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# Growth of *Cyclotella meneghiniana* Kutz. II. Growth and cell compositon under different growth rates with different limiting nutrient

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Keywords : *Cyclotella meneghiniana*, growth, cell composition, limiting nutrient, continuous culture.

The growth and cell composition of *C. meneghiniana* in P-, N- or Si-limited continuous cultures under different dilutions are discussed. Carbon, nitrogen and phosphorus content per cell or per unit cell volume increased with growth rate in all chemostats. Maximum growth rate depends on the type of limiting nutrient. The maximum growth rates are.1.65 ± 0.04, 0.73 ± 0.07 and 0.95 ± 0.08 $d^{-1}$ for P-, N- and Si-limited cultures respectively. These values are close to those calculated by Droop, Caperon, Goldman and Monod models. The concentrations of N and P in the river Danube are much higher than the requirements for growth and uptake of *C. meneghiniana*. This indicates that neither P nor N are limiting to the growth of *C. meneghiniana* in natural habitat.

**Croissance de *Cyclotella meneghiniana* Kutz. II. Croissance et composition cellulaire à différents taux de croissance en fonction de nutriments limitants**

Mots clés : *Cyclotella meneghiniana*, croissance, composition cellulaire, nutriment limitant, culture en continu.

La croissance et la composition cellulaire de *C. meneghiniana* soumis à des concentrations limitantes en P, N et Si en culture continue à des taux de dilution différents sont discutés. Le contenu cellulaire en carbone, azote et phosphore par cellule ou par unité de volume augmente avec le taux de croissance dans toutes les conditions précitées. Le taux maximum de croissance dépend du type de nutriment limitant. Ces valeurs sont très proches de celles calculées à partir des modèles de Droop, Caperon, Goldman et Monod. Dans le Danube, les concentrations en N et P sont beaucoup plus élevées que les besoins pour la croissance et l'assimilation de *C. meneghiniana*. Cela indique que ni N ni P ne sont limitants pour la croissance de *C. meneghiniana* en milieu naturel.

## 1. Introduction

In the River Danube, diatoms are important especially in spring and autumn when they become dominant over the other algae; up to 90 per cent of the total number may be diatoms (Kiss & Nausch 1988, Kiss 1994). *Cyclotella meneghiniana* Kutz. is one of the most frequently dominant potamoplankton in the river (V.-Balogh et al. 1994). The domination of diatoms is characterized by depletion of nutrients. The most important nutrients are P and N, in addition to Si in case of diatom development. Nutrient limitations not only affect the growth of algae but also the cell composition and nutrient uptake. There is a good coupling between growth rate and nutrient limitation (Sommer 1991). Tilman & Kilham (1976) and Tilman (1977) studied the growth and competition ability of *C. meneghiniana* in batch and semi-continuous cultures under P or Si limitation. The growth of this species under N-limitation and the effect of growth rate and nutrient limitation on cell composition, particularly nutrient ratio, may need more studies to give a complete outstanding of these relations.

Nutrient limitation can either be described by the Monod equation and by the Droop equation. Monod

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and Droop models are usually used under steady-state condition in continuous cultures. Monod's model depends on the concentration of the dissolved limiting nutrient.

According to Monod (1942) the relationship between growth and substrate consumption is the following:

$$\mu = \mu_{max} \frac{S}{K_s + S} \quad (1)$$

where $\mu$ is the growth rate, $\mu max$ is the maximum value of $\mu$, that is when S is the concentration of limiting nutrient, and $K_s$ is the half saturation constant.

Droop (1968) found that growth rate is a hyperbolic function of intracellular nutrient concentration. This is described by an empirical formula:

$$\frac{\mu}{\mu_{max}^*} = 1 - \frac{Q_{min}}{Q} \quad (2)$$

where $Q$ is the cell quota, $\mu_{max}^*$ is the maximum growth rate at which $Q$ is infinite and $Q_{min}$ is the minimum cell quota. $\mu_{max}$ is smaller than $\mu_{max}^*$ by the factor of ([1 - $Q_{min}$ / $Q_m$), where $Q_m$ is the cell quota at which this nutrient ceases to limit growth (Droop 1973, 1974).

Caperon (1968) found that growth rate was a direct function of cell quota expressed by:

$$\mu = \mu_{max}^* [(Q-Q_{min})/[Kq+(Q-Q_{min})]] \quad (3)$$

where $Kq$ is the internal nutrient level when $\mu$=1/2 $\mu_{max}^*$.

Under steady-state conditions the nutrient uptake is equal to the product of the specific growth rate and the cellular quotas (Droop 1968).

$$v = \mu Q \quad (4)$$

The uptake kinetic curves of v vs. $\mu$ are shown by Goldman (1977), Goldman and McMarthy (1978):

$$\mu = \mu_{max}^{**} \frac{v}{K_{up} + v} \quad (5)$$

where v is the uptake rate, $K_{up}$ is a half-saturation coefficient for uptake at steady-state, analogous to $K_s$ in equation (1), but with different units. $K_{up}$ is equal to $\mu_{max}^{**}$ x $Q_{min}$, ($Q_{min}$ = $K_{up}$ / $\mu_{max}^{**}$)

A strain of *C. meneghiniana* was isolated from the river. Series of experiments were run to discuss the factors controlling the growth (Shafik et al. 1997) and the changes of cell composition under P, N or Si limitation. The results were compared to different models.

## 2. Materials and methods

A strain of *Cyclotella meneghiniana* was grown in three identical chemostats. The used chemostat apparatus and the culturing conditions had been described in details (Shafik et al. 1997). All chemostats were run at 25°C, the optimum temperature of growth from the result of batch culture experiments (Shafik et al. 1997). The light intensity of 210 $\mu E\ m^{-2}\ s^{-1}$ for 16 hours light and 8 hours dark cycle was used, where light saturated and the same light period in nature at the time of isolation. Schlösser's medium (1982) with some modifications (Shafik et al. 1997) was used. The concentration of phosphorus was reduced to 0.125 mg P $l^{-1}$ in P-limited culture (7 % from the original concentration of P in Schlösser's medium). The concentration of nitrogen was reduced to 1.6 mg N $l^{-1}$ in N-limited culture (15 % from the original concentration of N). The concentration of silicate was reduced to 1.6 mg Si $l^{-1}$ in Si-limited culture (25 % from the original concentration of Si). After the cultures had reached a steady-state (dilution rate (D) = growth rate ($\mu$)) a definite volume of 120 ml from each culture was harvested for analysis. Samples were analysed in three successive days then the dilution rate was set at a new higher value and the population was again allowed to reach the steady-state. The cultures reached the steady-state within three weeks.

