Characteristics of Heterotrophic Growth in the Blue-Green Alga *Nostoc* sp. Strain Mac

By P. J. BOTTOMLEY AND C. VAN BAALEN

University of Texas Marine Science Institute, Port Aransas Marine Laboratory,
Port Aransas, Texas 78373, U.S.A.

(Received 22 March 1978)

The blue-green alga *Nostoc* sp. strain Mac was grown heterotrophically in the dark on glucose and fructose. Cell composition was similar under photoautotrophic and heterotrophic growth conditions and the efficiency of cell synthesis, in most cases, was similar to values obtained with other heterotrophic micro-organisms. Differences were seen in growth on the two sugars in response to sugar concentration and nitrogen source, and in the efficiency of cell synthesis in minimal medium, which indicated differences in the metabolism of the two sugars by the alga. Heterotrophic growth was stimulated by casein hydrolysate and dim light. Both effects were additive, indicating at least two different rate-limiting steps in the heterotrophic metabolism of the alga. Casein hydrolysate acted as a bulk nitrogen source for the alga and the response to dim light showed a chlorophyll *a*-like action spectrum.

INTRODUCTION

In recent years, several blue-green algae (cyanobacteria) have been shown to be capable of heterotrophic growth in the dark at the expense of various sugars which act as both the carbon and energy source for growth (Fay, 1965; Watanabe & Yamamoto, 1967; Hoare *et al.*, 1971; Rippka, 1972; White & Shilo, 1975; Wolk & Schaffer, 1976). Unfortunately, those strains so far found capable of growing heterotrophically do so at only a fraction of their photoautotrophic growth rates which has made it arduous to obtain information on physiological adaptations to, and characteristics during, this type of growth.

The blue-green alga *Nostoc* sp. strain Mac was originally isolated from the coralloid roots of the cycad *Macrozamia lucida* by Bowyer & Skerman (1968), and has been shown by several workers to be capable of heterotrophic growth in the dark at a reproducible rate (Hoare *et al.*, 1971; Pulich & Van Baalen, 1973). Since there is a lack of information on the basic characteristics of heterotrophic growth in this alga, and in the blue-green algae in general, the aim of this paper is to present quantitative data on this mode of growth in *Nostoc* sp. strain Mac. The data covers growth rates, growth efficiencies, cell composition, and partial characterization of two factors, dim light and casein hydrolysate, which stimulate heterotrophic growth in this blue-green alga.

METHODS

Organism and growth. *Nostoc* sp. strain Mac is a filamentous blue-green alga which was isolated from the coralloid roots of the cycad *Macrozamia lucida* (Bowyer & Skerman, 1968) and maintained in this collection in the light on slopes of medium Cg10 (Van Baalen, 1967), solidified with 1 % (w/v) Difco Bacto (0140) agar. In all growth experiments, inocula were grown from a slope to an absorbance of 0.16 to 0.2, equivalent to 0.07 to 0.09 mg dry wt ml⁻¹; 0.1 ml samples of such a suspension were used as the inoculum for each growth tube.

Photoautotrophic growth. The organism was routinely grown in Pyrex test tubes (175 × 22 mm) in 20 ml medium Cg10 at 39 ± 0.1 °C. The tubes were bubbled with air enriched with 1 ± 0.1 % (v/v) CO₂ at a rate of 5 to 6 ml min⁻¹ and illuminated by two F48T12/CW/XHO fluorescent lamps placed 10 cm from the growth tubes on either side of the growth bath. The method is essentially a modification of that of Myers (1950). Growth was routinely followed turbidimetrically using a model 402-E Lumetron colorimeter equipped with a narrow band-pass coloured glass filter set with peak transmission centred at 660 nm. The overall transmission of the filter set was similar to a Corning CS 2-78 filter. Growth was also measured on a dry weight basis and correlated with the absorbance of suspensions of the alga when required. For convenience, the specific growth rate constants (*k*) were converted to generation times (h).

