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SIGNIFICANCE OF CARBON DIOXIDE AND BICARBONATE-CARBON UPTAKE IN MARINE BIOMASS PRODUCTION

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ABSTRACT

The red alga Gracilaria tikvahiae is capable of extremely high rates of biomass production ($> 20 \text{ g vs/m}^2\text{.day}$ or 74 mt vs/ha.yr), but such yields require rapid seawater exchange rates (25 culture volumes/day) that would be uneconomical and nonenergy cost-effective in a land-based or coastal energy farm based on pumped water.

The requirements for high seawater exchange reflect CO_2 limitation which is exacerbated in species such as Gracilaria that cannot utilize bicarbonate as a substrate for photosynthesis, since free CO_2 is almost unavailable at the high pH levels (> 9.0) attained in dense cultures at low exchange rates. Bicarbonate users, such as Ulva are better adapted to growth under such relatively stagnant conditions where CO_2 is not rate-limiting.

At extremely low seawater exchange rates (< 1 exchange/day) even CO_2 may become growth limiting and high yields depend upon CO_2 -enrichment.

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INTRODUCTION

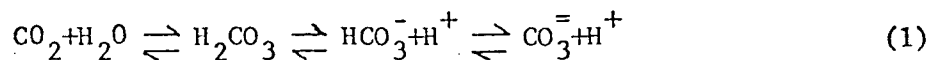
The red seaweed, *Gracilaria tikvahiae*, has been grown in culture at the Harbor Branch Foundation (Fort Pierce, FL) for four years. A asexual clone, isolated in 1978 and grown vegetatively throughout the year at its optimal density by frequent, routine cropping, was found capable of yields averaging as much as 35 g dry wt/m².day, equivalent to 129 dry tons or 75 ash-free dry tons/ha.year (52 and 30 tons/acre.year respectively) (6). However, such high yields were obtainable only by maintaining the seaweed in suspension through vigorous aeration and by exchanging the seawater in the culture as often as twenty-five times per day (detention 0.04 days) (10).

Subsequent experiments have shown that intermittent rather than continuous aeration, for as little as a few minutes each hour during daylight only, does not significantly reduce seaweed growth but greatly reduces cost and energy inputs for that function. However, pumping as much as twenty-five exchanges of seawater per day would be prohibitive, both economically and in terms of energy balance, and it has not been possible to date to reduce that requirement without sacrificing yields appreciably (Figure 1).

If the need for rapidly exchanging the seawater in the seaweed culture is to be alleviated, it is first necessary to understand what beneficial function the rapid seawater exchange serves. It is not believed to be enhancement of the supply of normal mineral nutrients (N, P, Fe, and the various trace elements normally used in algal culture), since these are added separately and independently of the influent seawater. It is also not associated with water motion *per se* and the associated diffusion gradients between water and algae, since both are kept in rapid turbulent motion by aeration, which was also constant and independent of water exchange in the early experiments (Lapointe and Ryther, loc.cit.). A major nutrient that is not added with the enrichment medium, however, is carbon dioxide, and its supply to the seaweed is directly proportional to the rate at which seawater is pumped through the culture system. The following research was undertaken to investigate the possibility that the relatively low seaweed yields that have been observed to be associated with low seawater exchange rates may have resulted from carbon dioxide limitation.

The Carbon Dioxide System in the Ocean

Carbon dioxide gas entering the ocean hydrolyzes to form the weak carbonic acid which, in turn, ionizes, yielding bicarbonate and carbonate in equilibrium, the reactions being summarized as:



In typical open-ocean seawater, total CO₂ in all forms in the equilibrium is approximately 2 mM in concentration. At the normal pH of seawater of 8.0, most of the CO₂ exists as bicarbonate. Free CO₂ (i.e., as dissolved gas and carbonic acid) is present at no more than 10 μM.

