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Nutrient uptake, chlorophyll *a* and carbon fixation by *Rhodomonas* sp. (Cryptophyceae) cultured at different irradiance and nutrient concentrations

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Abstract

The goal of this research was to study biomass production, nitrate and phosphate uptake and carbon fixation in batch culture of the marine microalgae *Rhodomonas* sp., which is used in aquaculture as food for commercially reared invertebrates. Cultures were grown for 7 days under four irradiance levels (52, 68, 103 and 142 μmol photon m^{-2} s^{-1}) and at three nitrate and phosphate concentrations (661–29, 882–39, 1323–58.5 μM of $NaNO_3$ and NaH_2PO_4, respectively) establishing 12 experimental treatments. During the exponential phase, growth was directly correlated with irradiance and the initial nitrate and phosphate concentrations. Significant differences were found in the low growth phase as a function of nutrient concentration, but not for irradiance. For the low nutrient concentration treatments, growth was limited after 2 days. Final cell density was influenced by the initial concentration of nutrients, independently of the irradiance level. The average maximum biomass production was reached in 7 days in cultures with high nutrients ($1.53 \pm 0.07 \times 10^6$ cells mL^{-1}). The total chlorophyll *a* content was directly related to cellular density and indirectly to irradiance level and concentration of nutrients. In conclusion, the optimal growing condition for *Rhodomonas* was up to the fourth day. However, after this time the cellular density, chlorophyll content and carbon uptake changed with the nutrient concentration and irradiance. Therefore we suggest that *Rhodomonas* culture could be used at the fourth day, either as inoculums for higher volume or as nourishment for invertebrate animals in marine aquaculture program.

Keywords: Growth rate; Nutrients uptake; Carbon fixation; Irradiance; *Rhodomonas* sp

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1. Introduction

Rhodomonas sp. is a flagellate unicellular red alga with a cell diameter between 9.2 and 9.9 μm. This marine microalga has been used as food for marine copepods (Jónasdóttir, 1994), oyster larvae and spat (Brown et al., 1998; McCausland et al., 1999; Muller-Feuga et al., 2003) and *Strombus gigas* veliger larvae (Aldana-Arana and Patiño-Suarez, 1998). At present, the increasing demand of sea urchin in Asian markets has caused a serious decline in their biomass for a variety of populations in coastal areas of Baja California, México (Palleiro-Nayar, 1994). To abate this decline management efforts have been focused on introducing laboratory raised sea urchins to these depopulated areas. The success of the culturing effort depends on the production of unicellular algae like *Rhodomonas* sp. which is used as a food source for the sea urchin larval stage (Rogers-Bennett et al., 1994), as well as for the penaeid shrimp larvae (Muller-Feuga et al., 2003). A better understanding of growth rates and nutrient uptake by *Rhodomonas* sp. under different environmental conditions is needed, including nutrients concentration, light intensity and quality, temperature, salinity and pH, among other factors (Richmond, 1986). Nutrients or light variability affects microalgal physiology include changing growth rates, cellular volume, biochemical composition (Claustre and Gostan, 1987; Lewitus and Caron, 1990), pigment concentration and composition (Eriksen and Iversen, 1995; Sciandra et al., 2000), photosynthesis, carbon metabolism (Turpin, 1991) and nutrient consumption (Cloern, 1977). Although some of these authors pointed out the importance of study the effect of two or more variables in physiological algae, therefore, the objective of the study was to study growth rates, nutrient uptake and carbon fixation by *Rhodomonas* sp. in batch cultures grown at different irradiances and nutrient concentrations.

2. Materials and methods

The marine microalga *Rhodomonas* sp. was obtained from the microalgae production laboratory at the Instituto de Investigaciones Oceanológicas of the Universidad Autónoma de Baja California. Non-axenic cultures were maintained at 20 ± 1 °C, a salinity of 32‰ and irradiance of 92 μmol photon m^{-2} s^{-1} in f/2 medium (Guillard, 1975). To evaluate the effect of the irradiance and nutrients on growth, a factorial design of 4×3 (12 experimental treatments) was carried out using four irradiance levels: 52, 68, 103 and 142 μmol photon m^{-2} s^{-1}, and three nitrate and phosphate concentrations on f/2 relatively low nutrients with 661-29; medium with 882-39 and relatively high nutrients with 1323-58.5 μM of $NaNO_3$ and NaH_2PO_4, respectively. The nitrate to phosphate ratio was maintained at 22.61. All treatments were performed in triplicate. Illumination was provided by four 8 ft lamps at 75 W power (General Electric Day-light; catalog number of F96T12-D-EX). The light intensity was measured with an irradiometer (model QSL-100, 4π sensor, Biospherical Instruments) in the center at the base of the culture containers. Cultures were growth for 7 days at 20 ± 1 °C. The seawater used for the medium was filtered through 1 and 0.65 μm porosity filter cartridges, and subsequently irradiated with ultraviolet lamps (25 W). We added Tris–buffer to the culture medium to minimize the pH effect. Experimental cultures were kept in Erlenmeyer flasks (190 mL of media, $n = 3$), which were autoclaved at 121 °C and 1.05 kg cm^{-2} for 10 min. Each Erlenmeyer flask received f/2 vitamins and 10 mL of *Rhodomonas* culture ($1.076 \pm 0.08 \times 10^6$ cells mL^{-1}). To minimize cell sedimentation, cultures were shaken by hand twice daily.

