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Optimum growth conditions and light utilization efficiency of *Spirulina platensis* (= *Arthrospira fusiformis*) (Cyanophyta) from Lake Chitu, Ethiopia

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Abstract

Spirulina platensis (= *Arthrospira fusiformis*) was isolated from Lake Chitu, a saline, alkaline lake in Ethiopia, where it forms an almost unialgal population. Optimum growth conditions were studied in a turbidostat. Cultures grown in modified Zarrouk's medium and exposed to a range of light intensities (20–500 μmol photons $m^{-2}s^{-1}$) showed a maximum specific growth rate (μ_{max}) of 1.78 d^{-1}. Quantum yield for growth ($\Phi\mu$) was 3.8% at the optimum light for growth of 330 μmol photons $m^{-2}s^{-1}$, and ranged from 2.8 to 9.4%. With increase in irradiance, the chlorophyll *a* concentration decreased, and the carotenoids/chlorophyll *a* ratio increased by a factor of 2.4. The phosphorus to carbon ratio (P/C) showed some variation, while the nitrogen to carbon ratio (N/C) remained relatively constant, thus causing fluctuations in the N:P ratio (7–11) of cells. An optimum N:P ratio of about 7 was attained in cells growing at the optimum light for growth. Results from the continuous culture experiments agreed well with maximum values of photosynthetic efficiency given in the literature for natural populations of *S. platensis* in the soda lakes of East Africa, Lake Arenguade (Ethiopia), and Lake Simbi (Kenya).

Introduction

Spirulina platensis (Gom.) Geitl., renamed as *Arthrospira fusiformis* (Voronich.) Komarek & Lund (1990), is a filamentous, helicoidal cyanophyte, and cosmopolitan in distribution. It flourishes very well in alkaline, saline waters where the pH (9–11) is too high for most other species to thrive in. It dominates the algal flora of some soda lakes, reaching an almost unialgal population in some, including the crater lakes Arenguade (Talling et al., 1973) and Chitu in Ethiopia, and lakes Simbi and Nakuru in Kenya (Melack, 1979). The alga is the major food source for the vast flocks of lesser flamingo (*Phoeniconaias minor* Geoffroy) which inhabit the shores of soda lakes in East Africa. The only fish species in Lake Nakuru (Vareschi, 1979), *Sarotherodon alcalicum grahami* (= *Tilapia grahami* Boulenger), also filter feeds extensively on *S. platensis* abundant in the lake (Vareschi & Jacobs, 1984).

For the past two decades, *S. platensis* has been a focus of interest among researchers in various fields because of its commercial importance as a source of protein, vitamins, essential amino acids and fatty acids (Ciferri & Tiboni, 1985; Tanticharoen et al., 1994; Vonshak, 1990; Vonshak & Richmond, 1988), and more recently, for its potential in therapeutic effects (Amha Belay et al., 1993). Basic studies on the species behaviour include studies on growth kinetics by Ogawa et al. (1971) and Iehana (1983), growth and growth yield by Aiba & Ogawa (1977) and Ogawa & Aiba (1978).

Light is an essential resource often limiting the distribution and growth of *S. platensis* in nature. It is also a major limiting factor in production of the alga for commercial purposes. When discussing growth of photosynthetic organisms, the efficiency of light utilization is also an important question which deserves to be addressed. Influence of light on production, photoinhibition and photosynthetic pigments has been studied by Olaizola & Duerr (1990), Vonshak et al. (1982), Jensen & Knutsen (1993) and Vonshak et al. (1994). In spite of the substantial number of studies made on dif-

ferent aspects of growth, studies incorporating growth conditions other than growth rate and photosynthesis, such as quantum yield, cellular content of nutrients and pigments, and cellular morphology, are very few. Differences in culture conditions, experimental procedure and measurements, especially of light, make it difficult for synthesis of results from separate studies.

The purpose of this study is to describe growth-irradiance relationships, make an assessment of light utilization efficiency and quantum yield, and optimum growth conditions of *S. platensis* in culture, in relation to light. The relevance of growth parameters estimated from culture experiments, to natural populations, is also evaluated by comparison with field data from the literature. Continuous culture was preferred over batch culture, as cells at steady state are adapted to the specific light treatment in the different experiments, giving a more accurate estimate of the specific growth rate.

Materials and methods

Isolation and culturing

S. platensis was isolated from Lake Chitu, Ethiopia, sampled from the open water just after the dry season, in March 1991. Lake water was diluted with culture medium to obtain a suspension of algae, and aliquots of single drop were transferred to culture flasks with medium. The isolate was not axenic. Zarrouk's medium (Zarrouk, 1966) modified by Ogawa & Teruyi (1970) and George (1976), was used for isolation and maintenance of culture as well as for growth experiments (Table 1). The modification was mainly in the microelement solution, where concentrations of some elements have been reduced to very low levels (ca 10–3000 fold lower), the elements V, Cr, Ni, W and Ti have been excluded, and more Fe added ($FeSO_4$, 3.5 mg/l).

Table 1. Spirulina medium (Zarrouk's medium, as modified by George, 1976).