When free CO₂ is removed from seawater, as occurs during photosynthesis, the pH rises and the equilibrium shifts towards the right, producing more bicarbonate and carbonate. At pH 9, the concentration of free CO₂ is no more than 1 μM and at pH 10, 0.1 μM (9). At very high pH and temperature, carbonate precipitates as CaCO₃ and carbon is thereby lost to the system.

Effect of pH on Photosynthesis

Österlind (7) demonstrated that the unicellular green alga Scenedesmus quadricauda was able to grow at pH levels as high as 9.5 while another unicellular green, Chlorella pyrenoidosa, was not. This was attributed to the ability of the former and the inability of the latter to assimilate bicarbonate ions directly and subsequently to utilize bicarbonate as a substrate for photosynthesis. Subsequent publications by Österlind and several other authors have confirmed his general observations, though the exact role of bicarbonate in this respect is still a matter of some uncertainty and debate (see for example, Raven [9]).

Blinks (1) appears to have been the first to show that macroscopic algae also differ in their ability to photosynthesize at high pH, again distinguishing between those that could and those that could not do so (Table 1), and attributing the difference to their respective ability to use bicarbonate.

The only other published work in this area with seaweeds that we are aware of is the series of papers by Tregunna and Thomas (13), Thomas and Tregunna (12), and Jolliffe and Tregunna (5). Using a rather more sophisticated technique of measuring photosynthesis by CO₂ analysis, those authors concluded that bicarbonate ion could be utilized by the Phaeophyta Alaria sp., Costaria costata, Nereocystis luetkeana, and Sargassum muticum, the Rhodophyta Iridaea cordata, and the Chlorophyta Ulva sp. and Enteromorpha lingua. Only the brown alga Desmarestia munda and red alga Porphyra schizophylla showed a significant reduction of photosynthesis at high pH levels, but the authors considered that this may have resulted from a direct effect of pH or other causes and were reluctant to attribute the effect to the inability of those plants to use bicarbonate alone. Incomplete studies with the browns Fucus gardneri and Laminaria saccharina and the red Gigartina sp. indicated that they, too, were bicarbonate users.

It is clear that the latter authors found that most of the seaweeds they examined could utilize bicarbonate in photosynthesis, while the opposite was true of Blinks results. The two studies appear to disagree on the two red algae, Iridaea and Gigartina. Examination of the data in all of these publications suggests that there is no clear cut distinction, but rather a gradation in the ability of different kinds of seaweeds to utilize bicarbonate or, at least, to photosynthesize at "high pH", a rather non-quantitative distinction in any case to add to the confusion. However both Blinks and Jolliffe and Tregunna epitomized Ulva as a bicarbonate user while the former found Gracilaria to be the most unsuccessful in this respect of all the algae he tested, "showing zero net photosynthesis at pH 9.0 and an actual excess of respiration at higher pH values" (Blinks, loc.cit.).

Those who have studied the phenomenon have considered bicarbonate utilization to be primarily of survival value to algae in isolated tide pools and brackish ponds. But it is, of course, of considerable significance to seaweed culturists faced with the need for high organic yields in relatively stagnant growth systems. Even at a water exchange of 10 volumes per day, Gracilaria cultures reach pH 8.8 and at one exchange per day, over 9.0 (Table 2). Values as high as 10.0 have been measured in the afternoon of bright sunny days. Under such conditions, algae such as Gracilaria that are reputedly able to use only free CO₂, may be unable to grow at all.

Because the experimental evidence has been somewhat inconclusive, additional experiments were conducted in which photosynthesis of Gracilaria and Ulva was measured by oxygen production at several different pH levels in buffered seawater.

