

Release of Organic Carbon by Cells of *Microcystis aeruginosa* (Cyanobacteria) in Culture under Different Irradiances.

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ABSTRACT: Release of organic carbon by cells of *Microcystis aeruginosa* in cultures under different irradiances. *Microcystis aeruginosa* is, nowadays, one of the most studied cyanobacteria due to its potential of toxic bloom formation and production of great quantities of organic matter in the water column. Once a bloom is formed, cells of *M. aeruginosa*, due to the presence of gas vesicles, can float reaching the water surface, becoming exposed for long periods to high irradiances. This exposure can injure the cells, inducing the release of dissolved organic matter (DOM), this harm being even more accentuated with aging of the cells. The aim of this work was to investigate the effect of exposure to different irradiances in cells of *M. aeruginosa*, isolated from Barra Bonita Reservoir, Tietê River - SP. The relationship between the photosynthetic rate and light intensities were obtained for cultures at two ages (8 and 20 days), using the ¹⁴C method. Also, the rates of total fixation and release (absolute and relative) of carbon, and analysis of released carbohydrates and molecular weight distribution of DOM as a function of the irradiances were performed. Carbon fixation by *M. aeruginosa* as a function of the irradiances were quantified (as DPM) showing that there was no photoinhibition, even when the cells were exposed to high irradiances as 2000 $\mu\text{mol m}^{-2}\text{s}^{-1}$. The rates of DOM released, even in the older culture cells, were surprisingly low and directly proportional to the photosynthesis rates in all irradiances tested. Older cells released higher quantities of high-molecular-weight compounds, including carbohydrates, whereas the younger ones released low-molecular-weight compounds, including carbohydrates. Chlorophyll-a concentrations indicated a slight photooxidation or photoacclimatation in the cells at the beginning of the stationary growth phase of the culture.

Key-words: *Microcystis aeruginosa*, irradiance, fixation and release of carbon, extracellular carbohydrates.

RESUMO: Liberação de carbono orgânico por células de *Microcystis aeruginosa* em cultura sob diferentes irradiâncias. *Microcystis aeruginosa* é, atualmente, uma das cianobactérias mais investigadas devido ao seu potencial de formação de florescimentos tóxicos e à produção de grandes quantidades de matéria orgânica na coluna d'água. Uma vez formado o florescimento, as células de *M. aeruginosa*, devido ao fato de poderem produzir vesículas de gás, podem flutuar e se posicionar à superfície e conseqüentemente ficar expostas por longos períodos a altas intensidades de luz, o que pode causar danos às células fazendo-as liberar matéria orgânica dissolvida (MOD). Esses danos tornam-se mais acentuados com o envelhecimento das células. O objetivo deste trabalho foi estudar o efeito da exposição a diferentes irradiâncias de células de culturas em fase exponencial e início da fase estacionária do crescimento (8 e 20 dias) de *Microcystis aeruginosa*, isolada do Reservatório de Barra Bonita, Rio Tietê, SP. A metodologia utilizada foi a do ¹⁴C, quantificando-se também as taxas de fixação de carbono total (em DPM) e de excreção relativa e absoluta, análises de carboidratos liberados e distribuição de massa molecular da MOD liberada em função da irradiância. Medidas de assimilação de carbono em função da irradiância não evidenciaram a ocorrência do fenômeno da fotoinibição, mesmo quando as células foram expostas a 2000 $\mu\text{mol m}^{-2}\text{s}^{-1}$. As taxas de excreção de MOD, mesmo nas células da cultura mais velhas, foram surpreendentemente baixas e diretamente

proporcionais às taxas de fotossíntese em todas as irradiâncias testadas. Entretanto, células da cultura mais velha liberaram maiores quantidades de compostos de alta massa molecular, incluindo carboidratos, enquanto as da cultura mais nova liberaram compostos de baixa massa molecular, incluindo carboidratos. O decréscimo na concentração de clorofila-a fornece indícios de ligeira fotooxidação ou fotoaclimatação em células no início da fase estacionária.

Palavras-chave: *Microcystis aeruginosa*, irradiância, fixação e liberação de carbono, carboidratos extracelulares.

Introduction

Microcystis aeruginosa is, nowadays, one of the main microalgae, or cyanobacteria, investigated as shown by phycological and sanitation literature, and even newspapers due to the problems caused by its toxic blooms. They are also in focus due to the fact that besides being very common in eutrophic and hypereutrophic waters throughout the world, they can cause serious problems due to its toxins which under certain circumstances can be lethal to people (Jochimsen et al., 1999). There are, therefore, considerable amounts of ecological and physiological data on this species. In fact, there are many works dealing with the effect of some environmental factors like irradiance, temperature and nutrients, which can influence the development of blooms, cell fluctuation, photosynthesis and other aspects of growth (Paerl, 1983; Paerl et al., 1985; Ibelings, 1992; Ibelings & De Winder, 1994; Klemmer et al., 1996; Brookes & Ganf, 2001; Brookes et al., 2003).

Cells at the beginning of bloom formation in the lower portions of the euphotic column and which were also adapted to low irradiances can quickly be exposed to high irradiances, due to the presence of gas vesicles, which allow them to float (Fogg, 1969) and reach the water surface. The sudden exposure of the phytoplanktonic organisms to irradiances higher than those they are adapted to, can lead to the phenomenon of photoinhibition (Powles, 1984; Ibelings, 1996). This would be more critical in tropical and sub-tropical areas, during bright days, both in winter and summer, when the cells can be exposed to irradiances reaching up to 2000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for several hours. In other words, a chronic photoinhibition can be settled up, injuring the cells (Ibelings, 1996). The high irradiances can cause irreversible photooxidation which might be involved in the sudden die-off observed in cyanobacterial blooms in nature (Abeliovich & Shilo, 1972; Eloff et al., 1976). Considering that the biomass of the blooms of *M. aeruginosa* is usually great, the release of DOC will be large, despite the irreversibility of injuries caused by photooxidation. This means a great potential of disturbance of physical and chemical properties of water, yet it also means that if the waterbloom is toxic, toxins will be directly released in the water (Charmichel, 1993).

