuations in temperature and pH, CO_2 limitation, or perhaps other chemical and/or physical stresses).

The achievement of high yields of Gracilaria at low water exchange rates is a major accomplishment, because the cost, economically and in terms of energy, of continuously pumping large volumes of water would represent a major constraint to an intensive seaweed culture operation, no matter how large the yield. New experiments will now be undertaken in which the rate of exchange of water will be reduced further, using both continuous and pulsed water supply, until a minimum flow rate consistent with high yields is established.

A discovery of similar significance to the economic and energy cost of intensive seaweed culture was the demonstration that yield is not affected by restriction of aeration to the 12 daylight hours (i.e., in contrast to continuous aeration). This finding is still tentative and will require further confirmation, but if the results of the preliminary findings are repeated, additional experiments in this area will also be undertaken, reducing both the quantity and period of aeration until the minimum requirement of that costly procedure is also determined.

Gracilaria has also been grown, using a variety of non-intensive culture methods, in a series of PVC-lined earthen ponds ranging in bottom areas from 10 to 20 m^2, in depth, from 0.4 to 0.8 m, and in volume from 5,400 to 24,000 liters.

In one series of such experiments, the seaweeds were floated on the pond surface in plastic-mesh trays, the plants just submerged below the water surface, and enriched seawater was continuously pumped from the pond bottom and sprayed, through conventional shower heads, onto the trays from above. The Gracilaria in these experiments became heavily epiphytized, primarily with the filamentous green alga Enteromorpha sp., and eventually died.

In another series of non-intensive culture experiments, pieces of Gracilaria 10-20 cm in length were inserted into the weave of polypropylene rope at intervals of roughly 10 cm and the ropes were suspended in the ponds at various distances off the bottom. These plants also became heavily infested with epiphytes and failed to grow.

Several attempts were made to grow the seaweeds passively on the bottoms of the ponds in a manner similar to that employed in the commercial Gracilaria culture industry of Taiwan. Mixed results have been obtained from the bottom culture experiments, which are continuing, but the tentative conclusions that have been reached to date are:

1) Using two exchanges of water per day and continuous enrichment (150 μmoles/l NO_3-N, 15 μmoles/l PO_4-P, chelated trace elements) gave poor results, the seaweeds becoming heavily infested with epiphytes and eventually dying.

2) Using the same flow rate but pulsing the nutrients, stopping the flow for 48 hours every two weeks and adding an amount of nutrients comparable to that which would be provided in (1) above over the two-week period, prevented epiphyte development and resulted in good growth of the Gracilaria.

3) The use of shallow (0.4 m) ponds resulted in good growth in winter and poor growth in summer. Deep (0.8 m) ponds produced yields that were intermediate throughout the year. Thus, use of deep ponds in summer and shallow ponds in winter is indicated.

4) Gentle aeration, insufficient to move the seaweed but enough to circulate the water and break down thermal stratification, appears necessary in summer but is not required in winter.

5) The use of a sand-soil substrate on the bottoms of the PVC-lined ponds did not enhance growth.

Mean yields of Gracilaria in the non-intensive pond-bottom cultures that have proved successful to date, since these experiments were initiated in May, 1979, has been about 3 g afdw/m^2.day (10 t/ha.year or 4 t/acre.year), about one-quarter of the yields obtained by the intensive culture methods. However, this research is still in a relatively early stage of development and is continuing, so some improvement in yields may be anticipated as more experience is obtained with these methods.

A major purpose of the non-intensive pond culture experiments is to develop the most promising method for growing Gracilaria in the one-quarter acre (10,000 ft^2) demonstration unit that was completed in the late summer of 1979. Initially it was planned to grow a sufficient quantity of the "ORCA" clone to stock the large pond, and the yields of four 2600-liter aluminum tank cultures and one 25,000 liter aluminum tank culture are presently being used for that purpose. However, to save time over that process, which could take over a year to stock the large pond at the desired density, approximately one ton of Gracilaria was collected from the Indian River in October and used as an initial seed culture, which will continue to be supplemented from the smaller experimental cultures of "ORCA" clone. Thus, it is hoped that sufficient stock will be available to initiate seaweed culture experiments in the quarter-acre pond by early 1980.