Heterotrophic growth. The system was similar to that described above, except that the culture bath was darkened and placed in a dark room. The sugars α -D(+)-glucose and β -D(-)-fructose (Sigma) were carefully dissolved in distilled water without heating, and then filter sterilized through Nucleopore filters (24 mm diam., 0.45 μ m pore size) and added aseptically to the growth tubes to give the required final concentration. Casein hydrolysate (Matheson, Coleman & Bell, East Rutherford, New Jersey, U.S.A.) was stirred in distilled water for several hours, heated gently to complete the solution and then filter sterilized and added aseptically to the growth tubes. Growth medium without nitrate was Cg10 with 750 mg KCl l⁻¹ and 13 mg CaCl₂ · 2H₂O l⁻¹ replacing KNO₃ and Ca(NO₃)₂, respectively.

Heterotrophic growth in dim light. These experiments were performed in a thermostated light-tight bath at 39 ± 0.1 °C containing four cells. Each cell was baffled from the next and had a front and rear window 30 mm in diameter. A piece of white bond paper was placed over the front window of each of the four cells, which were illuminated individually by four standard 500 Junior projectors operating at 55 to 60 V with 500 W DAY Sylvania projection lamps as light sources. Irradiance measurements were made with a Kipp-Zonen CA-1 thermopile operated with the 8 mm aperture placed against the rear window of each cell in turn, and the incident energy was recorded on a Keithley 150B microvolt ammeter. The output of each lamp was adjusted so that 30 to 35 μ W cm⁻² was recorded between the lower transmission limit of the lamp and the cut-off point of a Schott RG-10 filter (< 5 % transmittance at 690 nm and zero below 685 nm). Spectral distribution of the energy was determined using an ISCO spectroradiometer model SR with the scale expanded through an Omniscrite recorder.

Photosynthesis measurements. Measurements of oxygen exchange were made using a YSI (model no. 5331) Clark-type oxygen electrode. Samples (1.9 ml) of algal suspension, corresponding to 0.09 mg dry wt ml⁻¹, were added to the chamber which was thermostated at 39 °C and bubbled for 2 min with 1 % CO₂ in air. The chamber was then stoppered, illuminated, and the change in electrode current was detected on a Keithley 150A microvolt ammeter and recorded on a Heathkit recorder. To determine CO₂ fixation, 20 μ l (0.2 μ Ci) of radioactive NaH¹⁴CO₃ (sp. act. 10 μ g μ Ci⁻¹, New England Nuclear) was added to the algal suspension already equilibrated in the chamber as described above. The chamber was closed and the sample was illuminated. At intervals, 1 ml samples were removed, filtered (25 mm diam., 0.45 μ m pore size filters; Gelman Instrument Co., Ann Arbor, Michigan, U.S.A.) and washed with 5 ml ice-cold Cg10 containing 10 g unlabelled NaHCO₃ l⁻¹. Two drops of 2 M-HCl were then added to each filter and left to dry. The filters were placed in 1 ml Soluene 350 (Packard Instrument Co.) in a scintillation vial and the algal material was dissolved overnight. Scintillation fluid (10 ml Insta-fluor, Packard Instrument Co.) was then added and the samples were counted. Controls were treated identically but without illumination. Illumination was provided by a standard 500 Junior projector operating at 90 V; the light intensity was regulated by placing copper screens between the projector and the electrode chamber. To measure O₂ and CO₂ fixation in dim light, a lighting system of similar geometry to that on the growth bath was set up such that a similar irradiance fell on the electrode chamber. No correction was made, however, for the different optical properties of the chamber and the growth bath.

Dry weight determinations. Samples of cultures were passed through Nucleopore filters (47 mm diam., 0.4 μ m pore size) which had been pre-dried to constant weight by storing under vacuum in a desiccator. The algal samples were rinsed with 5 ml distilled water, dried at 50 °C over P₂O₅ in a vacuum oven for 2 d and then dried to constant weight ± 0.05 mg.

Sugar determinations. Glucose was determined using glucose oxidase (Sigma kit 510). Fructose was determined by the anthrone method (Ashwell, 1957). Standard curves were constructed for both sugars over the range 0 to 0.55 mM and were linear. All samples and controls were diluted such that they fell within this range.

Fig. 1. Growth curves in terms of absorbance of *Nostoc* sp. strain Mac grown under saturating light conditions as described in Methods. Glucose and fructose were added at 28 mM, NH₄Cl at 2 mM and casein hydrolysate at 1 g l⁻¹. (a) Δ , Cg10; $\square$, Cg10+glucose; $\circ$, Cg10+fructose. (b) ∇ , Cg10+glucose+NH₄Cl; $\square$, Cg10+glucose+casein hydrolysate; $\circ$, Cg10+fructose+casein hydrolysate.

