Assessment of Growth Yield of a Blue-green Alga, *Spirulina platensis*, in Axenic and Continuous Culture

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An axenic and continuous culture of a blue-green alga, *Spirulina platensis*, was established in a Roux bottle. The opalescent plate method was used to measure the light energy absorbed by the cells and to assess the cell growth yield, Y_{kcal} i.e. the amount of dry algal cells harvested per kcal light energy absorbed. Values of Y_{kcal} calculated ranged from 0.01 to 0.02 g cell kcal⁻¹.

INTRODUCTION

Since there is growing interest throughout the world in photosynthetic microbes as a potential source of food (Clément, 1968) or as agents to remove nutrients from waste waters (Kosaric, Nguyen & Bergougnou, 1974), an assessment of their efficiency of light energy conversion to cellular material is important. Many papers have been published on the efficiency of light energy conversion by photosynthetic microbes since the classical work of Warburg & Negelein (1923). If high concentrations of algae are used so that virtually all incident light energy is absorbed, as in the early work (Warburg & Negelein, 1923), the metabolism is claimed to be modified from that of individual cells exposed to uniform irradiation (Emerson & Lewis, 1943; Kok, 1972). In dilute suspensions the problem of how to correct for not only transmitted, but also reflected light must be solved. There have been several papers published on a simple use of the Lambert-Beer's law to estimate the light-energy absorption (Rabe & Benoit, 1962; Ragonese & Williams, 1968). The purpose of this work was to establish axenic and continuous cultures of *Spirulina platensis* to allow the calculation of Y_{kcal} of a dilute suspension of cells, using the opalescent plate method (Shibata, Benson & Calvin, 1954; Shibata, 1958).

METHODS

Strain and culture medium. The algal strain, *Spirulina platensis*, came from l'Université Libre de Bruxelles, Belgium. An axenic culture was established and maintained as described by Ogawa & Terui (1970). The culture medium contained (g l⁻¹): NaHCO₃, 13.61; Na₂CO₃, 4.03; K₂HPO₄, 0.5; NaNO₃, 2.5; K₂SO₄, 1.0; NaCl, 1.0; MgSO₄·7H₂O, 0.2; CaCl₂·2H₂O, 0.04; FeSO₄·7H₂O, 0.01; Na₂-EDTA, 0.08; and A₅ and B₆ oligoelements solutions (Ogawa & Terui, 1970), 1 ml l⁻¹. The bicarbonate/carbonate buffer (pH 9.4) together with the phosphate was sterilized separately at 121 °C for 10 min before adding to the medium.

Preculture. The alga was precultured in shaken 500 ml flasks containing 100 ml medium at 30 °C for 4 to 5 days. The cultures were illuminated with fluorescent tubes at an intensity of approximately 1.5 klux.

Continuous culture. Two systems of continuous cultures (light-limited) were used. In one, a constant cell concentration was achieved spontaneously by altering the dilution rate, the light intensity being kept constant. In the other system, the dilution rates were controlled automatically such that a constant low concentration of cells was maintained independent of the light intensity. The automatic control of dilution rate was achieved using a sensor tube, measuring cell concentration in the medium through extinction, which controlled the flow rate of a peristaltic pump. A diaphragm circulation pump (model DP-1, Iwaki Co., Tokyo, Japan) was used to avoid damage to the cells. The cell concentration was fixed at 0.039 g l⁻¹ ($E_{560} = 0.056$), whereas the light intensity was varied widely. The culture vessel used throughout was a Roux bottle

Fig. 1. Experimental correlation between absorption (I_a/I_0) and cell concentration (x) of *Spirulina platensis* for a light-path length of 50 mm and the halogen lamp described in Methods. The correlation was confirmed to be reliable by another experiment using an Integrating Sphere Spectrophotometer (UV-210; Shimadzu, Kyoto, Japan) (data not shown).

(230 × 50 × 102 mm). The area irradiated by incandescent light was 10.2 × 7.9 cm (= 80.6 cm²), the length of the light path was 50 mm, and so the working volume was 403 cm³. A magnetic stirrer was used to maintain a homogeneous cell suspension. In addition, CO₂-free air was applied to the glass walls by a miniature air pump (Shin-Osaka Seigi Co., Osaka, Japan) at a rate of 180 ml min⁻¹, to prevent adhesion of algal cells.

