NITROGEN UPTAKE AND STORAGE BY THE RED ALGA *GRACILARIA TIKVAHIAE* (McLACHLAN, 1979)

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ABSTRACT

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Nitrogen-starved plants of the red alga *Gracilaria tikvahiae* (McLachlan, 1979) assimilate ammonium-nitrogen very rapidly, doubling their total tissue nitrogen content in 8 h or less. Uptake of nitrate-nitrogen is less rapid. Ammonium uptake is initially the same in the dark as in full sunlight, but the light-exposed seaweeds continue to assimilate the nitrogen longer. N-starved plants soaked in a full nutrient medium including NH_4^+-N for as little as 6 h and returned to unenriched flowing sea water will grow at non-nutrient-limiting rates for as much as 2 weeks before they again become nutrient-deficient and their growth rate declines.

INTRODUCTION

Earlier studies on the culture of the red seaweed *Gracilaria tikvahiae* (McLachlan, 1979) have shown that healthy, well-nourished plants are capable of growth for as much as 2 weeks in unenriched running sea water, even when the latter contains low to undetectable concentrations of inorganic nitrogen, either as ammonia or nitrate. Eventually, however, the plants held under such conditions lose their dark reddish-brown coloration, assume a pale straw-yellow color, and cease growing. The change in color is thus indicative of nitrogen deficiency, in the course of which total nitrogen concentration in the plant tissues decreases from 3—4% to 1—2% of total dry weight, with carbon : nitrogen ratios increasing correspondingly from about 6 : 1 to nearly 30 : 1 (Lapointe and Ryther, 1979; Ryther and Hanisak, 1981).

Such nitrogen-deficient algae are capable of extremely rapid assimilation of nitrogen as nitrate, even more rapid uptake as ammonium-nitrogen, which most species preferentially utilize. D'Elia and DeBoer (1978) describe the

uptake kinetics of the two nitrogen forms by N-deficient (C/N > 10) *G. tikvahiae* (then known as *G. foliifera*) and *Neoagardhiella baileyi*, showing rates that did not follow the classical Michaelis—Menten equation of saturable uptake kinetics and were apparently independent of and far in excess of uptake associated with growth.

The same behavior has been demonstrated in the unicellular algae by a number of authors (see, for example, Bongers, 1956; Caperon and Ziemann, 1976; and the reviews by Meyers, 1951, and Syrett, 1962), several of whom have discussed the physiological and biochemical aspects of the phenomenon. Those processes will not be discussed here, but rather the magnitude of uptake and storage by N-deficient seaweeds will be considered together with the consequences of such storage capacity on subsequent growth of the seaweeds.

Chapman and Craigie (1977) have demonstrated the ability of the brown alga, *Laminaria longicruris*, to accumulate both inorganic and organic reserves of nitrogen in winter, when relatively high concentrations of that element, primarily as nitrate, occur in the water. That stored nitrogen may then be drawn upon for growth of the alga throughout much of the summer, when the supplies of dissolved nutrients in the water become depleted. The present article will show how this phenomenon also occurs in the red algae and how it may be employed as a strategy for those who would grow such seaweeds in artificial culture systems.

METHODS

The "ORCA" clone of *G. tikvahiae*, isolated from the Indian River near Vero Beach, FL in 1977 and grown vegetatively both in Florida and in Woods Hole, MA since that time, was used in all of the experiments described below. In Woods Hole, the seaweed was grown in 1.8 × 2.4 × 0.2—0.8 m deep plywood boxes with sloping bottoms through which sea water was circulated and compressed air was pumped, maintaining the algae in suspension, as described by Lapointe and Ryther (1978). At Harbor Branch Foundation, the seaweeds were similarly grown in suspended culture in 2.1 × 0.8 × 0.6 m deep flat-bottom concrete tanks.

The experiments to be described were carried out at both locations during the period August—November, 1980. Sea water at Harbor Branch Foundation aquaculture facility contained 8.0—12.0 μmoles/l of dissolved inorganic nitrogen as nitrate and ammonia, that at the Woods Hole Oceanographic Institution's Environmental Systems Laboratory contained 1.0—3.0 μmoles N/l. Temperatures at the Florida site ranged from 35°C in September to 16°C in November, in Woods Hole, from 21°C in August to 18°C in October. Salinities averaged $34^0/_{00}$ in Florida, $30^0/_{00}$ in Woods Hole.