Cell density was determined using a 0.1 mm deep Neubauer chamber. The growth and doubling rates per day were calculated according to Guillard (1975). The pH was measured daily with a Chemcadet Jr potentiometer, while temperature was taken with an Ertco thermometer. Eight aliquots from each treatment were filtered through glass microfiber filters GF/C (1.2 μm nominal porosity) and stored at −60 °C for spectrophotometer chlorophyll analysis (Parsons et al., 1985). Pigment concentrations were calculated using the equations of Millán-Núñez and Alvarez-Borrego (1978). The filtered water was used to determine nitrate and phosphate concentrations (Parsons et al., 1985). For each treatment, two additional replicates of 10 mL with a cellular density of 9.3×10^4 cells mL^{-1} were used for incubation with 2 μCi $NaH^{14}CO_3$ to determine primary productivity. A non-parametric Kruskal Wallis ANOVA ($P < 0.05$) was used to determine differences among treatments.

3. Results

For all treatments, the pH increased from 7.7 to 9.9 ± 0.2 during the first 4 days of the experiment, where as after that (5–7 days) it decreased slightly to 9.5 (±0.2). No significant differences in pH ($P > 0.05$) were found among treatments.

3.1. Microalgae growth

The initial cell density of *Rhodomonas* sp. in all 12 treatments was $5.64 \pm 0.69 \times 10^4$ cells mL^{-1} (Fig. 1). In all experimental conditions, the exponential phase was observed during the first 4 days, after which growth stopped and a reduced growth phase was observed. Independently of the irradiance level, treatments at low nutrient concentrations, cultures starting dying on the sixth day (Fig. 1A; Table 1). Significant differences among cellular densities were observed from the second to the seventh day ($P < 0.05$) and during this exponential phase, the average growth rate of *Rhodomonas* sp. at low nutrients was 0.63 day^{-1}, for f/2 medium it was 0.64 day^{-1}, and for high concentrations it was 0.68 day^{-1} (Table 1). The maximum biomass production was reached on the seventh day and was of $0.81 \pm 0.03 \times 10^6$ cells mL^{-1} (Fig. 1A) in cultures with low nutrients, $1.17 \pm 0.04 \times 10^6$ cells mL^{-1} in f/2 medium (Fig. 1B) and $1.53 \pm 0.07 \times 10^6$ cells mL^{-1} with high nutrients (Fig. 1C).

3.2. Nitrate uptake

During the exponential phase (on the fourth day), the average nitrate uptake by *Rhodomonas* sp. in cultures with low nutrients was 607 ± 26 μM, 92% from the initial nitrate concentration (Fig. 2A). For treatments with f/2 medium and high nutrients, the average uptake were 687 ± 46 μM NO_3^- (78%) and 683 ± 29 μM NO_3^- (52%), respectively (Fig. 2B and C). On the other hand, during the low growth phase, the average nitrate uptake observed was related to its initial availability, i.e. 42, 187 and 581 μM NO_3^- for treatments with low, f/2 medium and high nutrient concentration respectively. By the seventh day, the average residual nitrate concentration in the medium was 12 μM in treatments with low nutrient, 9 μM in f/2 medium and 59 μM in high nutrient (Fig. 2). On the other hand at the 7 days of culture, the cells production