In case of N and Si- limited culture, the medium was added into the culture vessels at six dilution rates between 0.125 and 0.75 $d^{-1}$, while P-limited culture was run up to 1.65 $d^{-1}$. At each steady-state, soluble reactive phosphorus (SRP), nitrate-nitrogen (NN) and soluble silicate in the culture vessels were measured. SRP was measured with the molybdate method (Murphy & Riley 1962) Nitrate was determined via reduction to nitrite according to Elliott & Porter (1971) and available silicon was measured by the method of Mullin & Riley (1955).

The internal N and C content were measured following filtration on GF/C glass fiber filters and calculated by Automated Nitrogen/ Carbon analyzer-Mass Spectrometer System (ANCA-MS system Europa Scientific Ltd., UK.). For the determination of intracellular P, three replicates from each culture were filtered through cellulose-acetate membrane filters of 0.45µm pore size, then washed by P-free medium. The algae onto the filter was used to determine particulate phosphorous (PP). The P content of the algae onto the filter was measured as total P after digestion by $HClO_4$ and $K_2S_2O_8$ (120°C, 1 h) (Mackereth et al. 1978). In addition to the intracellular P, N and C, the cell number, cell volume, as well as chlorophyll-*a* were measured. The count of cells and cell volume were detected by in-

verted microscope technique (Utermöhl 1958). The chlorophyll-*a* concentration was determined by extraction with boiling methanol according to Iwamura et al. (1970).

## 3. Results

Dead cells were recorded only at growth rate of 0.125 $d^{-1}$ in all cultures. Dead cells were about 51 %, 2 % and 23 % of the total cell number in P, N and Si-limited cultures respectively. At higher growth rates, dead cells were not more than 1 % in any of the culture vessels. Chlorophyll-*a* content per cell increased with growth rate (Fig. 1A). It reached its maximum value of about 5.6 pg chlorophyll-*a* $cell^{-1}$ at the maximum growth rate in both P and N-limited cultures. The maximum value was 4.5 pg chlorophyll-*a* $cell^{-1}$ at growth rate of 0.5 $d^{-1}$ in Si-limited culture. Maximum chlorophyll-*a* content per unit cell volume of about 6.1 fg $\mu m^{-3}$ was recorded in all chemostats (Fig. 1B).

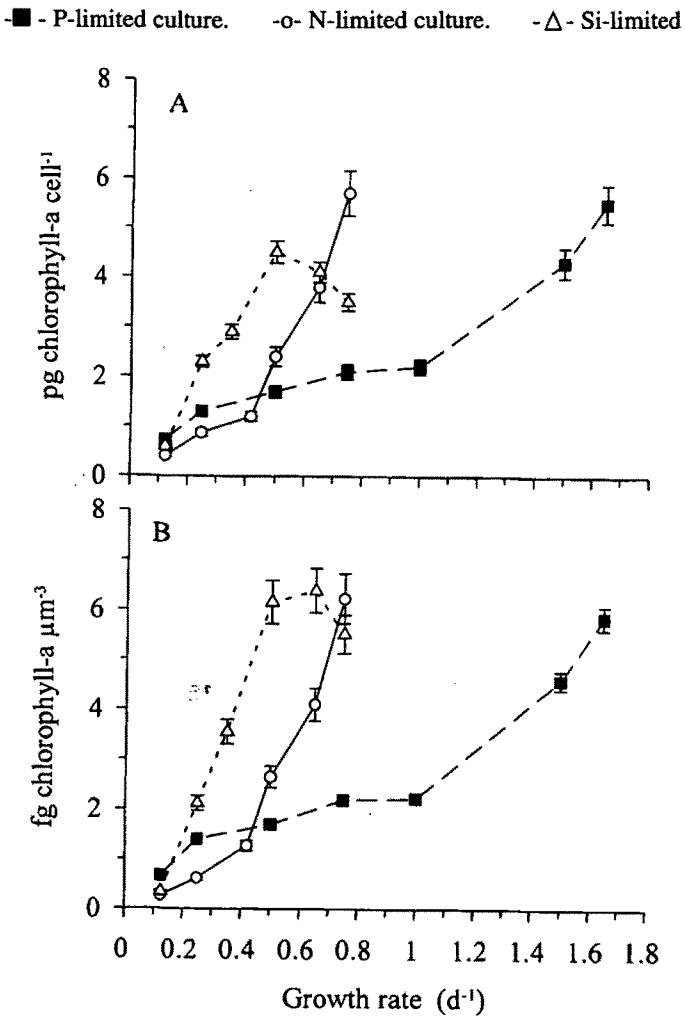


Fig. 1. Changes of chlorophyll-a content at different growth rate (steady-state) ± standard division, (A) per cell and (B) per unit cell volume in P, N or Si limited cultures.

Fig. 1. Variations du contenu cellulaire à différents taux de croissance (phase stationnaire) ± division standard (A) par cellule, (B) par unité de volume cellulaire dans des conditions limitées en P, N ou Si.

All cultures started with cell volume of about 1656 $\mu m^3$. The changes in cell volume with growth rates are shown in Fig. 2. Under Si limitation, the cell length increased, while its diameter decreased with increased growth rate. The lowest cell volume of about 636 $\mu m^3$ was recorded at the maximum growth rate in Si-limited culture. Under P or N limitations, both cell length and diameter decreased compared to the starting one.

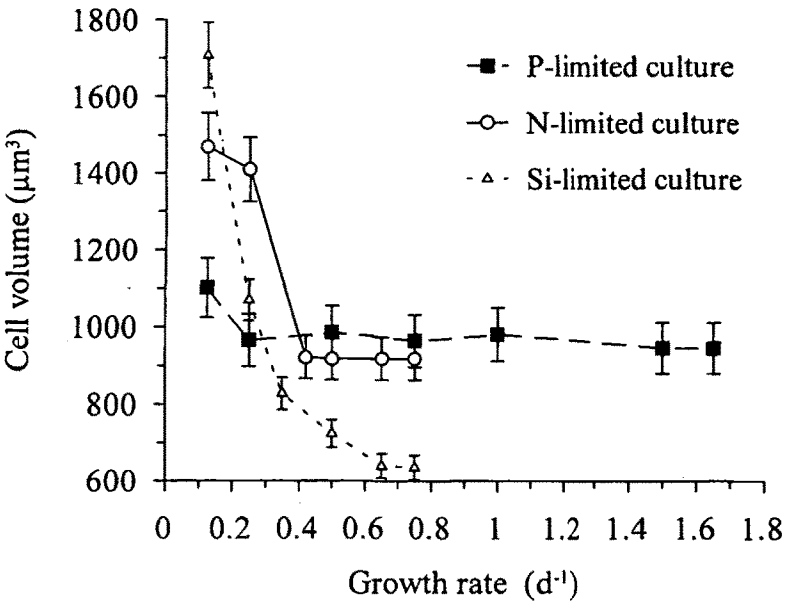


Fig. 2. Changes of cell volume ($\mu m^3$) versus growth rate (steady-state) ± standard division, in P, N or Si-limited cultures.

