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NOTE ON THE EFFECT OF HIGH NITRATE CONCENTRATION AND LIGHT INTENSITY ON THE GROWTH AND UPTAKE RATES OF *PHAEODACTYLUM TRICORNUTUM* (BOHLIN) CULTURE

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Synopsis

The effect of high nitrate concentration and light intensity on chlorophyll-a synthesis, cell number and nitrate assimilation on P. tricornutum culture, was determined. Growth and uptake rates were determined as a function of nitrate concentration ranging from 0.40 to 35.40 µg/l. The Ks showed high values, when compared with those obtained with lower nitrate concentration. The percentual variation of Ks was greater than that of Vmax.

Descriptors: Nitrates, Chlorophylls, *Phaeodactylum tricornutum*, Growth, Culture media, Nutrients (mineral), Light intensity, Cell.
Descritores: Nitratos, Clorofilas, *Phaeodactylum tricornutum*, Crescimento, Meio de cultura, Nutrientes minerais, Intensidade luminosa, Célula.

Introduction

The growth and uptake rates of marine phytoplankton are known to be under the influence of nitrogenous nutrients, light intensity and temperature Teixeira & Vieira (1976) showed that *P. tricornutum* growth could be limited by low nitrogenous nutrient concentration. Morris *et al.* (1974) related that *P. tricornutum* increases photosynthesis rate with decreasing light intensity, higher at 0.5 KLUX than at 9 KLUX. The constants Ks and Vmax are important ecological parameters (MacIsaac & Dugdale, 1969; Thomas & Dodson, 1974). The purpose of this work was to test the influence of high nitrate concentration and light intensity on these two parameters on *P. tricornutum* culture.

Material and methods

The clone of *P. tricornutum* was isolated from Ubatuba (SP) region and maintained in medium "f" (Guillard & Ryther, 1962), non-axenic culture, 3 KLUX intensity given by fluorescent day-light type lamps, continuous regime, in a BOD type incubator, at 22 °C temperature. The culture medium for the experiment was prepared with surface water from the same region. Table 1 shows hydrographic parameters and initial cell number, chlorophyll-*a* and nitrate concentration. Depleted nitrate cells were grown in Erlenmeyers flasks containing medium "f" without nitrogenous nutrient, until they reached the stationary phase. Then the organisms in a concentration of 6.9 x 10^4ml were put in a series of 7 Erlenmeyers flasks of 300 ml capacity, containing 200 ml of medium "f" to which crescent nitrate amounts were added, from 0.00 to 35.00 µg-at/l. The flasks were transferred to a BOD type incubator, 22 ± 0.5 °C and 12 KLUX light intensity, given by 6 fluorescent day-light lamps, 20 W each, continuous regime. Sampling was done every 24 hours, until the 5th day of the experiment. The active chlorophyll-*a* was determined according to Strickland & Parsons (1968). The cell number was counted with a Fucks-Rosenthal chamber. Nitrate concentration was determined according to Mullin & Riley (1955)'s hydrazine sulphate reduction technique, as described by Strickland & Parsons (*op. cit.*). The growth and uptake rates and the constants Ks and Vmax were calculated according to Thomas (1970). The slope of the straight line on the semi-logarithmic paper (Fig. 1*a*,*b*,*c*) was utilized to calculate the generation time (θ), that is, the time required for the population of *P. tricornutum* to double as cell number, synthesized chlorophyll-*a* and assimilated nitrate, according to Fairchild & Sheridan (1974).