Taking into consideration that the blooms of *M. aeruginosa* are ubiquitous and very common in eutrophic environments of tropical areas, the aim of this work was to verify if the cells of *M. aeruginosa* undergo damage which induces them to release dissolved organic matter when exposed to high irradiances such as those found during bright days.

Material and methods

Microcystis aeruginosa Kütz. (Cyanophyceae) was isolated (strain BB012) from the eutrophic Barra Bonita Reservoir (20° 31'S; 48° 32'W), formed mainly by the rivers Tietê and Piracicaba. Tietê River receives a large part of São Paulo City waste waters. This cyanobacteria is maintained at the Freshwater Microalgae Culture collection (WDCM 785) at the Botany Department of the Federal University of São Carlos. Stock batch cultures were cultivated in glass carboys of 2.5 liters using medium ASM-1 (Gorham et al., 1964) at $23 \pm 1^\circ \text{C}$. Cells were growing up in a unicellular way, not gathered in colonies. Stock batch cultures were kept under continuous aeration by gentle bubbling with filtered and moist air. Irradiance (E) of 150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ was provided by 40 W fluorescent tubes

in a 12:12 h light-dark cycle. The cultures were not axenic but bacterial contamination monitored by ASM-1 medium plus peptone and glucose added at concentrations of 250 mg l⁻¹ showed that the bacterial abundance was always very low.

Experimental procedure

An exponentially growing culture (8 days old) and other at the beginning of stationary growth phase (20 days old) were used in the experiments in which the cells were exposed to different irradiances. The values of irradiance were chosen based on the irradiance values frequently found in Barra Bonita Reservoir during shining summer days (without clouds): 1800 to 2050 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. In these conditions in the euphotic column of Barra Bonita reservoir 1.0 % of the incident light on the surface is usually found at 5.0 m depth. Nine irradiances levels (0, 65, 135, 200, 300, 530, 760, 1200, and 2000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) were obtained by combining neutral black cloth filters placed between the incubated flasks and the light source. The cells were exposed to a light source of 500 W halogen lamps, in 70 ml fulfilled flasks which were submerged in a running water system at a temperature of 23°C, stabilized using a thermostatic bath. The experiment was carried out in a glass device with nine interconnected boxes, one for each irradiance value. To each irradiance, 220 ml of *M. aeruginosa* culture at a specific age were inoculated with $\text{NaH}^{14}\text{CO}_3$ (10 mCi/250 ml) and then subdivided into three replicate-flasks of incubation. The flasks were incubated during four hours from 10 a.m. to 2 p.m. and shaken by hand every 15 minutes. After incubation, 10 ml (two replicates) of culture from each flask were filtered on 47 mm diameter and 0.45 μm pore size acetate membranes (Sartorius) at a low vacuum pressure (24 cm Hg). The filtrates were acidified to pH 3.0, bubbled with filtered air for 40 minutes to eliminate inorganic ¹⁴C, and then adjusted to pH 7.0. The membrane filters and 8 ml of each filtrate (three replicates) were placed into glass vials with 10 ml of liquid scintillation cocktail to quantify particulate labeled organic carbon (PO¹⁴C) and dissolved labelled organic carbon (DO¹⁴C), respectively. Different cocktails were employed for each type of sample: toluene + Renex-95 $\ddot{\text{a}}$ (Atlas Corporation) (7:6 v/v) + 150 mg l⁻¹ dimethyl POPOP (1,4-bis[2-(4-methyl-5-phenyloxazole)]-benzene) + 3.0 g l⁻¹ PPO (2.5 diphenyloxazole) for the aqueous samples and toluene + Renex-95 (2:1 v/v) + 150 mg l⁻¹ dimethyl POPOP + 3.0 g l⁻¹ PPO for the filters. The use of different Renex-95/toluene mixtures was required because 7:6 v/v results in a more stable emulsion, making it more effective for aqueous solutions. Renex-95 is a nonyl-phenol with an average number of nine ethylene oxide units molecule⁻¹. Sample radioactivity was determined using a Packard (Downers Grove, Illinois) Tricarb 1550 liquid scintillation counter. Absolute counting efficiency was determined by automatic external standard channels ratio quench correction, as the composition of samples was constant. Such standardization is performed by the scintillation counter through γ -irradiation of the samples by an external source (¹³³Ba, 20 mCi) followed by an automatic counting efficiency determination. Chlorophyll-a concentration was determined spectrophotometrically according to the method described in Talling & Driver (1963) using 90% acetone for extraction at temperature of -5°C. A saturating irradiance (E_k) was calculated as described by Talling (1957).

Molecular weight fractionation

Filtrates (40 ml) were concentrated four times under vacuum using a Büchi Mod 461 rotary evaporator at 40°C and submitted to gel filtration for molecular weight fractionation in a Frac 200 (Pharmacia, Uppsala, Sweden). Samples of concentrated filtrates (5 ml) were loaded onto a Sephadex G-100 (Pharmacia, Uppsala, Sweden) gel filtration column (69 x 1.5 cm). The samples were eluted with 1-butanol:water (1:50, v/v) using a flow of 12 ml h⁻¹, and fractions of chromatographed samples (2.0 ml) were collected to determine the radioactivity (DPM) and carbohydrate content by the method of Dubois et al. (1956).