Fig. 2. Variations du volume cellulaire ($\mu m^3$) en fonction du taux de croissance ± division standard dans des conditions de culture limitées en P, N ou Si.

SRP in the culture vessel was about 8 ± 2 µg P $l^{-1}$ at growth rate of 0.125 $d^{-1}$. It was undetected at higher growth rates in P-limited culture. It increased with growth rate in both N and Si- limited cultures. Nitrate-nitrogen increased in all cultures. It had a hyperbolic relationship with the growth rate in N-limited culture (Fig. 3). According to Monod's model, $\mu_{max}$ of *C. meneghiniana* under N-limitation was 0.86 ± 0.1 $d^{-1}$ and $K_s$ was 98.2 ± 36 µg N $l^{-1}$ and under Si-limitation they were 0.95 ± 0.08 $d^{-1}$ and 369 ± 73 µg Si $l^{-1}$ respectively (Fig. 4, Table 3).

The changes in cell composition calculated per living cell number or per unit cell volume are shown in Table 1.

According to Droop's, Caperon's and Goldman's models, the growth and uptake parameters were calculated per cell or per unit cell volume for all cultures. The growth and the uptake curves are shown in Figs 5 and 6. The calculated parameters are given in table 2 a

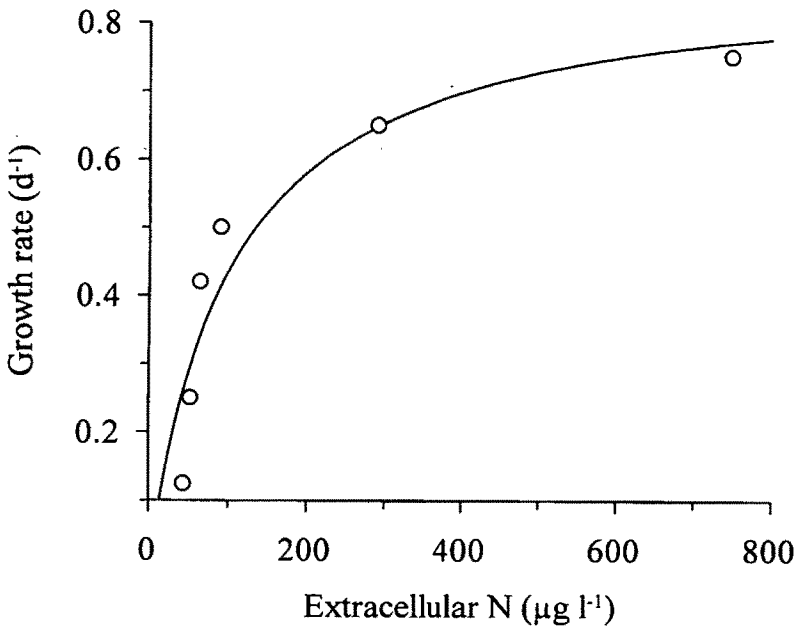


Fig. 3. Nitrogen limited culture hyperbolic relationship between extracellular N and growth rate (Monod's model).

Fig. 3. Culture limitée en azote. Relations entre l'azote extracellulaire et le taux de croissance (modèle de Monod).

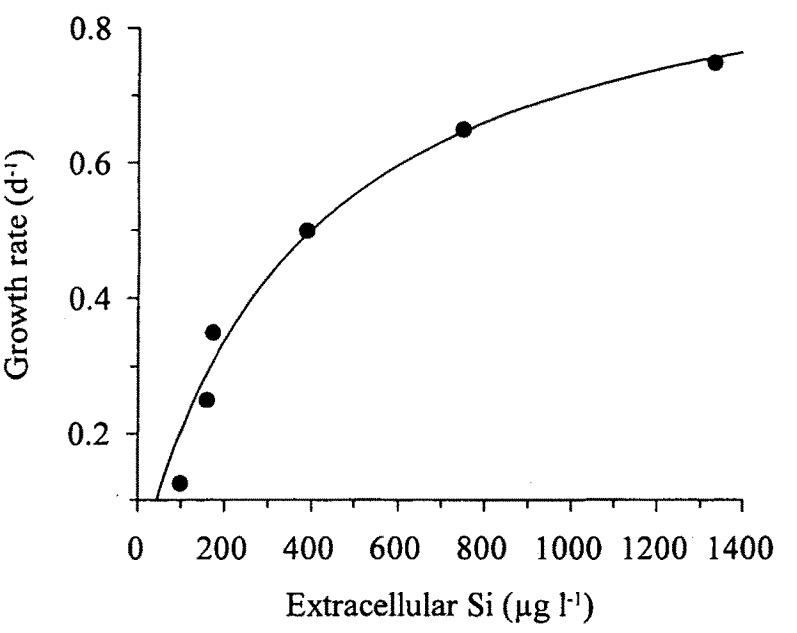


Fig. 4. Silicate limited culture hyperbolic relationship between extracellular Si and growth rate (Monod's model).

Fig. 4. Culture limitée en silice. Relations entre Si extracellulaire et taux de croissance (modèle de Monod).

and 2 b. Cell quota (pg Si cell$^{-1}$ or fg Si µm$^{-3}$) was calculated from the difference between the inflow and outflow Si concentration and then divided by cell number or by cell volume. From the Si-limited culture, figure 7A shows the relationships between the growth rate and cell quotas of Si calculated per cell. Droop's or Caperon's models give the same values of about 1 d$^{-1}$ and 11.4 pg Si cell$^{-1}$ for $\mu_{max}$* and $Q_{min}$ respectively. These values were significantly different when calculated per unit cell volume (Fig. 7B). The $\mu_{max}$* value was 0.70 ± 0.08 and 1.18 ± 0.3 d$^{-1}$ by Droop's and Caperon's models respectively. The $Q_{min}$ value was 7.15 ± 1.2 and 3.6 ± 3.3 fg µm$^{-3}$ by Droop's and Caperon's models respectively. Figure 7 C shows the relationships between the growth rate and the uptake rate of Si in Si-limited culture. From Goldman model, the $\mu_{max}$** value was about 1.01 ( 0.07 d$^{-1}$ (calculated per cell) and 0.87 ± 0.05 d$^{-1}$ (calculated per unit cell volume). The mean values of the half saturation constant for growth (11.0 ± 8.9 pg cell$^{-1}$) are very close to the half-saturation coefficient for uptake (11.2 ± 2 pg cell$^{-1}$) and not far from the minimum cell quota (about 11.3 pg cell$^{-1}$). The calculated parameters by different models are summarized in table 3.

