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The Feasibility of Mass Cultivation of *Porphyridium*

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ABSTRACT

The feasibility of growing Porphyridium *biomass outdoors was investigated. Laboratory studies indicated that although the optimum temperature for growth is 25°C no damage to the photosynthetic activity can be detected after exposure of the organism to higher temperatures, up to 35°C. Also, high O_2 evolution activity was observed even at relatively high cell concentrations. No inhibition of O_2 evolution was detected at high light intensity, i.e. 2000 $\mu E\ m^{-2}\ s^{-1}$. In cultures grown outdoors an output rate of up to 22 g dry wt $m^{-2}\ day^{-1}$ was obtained for several weeks. The biomass contained, on a dry weight basis, up to 40% polysaccharides and about 1·5% arachidonic acid. The cultures were maintained essentially monoalgal for over 3 months. The biomass can be readily removed from the medium by autoflocculation.*

Key words: Porphyridium, outdoor algal ponds, photosynthesis, algal biomass, polysaccharides, arachidonic acid.

INTRODUCTION

The potential use of algae cultivated outdoors as a source of biomass, chemicals, proteins and energy has already been discussed.[1–3] Nevertheless, only a limited number of algal species have been evaluated for their actual performance in outdoor cultivation.[4,5] Some *Porphyridium* species that grow in fresh water[6] or seawater[7] have been proposed as

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potential sources of polysaccharides. Gudin *et al.*[8] used an outdoor tubular reactor system for growing *Porphyridium* as a source of polysaccharides, while Ahern *et al.*[9] proposed using fermentors for growing this species as a source of arachidonic acid.

In this work, a series of laboratory tests was performed to evaluate the suitability of *Porphyridium* for growth outdoors. The possible use of this alga as a source of polysaccharides and arachidonic acid was studied, and preliminary attempts to simplify the harvesting procedure are reported. Finally, the performance of *Porphyridium* cultured outdoors was evaluated.

MATERIALS AND METHODS

Organism and growth medium

Porphyridium cruentum and *Porphyridium* sp., originally obtained from the University of Gottingen, were routinely maintained as bacteria-free cultures on an artificial seawater (ASW) medium, as described previously.[9]

Laboratory cultures

Cultures (500 ml) were grown in flat-bottomed 1 litre evaporating flasks placed in a transparent water bath illuminated from below with four cool-white fluorescent lamps providing 200 μE m^{-2} s^{-1} at the surface of the bath. Mixing was done with an air–CO_2 (95 : 5) mixture injected through a sintered glass bulb placed in the bottom of the culture flask, and the temperature was maintained constant within ±1°C.

Measurement of oxygen evolution

All kinetic measurements of O_2 evolution were performed with laboratory cultures. Cells were harvested at the logarithmic phase of growth and were resuspended in fresh ASW containing 1 mM $NaHCO_3$. Samples (5 ml) were placed in a well-stirred, thermoregulated glass cell illuminated by a tungsten–iodine lamp providing 3000 μE m^{-2} s^{-1}. The light intensity was modified by placing neutral-density filters between the

cell and the light source. The O_2 evolution was measured with a Yellow Spring 4004 clark-type oxygen electrode.

Outdoor cultures

The outdoor facilities for cultivation were the same as described previously.[10] For outdoor cultures the ASW medium was modified by omitting the Tris, and the pH of the cultures was kept constant at $7{\cdot}5 \pm 0{\cdot}2$ with a stream of CO_2 gas. The volume of the culture was 130 litres and the depth 13 cm. All growth conditions used were the same as described previously.[11]

Fatty acid analysis

The lipids of dried and weighed samples of *Porphyridium cruentum* were extracted with methanol–chloroform–water (10:5:4) according to the Bligh–Dyer method.[12] The dried extracts were transmethylated with methanol-HCl reagent according to Kates.[13] Heptadecanoic acid was added as an internal standard.

Gas chromatographic analysis was performed on a 6 ft $\times \frac{1}{8}$ in stainless steel column packed with 10% SP-2330 on Chromosorb W 100–120 mesh (Supelco).