Uptake experiments

G. tikvahiae was grown as described above for 2—4 weeks in running,

unenriched sea water (one volume exchange of water per day) until the algae were a pale yellow color and growth had ceased. Five kg (wet weight) were then placed in tanks containing 350 l (Florida) or 500 l (Woods Hole) of sea water enriched with NH_4Cl and $Na_3PO_4 .12H_2O$ to provide in both cases 2000 μmoles N and 150 μmoles P/l. The water in the Woods Hole experiment was gently aerated to provide motion of the water through the seaweed. Water samples were then withdrawn at intervals and analyzed for ammonium-nitrogen (Solorzano, 1969) and phosphate-P (Murphy and Riley, 1962). In one experiment, $KaNO_3$ was used in place of NH_4Cl and was analyzed at the same time intervals for NO_3^--N by the method of Wood et al. (1967). In one control experiment, no seaweed was added to check any loss of NH_3 to the atmosphere during the course of the enrichment. In another control, recently-enriched, dark reddish-brown *Gracilaria* was used to measure uptake rates in non-nitrogen-deficient seaweed.

Experiments in Woods Hole were carried out in the laboratory under continuous fluorescent illumination of approximately 0.1 ly or in darkened containers. Those in Florida were done out of doors in full sunlight or also in darkened tanks.

Samples of the seaweed were taken at the beginning and end of the enrichment period, usually 24 h, and in one case midway through the experiment. These were oven dried at 80° C for 24 h and analyzed for total C and N with a Perkin—Elmer Model 240 elemental analyzer.

Growth experiments

2.5 kg (wet weight) portions of yellow, nitrogen-deficient *Gracilaria* were placed in 2500 l aluminium tanks at the Florida site, in sea water enriched with NH_4Cl or $NaNO_3$ (100 μmoles N/l), $Na_3PO_4 \cdot 12H_2O$ (100 μmoles P/l) and a mixture of trace metals and chelated iron as described in Ryther and Hanisak (1981). The tank was gently aerated to provide movement of the enriched sea water through the seaweed. The seaweed was held in the tank for various periods of time, from 1 to 48 h, after which it was washed in clean sea water and placed in the 700 l concrete tanks described above, where it was grown in running, non-enriched sea water (one exchange per day), maintained in suspension by vigorous aeration. The experiment was run from September 15 to November 3, during which period the seaweed was taken from the tanks every 2 weeks, drained of excess water, weighed and harvested back to its starting weight (2.5 kg wet weight). The latter was then put into the enrichment tank again for the same length of time at each biweekly interval. A control was grown in the same manner but received no enrichment.

RESULTS

No measurable amount of ammonia was lost to the atmosphere from the

tank without seaweed in the Woods Hole experiment (Fig. 1A). Thus any loss from the tanks containing the algae could be assumed to have been due to assimilation by the plants.

There was also no significant removal of nitrogen from the tank containing the well-nourished *Gracilaria* and held under the same conditions (Fig. 1B). However, the ammonia-nitrogen in the tank socked with N-starved seaweed decreased rapidly, from 16.8 to 3.0 mg N/l in just 8 h, to nearly unmeasurable levels in 24 h (Fig. 1C). That loss represents a total uptake of some 8.4 gN by the 5 kg wet weight (0.55 kg dry weight) of seaweed in 24 h, a nitrogen-increase of 1.5% of total dry weight or a 2.5-fold increase over the starting concentration of 1.0% of dry weight. The total measured increase in the plant tissues, sampled at 7, 11 and 24 h (Triangles in Fig. 1C), agreed well with the increase calculated from the loss from the water (open circles, broken line, Fig. 1C), confirming the fact that the loss from the water was indeed due to assimilation by the plants.

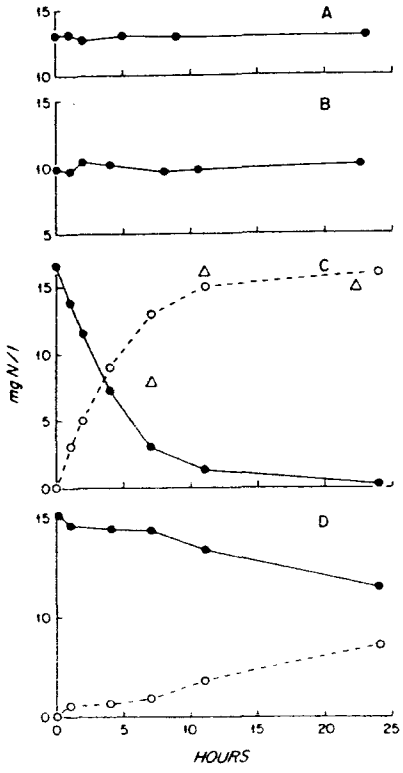

Fig. 1. (A) Change in ammonium-nitrate in tank containing no seaweed. (B) Change in ammonium-nitrogen in tank containing non-nitrogen-starved *Gracilaria*. (C) Removal of ammonium-nitrogen from water (solid circles) and calculated uptake (open circles) by nitrogen-starved *Gracilaria*. Triangles are measured increases in tissue nitrogen by the same plants. (D) Removal of nitrate-nitrogen from the water (solid circles) and calculated uptake (open circles) by nitrogen-starved *Gracilaria*.