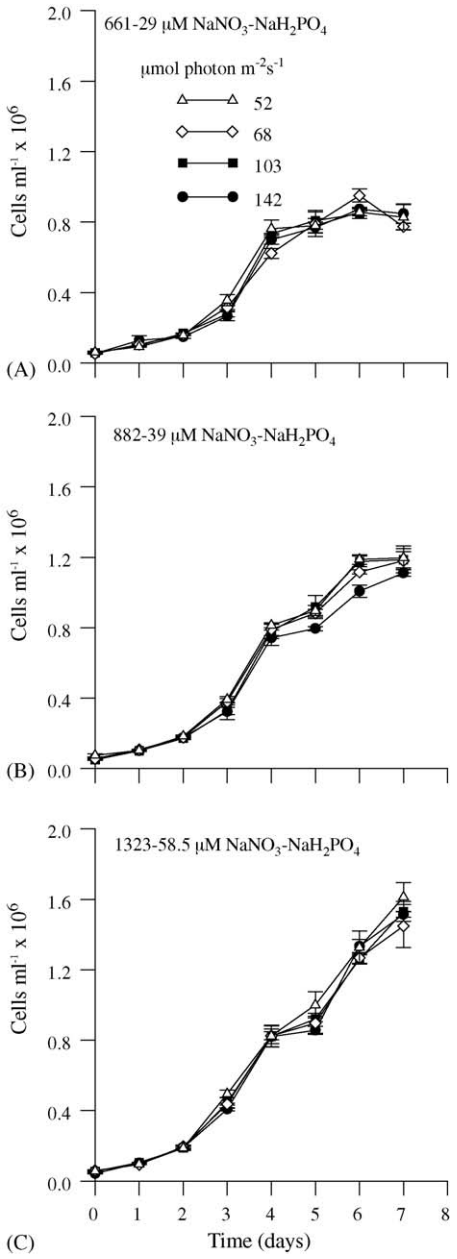

Fig. 1. Average growth of *Rhodomonas* sp. in cultures with different irradiance and three combination of nutrient. The vertical bars indicate standard error.

was limited at low irradiance and low nutrient. During the first 2 days the average uptake of nitrate at the four irradiances were 137.6, 110.3, 155.5 and 128.6 μM respectively and the cells production were 55.8, 60.7,

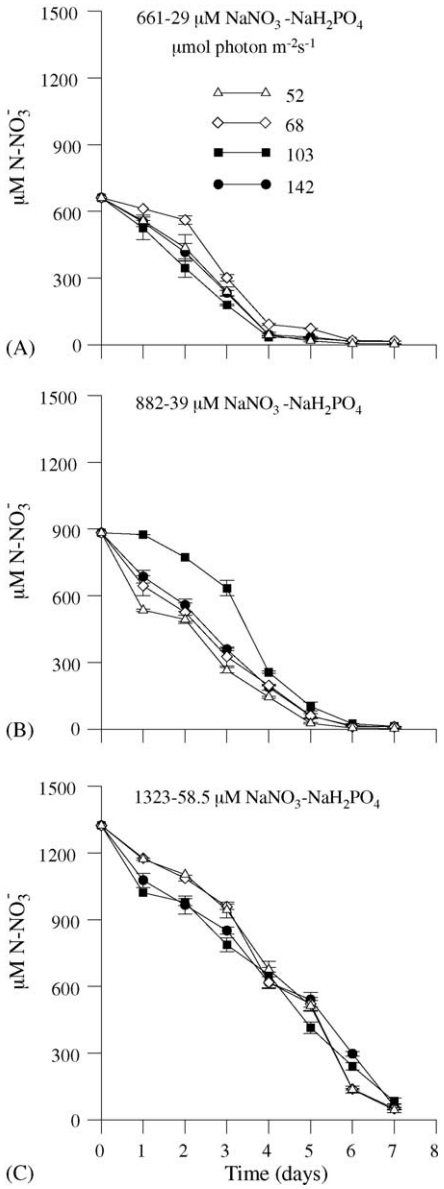

Fig. 2. Average nitrate concentration in the media in cultures of *Rhodomonas* sp., with different irradiance and three combination of nutrient. The vertical bars indicate standard error.

59.9 and 60.8 $\times$ 10^3 cells mL^{-1} (Table 2). However during the next 2 (3 and 4) days the average nitrate uptake were 196.3, 216.5, 180.8 and 192 μM, corresponding to an increase of 42.6, 96.2, 16.2 and 49.2% respectively, and the cells production were 312.0, 284.0, 291.2 and 301.2 cells mL^{-1} which is around of five times in relation to the production of the first 2 days.

3.3. Phosphate uptake

Phosphate uptake showed a significant decrease during the exponential growth (Fig. 3) and was directly related with the initial concentration of this nutrient in the cultures. As a result, by the fourth day, the phosphate was nearly exhausted in all treatments and the average uptake was 29.1 $\pm$ 0.04 μM (99%) in cultures with low nutrients, 38.7 $\pm$ 0.03 μM (99%) in f/2 medium and 55.7 $\pm$ 0.9 μM (95%) in high nutrients.