## 4. Discussion

*Cyclotella meneghiniana* showed different behaviors for its growth and cell composition under different nutrient limitations and at different dilution rates.

Chlorophyll-a contents per cell or per unit cell volume increased with growth rate, and the maximum values were within the range of other diatoms (Reynolds 1984). The increasing of chlorophyll-a with growth rate has been recorded for many algal species (Shafik 1991). C/chlorophyll-a ratios changed throughout the experiment. It increased with growth rate from 81 to 626 in P-limited culture, and oscillated between 42 and 405 (average 160 ± 130) in N-limited culture. While in Si-limited culture, it changed slightly (46 ± 6), except at dilution rate of 0.125 d$^{-1}$ it was 197. The ratio was high near the maximum growth rate in P and N limited cultures but, generally, it was not far from the reported values of other algae (see Villareal & Carpenter 1994). This indicated that the C/ chlorophyll-a ratio widely changed with growth rates and type of limited nutrient.

Cell volume was highly affected by both the growth rate and the limiting nutrient. Growth rates are really influenced by a variety of metabolic factors, and generally coupled to size (Laws 1975, Banse 1976). In the present study, cell volumes decreased when the growth rate is increasing as mentioned by Reynolds (1984). The most effective limiting nutrient was Si. The regression of the cell chlorophyll-a content values (pg cell$^{-1}$) versus the corresponding cell volume data (µm$^{-3}$) (log:log) has negative values of r = - 0.845, - 0.883 and - 0.980 for P, N and Si-limited cultures, respectively. The negative correlation is depending on the growth rate.

Table 1. Changes of cellular cell content with growth rate under different nutrient limitation calculated per cell and per unit cell volume (* = results not included in the hyperbolic curves).

Tableau 1. Variations du contenu cellulaire en fonction de différents taux de croissance dans des conditions limitantes en nutriments ; résultats exprimés par cellule ou par unité de volume cellulaire.

P-limited culture

| | Cell quota (Pg cell $^{-1}$) | | | cell quota (fg µm$^3$) | | |
|---|---|---|---|---|---|---|
| Growth rate (d$^{-1}$) | P | N | C | P | N | C |
| 0.125 | 1.3 ± 0.5 | 18.91 ± 4.6* | 86.2 ± 27 | 1.2 ± 0.7 | 8.1 ± 0.6 | 78.3 ± 2.9 |
| 0.25 | 1.5 ± 0.03 | 9.3 ± 1.3 | 106 ± 11.9 | 1.5 ± 0.3 | 9.6 ± 0.26 | 109 ± 2.1 |
| 0.5 | 2 ± 0.5 | 21.2 ± 0.4 | 226 ± 5.9 | 2.0 ± 0.2 | 21.5 ± 2.2 | 229 ± 3.6 |
| 0.75 | 2.2 ± 0.15 | 31.6 ± 1.0 | 298 ± 6.8 | 2.3 ± 0.4 | 32.8 ± 0.35 | 309 ± 48 |
| 1 | 2.7 ±.2.4 | 33.0 ± 1.5 | 328 ± 30 | 2.7 ± 2.3 | 31.8 ± 2.12 | 334 ± 6.2 |
| 1.5 | 20.3 ± 0.3 | 177.0 ± 6.6 | 1741 ± 60 | 21.5 ± 0.3 | 169 ± 8.4 | 1840 ± 7.5 |
| 1.65 | 50.6 ± 0.3 | 353.0 ± 6.2 | 3444 ± 61 | 53.4 ± 2.3 | 328 ± 80 | 3640 ± 74 |

N-limited culture

| | Cell quota (Pg cell $^{-1}$) | | | cell quota (fg µm$^3$) | | |
|---|---|---|---|---|---|---|
| Growth rate (d$^{-1}$) | P | N | C | P | N | C |
| 0.125 | 6.7 ± 4.7* | 9.3 ± 0.7 | 169 ± 4.7 | 4.6 ± 1.2 | 6.4 ± 1.8 | 102 ± 2.1 |
| 0.25 | 3.4 ± 0.3 | 9.4 ± 2.6 | 173 ± 4.6 | 2.4 ± 0.14 | 6.7 ± 1.4 | 98.3 ± 6.2 |
| 0.42 | 3.9 ± 0.2 | 15.4 ± 2.2 | 145 ± 11.9 | 4.2 ± 0.3 | 16.8 ± 0.4 | 158 ± 2.8 |
| 0.5 | 4.8 ±1.4 | 22.8 ± 2.9 | 222 ± 1.5 | 5.2 ± 2.1 | 25.1 ± 1.2 | 242 ± 90 |
| 0.65 | 8.7 ± 1.0 | 42.0 ± 10.2 | 390 ± 5.5 | 9.5 ± 1.6 | 52.3 ± 4.3 | 338 ± 35 |
| 0.75 | 72 ± 3.8 | 311 ± 9.3 | 2309 ± 11.7 | 78.9 ± 4.6 | 427 ± 13.8 | 2515 ± 88 |

Si-limited culture

| | Cell quota (Pg cell $^{-1}$) | | | cell quota (fg µm$^3$) | | |
|---|---|---|---|---|---|---|
| Growth rate (d$^{-1}$) | P | N | C | P | N | C |
| 0.125 | 4.4 ± 5.4 | 19.0 ± 2.8 | 116 ± 12.3 | 2.6 ± 2.3 | 11.1 ± 9.1 | 67.8 ± 60 |
| 0.25 | 5.2 ± 0.4 | 19.2 ± 2.7 | 123 ± 12.9 | 4.9 ± 0.2 | 18.0 ± 0.2 | 115 ± 19 |
| 0.35 | 5.9 ± 1.9 | 20.9 ± 5.7 | 133 ± 23.1 | 7.1 ± 2.1 | 24.1 ± 6.5 | 161 ± 36 |
| 0.5 | 6.8 ± 1.0 | 24.9 ± 2.3 | 170 ± 35.3 | 9.4 ± 0.8 | 29.5 ± 3.1 | 234 ± 27 |
| 0.65 | 12.6 ± 1.8 | 28.3 ± 3.2 | 172 ± 20.8 | 19.7 ± 2.0 | 34.2 ± 2.1 | 268 ± 13 |
| 0.75 | 25.6 ± 5.8 | 32.2 ± 4.1 | 177 ± 22.2 | 40.2 ± 3.5 | 50.6 ± 5.6 | 278 ± 28 |

In case of relatively high growth rates, more nutrients can be taken up and more leakage occurs under the same growth conditions (Mastala et al. 1996). This might be, because they have better metabolic cycle and good ion transport between the cell and the medium.