Fig. 2. Growth curves in terms of absorbance of *Nostoc* sp. strain Mac grown under dark heterotrophic conditions on (a) glucose or (b) fructose. Glucose and fructose were added at 28 mM, NH₄Cl at 2 mM and casein hydrolysate at 1 g l⁻¹. $\square$, Cg10+sugar; Δ , Cg10 without nitrate+sugar+NH₄Cl; $\bullet$, Cg10+sugar+casein hydrolysate.

RESULTS

Comparison of growth under phototrophic and heterotrophic conditions

The data in Fig. 1 show several features of phototrophic growth in *Nostoc* sp. strain Mac. The maximum growth rates obtained with CO₂ and KNO₃ as carbon and nitrogen sources, respectively, corresponded to a generation time of 5 to 6 h. Indeed, when more reduced forms of carbon, either alone (Fig. 1a), or in combination with more reduced forms of nitrogen (Fig. 1b), were added in the presence of CO₂ there was no enhancement of the growth rate. In contrast, several differences were seen during dark growth. First, glucose or fructose at a final concentration of 28 mM supported measurable growth, as previously noted (Hoare *et al.*, 1971), with generation times of 48 h (Fig. 2a) and 144 h (Fig. 2b) respectively. Second, the growth rate on glucose was similar over a concentration range from 56 to 0.56 mM, whereas concentrations of fructose of less than 28 mM supported growth at an exceedingly slow rate, corresponding to generation times in excess of 200 h.

Table 1. *Effect of various combinations of organic carbon and nitrogen on the growth rate of Nostoc sp. strain Mac in the dark*

To growth tubes containing 19 ml medium Cg10 with or without nitrate (10 mM) were added filter-sterilized glucose or fructose (28 mM) and the organic nitrogen sources indicated (at 1 g l⁻¹); 0.1 ml samples (8 µg dry wt) of photoautotrophically grown *Nostoc* sp. strain Mac were used as inocula. The cultures were incubated at 39 °C and bubbled with 1% CO₂ in air, and growth was followed turbidimetrically at 660 nm. The generation times were calculated from the exponential phase of growth.

Sugar	Inorganic nitrogen	Organic nitrogen	Generation time (h)
Glucose	KNO ₃	None	48
	KNO ₃	Trypticase	31
	KNO ₃	Trypticase + casein hydrolysate	28
	None	Casein hydrolysate	23
Fructose	KNO ₃	None	144
	KNO ₃	Trypticase	38
	KNO ₃	Trypticase + casein hydrolysate	22
	None	Casein hydrolysate	23
Glucose + fructose*	KNO ₃	None	48
	KNO ₃	Casein hydrolysate	23

* Each sugar was added at 14 mM.

Third, the growth rate on glucose could be stimulated by using ammonium chloride as nitrogen source in place of potassium nitrate, whereas the growth rate on fructose was not increased. These observations indicate that there are different rate-limiting steps during growth on these two similar sugars.

The greatest stimulation of growth was seen in the presence of casein hydrolysate, which halved the generation time on glucose at concentrations between 56 and 0.56 mM to 24 h (Fig. 2a), and caused a sixfold stimulation of growth on 28 mM-fructose to a similar value (Fig. 2b). Most remarkable was the fact that casein hydrolysate stimulated the growth rate on fructose at concentrations as low as 0.56 mM to give a generation time of 24 h, which was then maintained until the fructose was completely utilized. This growth stimulation was not seen when ashed casein hydrolysate was substituted, nor did casein hydrolysate alone support growth in the absence of the hexose sugar. Thus it is inferred that the organic constituents are the active components, although they cannot act as sole carbon, nitrogen and energy sources for the alga.

Vitamin-free Bacto Casamino acids (Difco) also stimulated growth, as did trypticase (BBL), an enzymic digest of casein (Table 1). However, since the growth stimulatory effects of these mixtures were not additive it suggested that they were acting on a common rate-limiting step which could be saturated by the acid digest alone. Similarly, when glucose and fructose were added together with casein hydrolysate there was no additive effect. This would imply that even when heterotrophic metabolism is stimulated by organic nitrogen there is a rate-limiting step which is common to the utilization of both sugars.