Without taking into consideration the irradiance level, the residual concentration of phosphate in cultures with low nutrients and f/2 medium increased slightly in the low growth phase (days 4–7). This increase was from 0.24 to 1.33 μM in the low nutrient treatment and from 0.33 to 0.89 μM in the f/2 medium one (Fig. 3A and B). In the treatments with high nutrients content, the increase was observed on the sixth day and it ranged from 0.52 to 0.94 μM PO_4^{3-} (Fig. 3C). The average uptake of phosphate was around 10 μM during the fourth days (Table 2).

3.4. Total chlorophyll a content

For all treatments, the concentration of chlorophyll *a* increased with time (Fig. 4), and was highly correlated with cell density (r = 92–99%). Significant differences in chlorophyll *a* content were observed from the third to the seventh day ($P < 0.05$). After the third day, the chlorophyll *a* concentration was inversely related to the irradiance level, but it was directly related to the nutrient concentration.

3.5. Chlorophyll a content per cell

During the exponential phase, chlorophyll *a* $cell^{-1}$ increased by nearly two times in low nutrient (from 1.1 to 2.1) and f/2 medium (from 1.1 to 1.7) cultures. But it decreased afterwards (Fig. 5A and B). On the other hand, the chlorophyll *a* at a high nutrient concentration was almost constant (1.35 $\pm$ 0.15 pg Chl *a* $cell^{-1}$) throughout the experiments (Fig. 5C). Chlorophyll *a* $cell^{-1}$ was significant among treatments ($P < 0.05$), however during the low growth phase for all nutrient

Table 1
Growth rate by *Rhodomonas* sp. per day, cultured in 200 mL with f/2 medium at different irradiance and three combination of nutrient

Irradiance (μmol photon m^{-2} s^{-1})	$NaNO_3/NaH_2PO_4$ (μM)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
52	661-29	0.40	0.53	0.82	0.76	0.02	0.09	–
	882-39	0.32	0.57	0.76	0.72	0.09	0.28	0.007
	1323-58.5	0.53	0.67	0.96	0.51	0.19	0.28	0.19
68	661-29	0.68	0.42	0.67	0.68	0.24	0.18	–
	882-39	0.65	0.50	0.76	0.73	0.11	0.23	0.05
	1323-58.5	0.45	0.73	0.80	0.63	0.08	0.34	0.13
103	661-29	0.56	0.51	0.50	0.97	0.09	0.04	–
	882-39	0.69	0.53	0.64	0.86	0.16	0.24	0.008
	1323-58.5	0.65	0.58	0.86	0.61	0.11	0.31	0.19
142	661-29	0.65	0.36	0.58	0.96	0.09	0.12	–
	882-39	0.56	0.55	0.61	0.83	0.06	0.23	0.09
	1323-58.5	0.88	0.60	0.75	0.70	0.04	0.44	0.12

Table 2
Nutrient uptake, cell production by *Rhodomonas* sp. per day, cultured in 200 mL with f/2 medium at different irradiance and three combination of nutrient

