Growth of *Cyclotella meneghiniana* Kutz. I. Effects of temperature, light and low rate of nutrient supply

H. M. Shafik[1,2]
S. Herodek[1]
L.Vörös[1]
M. Présing[1]
K. T. Kiss[3]

Keywords : *Cyclotella meneghiniana*, temperature, light, low growth rate, cell composition.

A strain of *Cyclotella meneghiniana* was isolated from the River Danube. The optimum irradiance and temperature for growth were determined in batch cultures. The optimum temperature for growth was 25 °C. The estimated saturated irradiance was near 125 µE m^{-2} s^{-1}. The calculated I_k for growth and photosynthesis were 30 ± 3 and 44 ± 6 µE m^{-2} s^{-1} respectively. A continuous culture technique was used to test the growth and cell composition at the transition state between batch, nutrient sufficiency, and a low rate of nutrient supply. Algal response was characterized by high intracellular N and C with negative growth rate. At a low dilution rate (0.06 d^{-1}), the continuous culture theory was not followed. This critical rate is characterized by low chlorophyll-a content per cell and may depend on the mode of nutrition.

Croissance de *Cyclotella meneghiniana* Kutz. I. Influence de la température, de la lumière et d'un faible apport de nutriments

Mots clés : *Cyclotella meneghiniana*, température, lumière, faible taux de croissance, composition cellulaire.

Une souche de *Cyclotella meneghiniana*, provenant du Danube, a été isolée. La température et la lumière optimales pour la croissance ont été déterminées en culture «batch» : 25°C et 125 µEm^{-2} s^{-1}. L'I_k calculé pour la croissance et la photosynthèse était respectivement de l'ordre de 30 ± 3 et de 44 ± 6 µE m^{-2} s^{-1}. La technique de la culture en continu a été également utilisée avec un faible apport de nutriments, correspondant à un stade en culture «batch» où les nutriments étaient encore non limitants. La réponse de la diatomée était caractérisée par un quota cellulaire de N et P élevé avec un taux de croissance négatif. Avec un faible taux de dilution (0,06 J^{-1}), la théorie de la culture en continu n'était pas vérifiée. Ce taux critique de dilution se caractérisait par un contenu cellulaire en chlorophylle a faible et peut dépendre du mode de nutrition.

1. Introduction

C. meneghiniana is one of the most frequently dominant potamoplankter in River Danube (V.-Balogh et al. 1994) and in European, American and Japanese rivers (Kiss & Nausch 1988, Murakami et al. 1994). To elucidate the essential factors of the growth and decline of mass populations, we have to know the influence of basic environmental factors such as light (Knowlton & Jones 1996) and temperature. It is well known that the growth and cell composition are highly affected by the available nutrients. *C. meneghiniana* may predominate over other diatoms in a silica-rich river environment (Hori et al. 1969). Tilman & Kilham 1976, Tilman 1977 studied the growth and competition ability of *C. meneghiniana* in batch and semi-continuous cultures under P and Si limitations. The changes of cell volume and cell composition throughout the nutrient gradients have not yet been clear. Therefore, this study was undertaken to find out the effect of low rate of nutrient

1. Balaton Limnological Research Institute of the Hungarian Academy of Science, Tihany, Hungary.
2. Suez Canal university, Faculty of Sciences, Botany Department, Ismailia-Egypt.
3. Hungarian Danube Research Station of the Hungarian Academy of Science, God, Hungary.

supply on growth, cell volume and cell composition of *C. meneghiniana*. A transition experiment among batch and a low addition rate with low nutrient concentrations was performed. The ability to use continuous culture theory at a critical low dilution rate was also investigated.

2. Materials and methods

A strain of *Cyclotella meneghiniana* was isolated from the Hungarian section of River Danube in summer 1994 by H. M. Shafik. Modified Schlösser's medium (1982) was used for the isolation and in batch and continuous culture experiment. The medium was modified by doubling the concentration of $Na_2 CO_3$, $Ca(NO_3)_2 \, 4H_2O$ and soil extract, while the concentration of Fe-citrate was reduced to half. The cultures were unialgal but not axenic. The count of bacteria on agar plates was very low in all bacteriological tests throughout the experiments.

2.1. Batch cultures

Erlenmeyer flasks of 500 ml capacity were used. The flasks were illuminated by cool-white fluorescent tubes. Lighting was unidirectional; triplicates were placed in a box with five black walls, one side being open toward the light (Healey 1983). The irradiance was measured with an LI-COR (LI-185 B) radiometer equipped with a flat $(2 \, \pi)$ sensor.

The temperature dependence of growth was determined at five different temperatures (10, 15, 20, 25 and 30 ° C). For temperature regulation a circulating water bath was used (Neslab RTE 210). Irradiance was adjusted to 210 $\mu E \, m^{-2} \, s^{-1}$ with a light: dark (16: 8) cycle.

