

A study was made of the requirements of microalgae for nitrogen and phosphorus, mineral nutritive elements. Data obtained on numbers of algae and substance assimilated, separately for nitrogen and phosphorus, were used to calculate cell quotas: minimum and maximum for cell division for five species of green algae and two species of blue-green algae.

CONCEPT OF REQUIREMENTS

The concept of requirements of microalgae for nutritive components is of the essence in theoretical and applied ecological problems. In practical applications, these are problems of controlling the structure of algocenoses; theoretical studies require including in a model the specific characteristics of a species that would match the actual assimilation of environmental resources by the species (Jørgensen, 1985; Kuchai, 1985; Levich, 1989).

Concepts of cells' requirements for nutritive components have been little studied in algology. The term, "requirements," is itself used in a wide variety of meanings (Levich, 1989). Qualitative concepts of requirements are linked with the methodology of biological research (Braginskii, 1961): If addition of a substrate results in additional production of phytoplankton, we admit that cells require this substrate. The measure of need of a species for a substrate factor may be assumed to be the constant of semisaturation (hyperbolic law of Michaelis-Menten) that characterizes the relation between rate of assimilation of substrate and its concentration (Fundamentals of Aqueous Ecosystem Research, 1979). Some authors (Maksimova, 1977) relate cell requirements to the concentrations of biogens in the environment that provide for optimum development of algae. Tilman (1982) defines cell requirement as that concentration of a substrate in the environment that ensures population increment, specifically, that neutralizes the demise of individuals. As phytoplankton requirement for a substance, we may consider the algological analog of the zoological ration - the specific rate of assimilation of the nutritive substance: This rate is related to the primary production phytoplankton. The cell's requirement for a certain substance may be considered as its content in a unit of biomass or in one cell. This value is also called the intracellular concentration of a substance or the cell quota. The "economic coefficient" $\frac{\Delta L}{\Delta b}$, the amount of substrate needed for a unit increment of cell biomass, also plays the role of a requirement.

We term as requirements the values q^* that are included in the balanced equations $\Delta L = \sum_i \Delta(q_i b_i)$. The values q_i^* are the amounts of substrate k that move from the environment into a biomass unit of the population of species i (ΔL^k is the amount of substrate k assimilated by the whole population, b_i is the biomass of the population of species i). As a rule, this value does not coincide with the content of the substance in the biomass because the substance assimilated from the environment is not only deposited in the cells, but is also excreted (in other chemical forms) from the cells in the processes of metabolism and is partially lost from the cell mass when the cell membranes break up in the process of mitosis.

Thus, the coefficient q_i^* is close to the cell quotas only when it is possible to justifiably disregard elimination of the substance from the cells. We suggest that the values q_i^* which are close to the economic coefficient be termed the requirements of the phytoplankton for the environmental substrate factor.

Biology Department, M. V. Lomonosov Moscow State University, Moscow. Translated from *Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya* No. 1, pp. 114-123, January-February, 1991. Original article submitted February 21, 1989.

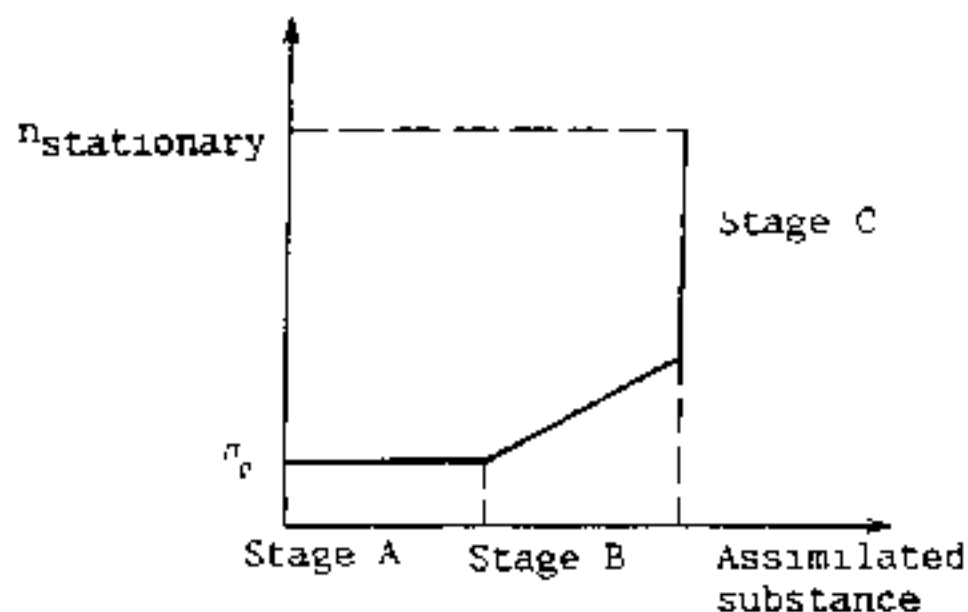


Fig. 1. Stages of the growth-assimilation process.

Frequently, we evaluate species requirements for some substances according to known requirements of other species, using stoichiometric ratios of elements in the environment or in the cells believing these ratios to be firmly established and stable. For example, handbooks on marine biology (Sverdrup et al., 1942) give the ratio C:C:Si:N:P = 212:106:20:15 (in the atomic form). Recently, however, it has been firmly established that owing to capability of phytoplankton to create reserves of biogens (even different proportions relative to the minimum requirements) and owing to mechanisms of conversion of the biochemical composition of cells when conditions of functioning are changed, the ratio of elements in the cells is extremely variable. Thus, for natural lake associations of fresh water microalgae, Jørgensen (1979) presents a range of ratios of N:P of from 4.1 to 291. With direct autoradiographic measurements on fresh-water lake phytoplankton, B. L. Gutelmacher et al. (1982) found that the ratio of carbons reserves to phosphorus reserves in the cells varied from 33 to 500.