B. Freshwater macrophyte culture:

In contrast to the seaweed research, where several important problems have remained to be resolved, relatively little effort was devoted to new research with freshwater plants during the present contract year. Water hyacinth remains the most promising species with respect to yield and relative freedom from major problems in its cultivation, though some new problems have emerged that will be discussed below.

Initial use of an inorganic trace element enrichment, discussed above in connection with the seaweed culture, was first found to be inadequate for the rapidly-growing water hyacinths, leading to obvious chlorosis of the foliage and eventually a cessation of growth. This could be corrected by spraying the foliage directly with the inorganic enrichment medium, but that practice was subsequently replaced by supplementing the medium, with chelated iron, which practice was later adapted for the seaweed culture.

Water hyacinths grown in PVC-lined ponds receiving a continuous flow of enriched well water (see previous report for details) exhibited a mean annual yield for the period March, 1978-March, 1979 of 25.7 g afdw/m^2.day, equivalent to some 94 t afdw/ha.year (37 t/a.yr). That is about 1/3 higher than the yield reported for the previous 11-month period (see previous report), but probably reflects the normal year-to-year variability in yield that may be expected, particularly for a species living near the limit of its range. In that connection, the higher yield during 1978-79 may have been due partly to the mild winter of that year, during which the plants were not once killed or visibly set back by frost.

A new problem in water hyacinth culture became obvious in May, 1979 with the infestation of the plants by weevils of the genus Neochetina, either or both of the species N. eichhorniae or N. bruchi that have been introduced to Florida from South American specifically for the purpose of water hyacinth control. Although apparently not lethal to the plants in themselves, the insects appear to stress them to the extent that other control methods may be effectively used.

The weevil infestation of the Harbor Branch Foundation population of water hyacinths visibly affected the plants and quantitatively reduced their yield until the insects were brought under control by aerial spraying of the foliage with an organophosphate systemic insecticide (Cygon 2E)*. Spraying must now be employed routinely to control the weevil infestation, a practice that could significantly affect the economics of large-scale water hyacinth cultivation.

A new series of experiments has been initiated during the present contract year to investigate the effects of season, nitrogen form and availability and plant stand density on the chemical composition of water hyacinths. Such variability in composition may have a significant effect upon the value of the plants as a feed or feed-supplement (i.e., through their nutritive value) or as a biomass source for conversion to fuel (i.e., through their energy content and digestability by anaerobic fermentation).

As growth of the plants decreases in winter, due to both reduced temperature and solar radiation, total nitrogen and ash content of the plants increase while lignocellulose and nonstructural carbohydrate levels decrease, while the reverse trend develops as the more active growing season begins in May and continues through the summer. Analyses of inorganic nitrogen levels in the plant tissues indicate that the plants are able to assimilate and store the nutrient in winter, when they are unable to grow or can grow only slowly and cannot convert the nitrogen to plant protein. In summer, when the

*American Cyanamid Co., Princeton, N.J.

plants can grow but environmental sources of nitrogen may be limiting, the hyacinths are then able to utilize their winter reserves of inorganic nutrients. In this respect, a striking similarity occurs in the strategy of these plants and of temperate species of seaweeds.

Both nitrate- and ammonium-nitrogen are assimilated equally well by water hyacinths, though ammonia is more readily incorporated into protein, as is true with most plant species. Nitrogen availability has a pronounced effect upon chemical composition of the plants, high availability resulting in correspondingly high levels of protein and ash and low availability resulting in plants with high levels of structural carbohydrates (which reduces their nutritive value and digestability). "High quality" plants, grown at the aquaculture facility with a non-limiting supply of nitrogen produced nearly three times as much biogas per unit weight as did nutrient-limited plants from a natural stand. Thus plants collected from the wild, where nutrients may be growth limiting, or those grown artificially under similar conditions, may give misleading information concerning the potential value of the species.

Finally, water hyacinths in very dense stands are able to grow only vertically with greatly elongated stems relative to those in sparsely populated stands. Vertical elongation requires a substantial increase in structural carbohydrates such as lignocellulose and the relative proportion of that substance increases correspondingly in dense stands of the species, to the detriment of its nutritive value and digestability to methane. Dense stands of water hyacinths are thus not only undesirable from the point of view of their organic yields (see Chapter 4) but also their composition and value.