A Packard Model 417 gas chromatograph fitted with a flame ionization detector and a spectra physics minigrator were used.

Samples of fatty acid methyl ester solutions in chloroform were injected into the column (N_2 20 ml min^{-1}, oven temperature 180–220°C, injector and detector temperatures 240°C). Identification and quantitation were based on injections of authentic samples.

Polysaccharide analysis

Exocellular polysaccharides

Samples (100 ml) of pond cultures were centrifuged and the supernatants were mixed with an equal volume of ethanol. After several minutes the gel-like precipitates were filtered and washed with a small volume of distilled water followed by ethanol, ethanol–ether (1 : 1) and ether. The samples were then air-dried and weighed.

Cellular polysaccharides

Water (20 ml) was added to 100 mg dry weight algal powder and the pH was adjusted to 12. The mixture was heated (80°C) and stirred for 1 h, then cooled and centrifuged. The precipitate was washed with water and centrifuged once again. The combined supernatants were acidified to pH 5. An equal volume of alcohol was added and the precipitate was filtered, dried as above and weighed.

RESULTS AND DISCUSSION

An algal species considered for outdoor cultivation should be able to survive the expected diurnal and seasonal changes in temperature and light intensity. It should be possible to grow the algae at high cell concentrations and obtain a high productivity. A major requirement is for the algal culture to exhibit steady continuous growth and undergo several harvesting cycles without becoming contaminated with other microorganisms and without deterioration caused by predators or by self-excreted growth inhibitors.

Effect of temperature

The growth response of *Porphyridium cruentum* to temperature is shown in Fig. 1. Cultures were adapted to the indicated temperatures for at least two generations and then diluted to the same cell concentration. Growth was assessed by measuring the increase in cell number, turbidity and chlorophyll concentration. The optimum temperature for *Porphyridium* was about 25°C, at which the cells divided every 10 h. Any deviation from the optimum temperature was associated with a relatively sharp decline in the growth rate. To determine whether this sharp optimum indicates that *Porphyridium* is particularly sensitive to fluctuations in temperature, cultures were grown at 25°C and then incubated for 1 h at other temperatures. The rate of oxygen evolution was used as an indication of possible cellular damage (Fig. 2). No harmful effects were detected at temperatures as high as 35°C, a temperature that is often reached during summer days in many arid zones. Only when the culture was exposed to 38°C was a decrease in O_2 evolution activity detected.

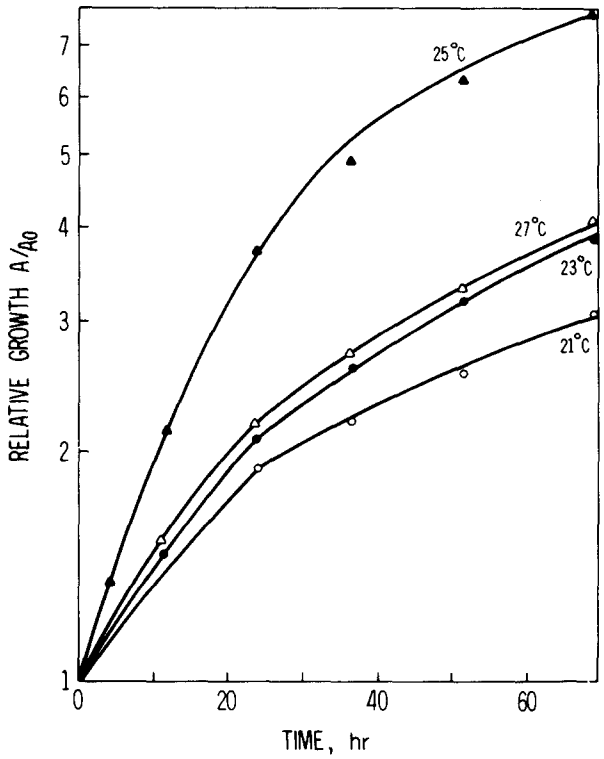

Fig. 1. The effect of temperature on the growth of *Porphyridium* under laboratory conditions. ○, 21°C; ●, 23°C; ▲, 25°C; △, 27°C. A = absorbance; A_0 = absorbance at time zero.