Nomenclature and definitions of photosynthetic parameters are according to Sakshaug et al. (1997). Hence, the former I_k , light saturation parameter ($(I_k - P_{\text{max}}) / a = I(d_{\text{isH}} - t)$), here is represented

by E_k ; the former PFD, photon flux density, by E = irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Other parameters cited are: $k_m = P_{\text{max}}/2$; $P_{\text{max}} = \text{mgC}_{(a)} \text{L}^{-1} \text{h}^{-1}$; $P_{\text{max}}^* = \text{mgC}_{(a)} \text{mg chl}^{-1} \text{h}^{-1}$ (a) = DPM in this work).

Results

Fig. 1 and 2 show the total ^{14}C fixation (in DPM), under different irradiances, by the exponentially growing culture (named 8 days old culture) and in the beginning of stationary growth phase (named 20 days old culture) of *M. aeruginosa*, respectively, without any sign of occurrence of photoinhibition, even under irradiances as high as $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. For the 8 and 20 days old cultures, the values of E_k (saturant irradiance) were $507 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and $464 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and the irradiances at K_m were $208 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and $204 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively. High E_k values found in this work are consistent with those obtained by Paerl et al. (1985) for cultures and natural populations of *M. aeruginosa*.

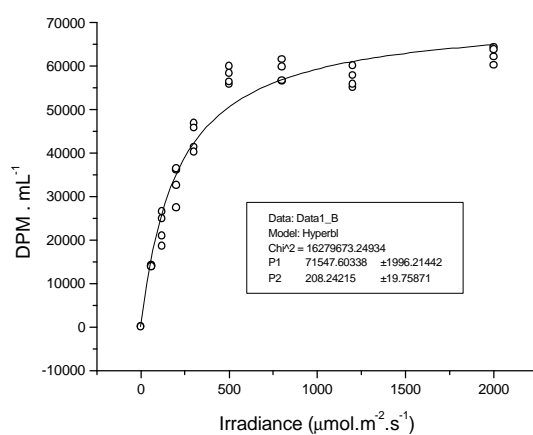


Figure 1: Total photosynthetic assimilation of ^{14}C (DPM mL^{-1}) by cells of *M. aeruginosa* at exponential growth phase culture (8 days), after 4 hours of incubation as a function of the irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$).

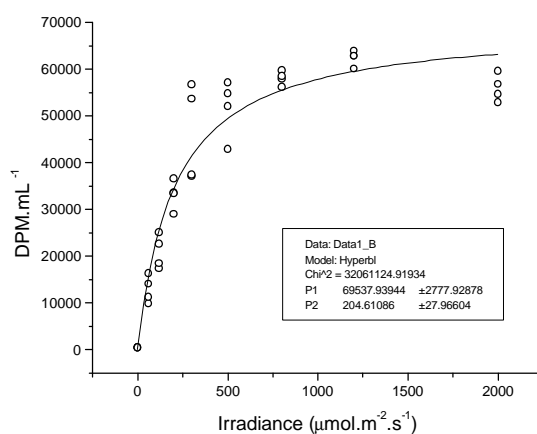


Figure 2: Total photosynthetic assimilation of ^{14}C (DPM mL^{-1}) by cells of *M. aeruginosa* at stationary growth phase culture (20 days), after 4 hours of incubation as a function of the irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$).

In the 8 days old culture, the initial chlorophyll-a concentration (the same found in cells exposed to darkness) did not vary (0.1471 ANOVA) in any irradiance tested during the four-hour period of exposure (Fig. 3). However, a 14.5% decrease in the initial chlorophyll-a concentration (0.41888 mg l⁻¹, 0.0011 ANOVA) in 20 days old culture exposed to 1200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and 29.5% at 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ are indications that these cells were under light stress. When the values of the particulate fraction of the fixed ¹⁴C (more than 99% of total fixation) are normalized to the chlorophyll-a data, the photosynthesis rate of cells of the 20 days old culture were reduced to around 19% of that obtained for 8 days old culture. Also, the P*_{max} was 255,1 and 48 DPM mg chlorophyll⁻¹ h⁻¹ to 8 and 20 days old cultures, respectively (Tab. 1). Since this reduction occurs at all irradiances we can deduce that this reduction due to the effect of aging.

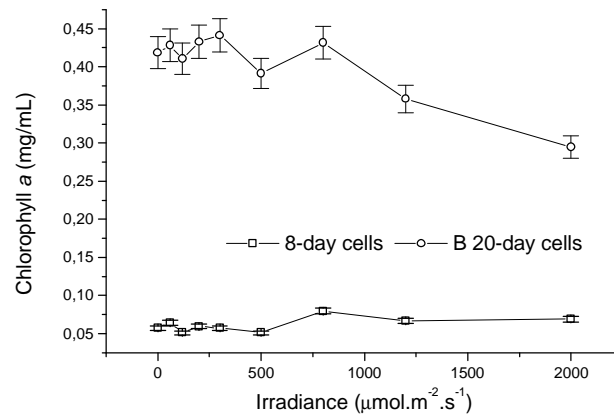


Figure 3: Chlorophyll-a concentration (mg ml⁻¹) of cells of *M. aeruginosa* at exponential growth phase (8 days), and stationary growth phase (20 days) cultures, after 4 hours of exposition to different irradiances ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$).

Table 1: Values of tested irradiance (E = $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$); total fixed labeled organic ¹⁴C; particulate labeled organic ¹⁴C (PO¹⁴C); released dissolved organic ¹⁴C normalized to chlorophyll (DO¹⁴C = DPM /mg chlorophyll); relative amounts of released DO¹⁴C (%); and P*_{max} obtained at 2000 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ (DPM of DO¹⁴C.mg chlorophyll⁻¹.h⁻¹).

