Micro-algal biotechnology

Edited by

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MICRO-ALGAL BIOTECHNOLOGY

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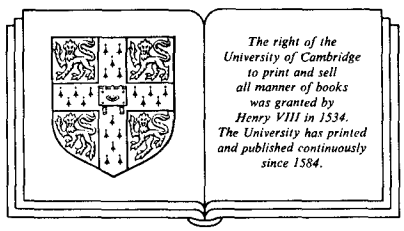

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Preface

Micro-algal biotechnology as we understand it today is a mixture of the 'old' and 'new' biotechnologies. In recent years there has been a great upsurge in work on micro-algae as sources of a wide range of fine chemicals, oils and polysaccharides, as well as in the use of micro-algae as soil conditioners and in waste-water treatment and aquaculture. The economic utilization of micro-algae has been explored for some time. In the 1940s there was some interest in Germany in micro-algae, especially diatoms, as sources of liquid fuels and since the 1950s there has been much interest in micro-algae as single-cell protein sources. Similarly, their use in high rate oxidation ponds and as food sources in aquaculture is not new. However, the commercial utilization of micro-algae gained impetus with the discovery that the extremely halophilic green alga *Dunaliella salina* was the best natural source of β-carotene. Work in this area was first begun in the 1960s in the USSR and several commercial and semi-commercial operations are now under way in Australia, Israel and the USA. Further interest in the micro-algae has been generated in recent years with the exploitation of the blue-green alga (cyanobacterium) *Spirulina*, wich is used mainly in the health food market.

The increase in interest in the micro-algae can be gauged from the appearance of major publications on the applied uses of micro-algae. The first major volume in this area was Algal Culture from Laboratory to Pilot Plant, edited by Burlew in 1953, which was concerned mainly with proteins. The next major volume, *Algae Biomass*, edited by Shelef & Soeder appeared in 1980 and covered a very wide range of topics. The present book is an attempt to provide a comprehensive and up-to-date introduction to algal biotechnology. It aims to illustrate the existing uses of micro-algae, and to point out the most interesting and promising areas for future development. Not only are the most important algal genera and the major products and processes treated in detail, but some of the engineering and genetic engineering aspects are also covered. There is also a chapter that considers the absolute limits to micro-algal growth. The wide scope of algal biotechnology

and its various ramifications preclude an exhaustive treatment of all topics; however, we hope that this book will serve as a guide.

The definition of algae used in this book includes prokaryotic and eukaryotic oxygenic organisms; it therefore includes the blue-green algae (Cyanophyta, cyanobacteria).

We hope that this book will stimulate further research and development in this exciting area of biotechnology.

Many people have assisted with the preparation of this volume, but in particular we would like to thank Dee Cahill, Carol Hooper, Michael Van Keulen and Kim Benjamin for their invaluable help and patience.

Michael A. Borowitzka
Lesley J. Borowitzka

Perth, WA, 1986

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Section I

The algae

1

Chlorella

T. OH-HAMA AND S. MIYACHI

1.1 Nutrient requirements

Chlorella was one of the first algae to be isolated as a pure culture: this was accomplished by Beijerinck in the 1890s. Otto Warburg (1919) introduced the use of *Chlorella* in the study of photosynthesis: cells were grown in a solution similar to Knop's solution, which had been used for hydroponics of higher plants. The solution for *Chlorella* contained $Ca(NO_3)_2$, $MgSO_4$, KH_2PO_4, KNO_3 (or KCl) and $FeCl_3$ in tap water.

Since the late 1940s scientific attention has been drawn towards the potential of micro-algae for mass cultivation. The algae, mainly *Chlorella* sp., were grown initially on defined mineral media, and nutritional and environmental requirements of a number of species were determined (Burlew, 1953*b*).

The inorganic elements considered to be required for the growth of green algae are N, P, K, Mg, Ca, S, Fe, Cu, Mn and Zn (Krauss, 1958; O'Kelly, 1968). Eyster, Brown & Tanner (1958) studied the growth of *C. pyrenoidosa* at various concentrations of each macronutrient (NO_3^-, K, Mg, S, P and Cl) and micronutrient (Fe, Cu, Zn, Mn, B and Mo). The mineral requirements for the autotrophic and heterotrophic (with sugar) growth are given in Table 1.1. It is evident that *C. pyrenoidosa* requires more Mn, Fe, Zn and nitrate and less Mg and K for autotrophic growth than for heterotrophic growth. Chloride is required only for autotrophic growth. Omission of Mb, Cu and B did not depress algal growth. In contrast, Walker (1953) showed that Ca, Cu and Mb were required when nitrate was the sole source of nitrogen in *C. pyrenoidosa*. Many investigators failed to demonstrate a requirement for B in *Chlorella* spp. (Bowen *et al.*, 1965; Gerloff, 1968; McBridge, Chorney & Skok, 1971).