Normal C, N and P content for some freshwater phytoplankton are 210-240 fg C µm$^{-3}$, 40 fg N µm$^{-3}$ and 5 fg P µm$^{-3}$ (Reynolds 1984). A close ratio was recorded for *Ethmodiscus rex* (Villareal & Carpenter 1994).

In all cultures, C, N and P content per cell or per unit cell volume increased with growth rate. Most of them had a hyperbolic relationship with growth rate (Fig. 5). In P-limited culture C:N:P ratios ranged between (136-66): (15-6): 1 suggest P-limitation. These ratios were (51-32): (5-4): 1 in N-limited chemostat suggest N-limitation. C and N content per cell or per unit cell volume slightly increased in Si-limited culture, but were lower than the other cultures. These values agree with

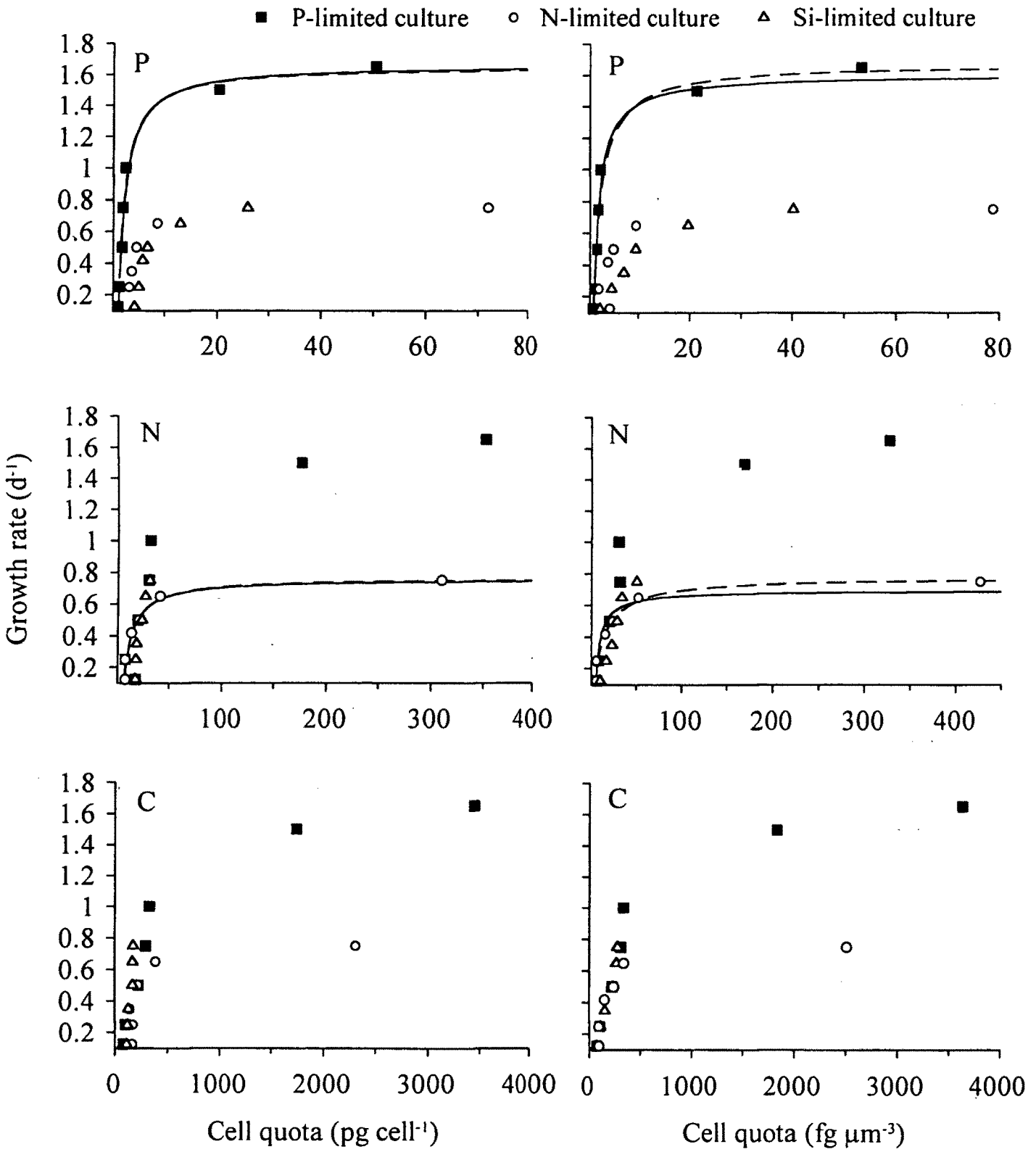


Fig. 5. The relationships between the cell quota (cell$^{-1}$ or cell volume ($\mu m^{-3}$)) and growth rates ($d^{-1}$) calculated by Droop's (——) and Caperon's (----) models for different nutrient in different cultures.

Fig. 5. Relations entre quotas cellulaires (cell/l ou volume cellulaire, $\mu m^3$) et taux de croissance calculés à partir du modèle de Droop (——) et de Caperon (----) pour différents nutriments dans des conditions différentes de cultures.

the normal values of other algae (Reynolds 1984). These ratios do not mean that the culture was C- or N-limited, but indicate that *C. meneginhiniana* accumulate P under Si- limitation, but it could not accumulate P-under N-limitation. At maximum growth rate of both P and N-limited cultures C, N and P contents were higher by one order of magnitude than the normal levels. Goldman (1980) suggested that the chemical composition of phytoplankton approaches the Redfield proportions (C:N:P 0f 41:7:1 by weight) near maximum

Table 2a. Growth parameters calculated per cell with different models.
Tableau 2a. Paramètres de croissance calculés par cellule à partir de différents modèles.