E	Total ¹⁴ C		PO ¹⁴ C		DO ¹⁴ C.mg chl ⁻¹ .h ⁻¹		%		P*max	
	8-day cells	20-day cells	8-day cells	20-day cells	8-day cells	20-day cells	8-day cells	20-day cells	8-day cells	20-day cells
2000	62978	56466	62694	56032	4599,7	1469,7	0.45	0.76	255	48
1200	57583	62938	57316	62442	4322,7	1383,5	0.46	0.78		
800	59129	58519	58724	58109	6555,9	948,8	0.68	0.70		
500	58096	52108	57747	51741	5656,2	938,3	0.60	0.70		
300	44054	46487	43682	46257	6023,5	521,7	0.84	0.49		
200	33506	33331	33251	33169	4117,5	372,1	0.75	0.48		
120	23088	21075	22869	20947	3545,6	312,1	0.94	0.60		
60	14323	13070	14154	12934	2730,8	318,6	1.17	1.04		
0	174	508	159	485	245,3	53,6	8.68	4.41		

The quantity of DO¹⁴C released by the cells of both ages was surprisingly low in all irradiances, not exceeding 1% of the total fixed ¹⁴C (Fig. 4 and Tab. 1). When the values of DOC ¹⁴C are normalized to the chlorophyll-a data (Fig. 4b), they show to be approximately proportional to the total assimilation. However, an increase in relative and absolute quantities of DOM ¹⁴C released by 20 days old culture at high irradiances also may indicate that cells could have been under light stress. The high values of relative quantities of DOM ¹⁴C released found in the samples that were kept in the darkness

and at 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ are due to very low rate of total assimilation, increasing the relative values of the very low absolute values of background DPM.

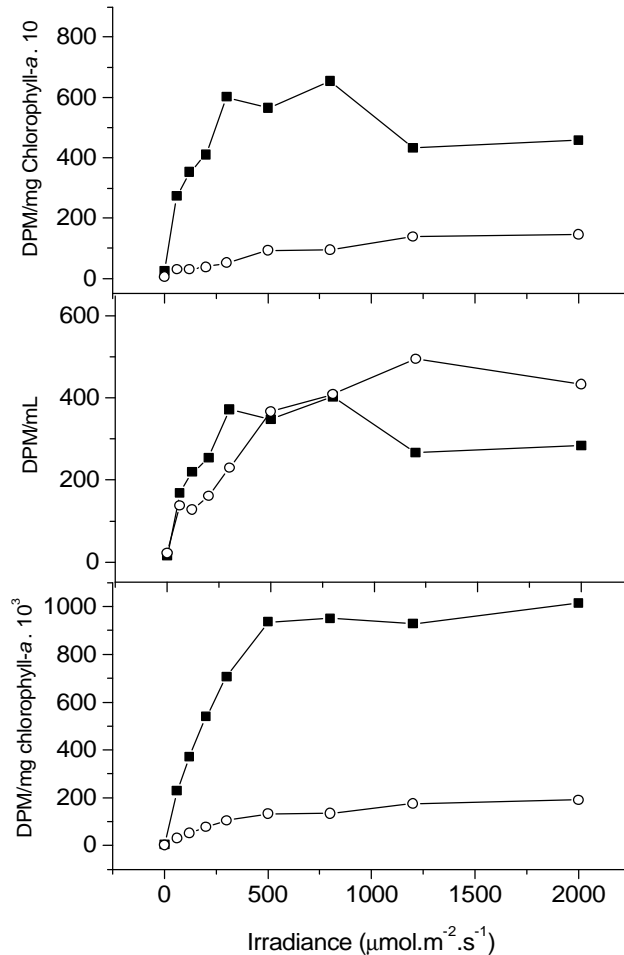


Figure 4: a) Released dissolved organic carbon in DPM normalized to chlorophyll-a (DPM.mg chlorophyll-a⁻¹); b) Absolute dissolved organic carbon (DPM ml⁻¹); c) Particulate carbon assimilation normalized to chlorophyll-a (DPM mg chlorophyll⁻¹.a).

The resulting fractions from the molecular weight fractionation of DO¹⁴C released by the 20 days old culture, when quantified by radioactivity (DPM) (Fig. 5a, b), show high-molecular-weight compounds released next to the void volume (³100 kd) (ve/vt = 0.27) with the highest concentrations under higher irradiances (500 - 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Inversely, in the 8 days old culture (Fig. 6a) under high irradiances, the low-molecular-weight compounds were predominant (ve/vt = 0.73).

The phenol-sulfuric acid method (Dubois et al., 1956), which was applied to all fractions, shows the occurrence of carbohydrates in both the high-molecular-weight fraction and in the low-molecular-weight one at both ages (Fig. 6a and b). However, in the matter released by the 20 days old culture, the high-molecular-weight carbohydrates were predominant (ve/vt = 0.27) in irradiances from 200 to 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, while in 8 days old culture the low-molecular-weight carbohydrates were predominant (ve/vt = 0.73) under all irradiances, in a similar pattern obtained with the DO¹⁴C measurements. Moreover, in the 20 days old culture exposed to high irradiances, the high-molecular-weight carbohydrates predominated, while in the 8 days old culture, the low-molecular-weight ones were predominant.