Day	$NaNO_3$/ NaH_2PO_4 (μM)	Irradiance (μmol photon m^{-2} s^{-1})							
		52		68		103		142	
		Uptake (μM)	Cells ($\times 10^3$ mL^{-1})	Uptake (μM)	Cells ($\times 10^3$ mL^{-1})	Uptake (μM)	Cells ($\times 10^3$ mL^{-1})	Uptake (μM)	Cells ($\times 10^3$ mL^{-1})
0–1	661-29	131/10.3	30.5	50/9.4	52.2	138/12.4	43.3	110/13.3	48.8
	882-39	329/16.6	28.3	272/14.8	51.1	8/11.2	50.5	197/13.0	43.8
	1323-58.5	152/14.3	39.4	147/16.2	33.8	302/21.4	50.0	247/20.4	60.5
1–2	661-29	90/6.2	64.4	50/8.2	55.0	178/6.7	67.7	136/5.9	45.5
	882-39	55/1.8	80.0	53/3.7	70.0	103/9.2	71.1	128/7.7	74.4
	1323-58.5	69/10.6	92.2	90/9.2	102.2	43/6.6	82.7	115/7.8	86.6
2–3	661-29	200/9.7	200.0	260/8.6	155.0	166/7.8	109.4	182/8.1	117.2
	882-39	234/11.9	209.4	232/11.9	202.2	138/11.9	155.5	199/12.1	147.7
	1323-58.5	159/9.0	306.1	128/14.1	241.1	191/15.4	257.2	107/17.5	215.5
3–4	661-29	196/2.5	406.1	209/2.4	307.2	144/2.0	456.1	189/1.6	433.8
	882-39	121/8.1	421.1	129/8.1	411.1	378/6.2	451.6	170/5.7	418.8
	1323-58.5	268/19.8	329.4	341/15.7	387.7	135/12.8	377.7	238/10.5	414.4
4–5	661-29	24/–	177.7	20/–	168.8	6/–	74.4	10/–	70.0
	882-39	117/0.0	833.3	137/–	92.2	154/–	137.7	130/–	52.2
	1323-58.5	164/3.3	1766.6	97/2.0	72.2	238/1.5	97.7	76/1.4	35.5
5–6	661-29	15/–	755.5	53/–	158.8	14/–	38.8	20/–	104.4
	882-39	21/–	290.0	48/–	233.3	77/–	25.8	43/–	212.2
	1323-58.5	3.75/0.2	325.5	3.82/–	370.0	174/0.1	338.8	244/–	476.6
6–7	661-29	1.0/–	–	1.0/–	–	1.0/–	–	2.0/–	–
	882-39	3.0/–	888.9	2.0/–	64.4	11/–	10.0	3.0/–	103.3
	1323-58.5	93/–	286.2	88.0/–	181.1	157/–	271.1	236/–	

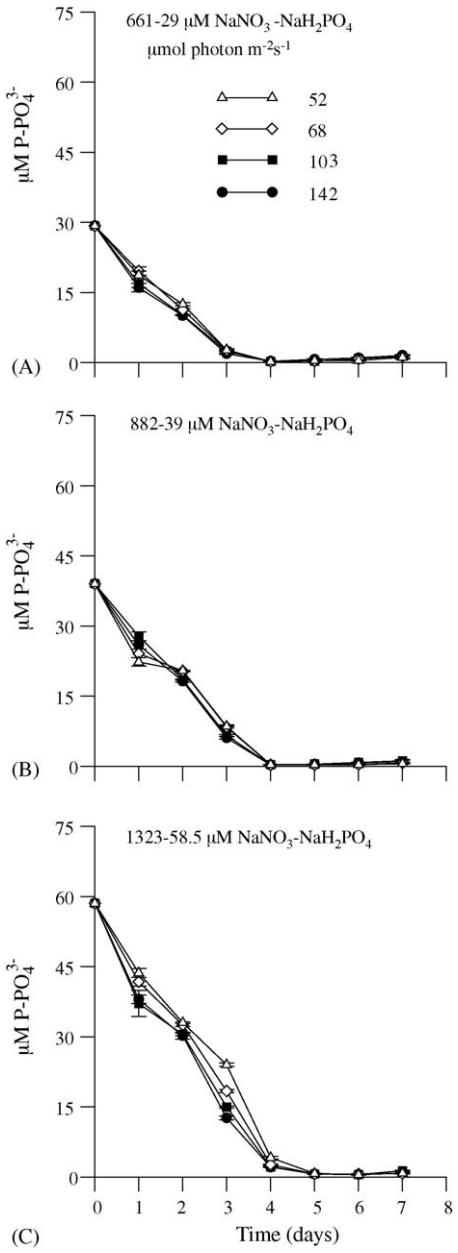

Fig. 3. Average phosphate concentration in the media in cultures of *Rhodomonas* sp., with different irradiance and three combination of nutrient. The vertical bars indicate standard error.

conditions, cells at low irradiance (52–68 μmol photon m^{-2} s^{-1}) had higher chlorophyll *a* content than those exposed to high irradiance (103–142 μmol photon m^{-2} s^{-1}) (Fig. 5).

3.6. Carbon fixation rate

During the first 2 days of culture, the mean carbon fixation rate per cell was 7.8 ± 2.1 pg C $cell^{-1}$ h^{-1} among treatments. On the third and fourth day, the rate of fixation in all treatments increased significantly, reaching maximum values of 45.7 ± 5.1, 33.8 ± 4.6 and 23.5 ± 4 pg C $cell^{-1}$ h^{-1} in low, f/2 medium and high nutrient respectively (Fig. 6). Furthermore, during the low growth phase, the carbon fixation rate decreased in all treatments reaching similar values to those reported for the beginning of the experiment (Fig. 6).