Growth- irradiance relationship was studied at the irradiance of 10, 20, 40, 70, 100, 200, 300 and 500 μE $m^{-2} \, s^{-1}$, at 25 ° C and the same light - dark cycle (16-8). The light dependence of photosynthesis and growth were described by Monod's model (1942) and by the exponential saturation equation (Webb et al. 1974):

$$\mu = \mu_{max} \, (1 - exp^{(-I/Ik)}) \tag{1}$$

where, μ_{max} is the maximum rate of photosynthesis or growth; I_k is the irradiance at which initial slope line reaches the photosynthesis or the growth of μ_{max}; and *I* is the irradiance at the front of the black box.

In preliminary experiments we found a significant correlation ($r = 0.994$, $n=19$) between cell number and light absorption at 750 nm. Accordingly, the growth was monitored intervals of 12 hours by measuring light absorption at 750 nm using SHIMADZU UV-VIS 160A Spectrophotometer.

The growth rate (μ) was determined by the following formula:

$$\mu = (\ln \chi_1 - \ln \chi_0) / (t_1 - t_0) \tag{2}$$

where, μ is the specific growth rate, χ_1 is the light absorption at time t_1, χ_0 is the light absorption at time t_0.

2.2. Continuous cultures

The chemostat apparatus has been previously described by Shafik (1991). Here, we shall give the basic details. The culture medium was supplied from the reservoir to the culture vessel at the desired flow rate by a peristaltic pump (Masterflex 7523-12) calibrated before use. The culture vessel has a conical form with capacity of 3 liters. The algal culture volume was 2 litres. The culture was aerated by pumping sterile air through a deeply inserted tube. The aeration also ensured the stirring of the culture. In addition, a teflon-coated magnetic stirring bar was used. All of the equipment and the culturing medium were autoclaved at 120 ° C for 2 hours. All chemostats were run at 25 ° C, the optimum temperature of growth obtained from the result of batch culture experiments. The irradiance was 210 $\mu E \, m^{-2} \, s^{-1}$ for 16 hours light and 8 hours dark of growth and cycle. This is higher than the saturated of growth and agreement light with the day length in nature at the time of isolation. The cultures were grown in three chemostats vessels, as batch cultures, for three days. At the end of exponential growth phase, fresh media were added into the culture vessels. The P, N and Si concentrations, in the original medium, were 1.78, 9.5 and 6.6 mg l^{-1} respectively. In the inflowing media, only the original concentration of the limiting nutrient was lowered to 0.125 mg P l^{-1} for P-limited culture; 1.5 mg N l^{-1} for N-limited culture and 1.6 mg Si l^{-1} for Si-limited culture. The inflowing medium were pumped at low dilution rate of 0.06 d^{-1}. This dilution rate was about 4 % of the maximum growth rate which had been calculated from the batch cultures (1.45 d^{-1}). The pH of the medium was between 7.2 and 7.4 throughout the experiment. A definite volume of 100 ml d^{-1} from each culture was harvested for different analysis. The experiment was repeated twice.

In continuous culture part of the experiment the growth rate ($\mu \, d^{-1}$) was determined by the following formula:

$$\mu = (\ln x_1 - \ln x_0) / (t_1 - t_0) + D \tag{3}$$

where, x_1 is the number of living cell at time t_1, x_0 is the number of living cell at time t_0, and D is the dilution rate = 0.06 d^{-1}.

2.3. Measurements

The internal N and C contents were measured following filtration on GF/C glass fiber filters by Automated Nitrogen/ Carbon analyzer-Mass Spectrometer System (ANCA-MS system Europa Scientific Ltd., UK.). For the determination of internal P content, algal cells were filtered through cellulose-acetate membrane filters of 0.45 μm pore size then washed with P free medium. 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 internal N, C and P content, the cell number, cell volume and chlorophyll-a were measured. The count of cells and cell volume were obtained by inverted microscope technique (Utermöhl 1958). At least 400 cells were counted, and the cell volumes of 20 cells were measured. Cell volume was calculated by assigning the cell to geometric shape (cylindrical shape). To determine the dry weight, 50 ml of the culture was filtered on dried and weighted Whatman GF/C glass fiber filters. The filters with the algae were dried at 105 ° C overnight, and they were reweighted. The chlorophyll-a concentration was determined by extraction with boiling methanol according to Iwamura et al. (1970). SRP (soluble reactive phosphorus) was measured with the molybdate method (Murphy & Riley 1962). Nitrate was determined via reduction to nitrite according to Elliott & Porter (1971) and the available silicon was measured by Mullin & Riley method (1955).

The photosynthetic activity was measured by the ^{14}C method (Vollenweider 1969). After 2 hours exposure the samples were filtered through cellulose-acetate membrane filters of 0.45 μm pore size. In order to remove radioactive contamination by non-assimilated ^{14}C, 10 ml unlabelled water was passed through the filters. Then the filters were exposed to the fume of concentrated HCl for 15 minutes. The filters were then dissolved in 10 ml Bray-solution. After 48 hours the radioactivity of the algae was measured using a LKB Rackbeta-2 liquid scintillation counter.