An experiment, initiated in 1978, was completed in 1979 in which water hyacinth yields at the Harbor Branch Foundation aquaculture facility, where the plants were grown in continuous-flowing, enriched well water, were compared with yields from a eutrophic natural environment. Growth of plants in the two locations was roughly the same during the late winter and early spring when light and/or temperature were presumably the limiting factors, but at other times the yield of the cultured plants increased by several fold while that of the natural stand decreased to levels one-third or less of the cultured plants. This annual study further substantiated the hypothesis, presented in the last report, that biomass yields at the Harbor Branch Foundation facility equalled or, at least, closely approached the maximum potential for the species for the climate and latitude of central Florida.

Some additional studies were carried out during 1978-79 on other species of freshwater macrophytes, though the effort made in that area was relatively minor. The pennywort, Hydrocotyle umbellata, which appeared to show some promise a year ago, particularly because it had been found elsewhere to grow at lower temperatures than does water hyacinth, proved to do rather poorly at Harbor Branch Foundation during the winter months, with periods of no growth alternating with periods of modest yields. Mean yield of pennywort over a 10-month period was 7.9 g afdw/m^2.day, only about one-third that of water hyacinths.

Water lettuce, _Pistia stratiotes,_ has been grown for a short period of time during the fall of 1979, during which it averaged 14.4 g afdw/m^2.day at its optimal density of 160 g afdw/m^2. _Pistia_ is reportedly even more of a tropical species than water hyacinths and its yields have, in fact, declined from early October to late November, but its growth will continue to be monitored through the winter of 1979-80 and, if it survives, through 1980, because it appears to be a highly nutritious plant that may have certain advantages over water hyacinth.

Finally, improved yields of duckweed over those reported in the last report have been described in the literature by means of frequent harvesting of the new growth so as to maintain a low density and prevent the deleterious effects of overcrowding. These experiments were repeated at Harbor Branch Foundation, comparing yields of cultures from which incremental growth was partially removed each day with that from cultures unharvested over periods of 5-10 days. No yield enhancement was found to result from the frequent harvesting regime.

A second PVC-lined, concrete-wall, one-quarter-acre (10,000 ft^2) pond, contiguous with that to be used for _Gracilaria_ culture, was also completed in the late summer of 1979 and will be used as a demonstration unit to assess the economic and energy cost:benefit ratio of a water hyacinth-based energy farm. The pond was stocked with approximately 3 tons (wet wt) of water hyacinths collected from a wild population during September-October, 1979, and the plants had grown to the extent that they covered approximately half the pond surface at the time this report was prepared.

C. Evapotranspiration:

The loss of water from evapo-transpiration of duckweed, water hyacinth and pennywort was measured over a period of nine months from January 17 through September 26, 1979 and compared with water loss from evaporation from a open water surface.

In contrast to the literature, in which most values of the ratio of evapotranspiration to evaporation ($^{ET}/E$) are of the order of 3-4 with some as high as 6, $^{ET}/E$ for water hyacinths for the above period was only 1.7 and that for pennywort, 2.0. Duckweed, with a $^{ET}/E$ of 0.9 serves as a water conservation device.

Extrapolation of the observed water loss from a solid cover of water hyacinths to a complete year gives an equivalent water loss of three million gallons per acre of plant surface, of which 1.2 million (41%) is in excess of the amount that would be lost from a bare water surface alone.

Linear regression of water loss from the three aquatic plant species with various meteorlogical and biological parameters showed a high correlation with incident solar radiation, temperature and plant yield (all of which are also correlated with each other) but no significant correlation with wind speed or relative humidity.

The above experiments will be continued so as to provide data for a complete year.

D. Recycling digester residues:

In recognition of the fact that supplying plants with essential nutrients is one of the more costly elements in any biomass production system, both in terms of economic and energy requirement, experiments were started last year to investigate the possibility of recycling the chemicals left in the solid and liquid residues following anaerobic digestion and methane production as a source of nutrients for new plant production. These experiments will be continued through the present contract year and beyond.

Stable, continuous anaerobic digestion of water hyacinths has now been maintained for longer than one year, with an average gas production of 0.4 l/g volatile solids, at 60% methane. The heat of combustion of water hyacinths of 3.8 kcal/g dry weight is equivalent to 4.6 kcal/g ash-free dry weight (since water hyacinths are on average 18% ash) or 19 kJ/g volatile solids. Since pure methane has an energy content of approximately 37 kJ/l, the above methane production represents an average bioconversion efficiency of about 47%.