| | P-limited culture | | | N-limited culture | | | Si-limited culture | | |
|---|---|---|---|---|---|---|---|---|---|
| | Droop model | Caperon model | Goldman model | Droop model | Caperon model | Goldman model | Droop model | Caperon model | Goldman model |
| $\mu_{max}$* or $_{\mu ma}$x**($d^{-1}$) | 1.65±0.06 | 1.66±0.08 | 1.65±0.04 | 0.81±0.05 | 0.77±0.02 | 0.81±0.07 | 0.91±0.06 | 0.81±0.04 | 0.87±0.03 |
| $Kq$ (pg P$cell^{-1}$) or $K_{up}$ (pg P$cell^{-1}$ $d^{-1}$) | - | 1.29±0.27 | 1.99±0.15 | - | 1.08±0.15 | 1.96±0.5 | - | 1.89±0.43 | 2.79±0.26 |
| $Q_{min}$ (pg P $cell^{-1}$) | 1.22±0.05 | 1.21±0.08 | 1.21 | 2.17±0.18 | 2.90±0.09 | 2.40 | 3.56±0.21 | 4.09±0.15 | 3.22 |
| $\mu_{max}$* or$\mu_{max}$**($d^{-1}$) | 1.42±0.15 | 1.79±0.2 | 1.68±0.06 | 0.76±0.03 | 0.77±0.05 | 0.77±0.01 | 1.53±0.12 | 1.13±0.4 | 1.50±0.06 |
| $Kq$ (pg N $cell^{-1}$) or $K_{up}$ (pg N$cell^{-1}$ $d^{-1}$) | - | 32.2±10 | 25.0±3.29 | - | 7.50±2.7 | 5.4±0.34 | - | 8.1±8.9 | 24.2±1.7 |
| $Q_{min}$ (pg N $cell^{-1}$) | 9.0±1.7 | 4.8±3.5 | 14.9 | 7.09±0.4 | 6.95±1.1 | 7.01 | 15.8±0.6 | 17±1.3 | 16.1 |
| $\mu_{max}$* or$\mu_{max}$**($d^{-1}$) | 1.41±0.15 | 1.79±0.05 | 1.69±0.05 | 0.81±0.11 | 0.82±0.17 | 0.81±0.04 | 1.64±0.18 | 1.89±2.3 | 2.01±2.3 |
| $Kq$ (pg C $cell^{-1}$) or $K_{up}$ (pg C$cell^{-1}$ $d^{-1}$) | - | 295±76.1 | 248±27 | - | 111±121 | 78±12 | - | 133±261 | 232±64 |
| $Q_{min}$ (pg C $cell^{-1}$) | 89.4±11 | 64.5±19.1 | 147 | 106±17 | 104±58 | 96 | 106±4.7 | 104±14 | 115 |

Table 2b. Growth parameters calculated per unit cell volume ($\mu m^3$) with different models.
Tableau 2b. Paramètres de croissance calculés par unité de volume cellulaire ($\mu m^3$) à partir de différents modèles.

| | P-limited culture | | | N-limited culture | | | Si-limited culture | | |
|---|---|---|---|---|---|---|---|---|---|
| | Droop model | Caperon model | Goldman model | Droop model | Caperon model | Goldman model | Droop model | Caperon model | Goldman model |
| $\mu_{max}$* or$\mu_{max}$**($d^{-1}$) | 1.61±0.06 | 1.67±0.08 | 1.65±0.04 | 0.71±0.13 | 0.82±0.21 | 0.79±0.05 | 0.69±0.07 | 0.81±0.04 | 0.81±0.03 |
| $Kq$ (fg P $\mu m^{-3}$) or $K_{up}$ (fg P $\mu m^{-3}$ $d^{-1}$) | - | 1.58±0.40 | 2.04±0.15 | - | 4.07±4.9 | 1.63±0.6 | - | 8.00±2.00 | 2.92±0.36 |
| $Q_{min}$ (fg P $\mu m^{-3}$) | 1.18±0.07 | 1.10±0.12 | 1.23 | 1.88±0.50 | 1.00±2.57 | 2.06 | 2.43±0.21 | 1.49±0.50 | 3.6 |
| $\mu_{max}$* or$\mu_{max}$**($d^{-1}$) | 1.39±0.15 | 1.80±0.1 | 1.69±0.07 | 0.69±0.04 | 0.78±0.05 | 0.74±0.03 | 0.83±0.09 | 1.78±0.03 | 1.06±0.07 |
| $Kq$ (fg N $\mu m^{-3}$) or $K_{up}$ (fg N $\mu m^{-3}$ $d^{-1}$) | - | 31.2±8.9 | 24.9±3.5 | - | 11.4±3.7 | 4.6±0.83 | - | 60±6 | 15.5±2.4 |
| $Q_{min}$ (fg N $\mu m^{-3}$) | 8.25±1.16 | - | 14.8 | 4.94±0.5 | 2.95±1.41 | 6.3 | 10.5±1.31 | 7.6±2.9 | 14.6 |
| $\mu_{max}$* or$\mu_{max}$**($d^{-1}$) | 1.38±0.16 | 1.79±0.12 | 1.68±0.05 | 0.78±0.05 | 0.78±0.07 | 0.78±0.02 | 0.78±0.10 | 1.65±0.65 | 1.11±0.16 |
| $Kq$ (fg C $\mu m^{-3}$) or $K_{up}$ (fg C $\mu m^{-3}$ $d^{-1}$) | - | 313±79 | 252±29 | - | 77±35 | 57.6±6.1 | - | 50.9±72 | 117±36 |
| Qmin (fg C $\mu m^{-3}$) | 84.4±13 | 59±20 | 150 | 75.7±5.6 | 75±13 | 73.8 | 63.5±9.9 | 72±19 | 106 |

growth rate. We never achieved the exact Redfield ratios throughout of the experiments. The hypothesis is stated by Sakshaug et al. (1983), that C:N:P ratio of 40:6:1 is a saturation level for 5 marine species is refuted here.

Si contents of cultured *Asterionella formosa* Hass. cells range from 11 to 52 pg $cell^{-1}$ (Paasche 1980), and values for *Cyclotella* spp. from natural planktonic samples are from 100 to 900 (Einsele & Grim 1938). Werner (1977) suspected that the plankton data were overestimated. The cell volume of *Asterionella formosa* ranged from 250 to 1000 $\mu m^3$ (Pavoni 1963, Besch et al. 1972). Recalculations of Si content per unit cell volume of *Asterionalla formosa* lead to a Si content

