Aquaculture, 72 (1988) 247–253
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Outdoor Cultivation of the Marine Microalga *Isochrysis galbana* in Open Reactors

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(Accepted 14 March 1988)

ABSTRACT

Boussiba, S., Sandbank, E., Shelef, G., Cohen, Z., Vonshak, A., Ben-Amotz, A., Arad, S. and Richmond, A., 1988. Outdoor cultivation of the marine microalga *Isochrysis galbana* in open reactors. *Aquaculture*, 72: 247–253.

Experiments concerning the optimization of growth conditions in the laboratory and mass production of the marine microalga *Isochrysis galbana* in open reactors are described. In the laboratory, a maximum specific growth rate (μmax) of 0.073 h^{-1} was obtained, which is equivalent to a doubling time of 9.5 h. In a small reactor (2.5-m^2 pond) the production rate of biomass was 29.5 ± 2 g m^{-2} day^{-1} and that of lipids was 6.5 ± 0.5 g m^{-2} day^{-1}. In a larger reactor (100-m^2 pond) in which algae were grown first by batch and then by semi-continuous mode, the average output rates of biomass and of lipids were 23.5 ± 1.5 g m^{-2} day^{-1} and 5.6 ± 0.5 g m^{-2} day^{-1}, respectively. A complete removal of the biomass was achieved in the 100-m^2 pond by means of flocculation with $FeCl_3$ (180 mg l^{-1}) and dissolved air flotation without a prior recycling of part of the effluent. Monoalgal cultures were maintained in the different outdoor experiments throughout the entire 4-month production period.

INTRODUCTION

Certain species of microalgae contain relatively large amounts of commercially valuable lipids and are commonly grown as a food source for commercial production in aquaculture (Rodhouse et al., 1983; Utting, 1985; Nesaratnam et al., 1986). Of special interest is the haptophyte *Isochrysis galbana*, a small

0044-8486/88/$03.50

(4–6 μm in diameter), free-living motile marine phytoflagellate. This marine microalga is known to accumulate alkenones (Scott and Middleton, 1979; Marlowe et al., 1984) and polyunsaturated fatty acids (PUFA) and has been used as part of the food chain for fish aquaculture (Rhodes and Landers, 1973; Walne and Helm, 1974). Indeed, due to its relatively high content of $\omega 3$ fatty acids, *I. galbana* biomass was successfully added to oyster and marine fish larval diet (Chu and Dupuy, 1980).

Presently, marine microalgae, including *I. galbana*, are produced in greenhouses or indoors in large tanks or transparent cylinders, sometimes under artificial light (Benemann et al., 1987). Such methods of cultivation often result in prohibitive production costs. In conventional bivalve hatcheries (Wilson, 1978), for example, the high costs involved in the production of algal food present a major obstacle to commercial application. Attempts to reduce production costs by producing these marine microalgae in open systems outdoors have generally been unsuccessful due to rapid contamination, especially by other algal species (Kaplan et al., 1986; Benemann et al., 1987).

In this work we studied the production of *I. galbana* grown in 2.5- and 100-m^2 pilot-size reactors to assess the feasibility of growing this alga as a source of PUFA-rich food.

MATERIALS AND METHODS

Organism

Isochrysis galbana strain S/ISOCH-1 (class Haptophyceae, order Isochrysidales) was obtained from the Culture Collection of the Solar Energy Research Institute (SERI), Golden, Colorado, U.S.A.

Growth conditions

Laboratory cultures

I. galbana was cultivated in artificial seawater (ASW) medium (Kaplan et al., 1986) in 500-ml glass tubes (3 cm in diameter), into which an air stream enriched with 1.5% CO_2 was bubbled to maintain the pH at 6.8 to 7.0. The columns were placed in a water bath at a constant temperature of 27°C, under a light intensity (at the surface of the vessel) of 175 μE m^{-2} s^{-1}. The same conditions of growth were used for preparing the inoculum for mass production outdoors, except that the algae were grown in 10-l flasks.

Outdoor cultures

For outdoor cultivation (same medium as above), two types of ponds were used:

(a) 2.5-m^2 ponds: The ponds were oval shaped with two channels forming a

single loop. The culture, 300 l in volume and 12 cm in depth, was stirred by a paddle wheel. CO_2 was supplied to maintain the pH in the range of 6.5–7.5. Six ponds of this type were used to provide the inoculum for the 100-m^2 pond.
(b) 100-m^2 pond: The pond consisted of two channels each 2.0 m wide and 25 m long in a single loop. The depth of the culture was maintained at 12 cm, and mixing was provided by a paddle wheel 100 cm in diameter, operating at 15 rpm. The pond was inoculated at a cell concentration of 3×10^6 cells ml^{-1} [240 mg ash-free dry weight (AFDW) ml^{-1}]. For the following 5 days, 40% of the surface of the pond was covered with a net providing 50% shade, to prevent photooxidation. The pH was maintained between 6.5 and 8.0 by injecting CO_2 into the culture. The ambient temperature was 23–24 °C in the morning, increasing to a maximum of 32–34 °C in the early afternoon, and dropping to 18–21 °C during the night.