		Element	mg/l
Solution I*			
$NaHCO_3$	13.61 g	N	412
Na_2CO_3	4.03 g	P	89
K_2HPO_4	0.50 g	C	2400
Distilled water	500 ml	S	184
		Na	6545
Solution II*		K	672
$NaNO_3$	2.50 g	Ca	11
K_2SO_4	1.00 g	Mg	20
NaCl	1.00 g	Cl	626
$MgSO_4 \cdot 7H_2O$	0.20 g	Fe	6
$CaCl_2 \cdot 2H_2O$	0.04 g		
$FeSO_4 \cdot 7H_2O$	0.01 g		
EDTA (Titriplex III, Merck)	0.08 g		
Microelement solution**	5 ml		
Distilled water	500 ml		

****Microelement solution**

	Stock solution (%)	Applied solution
$ZnSO_4 \cdot 7H_2O$	0.1	1.0 ml
$MnSO_4 \cdot 4H_2O$	0.1	2.0 ml
H_3BO_3	0.2	5.0 ml
$Co(NO_3)_2 \cdot 6H_2O$	0.02	5.0 ml
$NaMoO_4 \cdot 2H_2O$	0.02	5.0 ml
$CuSO_4 \cdot 5H_2O$	0.0005	1.0 ml
Distilled water		981 ml
$FeSO_4 \cdot 7H_2O$		0.7 g
EDTA (Titriplex III, Merck)		0.8 g

* Solutions I & II are mixed in equal proportions to make up 1 litre.
** Modified by George (1976) from Ogawa & Teruyi (1970).

Turbidostat culture

Growth experiments were run in turbidostats, a form of continuous culture where the biomass is internally controlled and the volume is maintained constant by an outflow device (Figure 1), under constant light and temperature (30°C). The culture density is controlled automatically by a photo cell fixed on the front wall of the culture vessel. The photo cell is connected to an electronic control unit adjusted to read up to a certain density level (ACC-50, Techtum instrument, Umeå, Sweden). When the preset density value is exceeded, the unit triggers the opening of a selenoid valve which dilutes the culture by adding fresh medium and removing an equal volume of excess culture When steady state is obtained, i.e. when biomass of algae and flow rate of excess volume are constant, the specific growth rate (μ) per day is equal to the dilution rate (D) which can be defined as

$$D = \mu = \frac{F}{V} \times \frac{24}{t}, \quad (1)$$

where F is the flow rate of excess volume, measured over t (hours), and V is the culture volume. Values of μ over the last 5–7 days of growth at steady state were

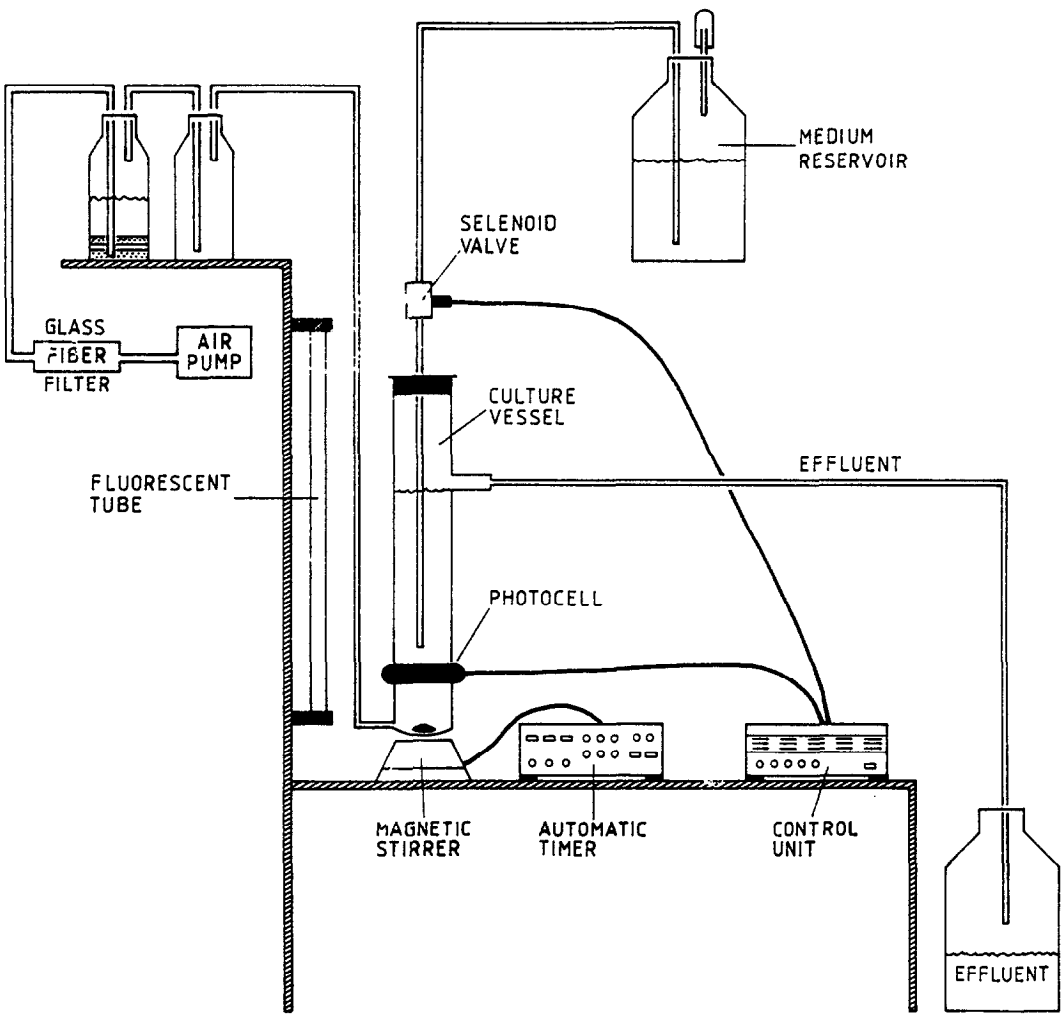

Figure 1. Diagrammatic sketch of the turbidostat.

averaged (coefficient of variation < 7% in all, except 3 cases [Iμ 43–53] with $\geq$ 10%).