METHODS

Photosynthesis of Gracilaria and Ulva in seawater maintained at several different pH levels was determined by measuring O₂ evolution in sealed, 2 l glass jars. Four jars were immersed in a 700 l concrete tank with flowing seawater to provide temperature control (25-30°C). Each jar was fitted with a lid containing two ports into which the probes from a YSI Model 51B oxygen meter and a VWR Model 55 Digital-mini-pH meter were inserted. A water seal limited oxygen exchange with the atmosphere when probes were inserted and removed. A 12 v submersible water pump provided water circulation within each jar. The seawater within the jars was adjusted to pH 7.5, 8.0, 8.5 and 9.0 with a range of dilute Tris buffers (0.005 M Tris with HCl or NaOH). Gracilaria (300 g wet wt) or Ulva (360 g) was stocked into each jar and held in the dark until initial readings were taken. Following initial measurements, the jars were placed in full sunlight. Oxygen concentrations, temperature and pH were determined every 20-30 min for 2-4 hr.

RESULTS

The results of the experiments are given in Figures 2 and 3, showing that photosynthesis of Gracilaria is inversely proportional to pH and is severely depressed at pH 9.0. Photosynthesis of Ulva, in contrast, was very slightly affected by increasing pH and remaining relatively high at pH 9.0-9.5. The results are summarized in Table 3, showing that oxygen production by Gracilaria at pH 9.0-9.1 was only 19% as much as at pH 7.5-7.6, while that of Ulva at pH 9.0-9.5 decreased only to 72% that at pH 7.5-7.6

In an earlier experiment, oxygen production of Gracilaria was measured for eight hours at two buffered pH levels, 7.5 (which increased to 8.0 during the eight-hour experiment) and 9.0. Oxygen production at the lower pH proceeded so rapidly that the oxygen recorder went off scale after two hours. At the higher pH, oxygen production proceeded slowly for 4.5 hours, when net O₂ production ceased and the alga remained at compensation level until 6.5 hours. At that point acid was added, reducing the pH to 7.0-7.3 which was simultaneously accompanied by renewed oxygen production at the high rate observed in the sample initially held at pH 7.5 (Figure 4). The immediate response of photosynthesis with the lowered pH suggests that the effect resulted from a

physical reaction in the water (i.e., shift in the CO₂ equilibrium) rather than a biochemical change within the plant tissue (i.e., recovery of an enzyme system) that might have resulted from some direct effect of the high pH.

Österlind (8) showed that a period of photoactivation is necessary for the induction of bicarbonate assimilation by Scenedesmus and Blinks (loc.cit.) found some evidence of the same phenomenon in Ulva. This was not evident in the experiments described above, but the possibility remained that Gracilaria conditioned to growth in a rapidly-exchanging seawater environment with a plentiful supply of free CO₂ might lack the mechanism for bicarbonate assimilation or utilization (i.e., carbonic anhydrase a/c Osterlind). An additional experiment was therefore conducted in which oxygen production at pH 7.5 and 9.0 was measured by Gracilaria which had previously been grown in 10 and one exchanges of seawater per day respectively. No difference was observed, both showing the same response as depicted in Figure 2. The effect is therefore apparently inherent in the species and not the result of environmental conditioning.

Implications for Seaweed Culture

It is certainly no coincidence that Gracilaria, like many seaweeds, thrives in nature where there are fast-moving currents continually re-supplying free carbon dioxide. Creation of an extensive culture facility for biomass production in such a dynamic natural environment would be difficult and costly from an engineering standpoint, while construction of an artificial system that would accomplish the same purpose, on land for example involving a series of raceways, would require pumping of seawater in quantities that would clearly be economically prohibitive and negatively energy cost-effective (Huguenin (3), Unpubl. ms. (4)).

In the latter report, Huguenin concluded that only a combination of high biomass yields at low seawater exchange (one volume per day or less) could provide a positive energy balance. Such a combination would appear unlikely from a non-bicarbonate using alga such as Gracilaria or Macrocystis (two of the prime candidate species that have been considered to date for marine biomass production). Algae such as Ulva, on the other hand, that can use bicarbonate and continue to grow at the high pH levels encountered in semi-stagnant, densely populated culture systems, would have a distinct advantage for the purpose.