Effect of cell concentration and light intensity

The potential yield obtainable under outdoor conditions depends to a large extent on the light saturation characteristics of the algal cells and on their resistance to photoinhibition at high radiation intensities. As shown in Fig. 3, the O_2 evolution activity of *Porphyridium* is saturated at a relatively high light intensity, i.e. one-third of the maximum incident light intensity outdoors. It is worth noting that light intensities higher than those occurring during summer days in the Israeli desert, i.e. $>2000\,\mu E\,m^{-2}\,s^{-1}$, did not inhibit the photosynthetic activity.

In addition to the intensity and duration of solar irradiance, the population density in the culture determines the amount of light avail-

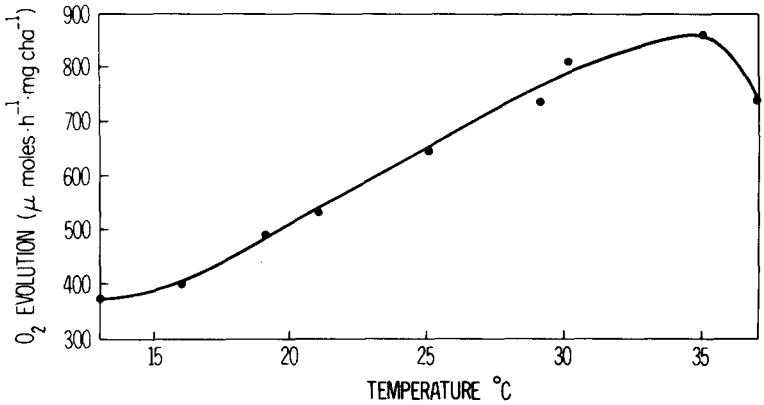

Fig. 2. The effect of incubation temperature (60 min) on the oxygen evolution activity of *Porphyridium* grown at 25°C under laboratory conditions.

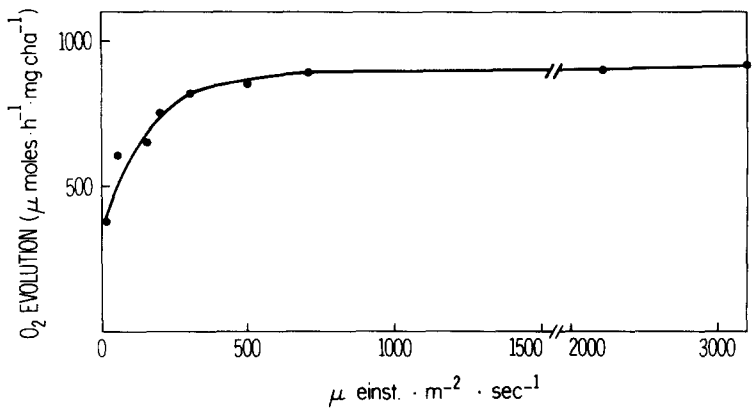

Fig. 3. The effect of light intensity on oxygen evolution activity of *Porphyridium.*

able to individual cells thus affecting the output rate of mass cultures. The output rate is a function of both the population density (x) and the specific growth rate (μ). If the cell concentration, therefore, can be increased with only a relatively small decrease in the specific growth rate, a net increase in the overall productivity per unit area will result. Figures 4 and 5 show that the specific photosynthetic activity decreases substantially only at fairly high concentrations of *Porphyridium* cells.