In respect to the nutrient concentration, the carbon fixation rate was inversely related during the 7 days of culture. However regarding the irradiance level, during the exponential phase the relationship was direct while for the stationary and death phases, this relationship was inverse (Fig. 6).

4. Discussion

Population growth and photosynthesis activity produce changes in pH. During the exponential growth phase, as biomass increases, a higher consumption of carbon occurred. Under the initial pH condition, approximately 95% of the inorganic carbon is in the bicarbonate form (Falkowski and Raven, 1997). When the carbon of the bicarbonate is assimilated in microalgal photosynthesis, the pH raises, while the concentration of inorganic carbon decreases (Uusitalo, 1996).

During the low growth phase, both the growth rate and the carbon fixation decreased significantly in all treatments and can be associated with pH effects on the availability of carbon. The relative concentrations of carbon chemical species are a function of the pH (Falkowski and Raven, 1997), where as Fogg and Thake (1987) showed that the microalgal growth could be affected by pH values between 10 and 11.

4.1. Microalgal growth

Irradiance level and nutrient availability are environmental variables that control the growth of algae. During the exponential phase, the growth rate of *Rhodomonas* sp. was directly correlated with both variables, but when the concentration of nutrients

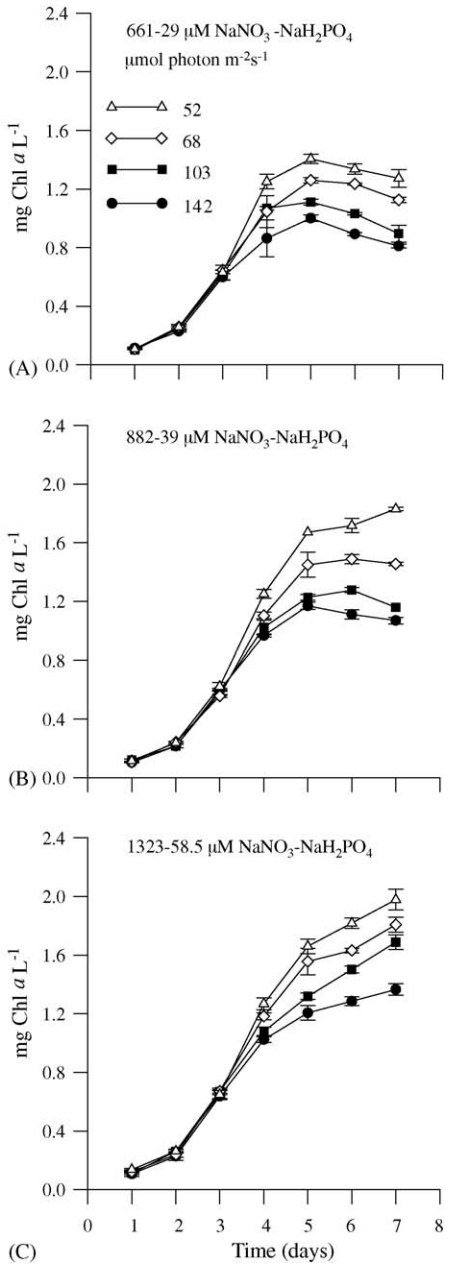

Fig. 4. Average total chlorophyll *a* content of *Rhodomonas* sp. in cultures with different irradiance and three combination of nutrient. The vertical bars indicate standard error.