3. Results

3.1. Batch cultures

The effect of temperature on growth is shown in Fig. 1. The optimum temperature for growth was 25 ° C. The maximum growth rate was 1.45 ± 0.06 d^{-1} and chlorophyll-a concentration was about 1261 ± 23 μg l^{-1}. These were enhanced with increasing temperatures till 25° C then decreased at temperature of 30° C. The effect of different irradiance on growth is shown in Fig. 2. The estimated saturated irradiance was about 125 μE

m^{-2} s^{-1}. The calculated I_k for growth and photosynthesis, using equation 1 were 30 ± 3 and 44 ± 6 μE m^{-2} s^{-1} respectively, compared to 23 ± 5 and 41 ± 8 μE m^{-2} s^{-1} by Monod's equation (Figs. 3a and b). The maximum growth rates were 1.47 ± 0.04 and 1.62 ± 0.08 d^{-1} as calculated by the Webb and the Monod equations respectively.

3.2. Continuous cultures

The cell numbers were increased in all cultures at the first 96 hours, then slightly changed in P-limited culture. They continuously increased in N-limited culture and decreased in Si-limited culture (Fig. 4). Dead cells were not observed in all cultures till 144 hours. At the next days dead cells were between 15 and 25 % of total cell number and were about 48% at the end of experiment in P-limited culture. The dead cells were found to be about 4 % in N-limited culture through the next period. While, in Si-limited culture dead cells were between 8 and 13 % and later increased to about 32 % (Fig. 4).

The maximum growth rates were recorded after 24 hours in all chemostats. The maximum growth rates were between 1.44 and 1.51 d^{-1}. These values were in accordance with those recorded in batch cultures.

The cell volume was slightly changed in the first 144 hours. In the next 96 hours, cell volume was unchanged in P-limited culture and slightly increased in N-limited culture. It was significantly increased in Si-limited culture (Fig. 5). The cell volume of the original inoculation was 1656 μm³ in all cultures. This volume was decreased to about 1197 μm³ in P-limited culture and slightly increased to about 1960 μm³ in N-limited culture. A much more significant increase in the Si-limited culture which was recorded as 2305 μm³ at the end of the experiment (Fig. 5).

The concentration of chlorophyll-a increased during the first three days and next decreased in all cultures. It showed higher values in Si-limited culture than both P- and N- limited cultures till 240 hours. Chlorophyll-a content per cell was increased to its higher value between 8 and 9.9 pg chlorophyll-a $cell^{-1}$ in batch culture phase (Fig. 7) and decreased afterwards. Chlorophyll-a has the lowest concentrations at the last period of experiment in all cultures (Fig. 6). The lowest values of 0.5, 0.2 and 1.2 pg chlorophyll-a $cell^{-1}$ were recorded in P, N and Si- limited culture respectively at the end of the experiment (Fig. 7).

P, N and C content per chlorophyll-a content (weight/weight) were changed throughout the experiment (Fig. 8). There were difficulties to calculate the actual cell quota because of the high number of dead
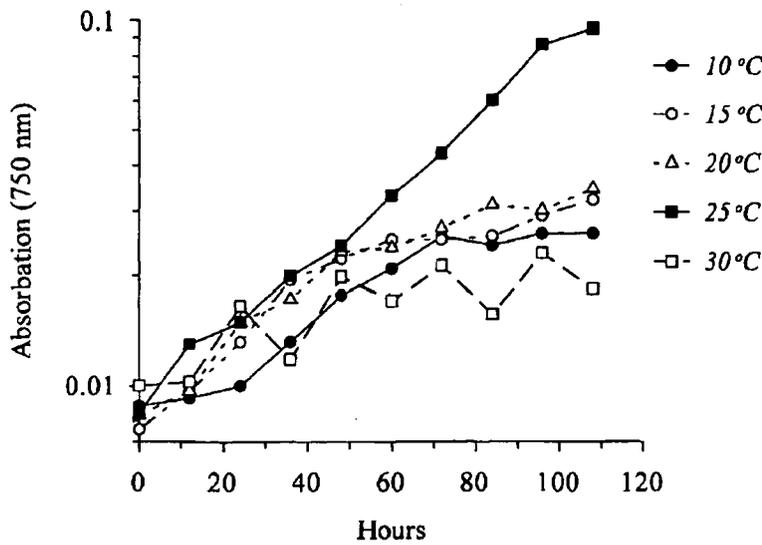
Fig. 1. Growth of *Cyclotella meneghiniana* at different temperatures (mean of triplicates) in batch culture.

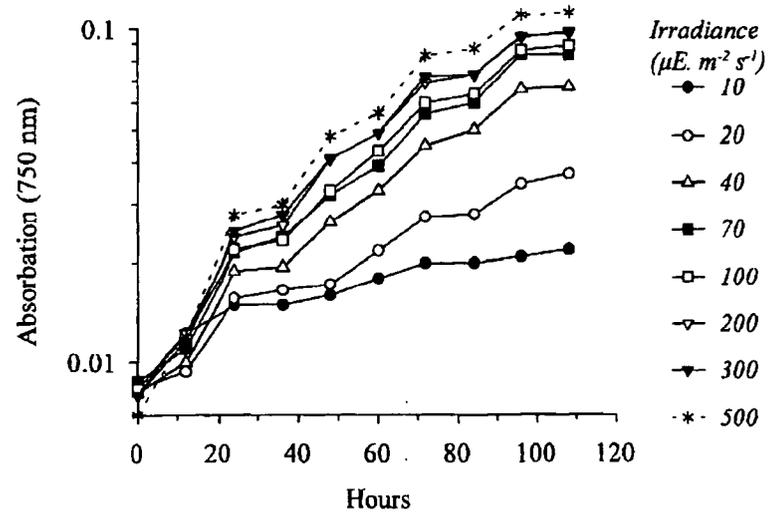Fig. 1. Croissance de *Cyclotella meneghiniana* en fonction de la température. Culture «batch».