An identical experiment conducted concurrently at Woods Hole but with nitrogen supplied at the same concentration as $NaNO_3$ showed a much slower rate of uptake by the *Gracilaria* (Fig. 1D). In that case, only 37% of the available nitrate-nitrogen was taken up, a total of 2.7 g by the 5 kg wet weight or an increase of only 0.4% of the dry weight (e.g., from 1.0 to 1.4 of total dry weight).

Ammonia-uptake in the light and in the dark were essentially the same in the Woods Hole laboratory experiments (Fig. 2B), where the seaweeds were exposed only to the weak illumination of fluorescent lamps. In Florida, where a similar experiment was conducted outdoors with the dark seaweed enclosed in a light-tight container but with the light-exposed plants held in full sunlight during daylight hours and in natural darkness at night, uptake in the 'light' and 'dark' were comparable for the first 5 h after which 'light' uptake was significantly higher than 'dark' uptake. The terms are placed in quotes because the experiment was started at about 9:00 AM and the

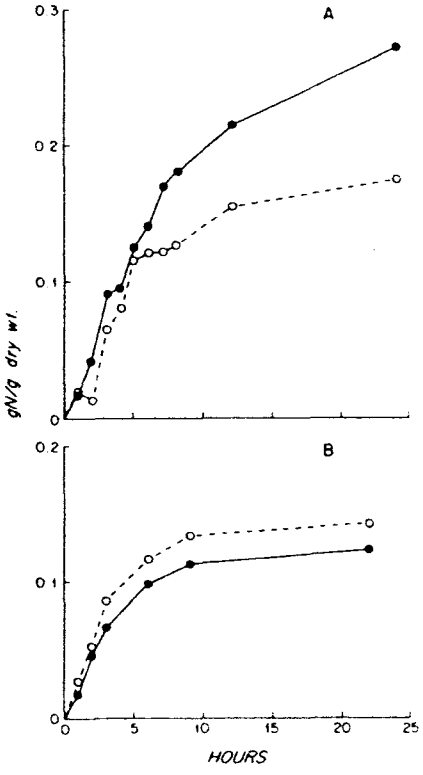

Fig. 2. Uptake of ammonium-nitrogen by nitrogen-starved *Gracilaria* in the light (closed circles) and in the dark (open circles). (A) Harbor Branch Foundation in natural daylight, experiment started at 09:00 a.m. (B) Woods Hole experiments with light provided by fluorescent lamps during entire period.

seaweed was in natural darkness from hour 10 to hour 22, the period when the greatest difference between the two was noted.

The growth experiments, carried out in Florida, showed that soaking *Gracilaria* in nutrient-enriched sea water for as little as 6 h every 2 weeks is sufficient to enable the plants to grow at the maximum possible rate under the conditions of the experiment (Fig. 3). Increasing the exposure to the nutrients beyond the 6 h, to a maximum of 48 h, had no additional stimulatory effect. Reducing the exposure to 1 h resulted in roughly half the yield of seaweed and the control showed no growth.

The companion experiment, in which nitrate rather than ammonium was the nitrogen source, showed a similar but less striking effect. Yields of *Gracilaria* soaked in the nitrate-enriched medium for durations from one to 24 h were consistently lower than those exposed to ammonium. Not until the nitrate-soaked plants were exposed for 48 h were their subsequent yields comparable to those obtained from the ammonium treatment for the same period of time.

DISCUSSION

The experiments described above shed little light on the physiological mechanisms or biochemical pathways associated with the uptake, storage and utilization of nitrogen by N-starved seaweeds. Such information is unfortunately generally lacking for the seaweeds. Though much more has been done in this respect with the unicellular algae (e.g., Syrett, 1962), it is not necessarily applicable to the macrophytic algae, at least some of which have quite different pigment systems, storage products and cellular constituents in general.

The rapid loss and reappearance of phycoerythrin, the reddish-brown pigment of *Gracilaria*, with the development of and recovery from nitrogen deficiency makes a connection between the two seem inescapable, particularly since the phycobilin pigments are protein-aceous. Yet there has been no suggestion in the literature that such pigments may function as nitrogen storage products.