Pond maintenance

Temperature, dissolved oxygen, and pH in the outdoor culture were monitored twice daily. To prevent the proliferation of zooplankton, $(NH_4)_2SO_4$ was added periodically to the culture to maintain a concentration of 2 m*M* during the entire period of cultivation. Ammonium concentration was determined by the Nessler method. To maintain steady-state growth and a constant population density, the culture was bled by removing an appropriate amount of culture volume and replacing it with fresh medium.

Harvesting

The culture grown in the 100-m^2 pond was harvested by flocculation with $FeCl_3$ followed by dissolved-air flotation (DAF) as previously described (Shelef et al., 1978). The inflow into the DAF basin was pumped from the pond at rates varying from 3 to 5 l min^{-1}.

Chemical analysis

AFDW, total chlorophyll, and cell number were determined daily as previously described (Kaplan et al., 1986; Boussiba et al., 1987). Total lipids were analyzed after repeated extraction with methanol–chloroform–water (10:5:4, *v/v/v*) by the method of Bligh and Dyer (1959), as modified by Kates (1964), and fractionated on a heat-activated silicic acid column (Unisil, Clarkson Chemical Co., Williamsport, PA), as described by Ben-Amotz et al. (1985).

RESULTS AND DISCUSSION

Isochrysis galbana was recently reported to attain a maximum specific growth rate (μmax) of 0.0347 h^{-1} under the following laboratory conditions: flask size 10 l, temperature 27 °C, pH 7.0, and agitation with an air stream enriched with 1.5% CO_2 (Kaplan et al., 1986). As evidenced from both cell number and chlorophyll concentration, a much higher μmax of 0.073 h^{-1}, which is equivalent to a doubling time of 9.5 h, was obtained in the present work under laboratory conditions (Fig. 1). This higher value apparently resulted from decreasing light limitation by using narrow culture columns, 3 cm in diameter. This is the fastest growth rate so far reported for this halotolerant microalga, which highlights the potential of *I. galbana* as a source of biomass rich in lipids essential for aquaculture.

In pilot-scale 2.5-m^2 ponds outdoors, the maximal daily output of algal biomass was 29.5 ± 2 g m^{-2} day^{-1} (AFDW) over a 30-day period. This production rate was achieved at a cell density of 350 mg AFDW l^{-1}, a culture depth of 12 cm, and a flow rate of about 30 cm s^{-1}. Since lipids constituted 24% of the total biomass, the calculated rate of lipid production was 6.5 ± 0.5 g m^{-2} day^{-1}. This value is relatively high compared with values obtained in outdoor cultivation of other oil-rich halotolerant microalgae (Boussiba et al., 1987).

The production of *I. galbana* was also tested in the larger, pilot-scale, 100-m^2 pond, which facilitates the testing of harvesting methods under conditions resembling those which may exist in commercial plants. The production in this reactor took place in two stages. The first, during which μmax and maximum cell density were determined, was run in a batch mode. The second stage, aimed at estimating the output rate, was run in a semi-continuous mode.

During the logarithmic phase of growth an approximate doubling time of 1.6

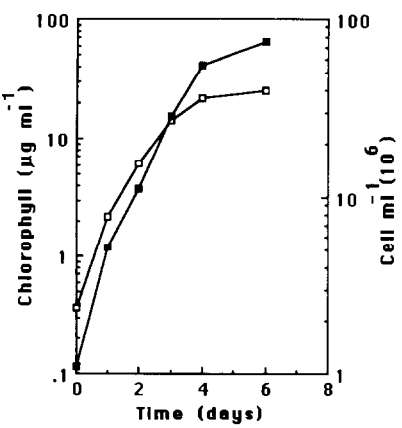

Fig. 1. Growth of *I. galbana* cultivated under optimum growth conditions in the laboratory. □-□ Chlorophyll *a* (μg ml^{-1}). ■-■ Cells ml^{-1}.

days was measured. The maximum calculated daily output of biomass at that stage was 22 g m^{-2} day^{-1} (Fig. 2). Cell number and chlorophyll content were closely correlated.

The culture entered the stationary phase of growth 12 days after inoculation, when the cell concentration reached 4.3×10^7 cell ml^{-1} (1.6 g AFDW l^{-1}) (Fig. 2). After the maximum cell concentration had been reached, a harvesting regime, involving removal of 2–3 m^3 (every second or third day, as required) and replacing this volume with the same volume of fresh growth medium, was established, and the biomass concentration in the pond was maintained between 0.9 and 1.3 g AFDW l^{-1} (Fig. 2). The average output rates of the biomass and lipids (which constituted 24% of the biomass) were 23.5 ± 1.5 g m^{-2} day^{-1} and 5.6 ± 0.5 g m^{-2} day^{-1}, respectively. It seems that scaling-up the system from the 2.5-m^2 to the 100-m^2 pond did not significantly affect the rate of biomass production. This is important for mass production of this microalga.