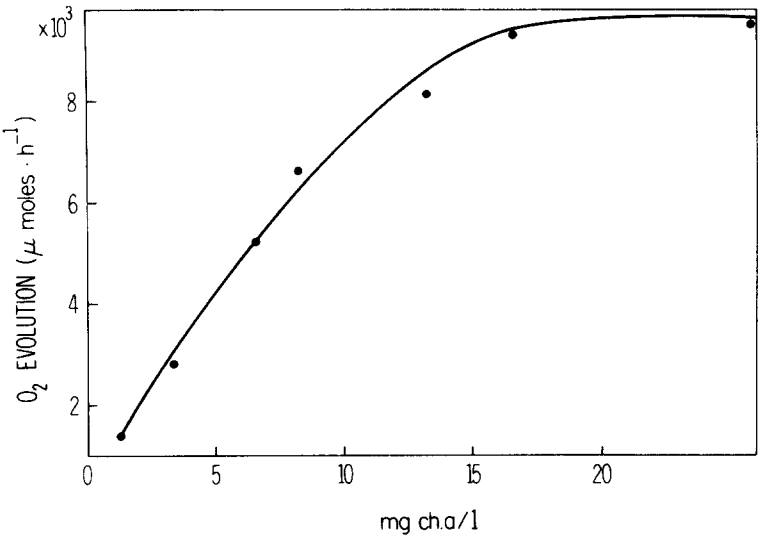

Fig. 4. The effect of chlorophyll concentration on the O_2 evolution activity of *Porphyridium*. Cells growing at the logarithmic phase were harvested and resuspended in fresh medium to the chlorophyll concentrations indicated.

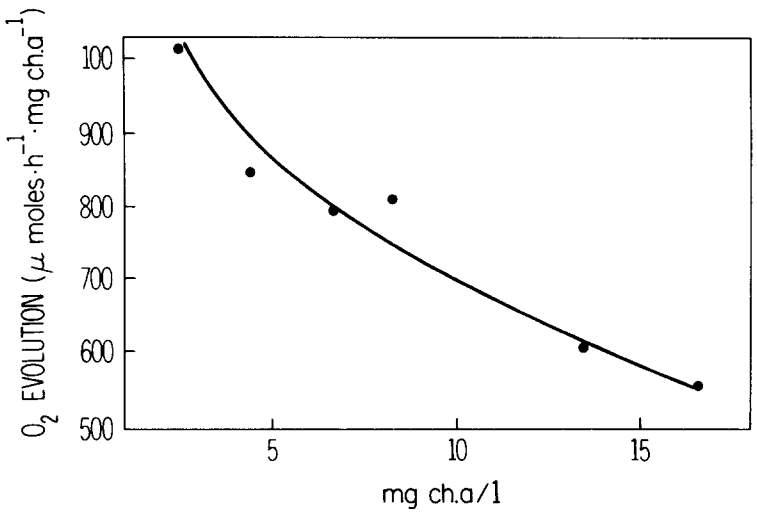

Fig. 5. The specific activity of O_2 evolution as a function of chlorophyll concentration (conditions as in Fig. 4).

A three-fold increase in cell concentration is associated with a decrease of only 50% in the specific photosynthetic activity, demonstrating the potential usefulness of *Porphyridium* as a highly productive species in mass cultivation.

Harvesting

A technology for cultivation of algal biomass requires a simple, inexpensive method of harvesting. For these reasons, autoflocculation seems to be a very promising approach.[14] Our preliminary experiments indicate that increasing the pH of the culture offers an easy method for the quick removal of biomass from the medium. It promotes settling of the cells and up to 80% of the biomass was precipitated within less than 10 min (Fig. 6).

Outdoor cultivation

Two cultures of *Porphyridium cruentum* and *Porphyridium* sp. were grown outdoors in a 1 m^2 pond containing 130 litres of culture medium.

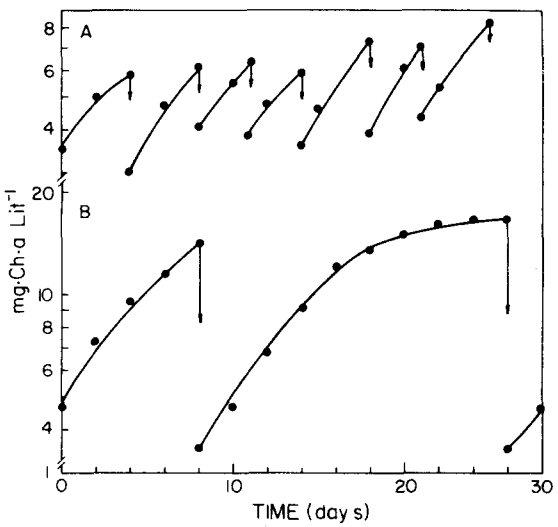

Fig. 6. Effect of harvesting regimes on growth pattern of *Porphyridium* cultivated outdoors. A – Culture kept in a semi-turbidostat mode and harvested by dilution. B – Culture harvested by dilution every time growth levelled off.