The role of casein hydrolysate

The alga showed a remarkable ability to extract and utilize the nitrogen available in casein hydrolysate. The generation times on glucose or fructose with casein hydrolysate were independent of the presence or absence of nitrate (Table 1). The yield of cells was proportional to the amount of casein hydrolysate and was independent of whether glucose or fructose was the sugar present (Table 2). Moreover, since there was only marginal growth in the absence of combined nitrogen, it can be calculated that the alga utilized about 40 to 50% of the available nitrogen whilst maintaining a generation time of 24 h. This suggests that

Table 2. Yield of cells of *Nostoc* sp. strain Mac grown with casein hydrolysate as the sole nitrogen source in the dark

To growth tubes containing medium Cg10 without nitrate were added filter-sterilized glucose or fructose (28 mM) and a filter-sterilized solution of casein hydrolysate to give the final concentrations shown; 0.1 ml samples of photoautotrophically grown *Nostoc* sp. strain Mac were used as inocula. Growth was followed turbidimetrically at 660 nm. The growth tube contents were harvested on tared Nucleopore filters after the absorbance had remained constant for 2 d.

Casein hydrolysate concn (g l ⁻¹)	Dry wt of algae (mg ml ⁻¹) grown on:	
	Glucose	Fructose
0	0.02	0.01
0.1	0.10	0.09
0.2	0.16	0.17
0.3	0.20	0.28
0.4	0.30	0.29
0.5	0.37	0.41

Fig. 3. Growth curves in terms of absorbance of *Nostoc* sp. strain Mac grown under heterotrophic conditions in darkness or dim light on (a) glucose or (b) fructose. The growth systems were as described in Methods. Glucose and fructose were added at 28 mM and casein hydrolysate at 1 g l⁻¹. Δ , Cg10 $\pm$ casein hydrolysate in dim light; $\bullet$, Cg10 + sugar in the dark; $\circ$, Cg10 + sugar in dim light; $\blacksquare$, Cg10 + sugar + casein hydrolysate in the dark; $\blacktriangle$, Cg10 + sugar + casein hydrolysate in dim light.

one role of casein hydrolysate is as a bulk nitrogen source from which the alga can efficiently transport and utilize the amino acids. This has not been seen previously in the blue-green algae. However, the observation that the growth rate in the presence of casein hydrolysate is independent of fructose concentration, whereas with nitrate the growth rate is influenced markedly by fructose concentration, implies that casein hydrolysate has other effects which are related specifically to fructose metabolism.

Table 3. *Observed rates of oxygen exchange by Nostoc sp. strain Mac in darkness and in dim light in the presence or absence of glucose*

(a) A sample (1.9 ml) of a suspension of *Nostoc* sp. strain Mac (0.17 mg dry wt ml⁻¹) was placed in the electrode chamber and bubbled for 2 min with 1% CO₂ in air. The chamber was sealed and darkened and the rate of oxygen uptake was monitored for 10 min. The chamber was then illuminated with dim light and the new rate of oxygen exchange was measured; glucose was added in 10 µl Cg10 (5.6 mM final concentration) and the rate of oxygen exchange was again measured. (b) The same procedure was followed except that glucose was first added to the culture in the dark, followed by illumination. All the rates are uncorrected except for electrode drift.

Treatment	Rate of O ₂ uptake [µmol h ⁻¹ (mg dry wt) ⁻¹]
(a) Endogenous rate (dark)	0.4
+ Dim light	0.3
+ Glucose	0.5
(b) Endogenous rate (dark)	0.4
+ Glucose	0.5
+ Dim light	0.5

Heterotrophic growth in dim light

The maximum dark growth rates obtained were only 25% of the photoautotrophic rate which suggested that there must be other rate-limiting steps during heterotrophic growth. The dark growth rates on glucose and fructose were stimulated to give generation times of 29 h (Fig. 3a) and 36 h (Fig. 3b), respectively, by a beam of light of insufficient intensity to support photoautotrophic growth. The growth rates were independent of sugar concentration between 56 and 1.4 mM and, furthermore, the presence of casein hydrolysate stimulated growth even further on either glucose or fructose to give generation times of 11 to 14 h. As in the dark experiments, there was no further increase in growth rate when both sugars were added together with casein hydrolysate. No growth was observed in dim light with other compounds such as acetate or glycerol.