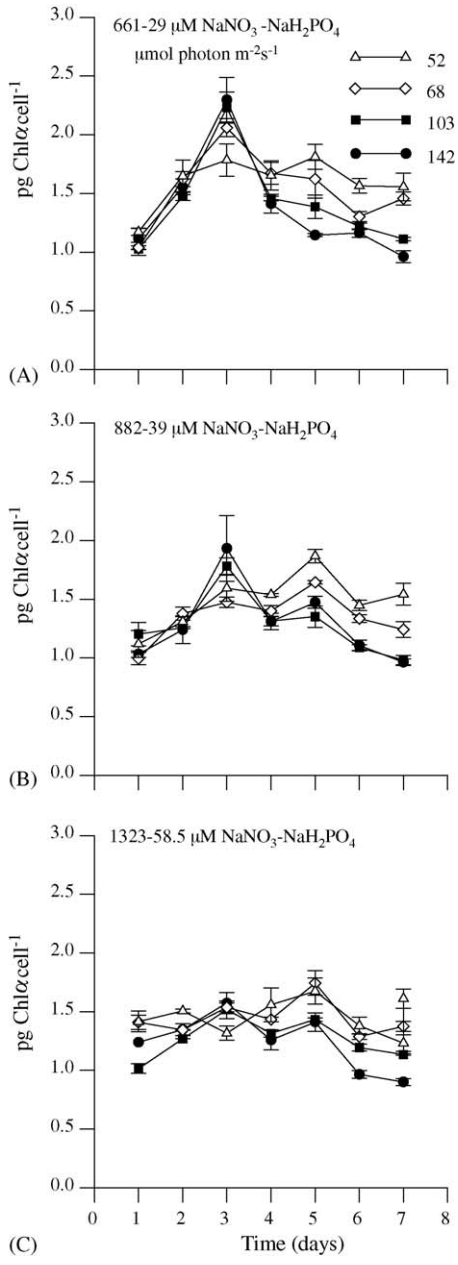

Fig. 5. Average chlorophyll *a* content per cell of *Rhodomonas* sp. in cultures with different irradiance and three combination of nutrient. The vertical bars indicate standard error.

decreased significantly, growth and maximum cell density were correlated with nutrient availability independently of the irradiance level. Lewitus and Caron (1990) found a similar response in cultures of *Pyrenomonas salina*. Fogg and Thake (1987) suggested that the death phase in batch culture is a result of the exhaustion of nutrients, and this was observed in treatments with low nutrient concentrations.

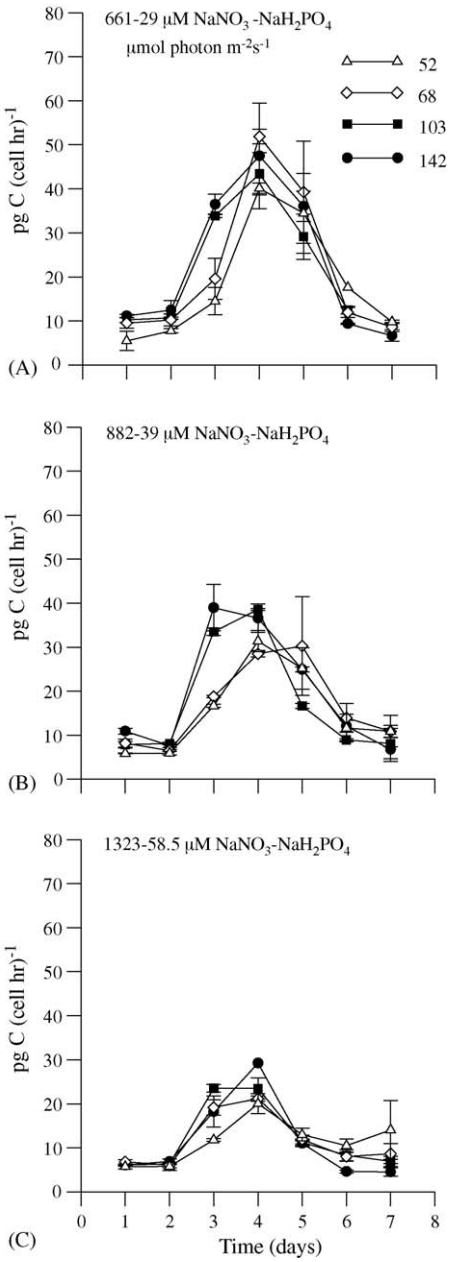

Fig. 6. Average of carbon fixation rate of *Rhodomonas* sp., in cultures with different irradiance and three combination of nutrient. The vertical bars indicate standard error.

4.2. Nutrient uptake

During the first 24 h of culture the nitrate and phosphate uptake was high. This immediate response to the supply of nutrients can be related with the increase in the activity of the enzymes involved in the uptake and assimilation of nutrients that has also been observed in algae (McCarthy, 1981). After this time, nutrient uptake tends to decrease with time as a result of the increase in the cellular density and its availability in the cultures. Although it has also been reported that light stimulates the nitrate uptake (Cloern, 1977), our data did not show this relationship.

On the other hand, the phosphate uptake among treatments was related with its concentration in the media. It has been documented that when the supply of phosphate is high, microalgae are able to accumulate the excess, as it is stored within the cells as polyphosphate granules (Fogg and Thake, 1987). Phosphate uptake increased slightly in all treatments during low growth phase.

This increase was observed when the residual concentration in the cultures was smaller than 0.5 μM and can be related to the presence of the alkaline phosphatase activity. Enzymatic activity facilitates the use of the organic phosphate in the immediate vicinity of the cellular surface (McCarthy, 1981). Graziano et al. (1996) found that the rate of phosphate production by the alkaline phosphatase activity was 2–3 times that of the maximum phosphate uptake rate in starved batch cultures of *Dunaliella tertiolecta* (0.05 μM of phosphate).