The pH was maintained at $7{\cdot}5 \pm 0{\cdot}3$ by bubbling CO_2 through the culture. The increases in chlorophyll content, cell concentration, dry weight and turbidity were followed. From our observations, cultures of *Porphyridium* can be maintained for several months, periodically harvested by dilution, without any noticeable changes in growth or contamination. As already reported for mass cultures of *Spirulina*, the population density should be maintained at a certain optimal concentration. Our experience indicates that too large a dilution may lead to inhibition of growth and contamination of the culture by flagellates.

Productivity outdoors

Biomass

When the outdoor culture was harvested at the stage where net growth had levelled off due to the high cell concentration, i.e. at extreme mutual shading and light limitation, the average daily output rate was estimated as 10–12 g m^{-2} day^{-1} (Fig. 7(b)). When, however, the culture was maintained in a semi-turbidostat, i.e. harvested more frequently to maintain the chlorophyll concentration at $5{\cdot}5 \pm 2{\cdot}0$ mg $litre^{-1}$, the output was almost 100% higher, i.e. 20–24 g m^{-2} day^{-1} (Fig. 7(a)).

Output of arachidonic acid

To determine the most suitable *Porphyridium* species for the outdoor production of arachidonic acid (AA), the performance of two species, i.e. *Porphyridium* sp. and *P. cruentum*, was compared (Fig. 8). The polyunsaturated fatty acid (PUFA) fraction was made up of almost equal concentrations of arachidonic acid and eicosapentaenoic acid (EPA). The PUFA concentration in *p. cruentum* was significantly higher (by over 0·45% of the total dry weight) than in *Porphyridium* sp. This difference was magnified when the total yield of AA in the two cultures was compared, since the growth rate and output rate of *P. cruentum* were higher than those of *Porphyridium* sp. Thus the total yield of AA by *P. cruentum* was approximately double that of *Prophyridium* sp., and *P. cruentum* was selected as the alga upon which to carry out further experimentation.

The AA concentration in a culture of *P. cruentum* varied greatly. One factor associated with these variations seems to be related to the concentration of biomass in the culture which directly affects mutual shading and the extent to which the radiation flux is available for an

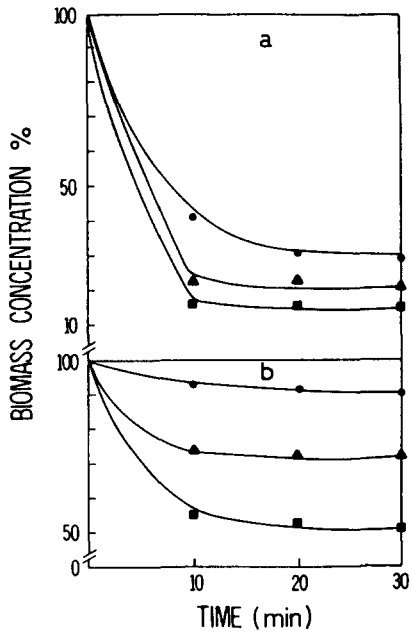

Fig. 7. The effect of pH on settling of *Porphyridium* biomass. The pH was modified by adding NaOH; 1 litre of outdoor-grown algae was placed in an Imhoff settling cone where samples were withdrawn from a constant depth for determination of the biomass concentration. (a) *P. cruentum*; (b) *Porphyridium* sp. ●, pH 7·5; ▲, pH 9·0; ■, pH 10.

average cell in the culture (Fig. 9). This correlation was not present over all 50 days of the experimental period, and factors other than cell density are apparently affecting the percentage of AA in *P. cruentum*. The maximum daily production of AA was 0·7 g m^{-2} day^{-1}, the average being 0·2 g m^{-2} day^{-1}.

Output of polysaccharides

Polysaccharide production in the culture arises from two sources, cellular and exocellular.