At extremely slow rates of water exchanges, however, even algae that can assimilate bicarbonate may become carbon-limited. For example, a culture one-meter-deep whose water is exchanged at the rate of one-tenth of its volume per day has access to only 2.4 g carbon/m².day. If the exchange is constant throughout the 24-hour day, roughly half of that amount or 1.2 g C/m².day would be available during daylight when the algae could use it. A yield of 1.2 g C/m².day is equivalent to about 2.4 g volatile solids or 4.1 g total dry wt/m².day, no more than 10-20% of light-limited production.

High-yield biomass production under such stagnant conditions can be achieved only by addition of CO₂ to the water. This can be accomplished to a limited extent by aeration. The observed yields of Gracilaria as a function of seawater exchange rate (Figure 1), parallel the availability of free CO₂ (in a one-meter-deep culture) but are consistently higher by some 5 g C (ca. 10 g volatile solids)/m².day. The difference is

probably due to the fact that the cultures were maintained in suspension by vigorous aeration which undoubtedly provided additional CO₂. Absorption of CO₂ from the air is dependent upon so many variables that it cannot be calculated in this case with any accuracy. But the increase in yield is at least roughly consistent with diffusion rates of CO₂ across the sea surface (11).

A much more effective method of enhancing CO₂ content would be to add pure or diluted CO₂ gas to the culture, a practice that has not, to our knowledge, been attempted in seaweed cultivation but that has proved highly successful in unicellular algal culture (e.g., Goldman et al. (2)). The economics of such a procedure remain to be evaluated and, in any event, must await experimental evidence of its efficacy. But it should be pointed out, in closing, that the production of methane gas by anaerobic digestion of biomass is accompanied by the recovery of half the CO₂ that originally went into the production of the digested material, and that the burning of the methane to produce energy for whatever purpose is accompanied by the release of the other half of the original CO₂. Thus, combination of the production and conversion systems at the same site could provide for the complete recycling of the CO₂ at no additional cost for its production or transport, a procedure that has previously been advocated for the recycling of the other essential nutrients for biomass production (10).

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Table 1. Relative ability of macroscopic algae to photosynthesize at high pH (after Blinks [1]).

I. Macroscopic algae that have little or no ability to photosynthesize at high pH.

Chlorophyta:

Cladophora
Codium
Monostroma

Phaeophyta

Desmarestia
Dictyoneurum
Egregia
Laminaria
Macrocystis

Rhodophyta:

Aghardiella
Botryoglossum
Endocladia
Gigartina
Gracilaria
Iridaea
Prionitis
Schizymenia

II. Macroscopic algae able to photosynthesize at high pH.

Chlorophyta:

Enteromorpha
Ulva

Phaeophyta:

Pelvetia
Fucus (?)

Rhodophyta:

Botryocladia
Centroceras
Gastroclonium

Table 2. Daily changes in pH of Gracilaria at different seawater exchange rates.

Time	Seawater exchanges/day	
	10	1
0800	8.2	8.1
1030	8.4	8.6
1315	8.7	9.0
1435	8.7	9.1
1530	8.8	9.1
1615	8.7	9.1

Table 3. Effect of pH on photosynthesis of Gracilaria and Ulva measured by oxygen increase after one hour. Figures are relative to increase at pH 7.5-7.6.

<u>pH</u>	<u>Gracilaria</u>	<u>Ulva</u>
7.5-7.6	100	100
8.0-8.2	74	104
8.6	46	92
9.0-9.1	19	72