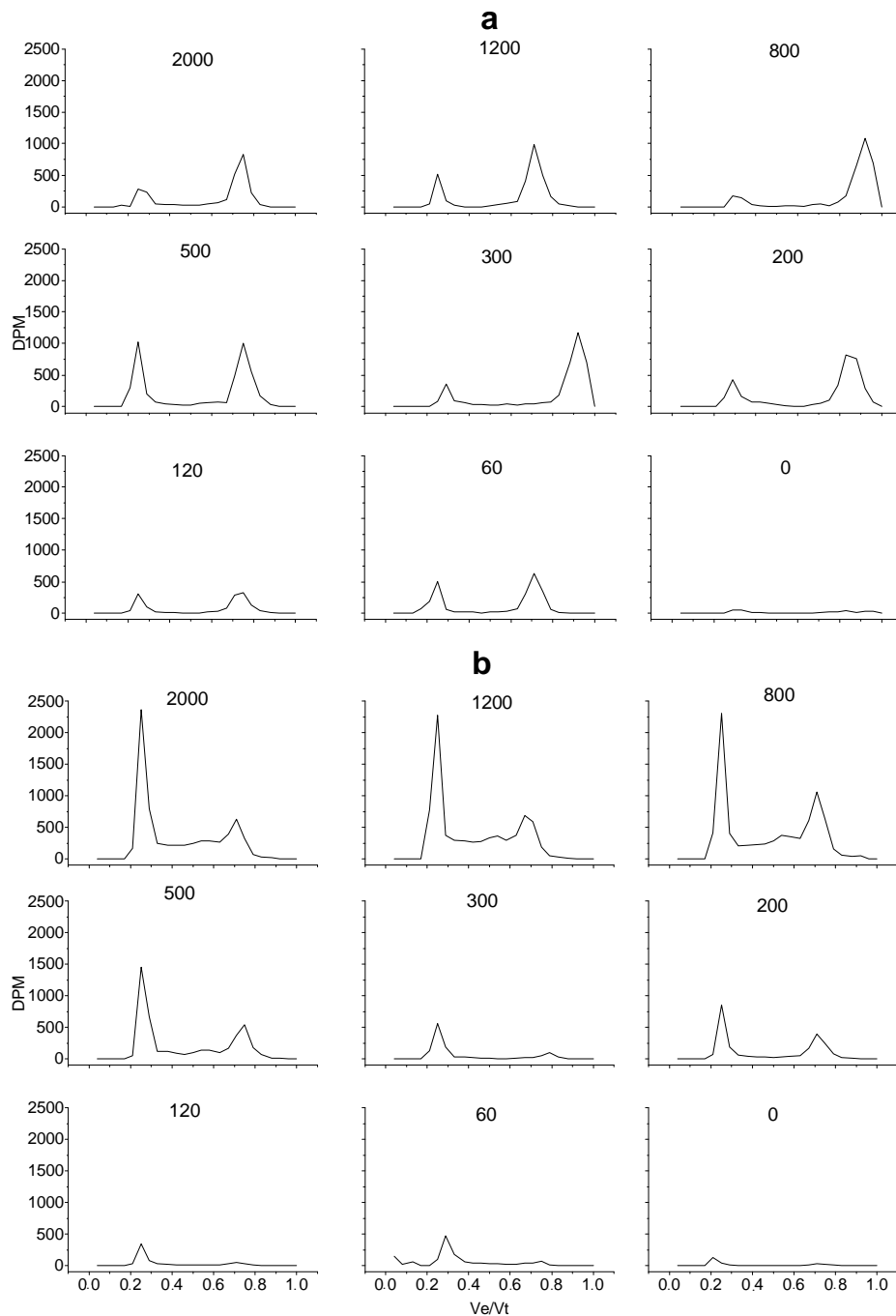


Figure 5: Radioactivity (DPM ml^{-1}) of eluted fractions (2.0 ml) of concentrated DO^{14}C released after 4 hours of exposition to different irradiances. The fractions were obtained by permeation in Sephadex G-100 gel column (69 x 1.5 cm). Elution (12 ml h^{-1}) was carried out on 1-butanol: water (1: 50 v/v). a) exponential growth phase (8 days); b) stationary growth phase (20 days).