Cellular polysaccharides. The cellular polysaccharides originate from the continuous excretion of polysaccharides which form an envelope around the outer cell wall. The outer layer of this envelope is gradually

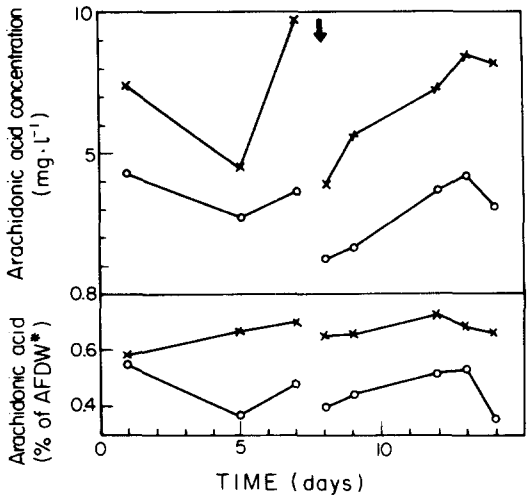

Fig. 8. Arachidonic acid content in *Porphyridium* cultures grown outdoors. ○, *Porphyridium* sp.; ×, *P. cruentum*. Arrow signifies harvest. * AFDW = Ash free dry weight.

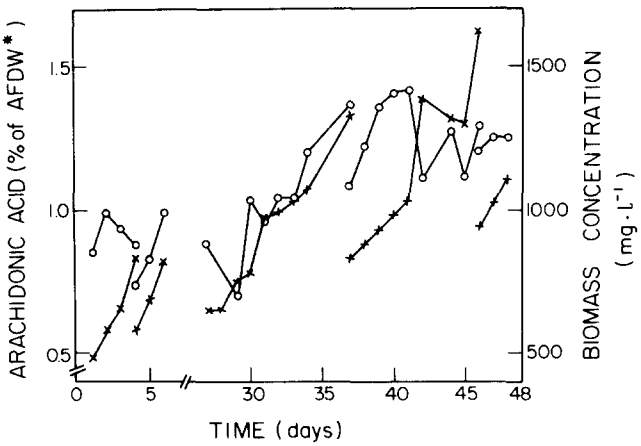

Fig. 9. The course of production of biomass and arachidonic acid in an outdoor culture of *P. cruentum*. ×, Biomass concentration; ○, arachidonic acid. *AFDW = Ash free dry weight.

sloughed off, enriching the growth medium and representing the exocellular polysaccharide fraction.

The concentration of cellular polysaccharides varied from 31·3 to 57·6% of the *P. cruentum* biomass (ash-free dry weight). These variations are as yet unexplained.

The total concentration in the culture varied from a low of 218 mg litre^{-1} (28·3 g m^{-2}), immediately after harvest, to a high of 901 mg litre^{-1} (117 g m^{-2}) 12 days later, reflecting an average daily increase in cellular polysaccharides of approximately 7·4 g m^{-2} day^{-1}.

Exocellular polysaccharides. Our preliminary findings show that the concentration of polysaccharides in a *P. cruentum* medium varied from a low of 240 mg litre^{-1} (31·2 g dry wt m^{-2}) immediately after culture dilution to a maximum value of 1490 mg litre^{-1} (193·7 g m^{-2}) within 19 days, levelling off after that.

The average daily production rate was estimated to be in the region of 55–75 mg dry wt litre^{-1} day^{-1} (7·15–9·75 g m^{-2} day^{-1}).

CONCLUSIONS

We have demonstrated that *Porphyridium* can be cultivated under outdoor conditions, harvested periodically, remain monoalgal, and yield high output rates of biomass, polysaccharides and arachidonic acid. The results suggest that, scaled up and operated on a yearly basis, an output of 40–50 tons ha^{-1} year^{-1} of dry biomass may be expected, from which approximately 1 ton of AA and 20–25 tons of polysaccharides may be extracted. Such yields and a simple autoflocculation procedure for harvesting the biomass should make the use of mass cultures of *Porphyridium* as a source of chemicals economically feasible.

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