In many aquaculture systems in which *I. galbana* biomass was used as a feed, it was applied as a suspension without any processing (Wilson, 1978). We tested the feasibility of harvesting the algae by a flocculation-flotation method, with the commonly used flocculant $FeCl_3$, as described by Shelef et al. (1978). Relatively high quantities of $FeCl_3$ (180 mg l^{-1}) at a retention time of 13 min were required to obtain a removal efficiency of 74% in the DAF unit, while 20% of the effluent from the DAF unit was recycled. This high requirement of flocculant was attributed to the high salt content of the ASW medium, particularly the rapidly formed $FePO_4$, which precipitates out, reducing iron availability. When the DAF unit was operated without recycling, the removal efficiency in the effluent was significantly higher (Table 1), being close to 100%, on both a

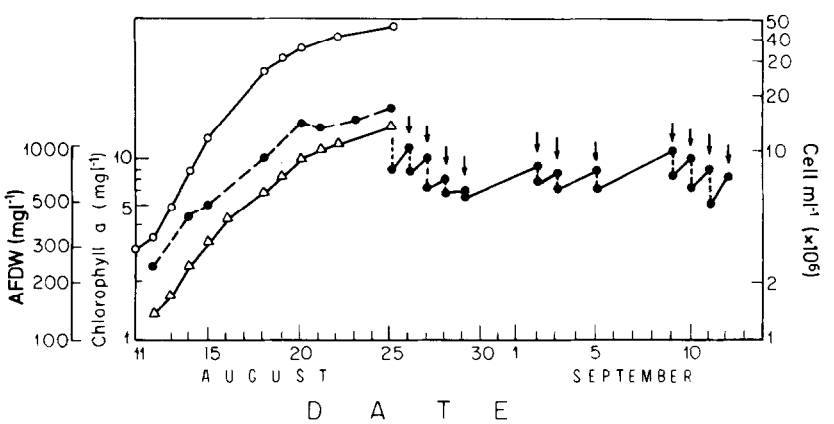

Fig. 2. Growth curve and harvesting regime of *I. galbana* culture grown outdoors in the 100-m^2 pond. ○-○ Cells ml^{-1}. ●- -● AFDW (mg l^{-1}). △-△ Chlorophyll *a* (μg ml^{-1}). Arrows indicate harvest of algal mass. The pH was maintained between 6.5 to 8.0. Temperature fluctuation: morning 23–25 °C, noon 32–34 °C.

TABLE 1

Effect of the method of harvesting in the dissolved-air flotation (DAF) unit on the removal efficiency[a]

Component	Pond medium	Effluent of DAF	
		Recycling[b]	No recycling
Chlorophyll *a* ($mg\ l^{-1}$)	6.3	1.55 (75.4[c])	0.12 (98.1[c])
Cell number (ml^{-1})	11×10^7	2.0×10^6 (81.8[c])	1.1×10^5 (99.1[c])

[a]$FeCl_3$ was used as the flocculant at a dose of 180 $mg\ l^{-1}$.
[b]20% recycling at 3 atm.
[c]Numbers in parentheses are percent removal.

chlorophyll and a cell number basis, as compared with 75 and 82%, respectively, when recycling was used. It appears that when the pond is supersaturated with oxygen, bubbles of oxygen enhance the flotation of the algal mass, facilitating its separation from the medium. Thus, essentially complete removal of the biomass was attained without pressurization of part of the effluent, which is found essential with other algae (Shelef et al., 1978). This finding may clearly have important practical implications.

As mentioned above, the average lipid content of the *Isochrysis* biomass in the 100-m^2 pond was 24%, a value similar to that in the smaller pond. The major components of this lipid fraction were the same as those reported earlier (Ben-Amotz et al., 1985), i.e. hydrocarbons in the benzene fraction, triglycerides in the chloroform fraction, glycolipids in the acetone fraction and phospholipids in the methanol fraction.

Throughout the entire period of outdoor cultivation (4 months), the cultures were essentially monoalgal as ascertained by daily microscopic observations. It is probable that the relatively high daily maximum temperatures (above 28°C) combined with the maintenance of a relatively high concentration of biomass in the ponds prevented the proliferation of other microalgae, as observed previously (Boussiba et al., 1987). Also, the addition of the ammonium salt (2 m*M*) precluded the proliferation of zooplankton. The maintenance of monoalgal cultures outdoors for a relatively long period of time and the high rate of lipid production (up to 6.5 $g\ m^{-2}\ day^{-1}$) obtained in open reactors indicate the potential of *I. galbana* as a source of biomass rich in special lipids essential for aquaculture.

ACKNOWLEDGEMENTS

This work was supported by grant No. XK-4-0410-1 from the Solar Energy Research Institute (SERI), U.S.A. We wish to thank Ms. Dorot Imber for editing the manuscript.

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