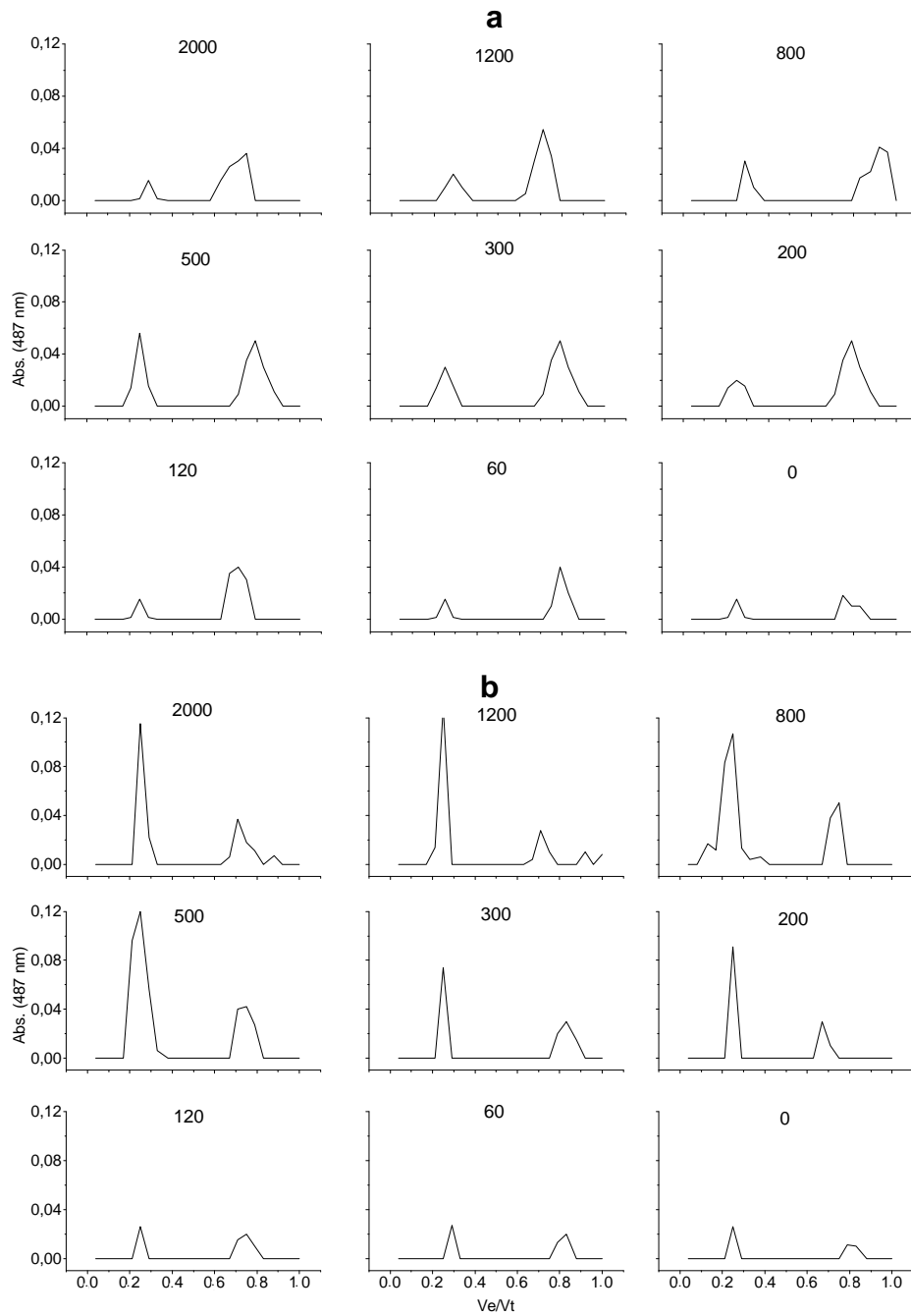


Figure 6: Carbohydrate (absorbance at 487 nm after phenol-sulfuric reaction) on eluted fractions (2.0 mL) of concentrated $DO^{14}C$ released after 4 hours of exposition to different irradiances. The fractions were obtained by permeation in Sephadex G-100 gel column (69 x 1.5 cm). Elution (12 ml h^{-1}) was carried out on 1-butanol: water (1: 50 v/v). a) exponential growth phase (8 days); b) stationary growth phase (20 days).

Discussion

The highest values of P^*_{\max} obtained at 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and the fact that there was neither a photoinhibition on the carbon fixation nor a reduction of the chlorophyll-a concentration at that irradiance in the 8 days culture indicate that this strain of *M. aeruginosa* isolated from a tropical hypereutrophic reservoir show a good tolerance to high irradiances. In fact, in Barra Bonita Reservoir the irradiance reaching the water surface is extremely high both in the summer and in the winter when characteristically bright skies occur, without clouds, for many consecutive weeks. Light intensities under these conditions can easily reach around 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ on the surface water at noon. However, natural populations of *M. aeruginosa* from non-tropical environments show, as well, high tolerances to high irradiances without the occurrence of photoinhibition (Eloff et al., 1976; Paerl et al., 1985). On the other hand, the same authors mention that populations of *M. aeruginosa* isolated and kept in culture show indications of photoinhibition and even destruction of the cells caused by photooxidation phenomena, what did not happen with our culture strains. In fact, the deep decrease in the fixation of carbon by 20 days old culture at all of the irradiances tested, despite the fact that there was no photoinhibition under 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, would be more related to the cell age than the effects of the stress caused by the high irradiances. Nevertheless, the decrease in the chlorophyll-a concentrations at the early stationary growth (20 days), and the increase in the DO^{14}C release are indications that with cell aging, the cells undergo some kind of stress triggered by high irradiances. It is well known that exposure to high irradiances in the surface water causes photooxidation of chlorophyll-a in strains of *Microcystis* in culture (Eloff et al., 1976). However, a decrease in the chlorophyll-a concentration at higher irradiances can also mean a photoacclimatation rather than photooxidation (Geider et al., 1986).

The increase in DO^{14}C release under high irradiances (800, 1200 e 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) by the culture at early stationary growth, however, was due to the increase in the release of high-molecular-weight compounds, which did not happen for the 8 days old culture. Analysis of these compounds showed that they are high-molecular-weight carbohydrates, certainly colloidal polysaccharides, because of the similarity between their v_e/v_t and that one obtained from Blue Dextran (2,000 kd) used as reference. The increase in the extracellular polysaccharides release in several species of microalgae normally occurs when some factors, like the cell age, nutrient limitation or other, which cause stress, inhibits the cytokinesis but not the carbon fixation. The increase in the rates of extracellular polysaccharides production and its release would be a mechanism of dumping the excess carbon of the cell (Angelis et al., 1993; Sutherland, et al., 1998; Staats, et al., 2000).

As in cells of 20 days the carbohydrate release is predominant even under 200, 300 e 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, the cause for the release of these compounds can be the fact that the cells were on the stationary growth phase instead of high irradiances effects, a common physiological feature to several microalgae taxa and already well-described in the literature (Myklestad, 1974; Vieira & Myklestad, 1986; Sutherland et al., 1998; Giroldo & Vieira, 1998). Moreover, the low rates of release in both ages and the uniformity of the peaks corresponding to the low-molecular-weight compounds concentration in both ages, are indications that the cells exposed to high irradiances for four hours, even in the beginning of the stationary growth phase, did not suffer damages that triggered the organic matter release. This kind of behavior – low rates of excretion and absence of photoinhibition – was also found in non-blue-green algae species *Synura petersenii* (Vieira et al., 1998) and *Peridinium willei* (Vieira et al., 2002), both from small shallow tropical lakes.