4.3. Chlorophyll a content

The total chlorophyll *a* content showed an inverse relationship with the irradiance and a direct relationship with the nutrient concentration. However, when standardizing the chlorophyll *a* content per cell, this relationship changed. Under the culture conditions evaluated, the total chlorophyll *a* was a good measure of the productivity of the system, but it did not reflect physiology state of the cells.

For all experiments the chlorophyll *a* per cell varied as a function treatment time. During the first 3 days, chlorophyll *a* per cell increased, while after this period it decreased. Eriksen and Iversen (1995) observed the same response in nitrogen-starved cells of *Rhodomonas* sp.; when fresh medium was added. They found that the content of chlorophyll *a* increased from 0.5 to 1.5 pg cell^{-1} during the first 23 h of incubation, but after the nitrate was exhausted, the chlorophyll

decreased to 0.3 pg cell^{-1}. The degradation of chlorophyll *a* and other accessory pigments in cryptophytes is probably the consequence of a simultaneous degradation of chlorophyll-binding proteins (Lewitus and Caron, 1990; Turpin, 1991; Sciandra et al., 2000). On the other hand, the content of chlorophyll *a* observed in low, f/2 medium and high nutrient treatments during the low growth phase was inversely related to irradiance level. This could be associated with the decrease in the growth rate, and as shown here pigment content was dependent on light availability (Fujiki and Tagushi, 2002). Reduction of the pigment content is considered a process of auto-regulation of the photosynthetic apparatus to reach a balance between the gain of light and the demand of energy necessary for the microalgal growth. This behavior has also been observed in other species of marine phytoplankton (Claustre and Gostan, 1987; Falkowski and La Roche, 1991).

4.4. Carbon fixation rate

For all treatments, at the beginning of the cultures experiment (day 1–2), carbon fixation rate per cell was constant, however the cells were in active growth and nutrients uptake per cell was significantly high. This behavior of *Rhodomonas* sp., can be due to the fact that the uptake and assimilation of nitrate is intimately bounded with the metabolism of the carbon fixation (Turpin, 1991; Falkowski and Raven, 1997). Also, the biochemical composition of the cell is modified by this process (Morris, 1981). Starting from the second day of the experiment, all treatments showed a direct relationship between growth and carbon fixation rates. In the exponential phase, both increased, while in the low growth phase rates decreased. Carbon fixation rate can be related with enzymatic activity in the process of carbon fixation and carbon availability. Falkowski and Raven (1997) showed that RubisCO content can vary as a function of the growth rate and the status of nutrients. For cultures of *Dunaliella tertiolecta*, Graziano et al. (1996), reported that the relative abundance of RubisCO, based on total proteins diminishes 30% as the growth rate decreased and starvation of phosphate increased.

In all treatments, carbon fixation rate was directly correlated with the irradiance level during the exponential phase, however during the low growth phase this relationship was inversed. The effect of the irradiance on the carbon fixation could be determined by the physiologic state of cells. During the exponential phase *Rhodomonas* sp. were actively growing and needed to synthesize proteins in order to continue with the cell division (Morris, 1981). In contrast, in the low growth phase the cellular division rate, the chlorophyll content and the carbon fixation decreased. In the treatments with low nutrients, the carbon fixation rate was higher than those obtained in the f/2 medium and high nutrient content. However, the growth rate was directly correlated with the concentration of nutrients. Morris (1981) found that cells cultured under these conditions promote the synthesis of carbohydrates and elevates rates of carbon fixation. The differences in our study can be caused by variability in the metabolic routes as discussed by Morris (1981). On the other hand, even though the cellular division and photosynthetic activity are intimately related, the response of these metabolic processes to variations in physical–chemical conditions, are not always the same. Under different condition of nutrients and light, *Rhodomonas* sp. has a different response for growth rate, chlorophyll content and carbon fixation.

5. Conclusion

No significant differences were found during the growth exponential phase for cellular density and chlorophyll concentration with the different irradiances and nutrient concentration. However, during low growth phase, changes in cellular density and the chlorophyll *a* concentration were observed.

The optimal growing condition for *Rhodomonas* was up to the fourth day. After this time, cellular density, chlorophyll content and carbon uptake changed with the nutrient concentration and irradiance. Therefore, it is suggested that *Rhodomonas* culture could be used at the fourth day, either as inoculums for higher volume or as nourishment for invertebrate animals in marine aquaculture program.

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