The culture vessels were illuminated continuously by fluorescent tubes (Philips TL 20W/33), and light intensity was varied by changing the number of tubes and/or adjusting the distance of vessels from the light source. Light intensity was measured in the center of the vessel filled with culture, with an immersible spherical sensor (QSL-100, Biospherical Instruments, Inc.; quantum response, 400–700 nm) which averages the light reaching the cells from all directions. The dimension of the scales on the meter is in quanta $cm^{-2}s^{-1}$. In this paper, the dimension is given in μmol photons $m^{-2}s^{-1}$, where all light readings have been divided with Avogadro's number (6.023×10^{23}) and multiplied with 10^4 (cm^{-2} changed to m^{-2}) and 10^6 (mol changed to μmol). The culture was aerated with air pumped through fibre glass and moistened by passage through distilled water. The culture was stirred every 5 minutes for 1 minute, with a magnetic stirrer controlled by an automatic timer.

Culture vessels filled with about 0.5 l of medium were inoculated with 10–15 ml from batch culture that have been kept growing in flasks with fresh medium for 2–3 days. In experiments where cultures were subjected to high light intensity, the vessels were shaded with a thin layer of paper for the first day and gradually exposed to full light over two days. In a series of experiments, growth rate in the culture medium was measured under different light intensities covering a range of 20–500 μmol photons $m^{-2}s^{-1}$. Cultures were harvested at steady state after about two weeks, and

subsamples were taken for analyses of pigments, particulate carbon, nitrogen and phosphorus. Methods for analyses are described in detail in Ahlgren & Ahlgren (1976). Light extinction by algae was calculated for every experiment by applying Lambert-Beer's Law to measurements of light transmission (with the same light source as above) through the harvested culture (I) and distilled water (I_0), in a cell of 5 cm length (l); i.e. $I = I_0 \cdot e^{-\varepsilon \cdot l}$, and solving for the extinction coefficient, $\varepsilon(m^{-1}) = 1/0.05 \times \ln(I_0/I)$. Algae were fixed with formalin for microscopic observation.

Calculations

The hyperbolic tangent equation defined by Jassby & Platt (1976), Platt & Gallegos (1980) and Gallegos and & Platt (1981) was fitted to the growth data:

$$P = P_m \tanh(\alpha I/P_m) \tag{2}$$

or, when normalized to Chl a,

$$P^B = P_m^B \tanh(\alpha I/P_m^B), \tag{3}$$

where P is C (carbon) production at light level I, P_m is production at saturating light, and α is the initial slope in the light saturation curve, a measure of production efficiency at low irradiance. As $P = \mu \times C$ where C is biomass in carbon, the equation can be written as

$$\mu \times C = (\mu_{max} \times C) \tanh(\alpha I/P_m). \tag{4}$$

As $P_m/\alpha = I_k$, the irradiance at which light saturation begins,

$$\mu = \mu_{max} \tanh(I/I_k). \tag{5}$$

Equation (5) is thus analogical to Equation (3), but normalized to carbon instead of chlorophyll.

Quantum yield for growth at steady state (Φ_μ), defined by the amount of mol C produced per mol quanta of light absorbed, was calculated using the equation derived by Falkowski et al. (1985; Equation 6):

$$\Phi_\mu = \frac{\mu}{\bar{k}_c \times (\text{Chl/C}) \times I\mu} \times f, \tag{6}$$

where μ = specific growth rate, $\bar{k}_c$ is the average absorption cross section normalized to chlorophyll concentration, I_μ is the growth irradiance in the culture, Chl/C is cellular chlorophyll/carbon ratio and f is a dimension factor. Since extinction of light (ε) is equal to the product of $\bar{k}_c$ and chlorophyll, the expression $\bar{k}_c \times$ Chl can be replaced by ε, and the equation rewritten as

$$\Phi_\mu = \frac{\mu \times C}{\varepsilon \times I\mu} \times f. \tag{7}$$

The dimension factor f varies depending on the dimensions applied for the different parameters. If μ is given in d^{-1}, C in mg l^{-1} (= g m^{-3}), ε in m^{-1}, $I\mu$ in μmol photon $m^{-2}s^{-1}$, then $f = 0.9645$. (The expression $d^{-1} \times$ g m^{-3} divided by m^{-1} and μmol $m^{-2}s^{-1}$, can be simplified to $d^{-1} \times$ g/μmol s^{-1}. Dividing by 12 to change g to mol C, by 10^6 to change μmol to mol, and by 86400 to change s^{-1} to d^{-1}, gives a factor of 0.9645 and the dimensions cancel out.)

Possible scattering of light by the algal cells was checked by measuring absorption (with the same cell and light source used for ε measurements, see above) as a function of chlorophyll concentration and extrapolating to zero chlorophyll ($\varepsilon = 0.20 + 12.8$ Chl, $R^2 = 1.000$, $n = 4$). Scattering (the Y-intercept) was about 1–2% of the ε values measured during the whole experiment, and thus negligible.

Results

Algae grown in the culture medium and exposed to increasing light intensity showed a typical growth response curve (Figure 2a), with the specific growth rate (μ d^{-1}) increasing from 0.23 to a maximum of 1.78 at about 330 μmol photons $m^{-2}s^{-1}$. Although the same rate was maintained at an even higher light intensity of about 500 μmol photons $m^{-2}s^{-1}$, the culture looked chlorotic with filaments forming clumps and sticking to the wall of the vessels; the culture could not be maintained at steady state for a long time. The rate of increase in the growth rate was highest below 80 μmol photons $m^{-2}s^{-1}$.