Both the liquid and the solid digester residues were a good source of nutrients for the growth of water hyacinths. Cultures grown on these residues were consistently more productive than those grown in a chemically-defined enrichment medium, with an average productivity of 65% and 47% higher over the entire period for the liquid residue and the solid residue respectively. The yield of plants grown in the enrichment medium and in the liquid residue were two and three times higher, respectively, than that of the unenriched control. The growth of water hyacinths grown on solid residue was 89% of those grown on liquid residues during the nine weeks these cultures were monitored concurrently.

Water hyacinths grown on digester residues have a composition similar to those grown on a chemically-defined enrichment medium in terms of percentage ash, carbon, and nitrogen. Cultures that did not receive nutrient enrichments had reduced levels of ash and nitrogen but enhanced carbon content and carbon:nitrogen ratios.

An approximate balance of the nitrogen recycled through the culture-digester-culture was made. Over the 39-week experimental period, one digester was loaded with a total of 532 kg wet weight of water hyacinths. This biomass was 21.3 kg in ash-free dry weight and contained 577 g N. Of this N, 48% (276 g N) was recovered in the liquid residue and 52% (303 g N) was recovered in the solid residue. In all, 525 l of liquid effluent was removed containing an average of 526 mg N/l (a total of 276 g N), of which about 50% was in the form of NH_4^+-N and the remainder was organic N of an unknown identity.

Addition of this liquid effluent to cultures of water hyacinths produced 4.7 kg ash-free dry weight which contained 179 g N over a 39-week period, a recycling efficiency of 65%. A total of 120.4 kg of solid residue was removed from the digester. This was equivalent to 4.6 kg ash-free dry weight and contained 303 g N. Recycling 24 kg wet weight (equivalent to 0.9 kg ash-free dry weight and containing 60 g N) of this material produced 1.1 kg ash-free dry weight containing 39 g N over an 8-week period, a recycling efficiency of 64%.

The red seaweed, Gracilaria tikvahiae, has also now been anaerobically digested for over one year, with an average gas production of 0.4 l/g ash-free dry wt, the gas containing 60% methane. Thus the efficiency of digestion and the energy value of the product gas is the same for water hyacinths and Gracilaria.

The liquid digester residue has also proved to be a highly successful source of nutrition for new growth of Gracilaria, there being no significant difference between the yields of cultures grown in seawater enriched with a chemically-defined medium normally used to grow the plants or in seawater enriched with liquid digester residue containing a comparable concentration of nitrogen, unenriched controls failing to grow at all in parallel cultures. Unlike water hyacinths, however, the nutrients contained in the solid residue from Gracilaria digestion, though not as great in quantity, were not available for assimilation and new growth of seaweed.

An approximate mass balance of the nitrogen recycled through the culture-digester-culture system was made. Over the course of the work completed to date, one digester was loaded 55 times with a total of 312.5 kg wet weight of Gracilaria. That biomass is equivalent to 20.2 kg ash-free dry weight and 0.89 kg nitrogen, of which 65% (0.58 kg) was recovered in the 305 l of liquid residue and 29% (0.26 kg) recovered in the 116 kg of solid residue. Concentration of total nitrogen in the liquid residue averaged 1.91 g/l of which about 66% was in the form of ammonia and the remainder was organic nitrogen of unknown identity.

Addition of the liquid residue over a 292 day period to cultures of Gracilaria at a rate of 0.5 l/week produced 0.58 kg afdw of seaweed containing 29.3 g N, an uptake efficiency of 73%. Overall efficiency through the complete cycle of seaweed-liquid residue-seaweed was 73% of 65%, or 48%.

The major difference between the nitrogen recycling of digester residues of water hyacinths and Gracilaria is the unavailability of the nutrient from the solid fraction of the latter. Visual observation of the solids from Gracilaria digestion suggests that these substances are not readily biodegradable. Indeed, one of the major constituents of Gracilaria, the hydrocolloid agar, depends for its major use in microbiological research and application upon its non-biodegradability by most bacteria. It seems quite possible, in other words, that *Gracilaria* may be fermented to produce methane, with the recovery of at least 65% of its nitrogen in the liquid residue from the digestion and available for recycling and with the bulk of its commercially-valuable product agar still available in the solid digester residue. This attractive possibility will be examined during the coming year.