The dim light beam was described physically using an ISCO model SR spectroradiometer; it was found that the emission was mainly in the red and far red regions of the spectrum, with zero output below 440 nm. The total energy in the beam was insufficient to compensate the respiratory rate of the alga [about 0.4 µmol oxygen uptake h⁻¹ (mg dry wt)⁻¹] by more than 25% (Table 3), and this was confirmed by a negligible rate of incorporation of ¹⁴CO₂ above the level of dark controls. Further characterization of the dim light response showed that the inhibitor of photosystem II, 3'-(3,4-dichlorophenyl)-1',1'-dimethylurea (DCMU), at a concentration (10 µM) which was sufficient to inhibit photoautotrophic growth, did not inhibit the stimulation of growth on glucose. However, DCMU did partially inhibit growth on fructose, extending the generation time from 36 to 50 h. Such data indicate that DCMU should be used with caution in studies concerned with photoheterotrophic growth in the blue-green algae. In both cases, the action of the light lay predominantly in the region of the spectrum where chlorophyll *a* absorbs (Fig. 4a, b).

Elemental analysis of cells

The chemical composition of an algal cell can be regarded as the overall product of its metabolism and can reflect stresses placed upon particular biosynthetic processes by environmental factors (Spoehr & Milner, 1949). There was little difference between the composition of the cells grown photoautotrophically and those grown heterotrophically (Table 4) which would indicate that even at very low growth rates the organism has a well balanced biosynthetic metabolism, which is reflected in a rather uniform carbohydrate, lipid and protein content. Furthermore, during heterotrophic growth the alga retained

Fig. 4. Growth curves in terms of absorbance of *Nostoc* sp. strain Mac grown on (a) glucose or (b) fructose as a function of the spectral region of the dim light. Glucose and fructose were added at 28 mM. $\circ$, Cg10+sugar in white light; $\triangle$, Cg10+sugar, and a Corning CS 2-64 cut-off filter, with transmission properties shown in (c, curve 1), placed over the window between the beam source and the growth tube; $\bullet$, Cg10+sugar and a Baird-Atomic band-pass optical filter, with transmission properties shown in (c, curve 2), placed over the window between the beam source and the growth tube. The initial beam intensity was approximately doubled to $65 \mu\text{W cm}^{-2}$ prior to placing the filter to correct for a 53% transmission at its peak.

Table 4. *Elemental analysis of Nostoc sp. strain Mac grown under photoautotrophic and heterotrophic conditions*

Nostoc sp. strain Mac was grown from a small inoculum to a density of about $0.4 \text{ mg dry wt ml}^{-1}$ under the various growth conditions. Several tubes were pooled, harvested by centrifuging and the pellets were rinsed once with filtered Cg10 and once with filtered distilled water. The cells were dried for 2 d at 50°C under vacuum over P_2O_5 and the samples were transferred to clean dry vials. Analyses were done by A. Bernhardt, Mikroanalytisches Laboratorium, 5231 Elbach über Ergelskirchen, Fritz-Pregl-Strasse 14-16, West Germany.

Growth conditions	C*	H*	N*	O*†	Ash	Carbo- hydrate‡	Lipid‡	Protein‡
Saturating light Air + 1% CO_2 KNO_3 (10 mM)	50.28	6.99	8.75	33.99	2.24	36.9	8.4	54.7
Dim light Glucose (28 mM) KNO_3 (10 mM)	50.03	7.01	7.93	35.03	3.05	41.2	9.2	49.6
Dark Glucose (28 mM) KNO_3 (10 mM)	49.63	7.06	7.81	35.50	2.00	42.4	8.8	48.8
Dark Glucose (28 mM) Casein hydrolysate (1 g l^{-1})	48.75	7.00	7.13	37.13	1.80	48.0	7.4	44.6
Dim light Fructose (28 mM) KNO_3 (10 mM)	49.78	7.09	7.85	35.28	1.40	41.7	9.2	49.1
Dark Fructose (28 mM) KNO_3 (10 mM)	49.17	6.98	8.63	35.22	3.01	40.2	5.9	53.9

* Calculated as percentages on an ash-free basis.

† Calculated by difference.

‡ Calculated using the procedure of Spoehr & Milner (1949).