The Equations 2, 3 and 5 were fitted (using SYSTAT, NONLIN program) to the data on P, P^B and μ vs. I, respectively, and parameters (μ_{max}, P_{max}, P_m^B, α, and I_k) estimated from the curve fittings are given in Table 2. The equation by Jassby & Platt (1976) fitted very well to the growth parameter μ (Figure 2a). Curve fittings for P (Figure 2b) and P^B (Figure 2c) were less accurate (Table 2), reflecting the error and variation introduced by the additional variables C and Chl, involved in determining the growth parameters. A sharper initial slope is evident when production, P (≡ harvest), i.e. biomass produced per day (μ $d^{-1} \times$ mg C l^{-1} = mg C $l^{-1}d^{-1}$), is plotted against light intensity (Figure 2b), than when production is normalized to Chl a, P^B (Figure 2c). The P^B curve gives, in fact, a better fit to a linear regression ($R^2 = 0.92$). Estimate for the initial slope α, from the linear regression of P^B vs I as in Jassby & Platt (1976), was about half of the value

Table 2. Growth parameters and light utilization efficiency for *S. platensis* estimated from the growth data μ (specific growth rate, d^{-1}), P (production = $\mu \times$ C, mg C l^{-1} d^{-1}) and P^B (production normalized to Chl *a*, mg C [mg Chl *a*]$^{-1}$), and I_k, with fitting of the appropriate equations. Confidence intervals are given within parentheses.

$\mu = \mu_{max} \times \tanh(I/I_k)$	$P = P_{max} \times \tanh(I/I_k)$	$P^B = P^B_m \times \tanh(I/I_k)$	$P^B = l + \alpha \cdot I$
$\mu_{max} = 1.76\ (1.57 - 1.94)$	$P_{max} = 104\ (76 - 132)$	$P^B_m = 141(77 - 206)$	
$I_k = 171\ (129 - 213)$	$I_k = 144\ (37 - 250)$	$I_k = 206\ (35 - 377)$	
$\alpha_\mu = 0.010(\mu_{max}/I_k)$	$\alpha_c = 0.73(P_{max}/I_k)$	$\alpha = 0.69(P^B_m/I_k)$	$\alpha = 0.38(P_B/I)^*$
$R^2 = 0.92; n = 13$	$R^2 = 0.74; n = 13$	$R^2 = 0.69; n = 11$	$R^2 = 0.92; n = 11$

* I and I_k = μmol photons $m^{-2}s^{-1}$

estimated from the curve fitting ($\alpha = P^B_m/I_k$). The initial slope of P vs. I, $\alpha_c (= P_{max}/I_k)$, was higher than α. Analogous to α, the parameter $\alpha_\mu (= \mu_{max}/I_k)$, the initial slope of μ vs. I_μ (Figure 2a), has been introduced here as a measure of growth efficiency, defining the maximum specific growth rate per amount of light available.

The compensation point (C.P.) defines the critical irradiance below which no net production or accumulation of biomass is possible (Reynolds, 1984). Values of the X-intercept from the regression lines of μ, P and P^B against irradiance at low light are taken as estimates of the C.P. as in Falkowski et al. (1985), who regressed μ vs. $\log I\mu$ in light limited regions. Estimates of C.P. based on growth rate and production were as expected about the same, 17 and 15 μmol photons $m^{-2}s^{-1}$, respectively, but doubled (34 μmol photons $m^{-2}s^{-1}$) when production was expressed per unit Chl *a*.

Quantum yield ranged between 2.8 and 9.4%, with the highest values shown at low irradiance (Table 3). With irradiance above about 100 μmol photons $m^{-2}s^{-1}$, μ increased with no apparent change in $\Phi\mu$. The mean quantum yield for growth at optimum light intensity was only 3.8%. The initial slope (α_c) of production plotted against $I\mu$ (Figure 2b) gives an estimate of the maximum quantum yield, Φ_m (Jassby & Platt, 1976). Since Φ is defined by biomass produced per amount of light absorbed, the slope α_c was divided by the mean value of extinction of light ($\bar{\varepsilon}$) for the points on the slope. Thus:

$$\alpha_c = \mu \times C/I\mu, \tag{8}$$

$$\Phi_m = (\alpha_c/\bar{\varepsilon}) \times f. \tag{9}$$

Φ_m calculated as such was 0.115 or 115 mmol C mol^{-1} photons, close to the theoretical limit of 125 mmol C mol^{-1} quanta (photons) for quantum yield of gross photosynthesis (Harris, 1978; Raven, 1984). Since quantum yield for growth (Φ_μ) represents net production, respiratory and excretory losses may contribute to the difference of 10 mmol C or 8%.

The nitrogen/carbon ratio (N/C, w/w) did not vary much (0.16–0.23) with increasing irradiance (Figure 3a). Cells growing at optimum light for growth (330 μmol photons $m^{-2}s^{-1}$) had a ratio of about 0.21. More variation was found in the phosphorus/carbon ratio (P/C, w/w) which ranged from 0.017 to 0.029, with a slight increase at higher irradiance (Figure 3b). Cells growing with the optimum light for growth had N:P ratio (by weight) of about 7, equal to the optimum Redfield ratio for healthy growing cells (Reynolds, 1984). Higher ratios of up to 11 were attained in cells growing at low irradiance. The chlorophyll *a*/carbon ratio (Chl/C, w/w) varied between 0.009 (0.9%) at the highest irradiance used (498 μmol photons $m^{-2}s^{-1}$), to 0.02 (2%) at intermediate level of irradiance (100–200 μmol photons $m^{-2}s^{-1}$) (Figure 4a). The carotenoids/carbon ratio (Caro/C, w/w) showed slight decrease at high irradiance and maximum values at about 175–250 μmol photons $m^{-2}s^{-1}$ (Figure 4b). With increase in light intensity, the ratio of Caro/Chl *a* (by weight) more than doubled (Figure 4c).