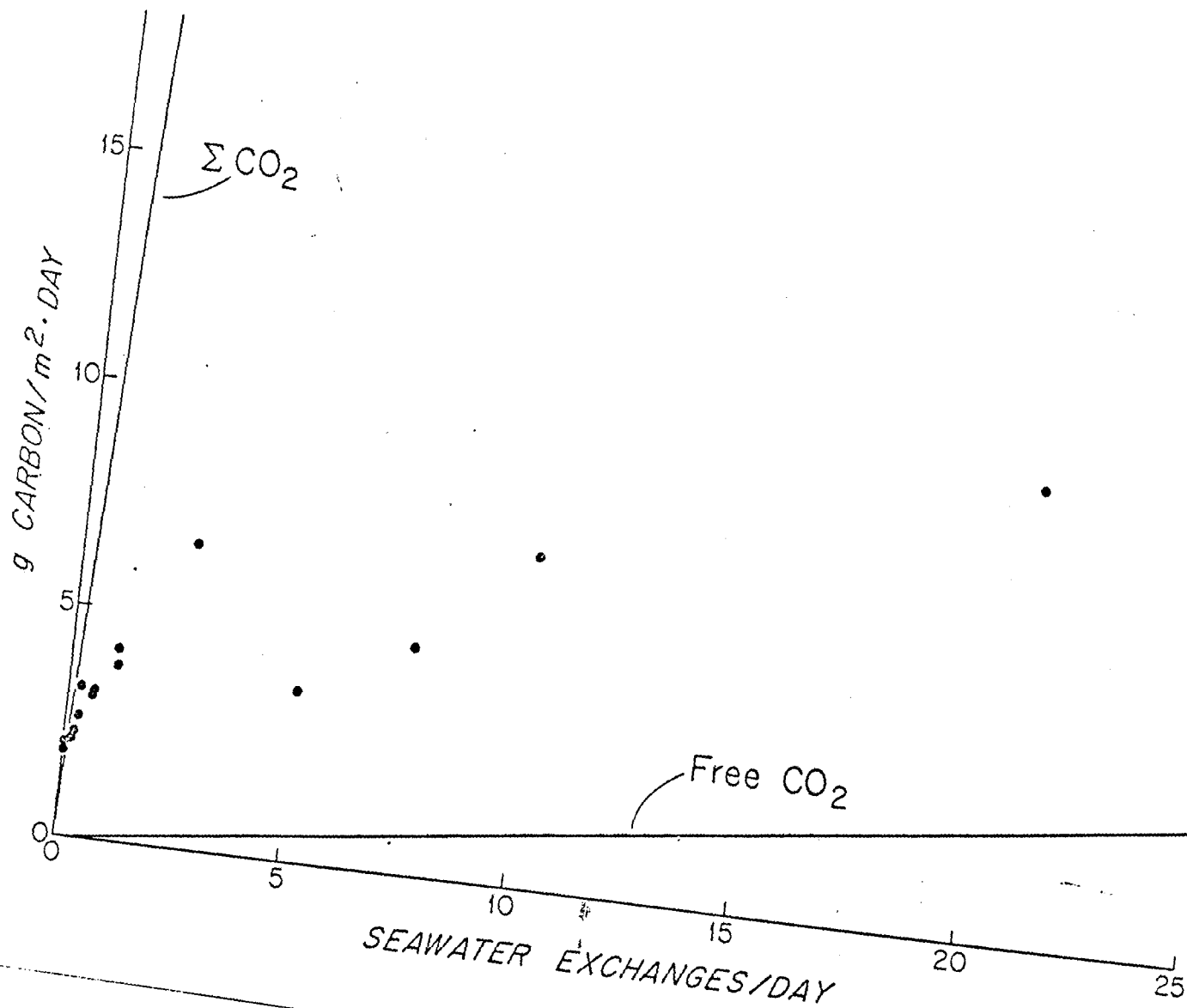


Figure 1. Free and ΣCO_2 available in a seaweed culture one-meter-deep and observed rates of production of *Gracilaria* in g C/m²·day (points) as functions of seawater exchange rate.