The rapid uptake of ammonium-nitrogen by N-starved unicellular algae is accompanied by an increase in respiration at the expense of endogenous carbohydrate reserves that apparently are not present in non-N-deficient cells (Bongers, 1956; Syrett, 1956 a). Whether or not this also occurs in the macroscopic algae is not known, though the accumulation of polysaccharides in N-starved seaweeds, the so-called 'Niesh-effect', appears to be a common phenomenon of at least the Rhodophyceae (Niesh and Shacklock, 1971; DeBoer, 1978). The fact that N-starved *Gracilaria* assimilated more ammonium-nitrogen following exposure to daylight than did plants held in the dark (Fig. 2A) appears to substantiate the observation of Syrett (1956 a,b) that rapid N-assimilation by starved algae depends upon carbohydrate reserves and ceases when those reserves are depleted.

The ecological advantage of storing nitrogen to be used for growth when it becomes scarce and limiting in sea water is obvious, particularly since dissolved inorganic forms of nitrogen are at their seasonal minimum in summer, when solar radiation and temperature are usually optimal for algal growtn. This was pointed out by Chapman and Graigie (1977), as mentioned earlier, and need not be discussed further here. However, the same phenomenon can also be used to advantage by the culturist interested in growing commercially-valuable seaweeds under controlled conditions.

The continuous feeding of seaweeds grown in culture by enriching the sea water that flows through the plants is costly and ineffecient. Plans to grow the giant kelp (*Macrocystis pyrifera*) in nutrient-rich deep ocean water, pumped up and dispersed through the kelp beds, appear at best to be marginally feasible economically, due largely to the projected high cost of pumping the deep water to the surface and spreading it through the seaweed. Utilization of the nutrients in that system was estimated at no better than 30% (Dynatech R/D Co., 1978).

The smaller, unattached seaweeds like *Gracilaria*, *Chondrus* and other red algae, on the other hand, may be removed from their culture system and soaked in a concentrated nutrient solution for only a few hours during which they are capable of more than doubling their nitrogen content. They may then be returned to the culture system where they will grow with no additional nutrients added to the water until they double their biomass, thereby halving their nitrogen content again. Harvesting the new growth may then be accompanied by another session of nutrient-soaking of the standing stock to be returned again to the culture unit.

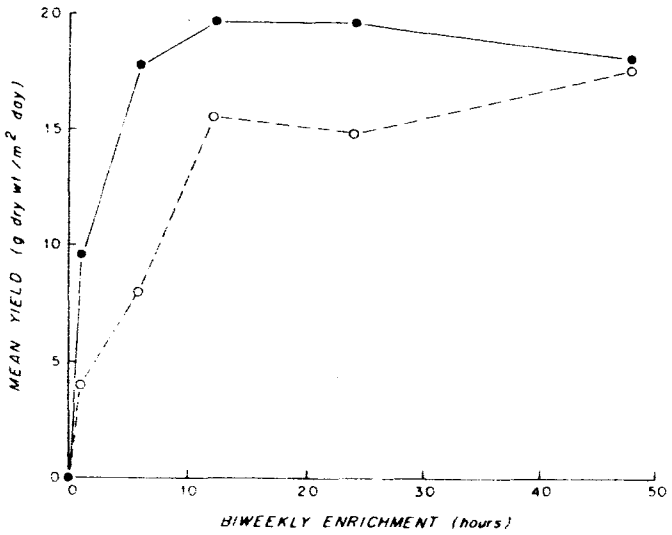

Fig. 3. Mean yield of nitrogen-starved *Gracilaria* immersed in nutrient medium containing ammonium-nitrogen (closed circles) and nitrate-nitrogen (open circles) every 2 weeks for the periods of time indicated.

Not only is such a system highly efficient in nutrient utilization, with no loss of unused nutrients, but it also solves simultaneously one of the chronic problems in seaweed culture, the growth of undesirable epiphytes on the cultured seaweeds. With no nutrients added to the sea water in which the algae are grown, epiphytes do not have an opportunity to become established and the externally-fed target species has the competitive advantage.

In the studies described above, only nitrogen was monitored in the water and plant tissues. The behavior of phosphorus, iron and other nutrients should also be studied in the same context. However, the subsequent growth of *Gracilaria* after nutrient-starvation followed by short-term enrichment (e.g., Fig. 3) would indicate either that other nutrients were not limiting in the sea water or that they behave similarly to nitrogen with respect to uptake, storage and utilization.

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