The culture was assumed to be in steady state when the cell concentration remained constant for at least 24 h after an initial three changes of the working volume. The pH of the medium remained almost unchanged at 9.4 to 9.8 in all the systems used and the temperature of the cultures remained at 35 ± 1 °C without the use of a controller. The specific growth rate of *S. platensis* was previously found to be largely unaffected by pH values from 8.5 to 10.5 (Ogawa, Kozasa & Terui, 1972).

Light source. A halogen lamp (National Halogen Lamp for Projector, Matsushita Denki-Sangyo Co., Osaka, Japan) was used through an infrared filter (Vacuum Optics Corporation, Tokyo, Japan). This illumination lacked any infrared component above 750 nm. Light intensity was measured with a compensated thermopile (Kipp and Zonen, Delft, The Netherlands) calibrated with a standard lamp (Matsushita Research Laboratories, Osaka, Japan).

Assessment of absorption, I_a/I_0 . The opalescent plate method (Shibata *et al.*, 1954; Shibata, 1958) was used to measure the light energy absorbed by the cells.

Cell concentration. The cell concentration was determined from the difference in protein concentrations of the cell suspension and the culture filtrate (Toyo Filter Paper, No. 2; Tokyo, Japan), since suspensions of cells in continuous culture were too dilute for direct measurement of dry cell concentration. The method of Lowry *et al.* (1951) was used throughout; before measuring protein in the cell suspension, cells were hydrolysed with 1 M-NaOH for 10 min in a boiling water-bath.

RESULTS AND DISCUSSION

Absorption as a function of cell concentration for this algal suspension is shown in Fig. 1. Data points (○) in the figure were from algal cells harvested during the exponential growth phase in a batch culture. The curve was used in the evaluation of light absorption by the cells, because it was confirmed that the absorption measured on samples from each experiment (see Table 1) fell on the curve. This implies that cell shape and pigments affecting the absorption could be considered as constant in these experiments.

Experimental data on continuous cultures of *S. platensis* are summarized in Table 1. The values of Y_{kcal} were determined from the equation:

$$Y_{\text{kcal}} = \frac{DVx}{AI_a} \quad (1)$$

Table 1. Cell concentration, light absorption and elemental composition of *Spirulina platensis* in light-limited continuous cultures

In all cultures, the pH was 9.4 to 9.8 and the temperature was $35 \pm 1^\circ \text{C}$.

Run no.	D (h^{-1})	x (g l^{-1})	$10^{-4} \times I_0^*$ ($\text{erg cm}^{-2} \text{s}^{-1}$)	$10^3 \times I_a^\dagger$ ($\text{kcal cm}^{-2} \text{h}^{-1}$)	$10^2 \times Y_{\text{kcal}}$ (g cell kcal^{-1})	Cell elemental composition ‡			
						C	H	O	N
1	0.014	0.272	1.89	1.50	1.27	5.06	8.63	2.02	1.0
2	0.022	0.246	1.89	1.44	1.88	4.96	8.50	2.05	1.0
3	0.023	0.177	1.89	1.25	1.63	4.97	8.30	2.21	1.0
4	0.029	0.169	1.89	1.21	2.03	5.04	8.96	2.16	1.0
5	0.034	0.122	1.89	1.05	1.98	4.95	7.50	2.35	1.0
6	0.038	0.057	1.89	0.61	1.78	5.01	8.16	2.42	1.0
7	0.013	0.039	0.793	0.184	1.38				
8	0.015	0.039	0.961	0.226	1.29				
9	0.030	0.039	1.47	0.346	1.69				
10	0.042	0.039	2.31	0.542	1.51				
11	0.040	0.039	2.55	0.600	1.30				
12	0.042	0.039	2.84	0.667	1.23				
13	0.048	0.039	3.19	0.750	1.25				
14	0.060	0.039	3.84	0.903	1.29				
15	0.056	0.039	3.92	0.920	1.19				
16	0.070	0.039	5.12	1.20	1.14				

* $1 \text{ erg cm}^{-2} \text{s}^{-1} = 0.305 \times 10^{-8} \text{ klux}$.