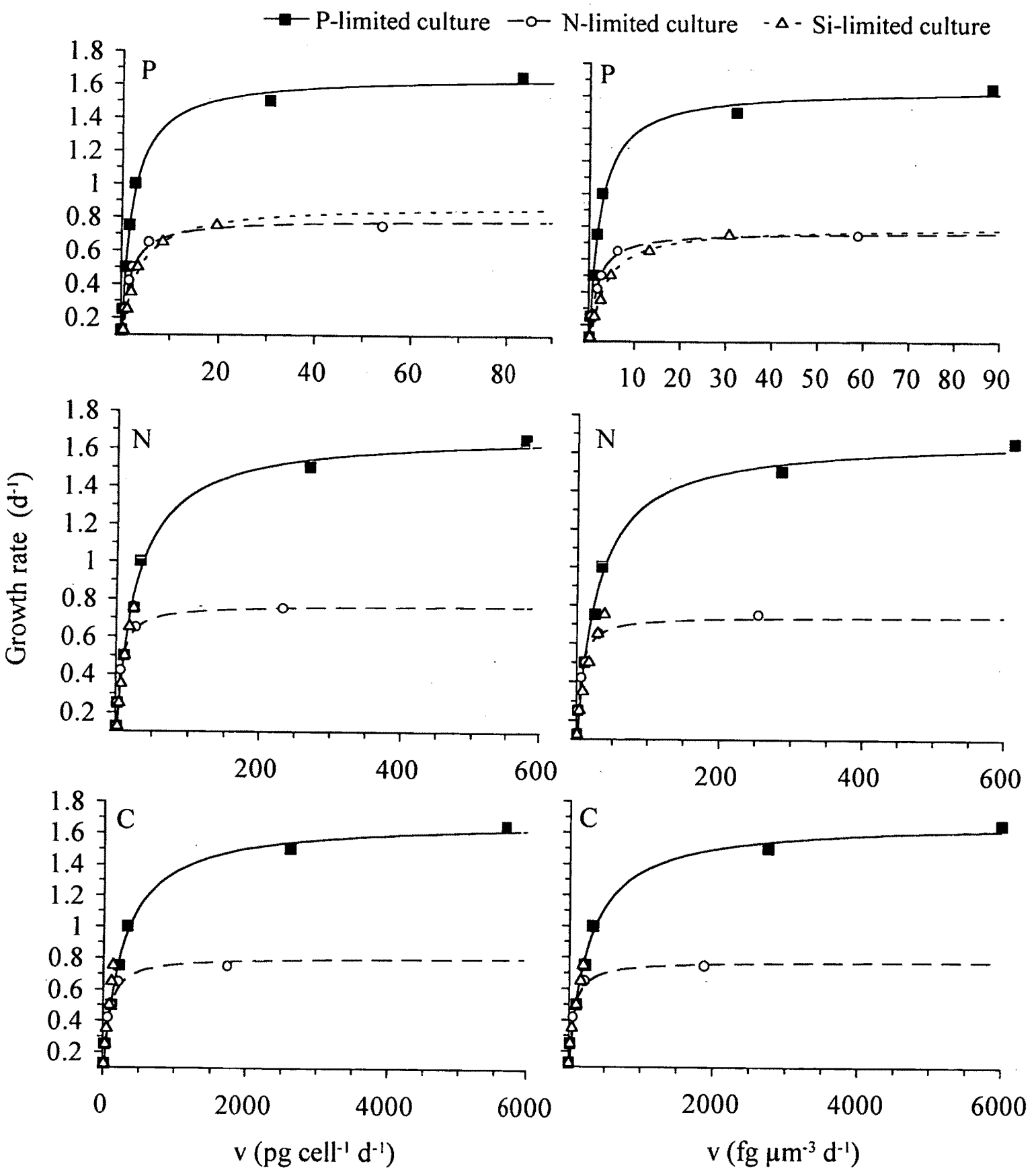


Fig. 6. The relationships between the nutrient uptake (cell$^{-1}$ d$^{-1}$ or cell volume (µm$^{-3}$ d$^{-1}$)) and growth rates (d$^{-1}$) calculated by Goldman's model for different nutrients in different cultures.

Fig. 6. Relations entre le taux d'assimilation (cell/1 ou volume cellulaire, µm$^3$) et taux de croissance calculés à partir du modèle de Goldman pour différents nutriments dans des conditions de culture différentes.

Table 3. The results of different parameters calculated by cell or unit cell volume ($\mu m^3$) ± standard deviation of Si-limited chemostat with different models.

Tableau 3. Résultats des différents paramètres obtenus en chemostat dans des conditions où Si est limitant. Les résultats sont calculés pour les différents modèles et exprimés soit par cellule soit en volume cellulaire ($\mu m^3$) ± déviation standard.

| | **Monod** | **Droop** | | **Caperon** | | **Goldman** | |
|---|---|---|---|---|---|---|---|
| | | $cell^{-1}$ | $\mu m^{-3}$ | $cell^{-1}$ | $\mu m^{-3}$ | $cell^{-1}$ | $\mu m^{-3}$ |
| $\mu_{max}$, $\mu_{max}$* or $\mu_{max}$ ** ($d^{-1}$) | 0.96 ± 0.09 | 1.00±0.08 | 0.70±0.08 | 0.99 ±0.27 | 1.18±0.3 | 1.01 ±0.07 | 0.87±0.05 |
| | $\mu g$ Si $l^{-1}$ | pg $cell^{-1}$ | fg $\mu m^{-3}$ | pg $cell^{-1}$ | fg $\mu m^{-3}$ | pg $cell^{-1}d^{-1}$ | fg $\mu m^{-3}d^{-1}$ |
| $K_s$ , $Kq$ or $K_{up}$ | 390 ± 89 | - | - | 11.0 ± 8.9 | 38.2±23.4 | 11.2 ±2.0 | 10.0±1.7 |
| | | pg $cell^{-1}$ | fg $\mu m^{-3}$ | pg $cell^{-1}$ | fg $\mu m^{-3}$ | pg $cell^{-1}$ | fg $\mu m^{-3}$ |
| $Q_{min}$ | | 11.3 ±0.9 | 7.15±1.2 | 11.4 ± 1.9 | 3.6±3.3 | 11.2 | 11.4 |

ranged from 44 to 52 fg $\mu m^{-3}$. In Si limited culture these values ranged from 13 to 41 pg $cell^{-1}$ and 17 to 64 fg $\mu m^{-3}$ for *C. meneghiniana*. Sommer (1988) suggested that the total Si content changed only by a factor of 1.6 between Si-rich and Si- starved cells. This factor was 3.1 when calculated per cell and 3.7 when calculated per unit cell volume in the case of *C. meneghiniana*.

The $\mu_{max}$* and $\mu_{max}$** per cell or per unit cell volume both had correspondence values of 1.65 ± 0.02 $d^{-1}$ for P- limited culture and 0.77 ± 0.04 $d^{-1}$ for N-limited culture. Goldman's and Caperon's models used for calculating $\mu_{max}$* and $\mu_{max}$** found close values for non-limited nutrient in case of P- or N-limited cultures. This means that the maximum growth rate can be calculated from the limited or non limited nutrient concentration, at least in some cases, and depending on the type of limiting nutrient. The maximum growth rate which recorded here (1.65 ± 0.2 d-1) was higher than that recorded by Tilman & Kilham (1976) 0.78 $d^{-1}$ for batch culture and 0.69 ± 0.09 $d^{-1}$ for semi-continuous cultures for P-limited culture of *C. meneginhiniana*. This may be a result in the difference of growth conditions of Tilman and Kilham's experiments (20°C) and the optimum temperature for growth of *C. meneginhiniana* (25°C) in our experiment (Shafik et al. 1997).