Table 1.1. *Nutritional requirement for the autotrophic and heterotrophic growth of* Chlorella pyrenoidosa *(from Eyster* et al., *1958)*

Nutrients	Critical concentration (M)[a] Autotrophic growth	Heterotrophic growth
NO_3	2.5×10^{-2}	2.5×10^{-3}
Mg	2×10^{-3}	2×10^{-2}
K	4.3×10^{-4}	4.3×10^{-3}
P	1.8×10^{-4}	1.8×10^{-4}
S	2×10^{-4}	2×10^{-4}
Fe	1.8×10^{-5}	$<1 \times 10^{-9}$
Zn	$>0.77 \times 10^{-6}$	$<0.77 \times 10^{-10}$
Mn	1×10^{-7}	1×10^{-9}
Cl	3.4×10^{-2}	

[a] The lowest concentration that gives maximum growth rate.

1.2 Efficiency in the utilization of light energy

1.2.1 *Efficiency of photosynthetic algal growth*

Kok (1948, 1960) reported that, in steady-state experiments of short duration, about eight quanta are used per molecule of O_2 evolved under optimal conditions. This agrees with the widely accepted two-step model of photosynthesis, which requires a minimum of 8 mol quanta of light to fix 1 mol CO_2 (i.e. to evolve 1 mol O_2).

Table 1.2 shows the efficiency of growth of *C. pyrenoidosa* (Myers, 1980), and gives the following on a per-day basis: (1) the amount of light absorbed by the cells during steady-state growth; (2) the increase (mg) in algal cells; and (3) the amounts of CO_2 fixed and O_2 evolved. The table shows also that, on average, the efficiency of energy conversion from a sodium light source into algal cells is 17.9% and the ratio CO_2/O_2 is 0.71. In this experiment, nitrate was used as the nitrogen source. Urea, on the other hand, increased the respective values to 20.4% and 0.84 (data not shown), indicating that the consumption of reducing power for nitrate reduction decreases efficiency. The table shows also that about 1 ml of CO_2 (at s.t.p.) is required to produce 1 mg (dry weight) of *Chlorella* cells.

Table 1.2. *Photosynthetic efficiency in* Chlorella pyrenoidosa *(modified from Myers, 1980)*

Expt no.	Light[a] energy absorbed (cal day^{-1})	Growth constant (k)	Yields day^{-1}				Efficiency for cell production (%)	Quantum yield ($O_2/h\nu$)
			$-CO_2$ (ml)	O_2 (ml)	AQ[b] (CO_2/O_2)	Cells produced (mg)		
1	1319	0.83	38.4	56.9	0.675	39.9	17.6	0.095
2	555	0.42	16.9	23.4	0.722	17.5	18.4	0.093
3	1304	0.88	38.1	52.9	0.720	39.6	17.7	0.089
Average					0.71		17.9	0.092

[a] 578 Hg line; nitrate was used as nitrogen source.
[b] Assimilation quotient.

1.2.2 *Limitation by light saturation*

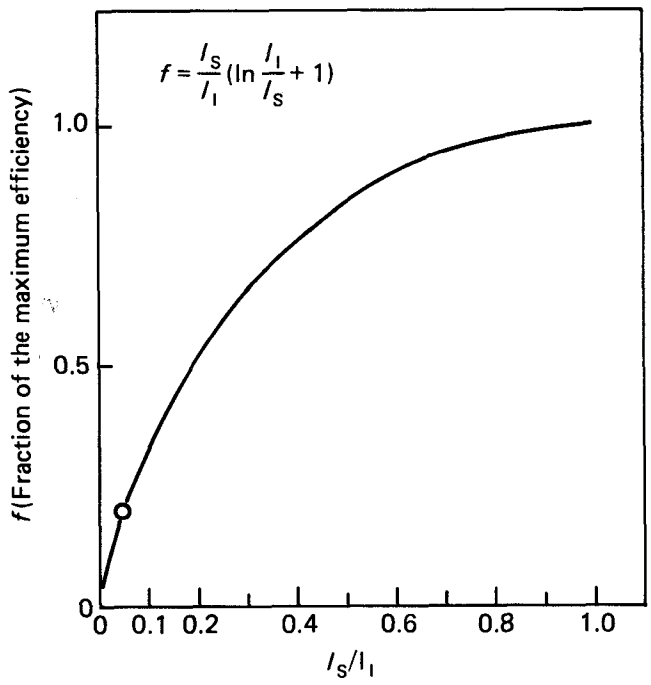
The overall efficiency of solar energy utilization is further limited by the fact that individual cells cannot utilize light of high intensity. The photosynthetic processes of *Chlorella* become saturated at relatively low illumination, ranging from 4000 to 30 000 lx, depending on the strain. The earliest attempt quantitatively to account for the effect of light saturation was made by Burlew, who adopted Bush's equation (Burlew, 1953*a*):

$$f = \frac{I_S}{I_I}\left(\ln \frac{I_I}{I_S} + 1\right), \tag{1.1}$$

where I_S represents the light intensity that saturates photosynthesis, I_I the incident light intensity, and f the fraction of the maximum efficiency that can be attained.