$^\dagger 1 \text{ erg s}^{-1} = 8.6 \times 10^{-8} \text{ kcal h}^{-1}$.

‡ Ash, 8.2 %.

in which $D = F/V$ (in h^{-1}), F is the flow rate of fresh medium into the glass vessel (in ml h^{-1}), V is the working volume of the glass vessel (403 ml), A is the area of the glass vessel exposed to light irradiation (80.6 cm^2), I_a is the intensity of light absorbed by the cells (in $\text{kcal cm}^{-2} \text{h}^{-1}$), and x is the cell concentration (in g l^{-1}).

Data for algal cell composition (Elementary Analysis Centre, Faculty of Engineering, Osaka University, Japan) are included in Table 1. The elemental cell composition hardly altered with dilution rate. By averaging the value of C, H, O and N for each dilution rate, the chemical formula of this alga was determined as $\text{C}_{5.0}\text{H}_{8.34}\text{O}_{2.2}\text{N}$, and so the stoichiometric equation for complete combustion of the algal cell is:

One 'mole' of cell material weighed 128 g when the ash content was taken as 8.2 %.

Y_{kcal} values (Table 1) were of the order of 0.01 to 0.02 g cell kcal^{-1} for a fairly wide range of dilution rates of *S. platensis*. Y_{kcal} values reported by other workers using heterotrophic micro-organisms (Payne, 1970) were larger than this by an order of magnitude.

On the basis of the elemental analysis of *S. platensis*, the heat of combustion of the cells was:

$$-\Delta H_0^* \times 7.235/128 = 5.99 \text{ kcal (g cell)}^{-1}$$

taking $-\Delta H_0^*$, the enthalpy change of oxygen, as $106 \text{ kcal (mol O}_2\text{)}^{-1}$ (Payne, 1970). If this heat content is multiplied by Y_{kcal} , the efficiency, Y , of conversion of light to chemical energy was 6 to 12 %. According to Emerson & Lewis (1943), quantum yield, defined as the number of quanta required to fix photosynthetically 1 mol CO_2 , varied from 11 to 16 when *Chlorella* was irradiated by visible light from 400 to 700 nm wavelength. Assuming the carbon content and heat of combustion of *Chlorella* cells are 50 % and $6 \text{ kcal (g cell)}^{-1}$, the chemical energy, E_1 , of the cellular material was:

$$E_1 = \left(12 \times \frac{1}{0.5}\right) \times 6 = 144 \text{ kcal (mol CO}_2\text{)}^{-1}$$

Light energy, E_2 , associated with this quantum yield (11 to 16 quanta per mol CO_2), taking the wavelength, λ , as 660 nm for convenience, is:

$$E_2 = (N_0 \times h \times c / \lambda) \times \xi \times (11 \text{ to } 16) = 475 \text{ to } 690 \text{ kcal (mol CO}_2\text{)}^{-1}$$

taking N_0 , Avogadro's number, as 6.0×10^{23} ; h , Planck's constant, as 6.62×10^{-27} erg s; light velocity, c , as 3×10^{10} cm s^{-1} ; and ξ , the factor to convert units of erg to kcal, as 2.39×10^{-11} kcal erg^{-1} . Consequently, the value of Y estimated from the quantum yield was:

$$Y = E_1/E_2 = 144/(475 \text{ to } 690) = 0.30 \text{ to } 0.21$$

Goedheer & Hammans (1975), using *Anacystis nidulans*, made an approximate determination of the Y value as 0.30; they obtained this result, not from the estimation of quantum yield, but from their observation on the cellular growth rate under a given light intensity without measuring the light energy actually absorbed by the cells.

The value of Y (6 to 12 %) estimated here was smaller than those of previous workers on *Chlorella* and *Anacystis nidulans*. Whether this discrepancy originates from the specific nature of *S. platensis* or not remains to be discussed when Y values for *Chlorella* and *Anacystis nidulans* grown in continuous culture are obtained.

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