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Results

Figure 1 shows: *a* - The cell number; *b* - synthesized chlorophyll-*a* and *c* - nitrate

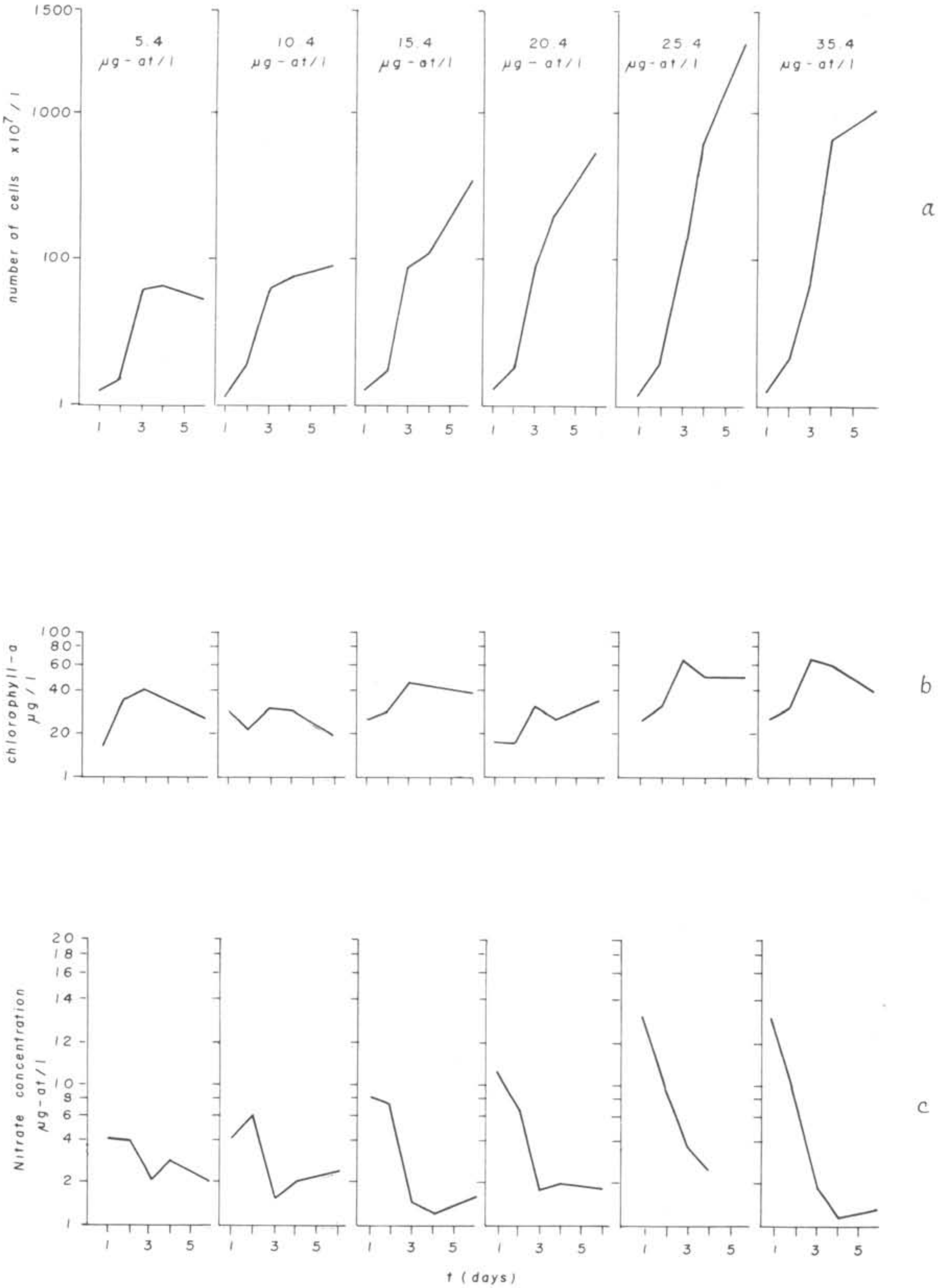

Fig. 1. *a*) Cell number; *b*) synthesized chlorophyll-*a*, and *c*) nitrate concentration of the culture medium, as a function of time, for each nitrate concentration; the initial nitrate concentration is shown at the top.