The $Q_{min}$ value of P in P-limited culture was very close when calculated with any of the three models. The $Q_{min}$ value was 1.21 ± 0.006 pg P $cell^{-1}$ and 1.17 ± 0.07 fg P $\mu m^{-3}$. This value is much higher than 2.9 and 38.8 fg $cell^{-1}$ which was reported for *Cyclotella nana* and *Thalassiosira fluviatilis* Hust, respectively (Fuhs 1969) and ranged from 0.5 to 1.4 fg P $cell^{-1}$ for six species of algae (Soeder et al. 1971). The $Q_{min}$ values of P in P-limited culture are lower than the $Q_{min}$ values of P recorded in N-limited cultures (2.5 ±0.4 pg $cell^{-1}$) and Si-limited cultures (3.6 ± 0.4 pg $cell^{-1}$). The same picture shows for N-limited culture (Table 2). Clearly, the calculated minimum cell quota depends mainly on the concentration of the limiting nutrient in the culture.

Table 3 shows the growth parameter calculated from Si-limited chemostat per cell or per unit cell volume by the different models. There is no difference in $\mu_{max}$, $\mu_{max}$* or $\mu_{max}$** when calculated per cell, and it is not far from the values recorded for *C. meneginhiniana* under Si-limited semi-continuous culture of Tilman & Kilham (1976). Very close values of $Q_{min}$ were recorded when calculated per cell by the different models. $K_s$ of 390 ± 89 $\mu g$ Si $l^{-1}$ was higher than the recorded value from batch culture by Tilman & Kilham (1976). $Kq$ and $K_{up}$ were 10.9 and 11.2 pg $cell^{-1}$ respectively. This means that the half saturation constant for growth is very close to the half saturation coefficient of uptake. The cell volume in Si-limited culture was significantly lowered with increasing growth rate. The growth parameters calculated per unit cell volume were changed significantly. The calculated values of μmax,

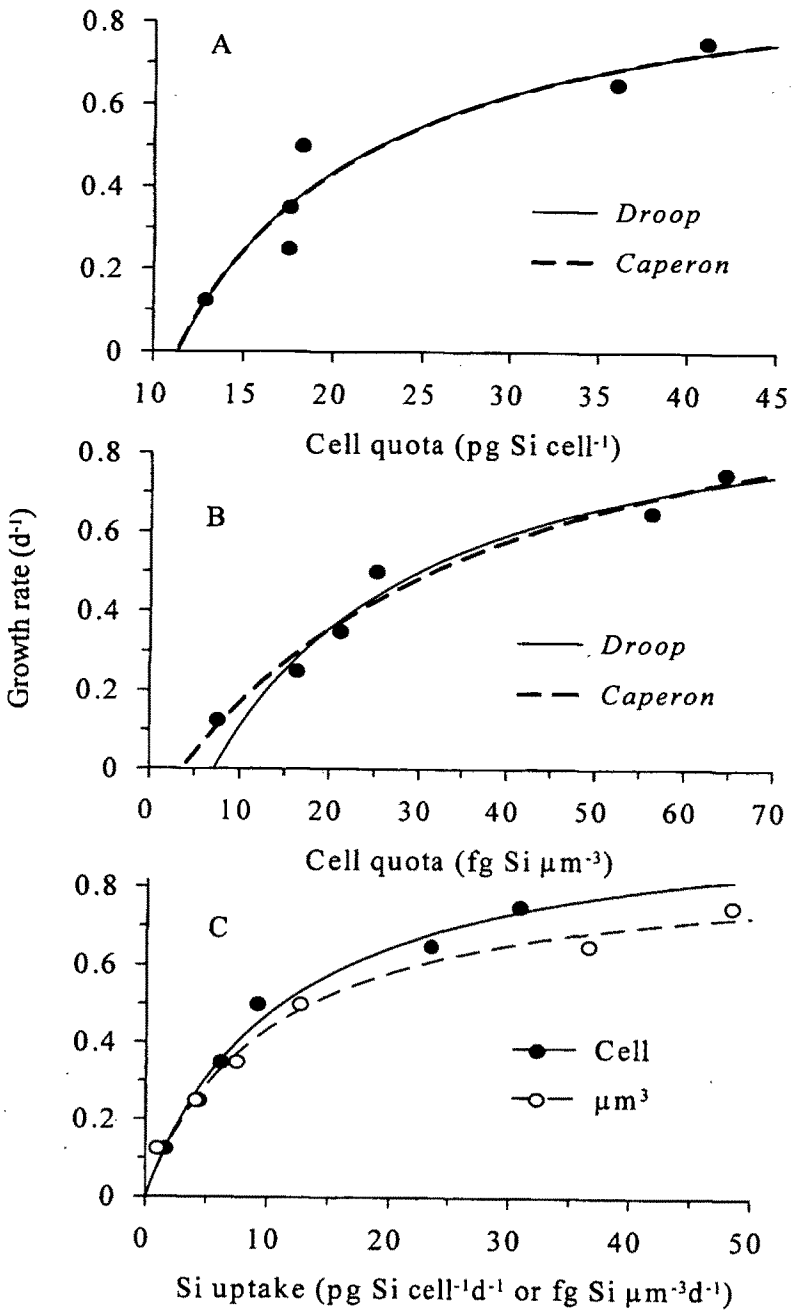


Fig. 7. Silicate limited cultures, the relationship between growth rate ($d^{-1}$) and cell quota of Si per cell (A), or per unit cell volume (B) by Droop's and Caperon's models and between growth rate and nutrient uptake by Goldman's model (C).

Fig. 7. Cultures limitées en Si - Relations d'une part entre taux de croissance et quota cellulaire en Si par cellule (A) ou par unité de volume (B) calculées à partir des modèles de Droop et de Caperon et d'autre part entre taux de croissance et taux d'assimilation calculés à partir du modèle de Goldman (C).

$\mu_{max}$* and $\mu_{max}$** from the cell volume gave different values by different models. So, $Q_{min}$ had significant difference in this values with different models. Clearly, when the cell volume of the algae changes, it is important to calculate the growth parameter per cell volume in relation to other biomass measurements.

The average trophic level of River Danube at Budapest is 2850 µg N $l^{-1}$ and 185 µg P $l^{-1}$ (Kiss 1994). These concentrations are much higher than the growth and uptake requirements for *C. meneginhiniana*. This indicates that the growth of *C. meneginhiniana* is not P- or N-limited in its natural habitat.

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