However, the carbon release as extracellular polysaccharides by cells of 20 days old culture could show interesting points in the buoyancy mechanism of *M. aeruginosa*. Besides there is still much to be learned, the buoyancy/sink mechanism of *M. aeruginosa*

cells is, in summary, based on the synthesis of gas vesicles that lead to positive buoyancy, and the accumulation of cellular carbohydrate, which increases the cell density producing negative buoyancy (Kromkamp & Mur, 1984).

In N-limited and optimal light conditions environments, cells of *M. aeruginosa* suffer a dilution in the volume of the gas vesicles and an increase in the carbohydrates content (Brookes & Ganf, 2001). Light limitation has an opposite effect to the nutrient limitation because when light is in low supply, gas vesicles tend to accumulate (Deacon & Walsby, 1990). Cells of *M. aeruginosa* in N-limited water but in optimal conditions of light keep the photosynthesis process. However, in these situations, the products of this process are changed to N-poor and C-rich compounds, the carbohydrates. The accumulation of soluble carbohydrates will contribute to both cell turgor and ballast, whereas insoluble forms of carbohydrate like starch only contribute to ballast, increasing the cell density but also lead to a dilution of gas vesicles if it causes growth in cell volume. In *M. aeruginosa* the loss of buoyancy is more due to increase in carbohydrate than to loss of gas vesicles by cell turgor (Kromkamp et al., 1988). With more ballast, the cells sink to scavenge nutrients in the deeper portions of the euphotic column, rich in nutrients but light-limited.

The fact that cells of *M. aeruginosa*, and others, can perform vertical migrations is advantageous when the water on the upper euphotic column are nutrient depleted. However, in eutrophic or hypereutrophic mixed environments like Barra Bonita Reservoir, the nutrients, mainly nitrate and phosphate and, naturally, inorganic carbon, might never be in severe limitation. It follows that abundant nutrient should promote a positive buoyancy for cells in a good physiological condition and optimal growth. In this condition, cells would not form ballast, which is important to their growth, tending to keep them on the water surface. Although cells did not accumulate carbohydrates enough to negate buoyancy, the gas vesicles dilution by the highest growth rates with synchronized cell division and the fact that they can accumulate more polyphosphate (density of 2170 kg m^{-3}), in hypereutrophic environments, part of the population can lose great buoyancy (Brookes & Ganf, 2001) and sink for migration to scavenge for nutrients reaching deeper water where there is low and limiting irradiance. Also, turbulence can bring cells to low irradiances in the deeper portions of euphotic column. Wallace & Hamilton (2000) postulate that when the irradiance increases, the *Microcystis* cells may require more time to increase their rate of carbohydrates accumulation. If the irradiance decreases before adjustment, what happens in a mixed environment like Barra Bonita Reservoir, when the colonies of *Microcystis* entraining in mixing goes to deeper portions of the euphotic column, the maximum rate of carbohydrates accumulation is not reached. But large colonies are able to overcome the entraining forces of turbulence due to higher buoyant velocities, ensuring that buoyancy can always be lost or gained through carbohydrate ballast regulation.

Aging cells with cellular division depressed certainly accumulate carbohydrates and loose buoyancy displaying vertical migration. However, if the extracellular carbohydrate release: accumulation of intracellular carbohydrate ratio increases, aging of the *M. aeruginosa* cells, when exposed to high irradiances, do not produce ballast. These non-dividing cells also can not dilute its gas vesicles, and consequently can not loose their buoyancy. Senescence is, among other factors, a cause of inability of the cells to regulate buoyancy (Reynolds, 1987). Persistent residence within the upper portions of the euphotic zone, or the formation of surface accumulations, may cause serious photooxidation and a significant decrease in the photosynthetic efficiency in aging cells with previously deficient photosynthesis. This could be one cause to the die-off of old surface scums.

Thus we can conclude that at least the *M. aeruginosa* strain here used does not seem to suffer great stress effects by the exposure to high irradiances in the experimental conditions used, that is, at a temperature of 23°C and with no UV radiation effects, which besides being low in halogen lamps, were certainly completely eliminated for the cells were incubated on polycarbonate flasks. The release of extracellular carbohydrates by senescent cells may signify great implications to their buoyancy mechanism.