concentration in the *P. tricornutum* culture medium, plotted in the abscissa and time in the ordinate for each nitrate concentration. The plotting of the cell number appeared as an almost perfect straight line, while synthesized chlorophyll-*a* and nitrate uptake lines increased and decreased with time. These variations will be commented later. The initial nitrate concentration in culture medium, generation time (θ), growth and uptake rate, μ, are listed in Table 2. Null hypothesis between growth rate estimated as synthesized chlorophyll-*a* and cell number medium values, that is, $H_o: \mu_1 = u_2$, was $t = -0.73$, for $N = 12$. The medium value differences were random, at 1 and 5% significance level. Figure 2*a* shows growth rate estimated as *P. tricornutum* culture synthesized chlorophyll-*a* in the abscissa and nitrate concentrations in the ordinate. The data did not present a perfect retangular hyperbole. The linear plot of S/μ versus S values is shown in Figure 2*b*, the Ks is in the negative intercept and Vmax in the slope of the regression equation. Figure 3 shows the growth rate of *P. tricornutum* culture, estimated as cell number in the culture medium, as a function of nitrate concentration. The S/μ vs S plot shows Ks and Vmax values. The nitrate uptake rate, as a function of the nitrate concentrations, is shown in Figure 4. The linearization plot shows Ks and Vmax values. Table 3 shows the Ks and Vmax values with 95% confidence interval. Ks and Vmax were estimated from growth and uptake rate values (Table 2). Ks and Vmax obtained with the synthesized chlorophyll-*a* values, 18.22 µM and 1.57 doublings/day, respectively, indicated that chlorophyll-*a* synthesis seemed to be the most sensible variable to the experimental conditions. Growth rate Ks and Vmax estimated as cell number were 3.14 µM and 1.71 doublings/day, and

Table 1. Hydrographic parameters, seawater nitrate, initial chlorophyll-*a* and cell number

Position 23°45'S 45°01'W	S°/oo	Sampling depth	Local depth (m)	Seawater nitrate (µg-at/l)	Chlorophyll-*a* (µg/l)	Cell number ($\times 10^7$/l)
	33.98	0.0	50.00	0.40	27.11	6.90

Table 2. Nitrate concentrations, generation time, θ, growth and uptake rates, μ, estimated as cell number, synthesized chlorophyll-*a* and assimilated nitrate in *P. tricornutum* culture

	N-NO_3 µg-at/l	Generation time(days) θ	Growth rate $\mu = \frac{0.693}{\theta}$ (doublings/day)
cell number	0.4	2.55	0.27
	5.4	0.77	0.90
	10.4	0.60	1.16
	15.4	0.43	1.61
	20.4	0.47	1.47
	25.4	0.42	1.65
	35.4	0.46	1.31
chlorophyll-*a*	0.4	1.91	0.36
	5.4	2.05	0.34
	10.4	1.51	0.46
	15.4	1.30	0.53
	20.4	1.10	0.63
	25.4	0.60	1.15
	35.4	0.55	1.26
			Uptake rate $\mu = \frac{0.693}{\theta}$
assimilation	0.4	2.10	0.33
	5.4	1.10	0.63
	10.4	0.50	1.38
	15.4	0.40	1.73
	20.4	0.30	2.31
	25.4	0.35	1.98
	35.4	0.31	2.23

Table 3. Growth rate K_s and V_{max}, with 95% confidence intervals, estimated as cell number and synthesized chlorophyll-*a*; uptake rate K_s and V_{max} with 95% confidence intervals, estimated as nitrate consumed from culture medium

Growth estimated as: Chlorophyll-*a*		Cell number	
Ks µM	Vmax doublings/day	Ks µM	Vmax doublings/day
18.22 ± 5.97	1.57 ± 0.55	3.14 ± 0.73	1.71 ± 1.46

Uptake estimated as nitrate consumed by the cells from culture medium	
Ks µM	Vmax doublings/day
10.10 ± 2.16	2.88 ± 0.46

these values are rather in agreement with the expected values for Ks and Vmax for *P. tricornutum* cells growing with nitrate as nitrogen source (MacIsaac & Dugdale, 1969; Collos & Slawyk, 1979). The Ks and Vmax for the uptake rate of nitrate were 10.10 μM and 2.88 doublings/day, respectively. The Ks and Vmax values obtained in this work, presented percentual of Ks greater than those of Vmax.