Table 5. *Maximum rates of photosynthesis of Nostoc sp. strain Mac grown photoautotrophically and heterotrophically in the dark with nitrate as nitrogen source*

Nostoc sp. strain Mac was grown under various conditions to equivalent densities of about 0.17 mg dry wt ml⁻¹. Samples (1.9 ml) of cells were carefully transferred in darkness to the electrode chamber, bubbled with 1% CO₂ in air and then illuminated as described in Methods. Rates of photosynthesis are expressed as $\mu\text{mol O}_2$ produced h⁻¹ (mg dry wt)⁻¹.

Growth conditions	Rate of photosynthesis
Photoautotrophically grown with air + 1% (v/v) CO ₂	5.9
Heterotrophically grown on glucose (28 mM)	5.4
Heterotrophically grown on fructose (28 mM)	5.2

Table 6. *Efficiency of utilization of sugar carbon by Nostoc sp. strain Mac under various heterotrophic conditions*

Nostoc sp. strain Mac was grown in Cg10 medium with or without nitrate (10 mM) or NH₄Cl (2 mM), with or without casein hydrolysate (1 g l⁻¹), and with glucose or fructose (28 mM) as carbon source. The amounts of carbon assimilated were calculated from the dry wt of cells and the percentage of carbon (from the elemental analysis). Hexoses were determined as described in Methods.

Hexose sugar	Inorganic nitrogen	Organic nitrogen	Growth conditions	Generation time (h)	Sugar carbon utilized Carbon assimilated
Glucose	KNO ₃	None	Dark	48	2.5
	NH ₄ Cl	None	Dark	31	1.6
	KNO ₃	None	Dim light	28	1.8
	KNO ₃	Casein hydrolysate	Dark	24	1.4
	KNO ₃	Casein hydrolysate	Dim light	14	1.5
	None	Casein hydrolysate	Dark	23	1.5
Fructose	KNO ₃	None	Dark	144	4.9
	KNO ₃	None	Dim light	36	1.6
	KNO ₃	Casein hydrolysate	Dark	24	1.4
	KNO ₃	Casein hydrolysate	Dim light	11	1.6
	None	Casein hydrolysate	Dark	24	1.3

photosynthetic capacity which was immediately and fully manifested on returning to a high light intensity (Table 5).

Cell yield studies

With a knowledge of the composition of cells grown under heterotrophic conditions, the efficiency of cell synthesis can be obtained and expressed as the percentage of substrate carbon assimilated. Table 6 shows values obtained for *Nostoc* sp. strain Mac grown under the various heterotrophic conditions. To make the calculations, extracellular products were assumed to be negligible.

In most cases, the efficiency of assimilation of sugar carbon is similar both to values obtained for other heterotrophic micro-algae grown on minimal medium (Samejima & Myers, 1958) and to values obtained for heterotrophic bacteria (Payne, 1970). However, there are several salient points. First, the efficiency of assimilation of sugar carbon in dim light was similar to values obtained in total darkness, suggesting that the alga is processing sugar carbon by an oxidative mode of metabolism. This is in contrast to data obtained at higher light intensities with another blue-green alga, *Agmenellum quadruplicatum* (strain BG-1), where all of the organic substrate taken up was assimilated (Ingram *et al.*, 1973). Second, the lowest efficiencies were obtained during growth with nitrate as nitrogen source

in the dark, and, since the efficiency increased when nitrate was replaced by ammonium, this is in accord with the energy demand for the reduction of nitrate to ammonia. However, in the case of fructose, the 20 % efficiency indicates that oxidation is proceeding uncoupled from biosynthesis, and suggests that biosynthesis is being reduced to satisfy the maintenance requirements at this low growth rate. Third, approximate calculations from the growth rates and yield data indicated that the rate of hexose utilization is fairly constant under most heterotrophic conditions, except for the high growth rates obtained with a combination of casein hydrolysate and dim light. It remains to be seen how the growth stimulating factors of casein hydrolysate and dim light control the partitioning of sugar carbon between anabolic and catabolic pathways in this blue-green alga.