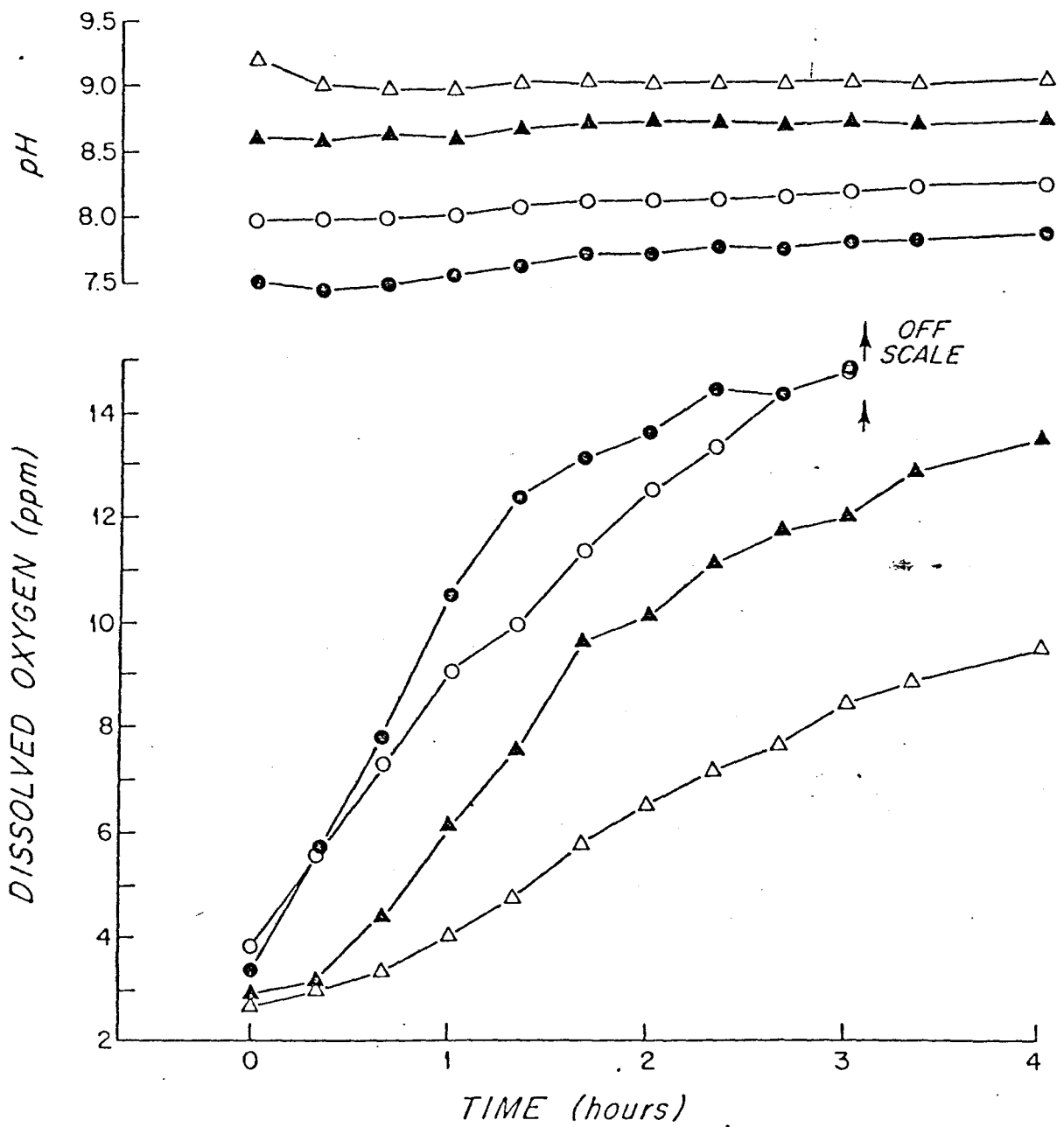


Figure 2. Oxygen production by Gracilaria at four pH levels.