Fig. 2. Growth of *Cyclotella meneghiniana* at different irradiance ($\mu E.\ m^{-2}\ s^{-1}$) (mean of triplicates) in batch culture at 25 ° C.

Fig. 2. Croissance de *Cyclotella meneghiniana* en fonction de l'intensité lumineuse ($\mu E.\ m^{-2}\ s^{-1}$). Culture «batch» à 25 ° C.

Table 1. Changes of cell composition calculated per cell number of living cell (pg cell^{-1}) during chemostat experiment (± standard deviation).
* = the replicate sample was lost ; - = no data (the sample was lost)

Tableau 1. Variations de la composition cellulaire des cellules vivantes (pg cell^{-1}) au cours d'une expérience en chemostat (± déviation standard).
* = le répliquat n'a pu être exploité ; - = absence de données

	P-limited culture			N-limited culture			Si-limited culture		
Hours	P	N	C	P	N	C	P	N	C
0	-	30.97±2.1	201.4±8.1	-	31.56±2.0	205.4±13.0	-	19.57±1.2	184.5±12.5
24	6.01±0.52	25.99±1.9	180.2±12.2	8.02±0.3	27.05±2.1	245.7±6.514.2	10.10±1.4	18.94±2.1	132.8±5.8
96	5.77±0.32	21.54±1.9	256.3±10.2	7.85±0.1	23.12±1.1	316.1±10.1	8.10±0.1	26.42±2.1	321.8±21.0
144	5.74±0.2	17.12±1.3	225.2±5.1	7.61±0.1	18.80±1.3	352.3±10.0	7.32±0.3	21.82±2.2	325.9±14.1
168	5.81±0.1	21.57±2.2	219.6±6.0	6.32±0.5	19.31±0.5	359.6±12.5	9.58±0.4	26.88±1.3	500.7*
192	5.20±0.23	23.31±1.6	172.0±1.2	4.45±0.5	11.91±1.0	228.5±13.2	6.75±0.1	19.63±0.9	376.4±37.4
216	4.48±0.5	26.51±1.5	180.2±1.1	3.77±0.2	10.76±0.5	191.9±1.2	7.75±0.6	28.36±2.1	346.7±33.6
240	3.49±0.31	27.13±2.1	166.9±2.4	3.99±0.3	10.68±0.3	176.7±2.8	8.96±0.2	28.06±0.3	343.1±25.0
336	5.66±0.21	43.14±1.1	232.7±3.1	4.67±0.2	9.86±1.0	172.0±12.3	16.91±0.2	38.51±0.5	382.3±10.0
360	5.41±0.23	31.53±2.1	162.8±13.5	4.02±0.3	13.40±0.4	78.9±0.3	16.80±0.4	36.56±1.9	716.7*