Discussion

Growth rate

The maximum specific growth rate (μ_{max}) of 1.78 d^{-1} found at 330 μmol photons $m^{-2}s^{-1}$ and 30°C in present work agrees very well with all but one of the previous estimates for the species (Table 4). Growth rates at comparable irradiance levels below 600 μmol photons $m^{-2}s^{-1}$ in batch culture experiments by Tedesco & Duerr (1989) were only about half the values found in continuous culture experiments by Alba & Ogawa (1977) and in the present study (Figure 5); the latter two studies gave very similar μ values at the

Table 3. Specific growth rate (μ), extinction of light (ε), carbon (C) and pigment concentrations, production ($P = \mu \times C$), and calculated quantum yield (Φ_μ) of *Spirulina platensis* grown in turbidostats at steady state growth irradiance levels $I\mu$ (μmol photons $m^{-2}s^{-1}$). Values in bracket are probably an overestimation because the algae were clumpy and sticking to the wall of the culture vessel.

$I\mu$	μ (d^{-1})	ε (m^{-1})	C	Chl *a*	Carotenoids	P	Φ_μ (%)
			——— (mg l^{-1}) ———			(mg C l^{-1} d^{-1})	
20	0.23	11.0	36.3	1.00	0.27	8.3	3.7
43	0.57	11.5	63.8	1.17		36.2	7.0
50	0.69	10.3	72.4	0.98		49.9	9.4
53	0.55	14.1	99.9			55.4	7.1
95	1.02	14.7	78.6			80.1	5.5
100	0.79	14.7	67.5	1.55	0.45	53.3	3.5
125	1.09	11.4	51.8	1.23	0.33	56.7	3.8
166	1.25	14.7	72.5	1.76	0.48	90.6	3.6
216	1.33	11.0	52.6	0.85	0.37	69.9	2.8
249	1.47	11.0	53.8	1.05	0.37	79.9	2.8
332	1.78	7.8	57.1	0.71	0.36	102	3.8
498	1.77	7.8	(76.6)	0.69	0.44	(136)	(3.4)

Table 4. Maximum specific growth rate (μ_{max} d^{-1}) of *Spirulina platensis* from available literature and present work.

I_μ	μ_{max} (d^{-1})	Culture	Temp. (°C)	Medium	Reference
438	1.80	Batch	35	Zarrouk's	Ogawa & Teruyi (1970)
273	1.68	Continuous	35	Zarrouk's	Aiba & Ogawa (1977)
600	0.69*	Batch	25	50% Zarrouk's	Tedesco & Duerr (1989)
600	1.39*	"	33	"	" "
1400	1.53	"	37	"	" "
465	1.87	"	35	50% Zarrouk's	Olaizola & Duerr (1990)
330	1.78	Continuous	30	Zarrouk's	Present work

* $\leq \mu_{max}$

same irradiance levels, in spite of the higher temperature (35°C) employed by Aiba & Ogawa (1977). Some differences lie in the irradiance level applied to reach similar μ_{max} values.

Comparing growth estimates from batch and continuous culture experiments (Table 4), it appears that higher rates are achieved in the latter and μ_{max} can be reached at lower irradiance. Batch cultures often give lower μ_{max} than continuous cultures (Van Liere et al., 1975; Van Liere, 1979). This can be due to circulation of algae under a constant light environment and nutrient replenishment in continuous culture, a condition lacking in batch culture where available light and nutrients decrease with time as density of the culture increases. Unless available light for growth is constantly adjusted over the growth period, onset of light saturation may be prolonged in batch cultures, thus increasing I_k, compared with continuous culture exposed to the same irradiance level. At about the same irradiance levels, growth rates found by Olaizola & Duerr (1990) in batch culture were higher than those found by Tedesco & Duerr (1989), even though the cultures were grown in the same media at about the same temperature. Differences in methods, especially methods of measuring available light, can be an important cause for such discrepancies in experimental results. Position of light sensor in relation to culture flasks, e.g. whether spherical sensors are immersed in culture flasks or flat sensors are placed outside, and the distance from the flasks if measured on the outside, is rarely mentioned in methods of experiments. Additionally, light meters should be recalibrated regularly, a recommendation seldom

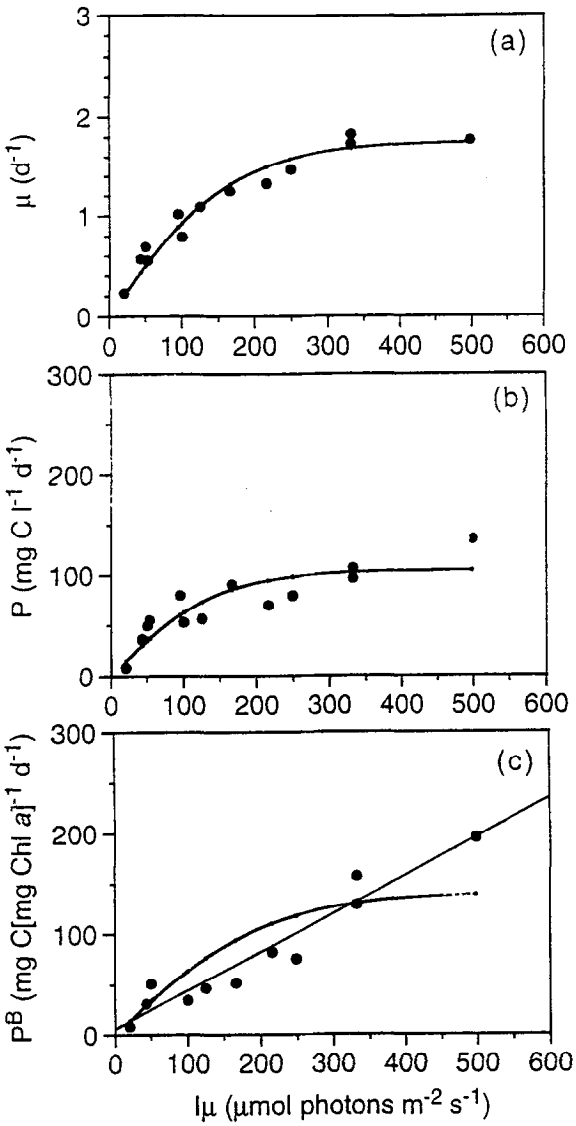

Figure 2. Growth response of *S. platensis* to irradiance level $I\mu$: (a) μ, specific growth rate, (b) P, production (harvest), and (c) P^B, production normalized to Chl *a*. The curves were fitted to the points using the hyperbolic tangent equation by Jassby & Platt (1976). Linear regression in (c) gives the equation: $P^B = 5.2 + 0.38I$ ($R^2 = 0.92$).