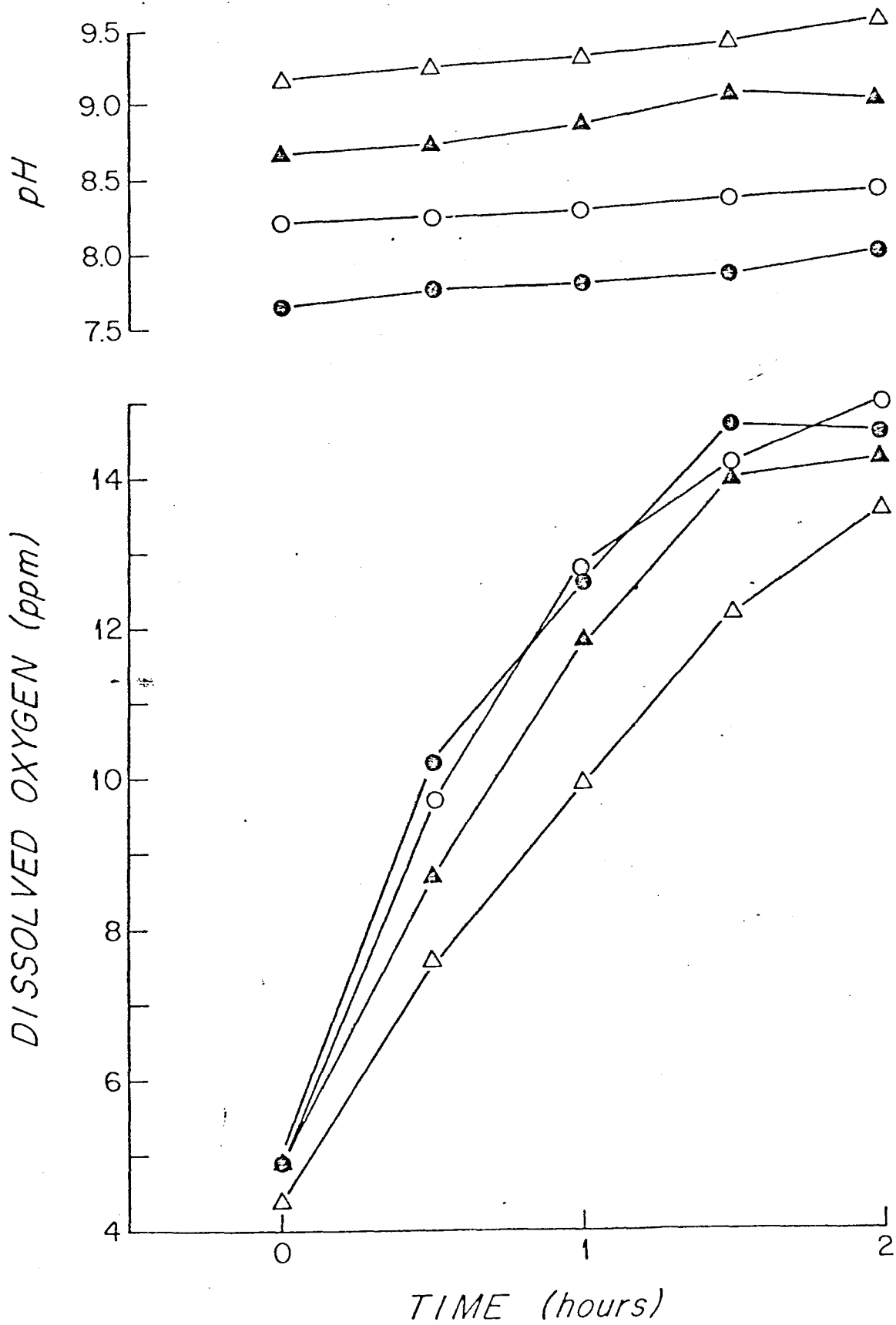


Figure 3. Oxygen production by *Ulva* at four pH levels.