cell found in the culture vessels. Table 1 gives the changes of P, N and C contents per living cell. Comparing P and N content per cell at the beginning and the end of the experiment showed that, there was slight change in P-limited culture, a decrease in N-limited culture and an increase in Si-limited culture. Carbon per cell decreased except in the case of Si-limited culture, where it was highly increased.
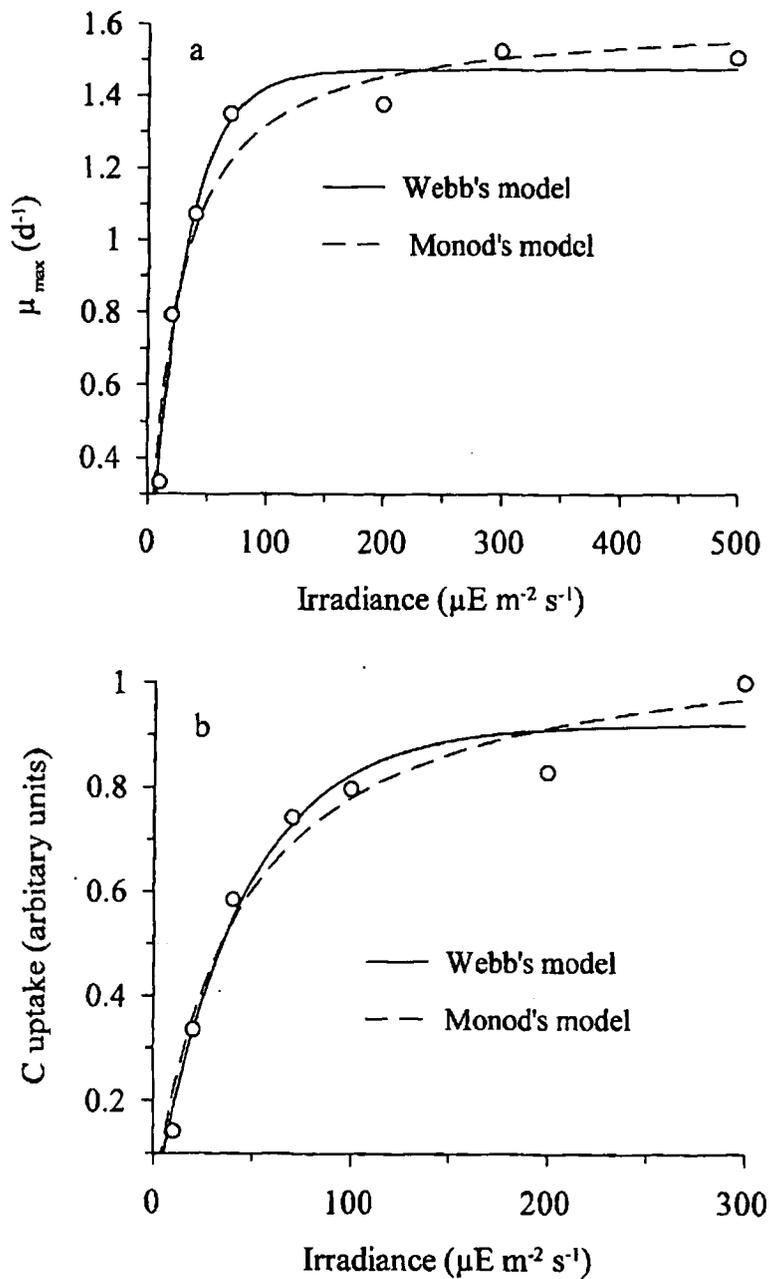
Fig. 3. Effect of irradiance on maximum growth rate (a) and on Carbon uptake (b). Results (mean of triplicates) from batch cultures and calculated by Webb's and Monod's model.

Fig. 3. Effet de l'intensité lumineuse sur le taux maximum de croissance (a) et sur l'assimilation du carbone (b). Les résultats en culture «batch» ont été calculés à partir du modèle de Webb et de Monod.

Fig. 4. Changes of cell number and dead cells (black) (± SD) during differet phases of growth in P-limited chemostat culture, N-limited chemostat culture and Si-limited chemostat culture.

Fig. 4. Variations du nombre de cellules vivantes et mortes (± SD) lors de différentes phases de croissance en chemostat : P-limitant ; N-limitant ; Si-limitant.

Fig. 5. Changes of cell volume (± SD) during different phases of growth in different chemostat cultures.

Fig. 5. Variations du volume cellulaire (± SD) pendant différentes phases de croissance. Cultures en chemostat.