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References

- Abeliovich, A. & Shilo, M. 1972. Photooxidative death in blue-green algae. *J. Bacteriol.*, 111:682-689.
- Angelis, F.D., Barbarulo, M.W., Bruno, M., Volterra, L. & Nicoletti, R. 1993. Chemical composition and biological origin of dirty sea mucilages. *Phytochemistry*, 34:393-395.
- Brookes, J.D. & Ganf, G.G. 2001. Variations in the buoyancy response of *Microcystis aeruginosa* to nitrogen, phosphorus and light. *J. Plankton Res.*, 23:1399-1411.
- Brookes, J.D., Regel, R.H. & Ganf, G.G. 2003. Changes in the photo-chemistry of *Microcystis aeruginosa* in response to light and mixing. *New Phytol.*, 158:151-164.
- Charmichel, W.W. 1993. The toxins of cyanobacteria. *Sci. Am.*, 270:64-72.
- Deacon, C. & Walsby, A.E. 1990. Gas vesicles formation in the dark, and in the light of different irradiances, by the cyanobacterium *Microcystis* sp. *Br. Phycol. J.*, 25:133-139.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 28:359-356.
- Eloff, J.N., Steintz, Y. & Shilo, M. 1976. Photooxidation of cyanobacteria in natural conditions. *Appl. Environ. Microbiol.*, 31:119-126.
- Fogg, G.E. 1969. The Leeuwenhoek lecture, 1968: the physiology of an algal nuisance. *Proc. R. Soc. Lond. B*, 173:175-189.
- Geider, R.J., Osborne, B.A. & Raven, J.A. 1986. Growth, photosynthesis and maintenance metabolic cost in the diatom *Phaeodactylum tricornutum* at very low light levels. *J. Phycol.*, 22:339-348.
- Giroldo, D. & Vieira, A.A.H. 1998. Release of dissolved organic matter (DOM) in a tropical strain of *Cryptomonas obovata* exposed to several irradiances. *J. Plankton Res.*, 21:1911-1921.
- Gorham, P.R., McLachlan, J., Hammer, U.T. & Kim, W.K. 1964. Isolation and culture of toxic strains of *Anabaena flosaquae* (Lingb.) Verh. Int. Verein. Limnol., 15:769-780.
- Ibelings, B.W. 1996. Changes in photosynthesis in response to combined irradiance and temperature stress in cyanobacterial surface waterblooms. *J. Phycol.*, 32:549-557.
- Ibelings, B.W. & Mur, L.R. 1992. Microprofiles of photosynthesis and oxygen concentration in *Microcystis* sp. Scums. *FEMS Microbiol. Ecol.*, 86:195-203.
- Ibelings, B.W. & DeWinder, B. 1994. The effects of irradiance, temperature and desiccation on cyanobacterial photosynthesis: a possible explanation for the diurnal changes in surface waterblooms. In: Stal, L.S. & Caumette, P. (eds.) *Structure, development and environmental significance of microbial mats*. Springer-Verlag, Berlin. p.311-318. (NATO ASI Series, v.G35).
- Jochimsen, E.M., Charmichael, W.W., An, J.S., Cardo, D.M., Cookson, S.T., Holmes, C.E.M., Antunes, M.B.C., Melo Filho, D.A., Lyra, T.M., Barreto, V.S.T., Azevedo, S.M.F.O., Jarvis, W.R. 1998. Liver failure and death following exposure to microcystins toxins at a hemodialysis center in Brazil. *N. Engl. J. Med.*, 338:873-888.
- Klemer, A.R., Cullen, J.J., Mageau, M.T., Hanson, K.M. & Sundell, R.A. 1996. Cyanobacterial buoyancy regulation: the paradoxal roles of carbon. *J. Phycol.*, 32:47-53.
- Kromkamp, J.C. & Mur, L.R. 1984. Buoyant density changes in the cyanobacterium *Microcystis aeruginosa* due to changes in the cellular carbohydrate content. *FEMS Microbiol. Lett.*, 25:105-109.
- Myklestad, S. 1974. Production of carbohydrates by planktonic diatoms. I. Comparison of nine different species in culture. *J. Exp. Mar. Biol. Ecol.*, 15:261-274
- Paerl, H.W. 1983. Partitioning of CO₂ fixation in the colonial cyanobacterium *Microcystis aeruginosa*: mechanism promoting formation of surface scums. *Appl. Environ. Microbiol.*, 46:252-259.

- Paerl, H.W., Bland, P.T., Bowles, D. & Haibach, M.E. 1985. Adaptation to high intensity, low wavelength light among surface blooms of the cyanobacterium *Microcystis aeruginosa*. *Appl. Environ. Microbiol.*, 49:1046-1052.
- Powles, S.B. 1984. Photoinhibition of photosynthesis induced by visible light. *Annu. Rev. Plant. Physiol.*, 35:15-44.
- Reynolds, C.S. 1987. Cyanobacteria waterbloom. In: Callow, J.A. (ed.) *Advances in botanical research*. Academic Press, London. v.13, p.67-143.
- Staats, N., Stal, L.J., De Winder, B. & Mur, L.R. 2000. Oxygenic photosynthesis as driving process in exopolysaccharide production of benthic diatoms. *Mar. Ecol. Prog. Ser.*, 193:261-269.
- Sutherland, T.F., Grant, L. & Amos, C.L. 1998. The effect of carbohydrate production by the diatom *Nitzschia curvilineata* on the erodibility of sediment. *Limnol. Oceanogr.*, 43:65-72.
- Sakshaug, E., Bricaud, A., Dandonneau, Y., Falkoski, E.G., Kiefer, D.A., Legendre, L., Morel, A., Parslow, J. & Takahashi, M. 1997. Parameters of photosynthesis: definitions, theory and interpretation of results. *J. Plankton Res.*, 19:1637-1670.
- Talling, J.F. 1957. Photosynthetic characteristics of some freshwater plankton diatoms in relation to underwater radiation. *New Phytol.*, 56:29-50.
- Talling, J.F. & Driver, D. 1963. Some problems in the estimation of chlorophyll-a in phytoplankton. In: Doty, M.S. (ed.) *Proceedings of the conference of primary productivity measurements, marine and freshwater*. University of Hawaii, Hawaii. p.142-146. (UA Atomic Energy Commission, TID-7633).
- Vieira, A.A.H. & Mykkestad, S. 1986. Production of extracellular carbohydrates of *Ankistrodesmus densus* Kors.(Chlorophyta). *J. Plankton Res.*, 8:985-994.
- Vieira, A.A.H., Colombo, V. & Rocha, O. 2002. Release of extracellular carbohydrates by *Peridinium willei* (Dinophyceae) under different irradiances. *Hoehnea*, 29:241-247.
- Vieira, A.A.H., Lombardi, A.T. & Sartori, A.L. 1998. Release of dissolved organic matter in a tropical strain of *Synura petersenii* (Chrysophyceae) grown under high irradiances. *Phycologia*, 37:357-362.
- Wallace, B.B. & Hamilton, D.P. 2000. Simulation of water-bloom formation in the cyanobacterium *Microcystis aeruginosa*. *J. Plankton Res.*, 22:1127-1138.

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