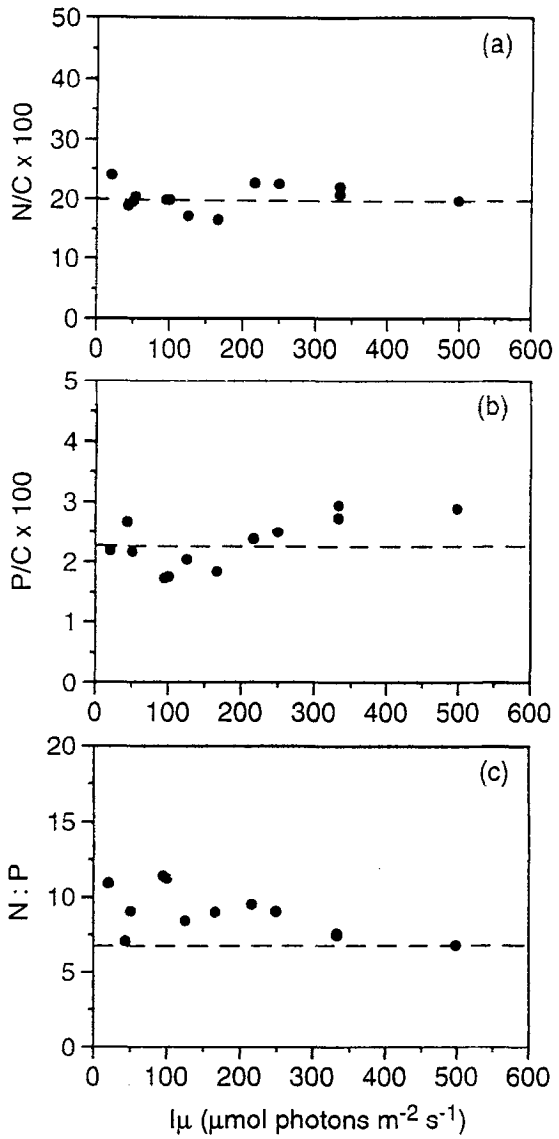

Figure 3. Nutrient ratios (by weight) under various irradiance levels, $I\mu$: (a) N/C, (b) P/C, and (c) N:P. The dotted lines show the mean ratios in (a) and (b), and the Redfield ratio in (c).

followed. Variations in light response by the use of different clones of *S. platensis* in the separate experiments also can not be excluded.

Summary of the results from the literature (Table 4) on the maximum specific growth rate (μ_{max} d^{-1}) of *S. platensis* gives a mean of 1.73 ($s = 0.13$; $n = 5$) in the temperature range of 30–37°C. This is relatively high compared to growth rates of other filamentous cyanophyte species listed in the review of data by Reynolds (1984). Growth rates varied between 0.27 and 1.56 d^{-1} for species of *Anabaena*, *Aphanizomenon* and *Oscillatoria*. But almost all algae in the review were grown at lower temperatures (20–25°C). As long as the optimum temperature for growth is not exceeded, which is most probable considering the species reviewed (Reynolds, 1984), growth rates can be higher with raised temperature.

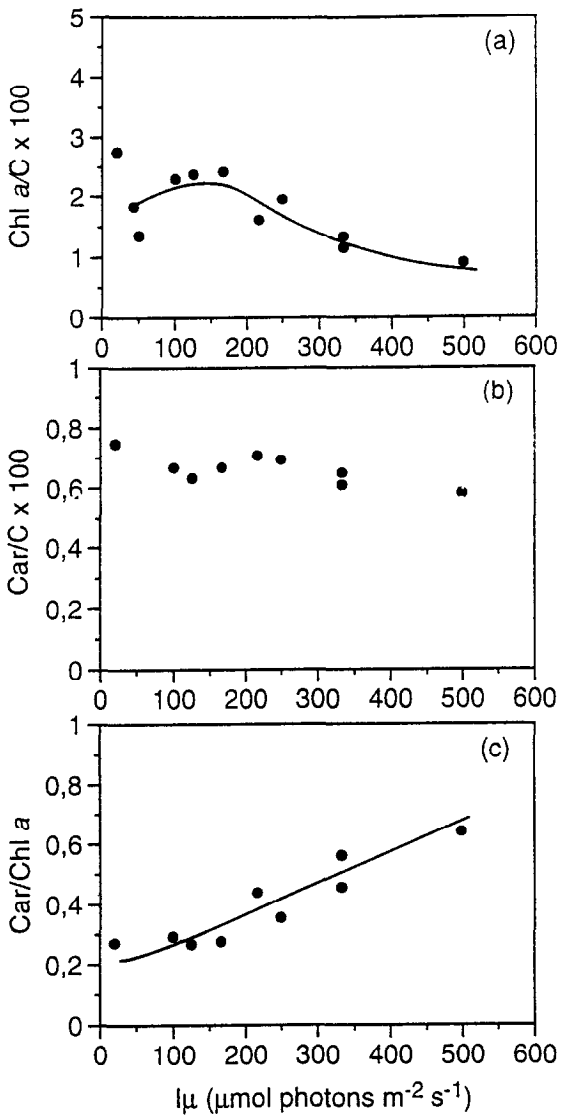

Figure 4. Pigment content expressed in ratios (by weight): (a) Chl *a*/C, (b) carotenoids/C, and (c) carotenoids/Chl *a*. The lines were fitted by eye.