DISCUSSION

The data presented here confirm and extend the earlier observations that *Nostoc* sp. strain Mac is a *bona fide* heterotrophic blue-green alga (Hoare *et al.*, 1971). The cell composition and the efficiency of cell synthesis data attest to the 'soundness' of heterotrophic metabolism in this organism and suggest, in the case of glucose, that growth is limited on the nitrogen side of metabolism. Furthermore, the observations are in agreement with data of other workers which suggest that the respiratory systems of blue-green algae capable of heterotrophic growth are efficiently coupled, though probably of limited capacity (Padan *et al.*, 1971; Pelroy & Bassham, 1973; Ginzberg *et al.*, 1976). However, in the case of fructose, since there was a concentration limit below which the alga scarcely grew, and ammonium nitrogen did not increase the growth rate, the data suggested that growth was limited on the carbon side of metabolism. The low efficiency of cell synthesis on fructose/nitrate medium is in accord with the alga having limited ability to transport fructose, the major portion of which is then oxidized to provide maintenance energy at these low growth rates (Stouthamer & Bettenhausen, 1973).

The ability of casein hydrolysate to stimulate growth on fructose as much as 15-fold and also to act as the bulk nitrogen source for growth is evidence that *Nostoc* sp. strain Mac may respond to heterotrophic conditions in a manner not seen previously in the blue-green algae. In contrast, the heterotrophic unicellular blue-green alga *Aphanocapsa* sp. strain 6714 is less flexible in its heterotrophic capabilities (Stanier *et al.*, 1971). The alga grows on glucose in the dark, as observed by others (Rippka, 1972). However, in contrast to *Nostoc* sp. strain Mac, we have observed that dim light stimulated this rate by only 20 % and the efficiencies of cell synthesis were similar under both conditions (40 and 43 % respectively). Furthermore, casein hydrolysate did not stimulate growth on glucose either in the dark or in dim light and there was no growth on fructose under any of the heterotrophic growth conditions. This comparative data suggests that the blue-green algae capable of making the autotrophy-heterotrophy transition have adapted in different ways.

The ability of dim light to stimulate heterotrophic growth in *Nostoc* sp. strain Mac supports previous observations made with two other blue-green algae, *Lyngbya lagerheimii* strain Montauk and *Agmenellum quadruplicatum* strain PR-6, both of which were incapable of dark growth (Van Baalen, Hoare & Brandt, 1971), and data of Hoare *et al.* (1971) with strain Mac, albeit at a higher light intensity. The data reported here show that the action of dim light lies in the region of the visible spectrum where chlorophyll *a* absorbs. This is different from the report of stimulation of growth of *Chlorella vulgaris* Beyerinck (Emerson's strain) in dim light, where the action was in the blue region of the spectrum suggesting that a porphyrin was involved (Karlander & Krauss, 1966). Several light-stimulated reactions of the blue-green algae are known to have chlorophyll *a*-type action spectra. These include nitrate reduction (Stevens & Van Baalen, 1973), acetylene reduction (Fay, 1970) and photoinhibition of respiration (Hoch *et al.*, 1963; Jones & Myers, 1963). Furthermore, the role of chlorophyll *a* in these phenomena has not always been attributable

to ATP production. Obviously, further studies need to be done to enhance our knowledge of the effects of light in the microalgae at levels below the compensation point, which could have important ecological implications.

P.J.B. acknowledges receipt of an N.E.R.C. overseas post-doctoral fellowship during the period of this work. We thank Mrs R. O'Donnell for excellent technical assistance and Professor Jack Myers, Dept of Zoology, University of Texas at Austin, for loan of the Baird-Atomic band-pass filter. University of Texas Marine Science Institute Contribution No. 284.