In a *Chlorella* culture, light intensity decreases with depth and algal concentration, according to the Beer–Lambert law, and light energy greater than I_S is not utilized. As seen from the graphical representation of equation (1.1) (Fig. 1.1), f is 1 at low light intensity, which corresponds to $I_S/I_I = 1$, while f decreases as I_I increases. If $I_I = 100$ klx and $I_S = 5$ klx, f is 0.2, which means that only 20% of light energy is utilized photosynthetically. In this respect *Chlorella* strains having high I_S value are suitable for mass culture.

Fig. 1.1. Light utilization efficiency according to the Bush equation (see the text).



In practice, the photosynthetic efficiency is determined by the product of the Bush factor (f) and the quantum yield factor.

Average yields of *Chlorella* from various types of outdoor mass culture were listed by Tamiya (1957) and Zahradník (1968). The efficiencies of light energy conversion into cell materials ranged from 7.1% to 2.6%, which corresponded to 1.9 to 4.9 g (dry weight) m^{-2} day^{-1} for cell biomass production. There is an optimum cell density in each culture to obtain best growth yield in sunlight; above this concentration, the yield decreases due to increased respiration. Davis *et al.* (1953) improved the yield by lowering the culture temperature at night.

1.3 Growth constant k

The growth rates of exponentially growing algae have been expressed in various ways (Hoogenhout & Amesz, 1965). Usually it is most convenient to calculate the growth rate K from the following equation:

$$K = \log_2 \frac{N_2}{N_1} \times \frac{1}{t}, \tag{1.2}$$

where N_1 and N_2 are cell numbers at the start and after time period t, respectively. By using $\log_2$, and selecting t as 1 day, the growth constant, k, becomes equivalent to the number of doublings per day.

The growth constants are also often expressed using 1 day for t and with $\log_e$ units (K_e) or $\log_{10}$ units (K_{10}) in equation (1.2). The number of doublings per day (k) can be obtained from either K_e or K_{10} by equation (1.3):

$$k \text{ (div./day)} = \frac{K_e}{0.69} = \frac{2.30}{0.69} K_{10} = 3.32\, K_{10}. \tag{1.3}$$

In this chapter the growth rates will be expressed by k, unless otherwise noted.

In Fig. 1.2 the growth rates of *C. vulgaris* 211-8b, *C. pyrenoidosa* van Niel strain and thermophilic *C. pyrenoidosa* 7-11-05 are presented as a function of light intensity (Sorokin & Krauss, 1958). There are three phases in k in response to incident light intensities: light-limiting, light-independent and light-inhibitory phases. In Fig. 1.3 the growth rates of the high- and low-temperature strains are plotted against growth temperature at two levels of illuminance. At around 25 °C, the growth rates are almost the same irrespective of differences in strains and illuminance. Above this temperature the growth rate of the thermophilic strain was much higher than that of the mesophilic strain. At low temperatures the growth in thermophilic strain is poor, especially at high illuminance. These factors would, in some cases, restrict usefulness of thermophilic strains for outdoor mass culture. Indeed,

Fig. 1.2. Growth rates of three different species of *Chlorella* as a function of light intensity: ○, ●, *Chlorella pyrenoidosa* (7-11-05); ×, *C. vulgaris* 211–8b; △, *C. pyrenoidosa* van Niel strain. – – – –, 39 °C; ——, 25 °C. (Redrawn from Sorokin & Krauss, 1958.)

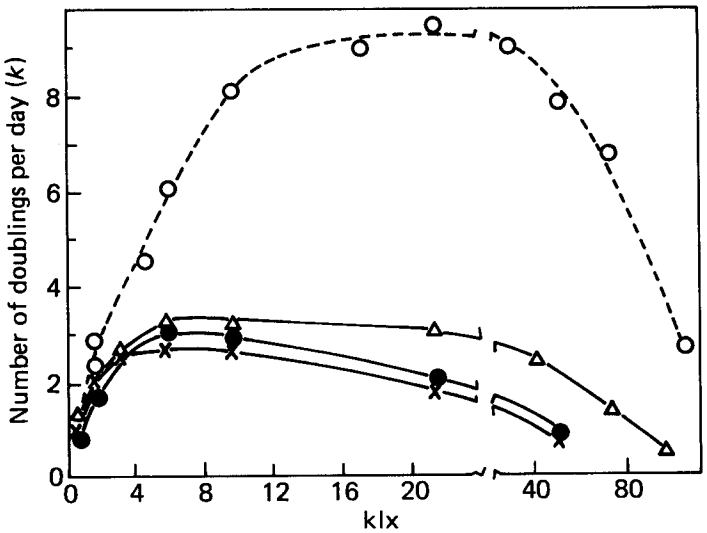


Fig. 1.3. Growth rate of two different species of *Chlorella* as a function of temperature: ○, ●, *Chlorella pyrenoidosa* 7-11-05; ×, *C. pyrenoidosa* Emerson strain. ——, 17.3 klx; – – – –, 4.7 klx. (Redrawn from Sorokin, 1960.)