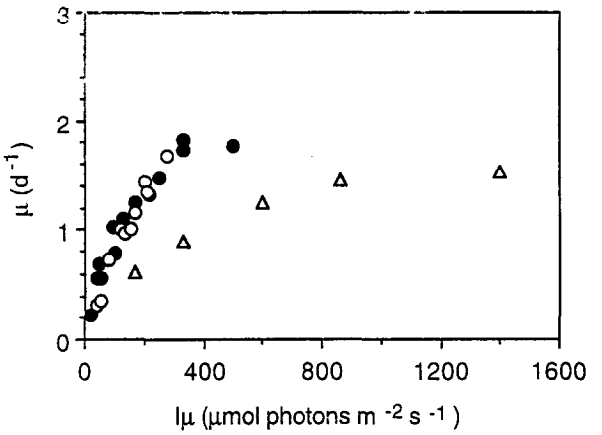

Figure 5. Specific growth rate of *S. platensis* at various irradiance levels ($I\mu$) from data in (●) present work, (○) Aiba & Ogawa (1977), and (Δ) Tedesco & Duerr (1989).

Light utilization

Efficiency of light utilization based on chlorophyll (α) was 0.68 (from curve fitting) and 0.38 (from linear regression) C (mg Chl *a*)$^{-1}$ d^{-1} (μmol photons m^{-2}s^{-1})$^{-1}$ which can be written as 7.9 and 4.4 mg C (mg Chl *a*)$^{-1}$ E^{-1} m^{-2}, respectively. The values lie in the range of observed photosynthetic efficiencies (2–37, peaking between 6 and 18 mg C [mg Chl *a*]$^{-1}$ E^{-1} m^{-2}) reviewed from the literature by Reynolds (1984). Interspecific variations in α can be marked since the average light-harvesting ability of a unit of Chl *a* depends on the cellular architecture, chloroplast arrangement and pigment composition of species (Platt & Jassby, 1976). Harris (1978) and Reynolds (1984) however, indicate that the effect of low light on growth would be clearly revealed and interspecific differences in efficiency would be more obvious, if light limited rate of photosynthesis is expressed per cell, per unit volume or per unit dry weight, in stead of per chlorophyll. The argument is that cells are able to regulate their photosynthetic efficiency by varying their pigment content. Thus, low chlorophyll content of cells at high light may result in extension of the linear portion of the $P^B - I$ curve (Figure 2c).

I_k estimates based on the three parameters (Table 2), are expected to be similar (cf. Equations 2, 3 and 5) and they show an overlap. They also fall within the range of I_k values (20–300 μmol photons m^{-2}s^{-1}) reviewed in Harris (1978). Variations can possibly occur due to differences in the photosynthetic behaviour of natural, mixed phytoplankton populations, in contrast to laboratory monoculture growing at steady state, as well as due to interspecific differences. The cyanophyte *Anacystis nidulans* grown in turbidostats at 30°C had an I_k value of 91 μmol photons m^{-2}s^{-1} (Ahlgren, unpublished data), a markedly lower value than for *S. platensis*. I_k estimate based on growth curve data in Tedesco & Duerr (1989), with a clear light saturation phase (Figure 5), is about 2 times higher (350 μmol photons m^{-2}s^{-1}) than present estimate of I_k (171 μmol photons m^{-2}s^{-1}). This marked difference can be due to higher growth temperature (37°C) used in Tedesco & Duerr (1989), since I_k is

temperature-dependent (Harris, 1978; Talling, 1957). I_k may also be exaggerated due to the relatively higher irradiance level required to reach μ_{max} in batch compared to continuous culture, a point discussed earlier in the discussion, or simply due to different methods of measuring irradiance level.

Compensation point (C.P.) for *S. platensis*, estimated from μ and irradiance values given in Tedesco & Duerr (1989), was about 53 μmol photons $m^{-2}s^{-1}$, much higher than C.P. found in present work (17 μmol photons $m^{-2}s^{-1}$). Batch culture by Tedesco & Duerr could contribute to higher C.P. In contrast, estimation from the growth curve (μ vs. $I\mu$) in the literature shows C.P. of less than 5 μmol photons $m^{-2}s^{-1}$ for the cyanophytes *Oscillatoria agardhii* (Van Liere, 1979), *Aphanizomenon flos-aquae* (Zevenboom et al., 1981) and *Microcystis aeruginosa* (Kappers, 1984), all grown in continuous culture, in 20°C. Even though the influence of temperature on respiration rates can be high (Grobbelaar & Soeder, 1985), under low and limiting light conditions, light is the main controlling factor (Talling, 1966; Tilzer, 1987), and growth temperature difference of 10°C is not expected to account for a 3-fold difference in C.P. levels. Higher growth rate in *S. platensis* (2–3.6×) than in the three species (μ_{max} = 0.5 – 0.9 d^{-1}) would contribute to higher respiration rate, and probably also contribute to raised C.P., since growth respiration is assumed to be in some way proportional to the rate of increase in biomass (Harris, 1978). It is apparent from the growth curves that *S. platensis* requires much more light for photosynthesis and growth than the other cyanophyte species cited here. *S. platensis* shows in fact, more similarities with some marine phytoplankton species which showed C.P. of 17–23 μmol photons $m^{-2}s^{-1}$ (Falkowski et al., 1985). Energy demanding physiological processes in *S. platensis* growing at high pH and salinity probably increase the rate of respiration relative to that of growth. These include enzyme regulated activities, such as osmoregulation in hypertonic environment (Martel et al., 1992; Warr et al., 1985), and extra- and intracellular uptake and conversion of HCO_3^- to CO_2 for photosynthesis (Espie et al., 1991).