Discussion

Chlorophyll-*a* concentrations of *P. tricornutum* culture are shown in Figure 1*b*. At the 3rd day of the experiment the chlorophyll-*a* presented a maximum value, followed by a decrease. Only at the concentration 20.40 μg/at/l $N\text{-}NO_3$, the chlorophyll-*a* increased, on the 5th day. According to Sournia (1974) and Meeks (1974) light is the main influence on the cellular chlorophyll concentration and high light intensity could inhibit synthesis and promote pigment bleaching. The relatively low chlorophyll-*a* content of *P. tricornutum* culture showed in Figure 1*b* and, consequently, the low growth rates (Table 2) could, probably, be explained by the high light intensity (12 KLUX) used in the experiment. The cells had been cultured at 3 KLUX light intensity and, according to Beardall & Morris (1976), *P. tricornutum* cells growing at 0.7 KLUX presented a decrease in chlorophyll-*a* contents when exposed to a 12 KLUX light intensity source. In this work, the cellular chlorophyll-*a* concentration values were ten times

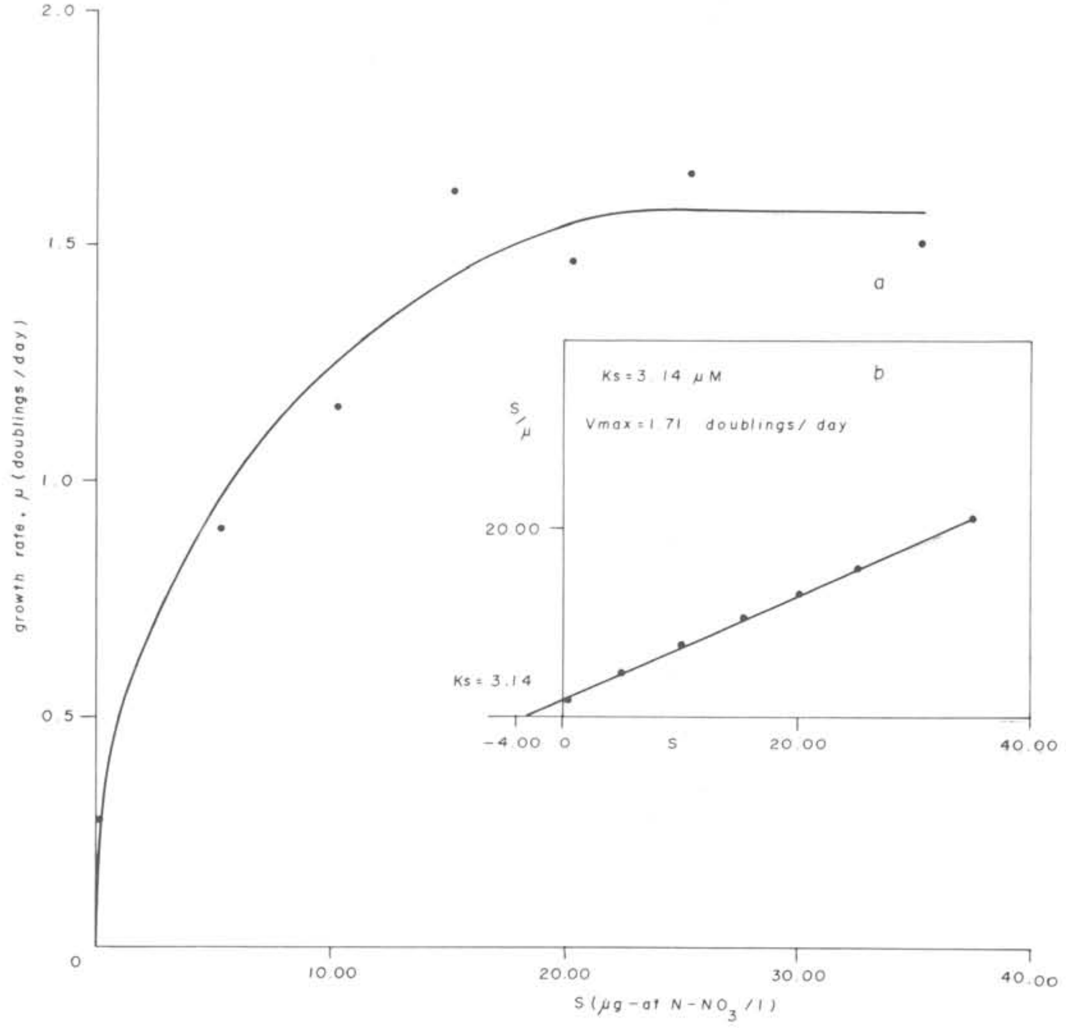

Fig. 2. *a*) Growth rate of *P. tricornutum*, estimated as cell number, as a function of culture medium nitrate concentration; *b*) linearization by plotting S/μ vs S, showing K_s and V_{max}.

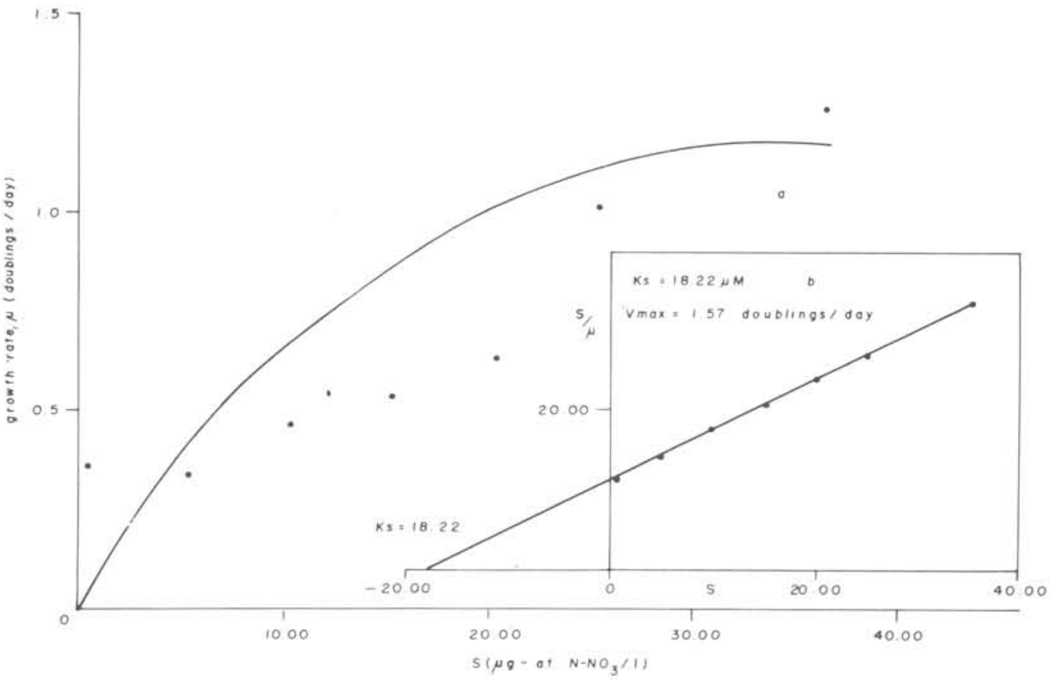

Fig. 3. *a*) Growth rate of *P. tricornutum*, estimated as chlorophyll-*a*, as a function of culture medium nitrate concentration; *b*) linearization by plotting S/μ vs S, showing K_s and V_{max}.