4. Discussion

The optimum temperature of growth was 25 ° C with a maximum growth rate of about 1.45 d^{-1}. In summer the water temperature of the River Danube rises to 25 ° C at Budapest, Hungary (Vituki 1993) where *C. meneghiniana* grows faster and become dominant.
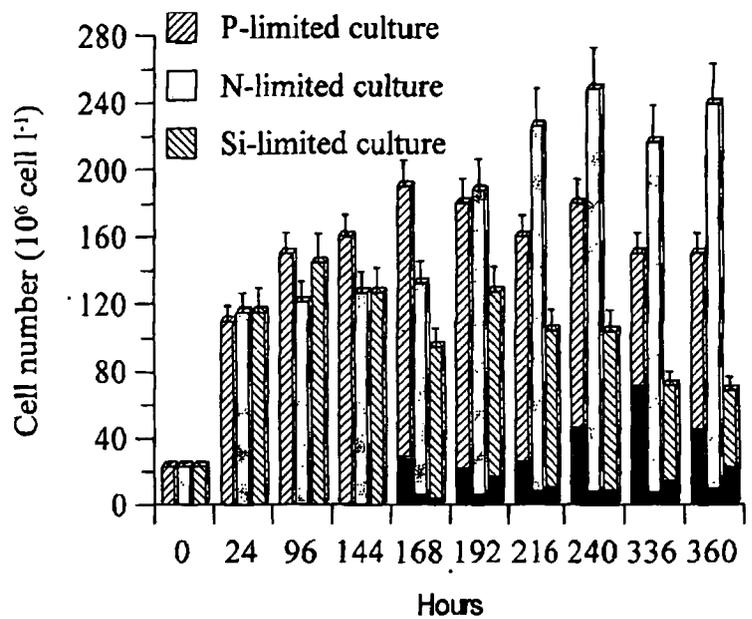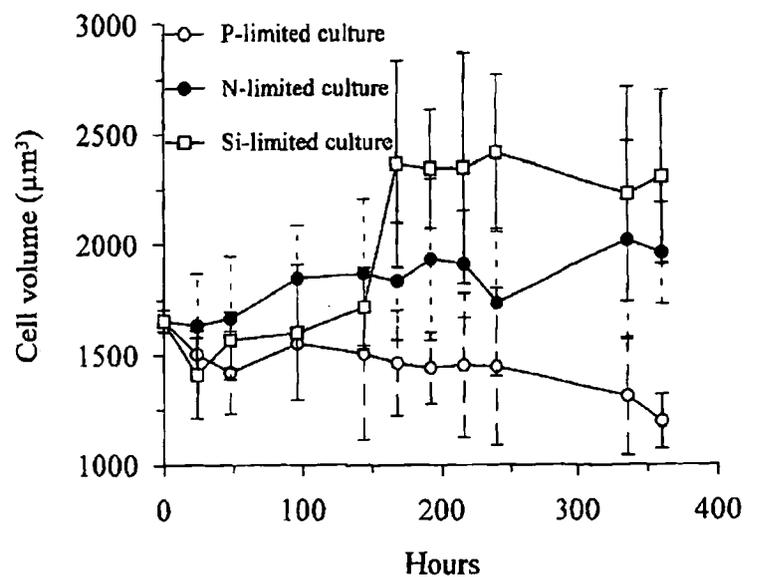
Other diatom species, such as *Stephanodiscus hantzschii, S. invisitatus* Hohn et Hellerman, *S. minutulus* (Kütz.) Cleve et Müller and *Cyclotella pseudostelligera* Hustedt were dominant in early summer (Kiss & Nausch 1988) and/or late summer (Kiss 1986), at relatively low temperature.
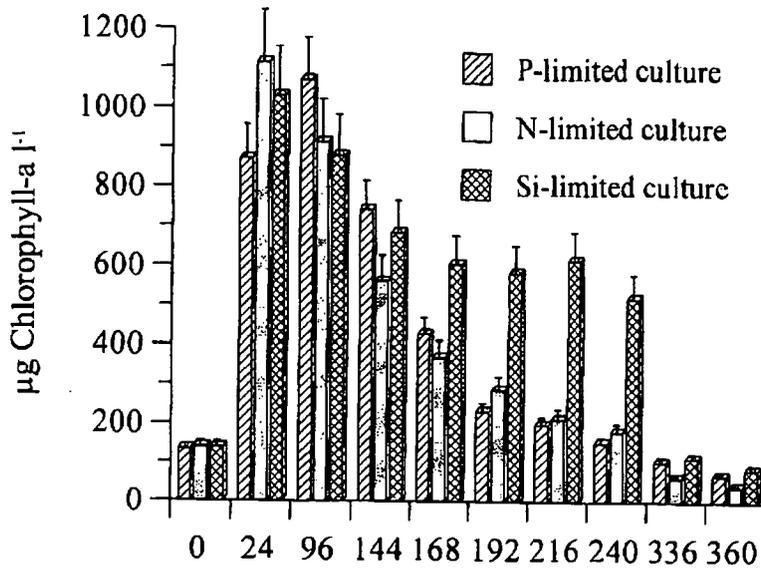
Fig. 6. Changes of Chlorophyll-a concentration (μg l^{-1}) ($\pm$ SD) during different phases of growth in the three chemostat cultures.

Fig. 6. Variations du quota cellulaire en chlorophylle a (μg l^{-1}) ($\pm$ SD) à différentes phases de croissance lors de 3 expériences en chemostat.
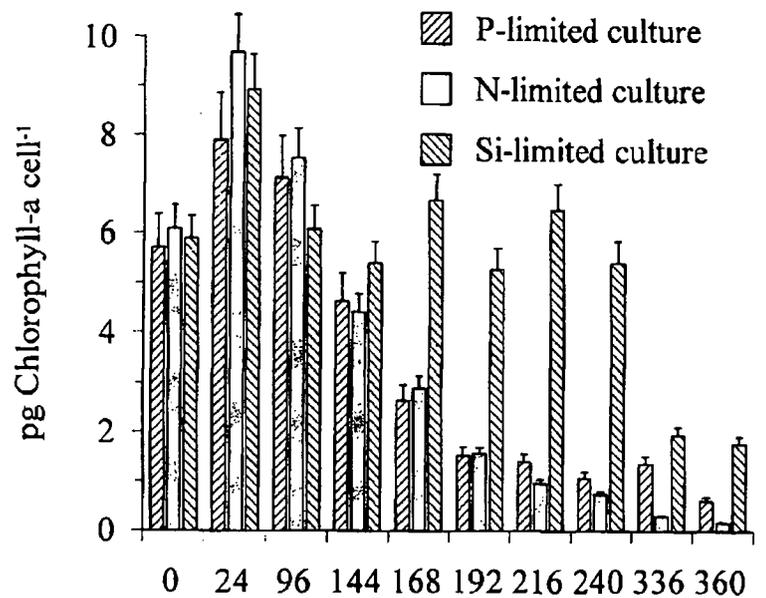

Fig. 7. Changes of Chlorophyll-a content per cell ($\pm$ SD) during different phases of growth in the three chemostat cultures.

Fig. 7. Variations du quota cellulaire en chlorophylle a ($\pm$ SD) à différentes phases de croissance lors de 3 expériences en chemostat.