$\Phi\mu$ showed about 3.4-fold difference, with the highest yield at low light levels (Table 3). Quantum yield at steady state growth should approach maximal values at low irradiance, while at high irradiance growth rates are maximum and quantum yield should be inversely proportional to irradiance (Kiefer & Mitchell, 1983). Ogawa & Aiba (1978) found an average $\Phi\mu$ of 5% for *S. platensis* grown at 6.8 klux ($\sim$ 119 μmol photons $m^{-2}s^{-1}$), which is slightly higher than $\Phi\mu$ found in this work at about the same irradiance (3.85% at 125 μmol photons $m^{-2}s^{-1}$). The maximum quantum yield of 9 and 11% (from both estimates of Φ_m) is much higher than values found for three species of marine phytoplankton by Falkowski et al. (1985) which had maximum values of about 1.5 to 5.3%. Values of 5 to 9% were found for five species of marine macroalgae (Markager, 1993), with relatively low growth rates varying between 0.07 and 1.2 d^{-1}. Differences in $\Phi\mu$ among the five species were believed to be mainly due to differences in light absorption which is tightly coupled to thallus specific carbon content.

Nutrient status and pigments

A relatively constant N/C ratio was maintained by cells exposed to the range of irradiance (Figure 3a); the mean ratio was 0.20 (standard deviation s = 0.02, n = 13) which agrees well with the mean N/C of 0.20 (s = 0.017, n = 6) found for *S. platensis* by Aiba & Ogawa (1977). Slight increase in P/C ratio (mean = 0.02, s = 0.004, n = 12) with increasing irradiance, causes decrease in N:P ratio (Figure 3c). Higher N:P ratio at low light is probably associated with relatively enhanced synthesis of chlorophyll during light limited growth, and/or lower capacity to utilize P (ATP) when the algae are energy-limited. In cells growing at irradiance below the optimum light for growth, the N/P ratio was between 8 and 11, deviating from the optimal Redfield ratio of 7 (w/w). Concentrations of N and P in the culture medium were non-limiting, and changes in ratios were due to physiological response to changing light environment.

Chl/C ratio of *S. platensis* was maximum (0.024 or 2.4%) at intermediate irradiance between 100 and 170 μmol photons $m^{-2}s^{-1}$ (Figure 4a). Olaizola & Duerr (1990) found a maximum chlorophyll content of 2.3% of dry weight (Chl/C ca 4.6% or 0.046) at 66 μmol photons $m^{-2}s^{-1}$, and a rapid decline with increasing irradiance. Markager (1993) found absorption peaks of chlorophyll at intermediate irradiance levels for five macroalgae, and also cited previous works on species of microalgae and submerged macrophytes which agree with his findings. Under light limiting conditions, high pigment content increases the capacity of cells to absorb photons. But as suggested by Raven (1984), maximum chlorophyll content may be found at intermediate light levels, where the gain from investment in new chlorophyll balances the cost of syn-

thesis. Variation in Caro/C ratio was not marked (Figure 4b), but the ratio of carotenoids to Chl *a* increased from 0.27 to 0.64 (2.4-fold) with increasing irradiance (Figure 4c), possibly due to reduced chlorophyll synthesis, as increase in carotenoids was not evident. It is generally known that adaptation of phytoplankton to higher irradiances results in a lower chlorophyll content and a higher proportion of carotenoids compared to chlorophyll (Halldal, 1970 in Platt & Jassby, 1976). Olaizola & Duerr (1990) found that carotenoids content of *S. platensis* decreased about 3-fold as irradiance increased from 66 to about 750 μmol photons $m^{-2}s^{-1}$. It started to increase above this level of irradiance, which they believed to be an adaptation to protect chlorophyll molecules from photooxidation. Information about other accessory pigments, e.g. phycobillins, would probably give a better picture of algal response in pigment content to different light levels, specially at limiting light levels where cells have to optimize pigment synthesis to capture available photons.

Comparison with field data

In the text table below, is shown production at light saturation, P_{max} ($\mu \times$ C, mg C l^{-1} hr^{-1}) and P_m^B (mg C [mg Chl *a*]$^{-1}$ hr^{-1}) from continuous culture, which are analogous to the maximal photosynthetic rate, A_{max} (mg O_2 m^{-3} hr^{-1}), and photosynthetic efficiency (mg O_2 [mg Chl *a*]$^{-1}$ hr^{-1}) at light saturation, in Talling et al. (1973) and Melack (1979). Values in O_2 have been converted to C assuming an equimolar exchange between C and O_2 as in Reynolds, 1984 (p. 129):

	P_{max} (mg C l^{-1} hr^{-1})	P_m^B (mg C [mg Chl *a*]$^{-1}$ hr^{-1})	Reference
Continuous	4.33	6	Present work
L. Arenguade	3.75–11.20	4.12–6.75	Talling et al. (1973)
L. Simbi	0.36–4.84	0.37–7.12	Melack (1979)

The values from continuous culture in the laboratory fall well within the ranges of primary production measurements for natural populations of *S. platensis* in Lake Arenguade (Ethiopia) and Lake Simbi (Kenya), where the species forms an almost unialgal population. Some degree of genetic differences are always exhibited within species (cf. Medlin et al., 1991). Isolation of the species from the natural population and taking a relatively large inoculum for the culture experiments in the present case, should minimize the risk of isolating a single clone of extreme qualities, different from the population mean. It could contribute to the agreement of maximum estimates of photosynthetic efficiency from experiments and field values (6–7 mg C [mg Chl *a*]$^{-1}$ hr^{-1}). This agreement allows to make reasonable comparisons of results from continuous culture studies to natural populations, with due consideration to other growth conditions, e.g. nutrients; it can also be useful in quantifying the requirement of light to support optimal growth in mass cultivation, which is a continuous or semicontinuous system where a continuous nutrient input and removal of biomass maintains a steady state of algal growth.

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