lower than those obtained by Beardall & Morris (*op. cit.*). The high Ks growth rate value, 18.22 μM, and low Vmax 1.57 doublings/day (Table 3; Fig. 2) presented by *P. tricornutum* culture could be attributed to the low cellular chlorophyll-*a* contents. According to Dugdale (1967) low Ks and high Vmax values are conditions of maximum production. Figure 1*c* shows the nitrate uptake by *P. tricornutum* during the experiment. Nitrate uptake was considered as the nitrate consumed from the culture medium every day, during the experiment. The concentration increase on the last day can be an irregularity that could be attributed to nitrite releases to the medium by *P. tricornutum* cells (Collos & Slawyck, 1979; Collos, 1982). Collos (*op. cit.*), working with *P. tricornutum*, reported that nitrite appeared in the medium after a few hours of nitrate addition. The increase of cell number during the experiment is shown in Figure 1*a*. The cell division rate did not seem to be affected by the light intensity. Paasche (1968), working with *Nitzchia turgidula*, related that this diatom did not have the cell division rate affected by 24 KLUX light intensity. The Ks and Vmax for growth rate estimated as cell number values were 3.14 μM and 1.57 doublings/day, respectively (Table 3; Fig. 3). MacIsaac & Dugdale (1969) observed that Ks and Vmax obtained for eutrophic regions, are comparable to those for laboratory cultures. These authors found 4.21 μM for NO_3 Ks of an eutrophic region population and 2.83 μM for NO_3 growth rate of *Isochrysis galbana* growing in a chemostat culture. The nitrate concentrations utilized in this work (Table 2) can be considered as of nutrient rich water, since Thomas (1970) listed a mean value of 5.6 μg-at/l N-NO_3 as characterizing an eutrophic seawater region. The Ks for uptake can exceed the Ks for growth rate by one order of magnitude (Eppley & Thomas, 1969). In this work, the Ks for growth rate estimated as cell number was 3.14 μM and the Ks for nitrate uptake rate was 10.10 μM. According to Collos (1980) eutrophic waters have high Ks values and oligotrophic low Ks ones. The Ks and Vmax showed in Table 3 could, perhaps, be compared to those obtained for nutrient rich seawaters. The author of this study, working with the same clone of *P. tricornutum*, found a NO_3 Ks of 0.37 μM and Vmax of 2.21 doublings/day for growth rate estimated as cell