The estimated saturated irradiance was about 125 μE m^{-2} s^{-1}. I_k values were between 23 and 30 μE m^{-2} s^{-1} for growth and 41 and 44 μE m^{-2} s^{-1} for photosynthesis. These values indicate that the light requirement is not high. The irradiance at water surface layer of the river neither limit the growth nor the photosynthesis activity of *C. meneghiniana*. However, according to Kiss (1994) the suspended solid content greatly changed the light conditions, which may cause a general inhibition of the growth of phytoplankton.

The growth of *C. meneghiniana* in the chemostat experiment can be classified into three growth phases:

1) *Batch culture with sufficient nutrient concentrations*

This phase of growth had the same trend in all cultures, as in batch cultures experiments. Through this period all growth parameters increased, except the cell volume which was more or less unchanged. The maximum growth rate was between 1.44 and 1.51 d^{-1} in accordance with recorded values in batch culture experiments. The changes of P, N and C per chlorophyll were not significant in all cultures. At the end of exponential growth phase the fresh medium was added into each P-limited chemostats described above.

2) *The transition phase between batch and lower dilution rate*

This phase lies between 144 and 240 hours. The growth rate was near zero in P-limited chemostat and still positive but low in N-limited chemostat. It has low negative and positive values in Si-limited chemo-

stat. Growth rates diverge during transient non-steady state. This phenomenon has reported by other authors (e.g. Gotham & Rhee 1981). When the P concentration was reduced to 125 μg l^{-1} (P-limited chemostat) total cell number was slightly affected but dead cells were increased with time. C and P contents per cell were decreased, while N content increased. The cell number was increased in N-limited chemostat, but it decreased in Si-limited chemostat. This means that the cell mortality increased in chemostats A and C.

3) *The effect of lower dilution rate*

Such a phase was characterized by low chlorophyll-a content per cell in all cultures. The inhibition of chlorophyll synthesis has been reported under Si stress (Werner 1977), here it depends on ill metabolism of slow growing cells. Negative growth rate (cell mortality) was recorded in all cultures. The total cell number was slightly changed in P-limited chemostat, while decreased in Si-limited chemostat. Dead cells comprised about 48 %, 4 % and 32 % of the total cell number in P, N and Si limited cultures respectively. Werner (1977) reported that cell division of exponentially growing *Cyclotella cryptica* cells stopped cell division when transfer into a Si - free medium. The experiment of Moed (1973) showed that *Asterionella formosa* undergo one more cell division. The later cell division is accompanied by increased cell mortality. Other diatoms, *Melosira granulata, Fragilaria crotonensis* and *Stephanodiscus binderanus*, species that stop cell division under Si stress did not similarly experience mortality
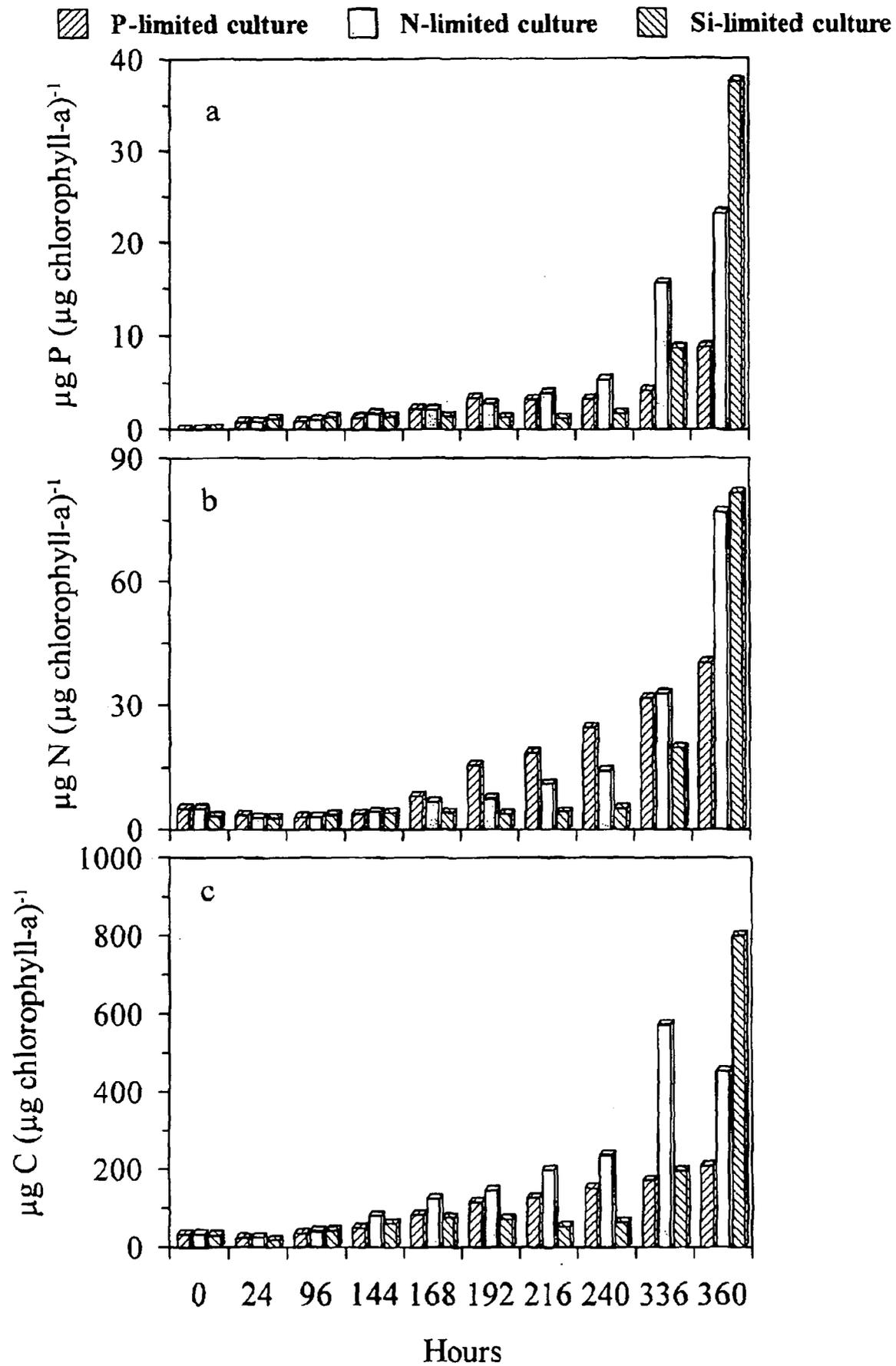
Fig. 8. Changes of cell composition calculated per chlorophyll-a content ($\mu g/\mu g$ chlorophyll-a^{-1}) during different growth phases (a) Phosphorus content, (b) Nitrogen content and (c) Carbon content in the three chemostat cultures.