REFERENCES

- ASHWELL, G. (1957). Colorimetric analysis of sugars. *Methods in Enzymology* **III**, 73–105.
- BOWYER, J. W. & SKERMAN, V. B. D. (1968). Production of axenic cultures of soil-borne and endophytic blue-green algae. *Journal of General Microbiology* **54**, 299–306.
- FAY, P. (1965). Heterotrophy and nitrogen fixation in *Chlorogloea fritschii*. *Journal of General Microbiology* **39**, 11–20.
- FAY, P. (1970). Photostimulation of nitrogen fixation in *Anabaena cylindrica*. *Biochimica et biophysica acta* **216**, 353–356.
- GINZBERG, D., PADAN, E. & SHILO, M. (1976). Metabolic aspects of LPP cyanophage replication in the cyanobacterium *Plectonema boryanum*. *Biochimica et biophysica acta* **423**, 440–449.
- HOARE, D. S., INGRAM, L. O., THURSTON, E. L. & WALKUP, R. (1971). Dark heterotrophic growth of an endophytic blue-green alga. *Archiv für Mikrobiologie* **78**, 310–321.
- HOCH, G., OWENS, O. H. & KOK, B. (1963). Photosynthesis and respiration. *Archives of Biochemistry and Biophysics* **101**, 171–180.
- INGRAM, L. O., VAN BAALEN, C. & CALDER, J. A. (1973). Role of reduced exogenous organic compounds in the physiology of blue-green bacteria (algae): photoheterotrophic growth of an 'autotrophic' blue-green bacterium. *Journal of Bacteriology* **114**, 701–705.
- JONES, L. W. & MYERS, J. (1963). A common link between photosynthesis and respiration in a blue-green alga. *Nature, London* **199**, 670–672.
- KARLANDER, E. P. & KRAUSS, R. W. (1966). Responses of heterotrophic cultures of *Chlorella vulgaris* Beyerinck to darkness and light. II. Action spectrum for and mechanism of the light requirement for heterotrophic growth. *Plant Physiology, Lancaster* **41**, 7–14.
- MYERS, J. (1950). The culturing of algae for physiological research. In *The Culturing of Algae*, pp. 45–51. Edited by J. Brunel, G. W. Prescott & L. H. Tiffany. Yellow Springs, Ohio: Charles F. Kettering Foundation.
- PADAN, E., RABOY, B. & SHILO, M. (1971). Endogenous dark respiration of the blue-green alga, *Plectonema boryanum*. *Journal of Bacteriology* **106**, 45–50.
- PAYNE, W. J. (1970). Energy yields and growth of heterotrophs. *Annual Review of Microbiology* **24**, 17–72.
- PELROY, R. A. & BASSHAM, J. A. (1973). Efficiency of energy conversion of aerobic glucose metabolism in *Aphanocapsa* 6714. *Journal of Bacteriology* **115**, 937–942.
- PULICH, W. M. & VAN BAALEN, C. (1973). Pyridine nucleotide-dependent glucose dehydrogenase activity in blue-green algae. *Journal of Bacteriology* **114**, 28–33.
- RIPPKA, R. (1972). Photoheterotrophy and chemoheterotrophy among unicellular blue-green algae. *Archiv für Mikrobiologie* **87**, 93–98.
- SAMEJIMA, H. & MYERS, J. (1958). On the heterotrophic growth of *Chlorella pyrenoidosa*. *Journal of General Microbiology* **18**, 107–117.
- SPOEHR, H. A. & MILNER, H. (1949). The chemical composition of *Chlorella*: effect of environmental conditions. *Plant Physiology, Lancaster* **24**, 120–149.
- STANIER, R. Y., KUNISAWA, R., MANDEL, M. & COHEN-BAZIRE, G. (1971). Purification and properties of unicellular blue-green algae (Order Chroococcales). *Bacteriological Reviews* **35**, 171–205.
- STEVENS, S. E. & VAN BAALEN, C. (1973). Characteristics of nitrate reduction in a mutant of the blue-green alga *Agmenellum quadruplicatum*. *Plant Physiology, Lancaster* **51**, 350–356.
- STOUTHAMER, A. H. & BETTENHAUSEN, C. (1973). Utilization of energy for growth and maintenance in continuous and batch cultures of microorganisms. *Biochimica et biophysica acta* **301**, 53–70.
- VAN BAALEN, C. (1967). Further observations on growth of single cells of coccoid blue-green algae. *Journal of Phycology* **3**, 154–157.
- VAN BAALEN, C., HOARE, D. S. & BRANDT, E. (1971). Heterotrophic growth of blue-green algae in dim light. *Journal of Bacteriology* **105**, 685–689.
- WATANABE, A. & YAMAMOTO, Y. (1967). Heterotrophic nitrogen fixation by the blue-green alga *Anabaenopsis circularis*. *Nature, London* **214**, 738.
- WHITE, A. W. & SHILO, M. (1975). Heterotrophic growth of the filamentous blue-green alga *Plectonema boryanum*. *Archives of Microbiology* **102**, 123–127.
- WOLK, C. P. & SHAFFER, P. W. (1976). Heterotrophic micro and macro cultures of a nitrogen fixing cyanobacterium. *Archives of Microbiology* **110**, 145–147.