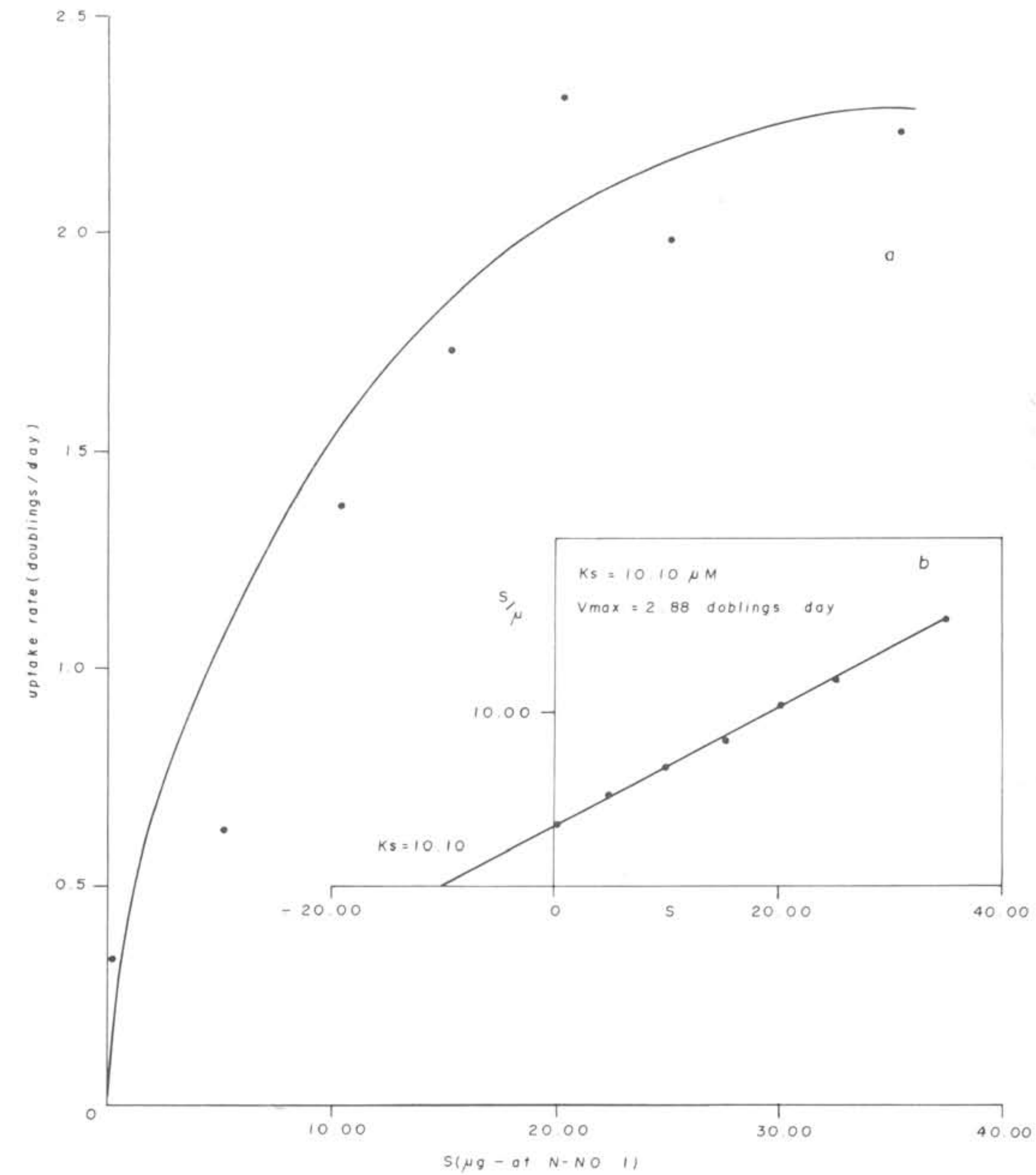

Fig. 4. *a*) Uptake rate of *P. tricornutum* estimated as nitrate assimilated, as a function of culture medium concentration; *b*) linearization by plotting S/μ vs S, showing K_s and V_{max}.

number, and Ks of 2.30 μM and Vmax of 1.07 doublings/day for nitrate uptake rate, nitrate concentrations ranging from 0.90 to 10.00 μg-at/l, 5 KLUX light intensity (Schmidt, 1982). The *P. tricornutum* kinectics values presented here could be explained by: a) instability of chlorophyll-*a* synthesis (Sournia, 1974); b) physiological stress of cells, due to the large starvation period (Collos, 1980); c) high light intensity, near to the saturation point and self-shading (Vieira, 1975). Other factors would influence these results and new experiments must be carried on so as to confirm or not these data.

Resumo

Foi determinado o efeito de uma alta concentração de nitrato e alta intensidade luminosa na síntese de clorofila-*a*, número de células e assimilação de nitrato em uma cultura de *P. tricornutum*. As velocidades de crescimento e de assimilação foram determinadas em função de

concentrações de nitrato variando de 0,40 a 35,40 µg-at/l e intensidade luminosa de 12 KLUX. A Ks e a Vmax apresentaram valores altos, comparativamente com valores obtidos em concentrações mais baixas de nitrato e menor intensidade luminosa. A variação porcentual da Ks foi maior que a variação porcentual da Vmax.

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