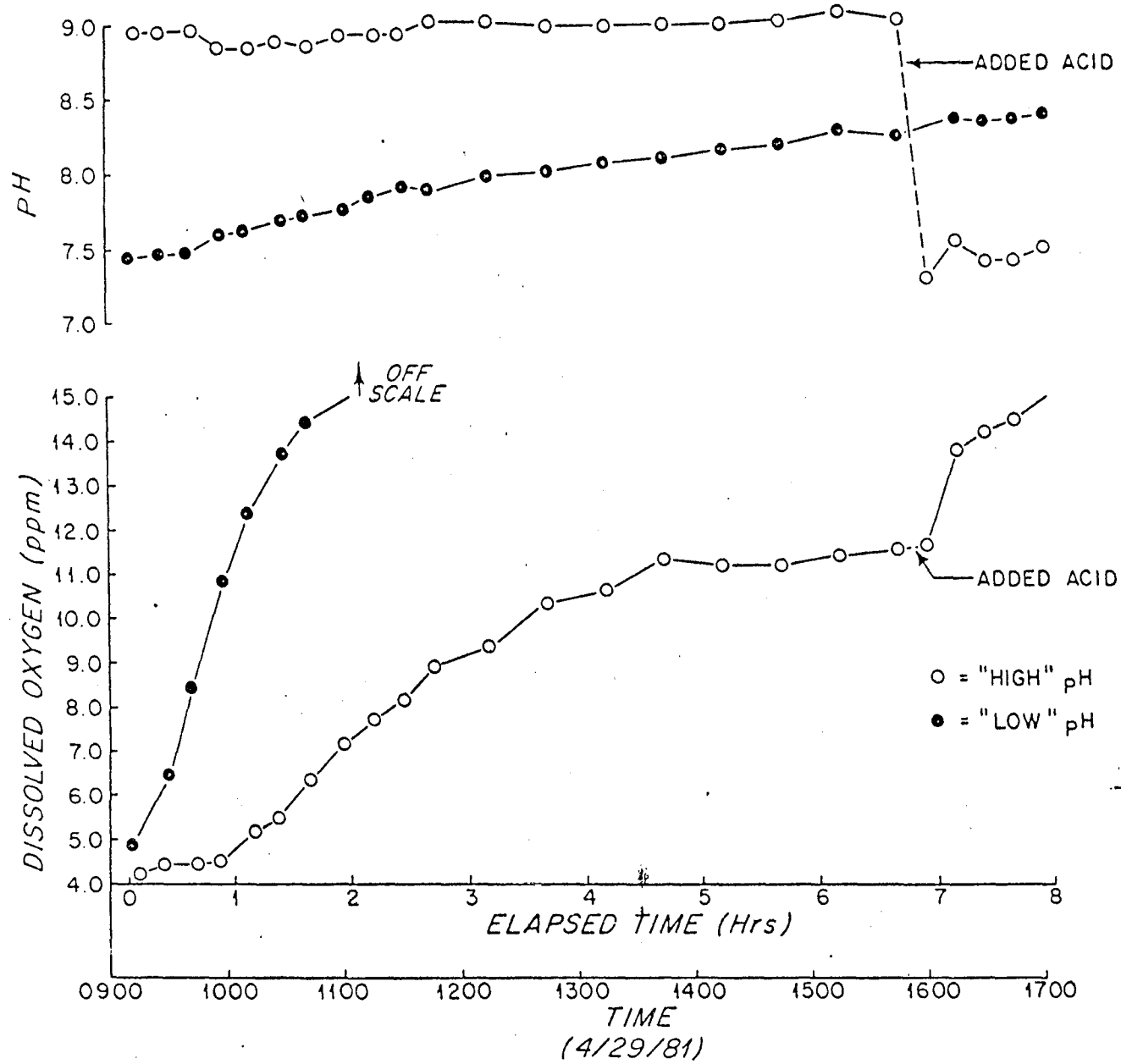


Figure 4. Oxygen production by Gracilaria at two pH levels, with the higher level