Fig. 8. Variations du quota cellulaire en P (a), N (b) et C (c) à différentes phases de croissance (3 expériences en chemostat) par rapport au quota cellulaire en chlorophylle a ($\mu g/\mu g$ chlorophylle-a^{-1}).

increase (Sommer & Stabel 1983). *C. meneghiniana* show an increase in cell mortality under both of P and Si stress at the low dilution rate. P and N contents per cell or per unit cell volume have little changes in P-limited chemostat, they were decreased in N-limited chemostat, while increased in Si-limited chemostat. N content per cell was increased in both P and Si-limited, while decreased in N-limited culture (Table 1). C, N and P per chlorophyll-a or per cell were increased in P and N- limited cultures but slightly changed in Si-limited culture (Fig. 8). They were high in comparison to the other phases.

Chlorophyll-a contents per cell (8 to 9.9 pg cell^{-1}) or per unit cell volume (5.5 and 6.2 μg mm^{-3}) were in agreement with the mean chlorophyll-a content of other diatoms (see Bailey-Watts 1978, Reynolds 1984) in the batch culture phase. These values were reduced to critical values of 0.5, 0.2 and 1.2 pg cell^{-1} or 0.5, 0.09 and 0.092 μg mm^{-3} in P-, N- and Si-limited cultures, respectively at lower dilution rate. This was interpreted as indicative of the formation of carbohydrate reserves at the slower growth rate. Chlorophyll-a content was also a good indication of ill metabolic cycle which could occur at the last period of the experiment. The cultures were not axenic but count of bacteria was very low (less than 2 % of total biomass). Therefore, their effect on metabolis of culture can be neglected. This means that the low chlorophyll-a content per cell is a result of slow metabolic rate and it is not a result of infection by bacteria. In N-limited chemostat dead cells were not more than 4 % of the total cell and have the lowest chlorophyll-a content per cell.

Under sufficient nutrient, dead cells were not recorded (batch culture phase of growth). At extremely low dilution rate, the nonviable cells, in bacterial studies, were accounted as 60 - 70 % of the total cell number (e.g. Tempest et al. 1967). Determination of viable cells is not an easy task with algae (Rhee 1980). There is no effective method for measuring viable cells. In the present study it might be easier to observe and count the living and non-living cells (empty cells). There is no data in literature about counting living and non-living cells of diatoms under critical low dilution rate. When dead cells are present in significant number, particularly at low dilution rate, the growth rate of viable cells is higher than the dilution rate. Cryptic growth (i.e., growth utilizing the autolysis products of other cells) may also occur at low dilution rates, which can alter the surviving cells (Rhee 1980). P and Si-limited cultures were characterized by high number of dead (empty) cells at the last phase of our experiment. The growth rate has negative value in all chemostats at

the last day of the experiment. In fact, under the low dilution rate, we can not be sure of the growth of *C. meneghiniana* whether is equal to the dilution rate or not, because high numbers of dead cells were found with low chlorophyll-a per cell. In addition, low cell density at low dilution rate is inferior to the theoretical bases of chemostat theory. Therefore, there is a lower limit dilution rate, below which simple continuous culture theory is not followed. This hypothesis had been reported by Tempest et al. 1967 and Sciandra & Ramani 1994. This critical rate probably is species specific (Sciandra & Ramani 1994) and may depend on the limiting nutrient. The population of cells are decreased by the emergence of unfavorable factor. In addition, cell quotas are not solely regulated by the limiting factor.

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