Unfortunately, in the literature there are no systematic data on the quantitative characteristics of intracellular concentration of nutritive substances for individual species of phytoplankton. Also, the dynamics of cell quotas is little known. Experimental studies are needed to elucidate the limits of stability of selected characteristics and to measure quantitatively the requirements of phytoplankton species for components of mineral nutrition.

In experiments in which preliminary measurements of requirements were made, quotas were computed for 10 (Levich et al., 1986a) and for 4 (Artyukhova et al., 1988) species of fresh-water green algae as amounts of biogen assimilated from the environment per cell. These determinations showed that requirement indices are species specific; a quantitative study is needed to determine the relation between requirements and environmental factors, depending on the background of culture growth and change in biogens in the medium, requirements are extremely variable and isolation of the stages of assimilation and growth processes at which the index values are most stable must be isolated.

2. STAGES OF THE "ASSIMILATION-GROWTH" PROCESS IN MICROALGAE

Many experiments with cumulative cultures of phytoplankton established (Levich et al., 1986b) that, in cell populations, the process of increase in numbers, promoted by the consumption of biogenic substrates, consists regularly of three stages (Fig. 1):

- Stage A. Accumulation of the biogen in the cells without an increase in cell number.
- Stage B. Cell division due to assimilation of substances from the environment.
- Stage C. Cell division due to internal reserves of nutritive substance.

The stages may be reduced and overlapping. The stages of the "growth-assimilation" process only partially coincide with growth phases indicated in growth curves. Specifically, stage A is the concluding part of the lag phase (at the initial part of the lag phase, there is neither growth of the culture nor assimilation of substances), and stages B and C are part of the exponential growth phase of the culture.

An analysis of the stages shows that the regular change in stages is accompanied by a regular change in biogen assimilated by the cell (for this value, we will retain the traditional, laconic term from English-language literature, "cell quota"): The substance is ac-

cumulated in cells at stage A, changing from q_{init} to q_{max} ; at stage B, the assimilation of the substances from the environment per newly formed cell remains constant; this value is termed the division quota. Usually the division quota is less than the maximum quota, which means that absence of growth at stage A is not connected with an intracellular content of the element inadequate for division; at stage C, the cell quota decreases and cell division stops upon reaching a quota value of q_{min} .

3. MEASURING REQUIREMENTS

The content of substances in the cells could be measured by direct chemical methods of microanalysis. However, suitable methods are labor-intensive, require a great amount of cell material, and are sensitive to errors in determining dry weight of the substance. Hydrobiological determination of the quota is not a direct, but an indirect method (Dauta et al., 1982; Revkova, 1987). Actually, what is measured is the disappearance from the medium of the substance assimilated by the cells and the biomass of the growing algal cultures. Quotas are computed on the basis of the law of conservation of matter. Decrease of a substance in the medium is compared to the amount of substance assimilated by the cells. For determining the initial and minimum quota, the culture must be brought to the stationary phase of growth at two different initial biogen concentrations:

$$\begin{aligned} b_{init}q_{init} + \Delta L_1 &= b_{1\text{ end}}q_{min} \\ b_{init}q_{init} + \Delta L_2 &= b_{2\text{ end}}q_{min} \end{aligned}$$

Since the values b_{init} , ΔL_1 , ΔL_2 , $b_{1\text{ end}}$, $b_{2\text{ end}}$ are known from measurements, the given balanced equations comprise a system of two equations for computing two unknown values q_{init} and q_{min} . Here, we understand that in both media, cessation of culture growth occurs because of unavailability of substrate L specifically. If different biogenic elements are the limiting elements in the two experiments, then the end quota at the stationary phase according to the element of interest will not be the minimum and will be different in each of the equations. Usually one of the concentrations (let us say, ΔL_1) will equal 0. Then

$$q_{min} = \frac{\Delta L}{b_{1\text{ end}} - b_{2\text{ end}}}, \quad q_{init} = \frac{b_{1\text{ end}}}{b_{init}} q_{min} \quad (1)$$

According to empirical generalization (Fig. 1), the ratio of assimilated biogen to size of accrued biomass at stage B is constant. Above, this ratio is termed the division quota and computed according to values ΔL and Δb at stage B in the experiment:

$$q_{div} = \frac{\Delta L}{\Delta b} \quad (2)$$

Let us repeat once again that values q_{init} , q_{div} and q_{min} computed in this way do not coincide with the precise content of the substance in the cells since cell division is accompanied not only by assimilation of biogens, but also their excretion. Also, at stage B the content of the substance in the cell

$$q = \frac{b_{init}q_{init} - \Delta L}{b} \quad (3)$$

corresponds only approximately to q_{div} at $\Delta L \gg b_{init} \times q_{init}$ and $b \gg b_{init}$. Thus, it would be more correct to speak of the values q_{div} and q_{min} estimated according to the decrease of the biogen in the medium as amounts of the substance assimilated from the medium and not as substances contained in the cells and call these cell requirements and not cell quotas.

Measuring the requirements involves not a few methodological difficulties. Thus, let us stress once again, that in determining the minimum quota, it is necessary to create conditions that guarantee limitation of the substance whose quota is being measured. Otherwise, the required quota will not be minimal at the stationary phase of growth. However, a correct determination of the limiting factor is based on knowing the minimum quotas, this is why the limiting agent of the medium is unknown before the experiment. For this reason, the design of an experiment on determining minimum quotas requires special efforts to provide and test the limiting conditions.

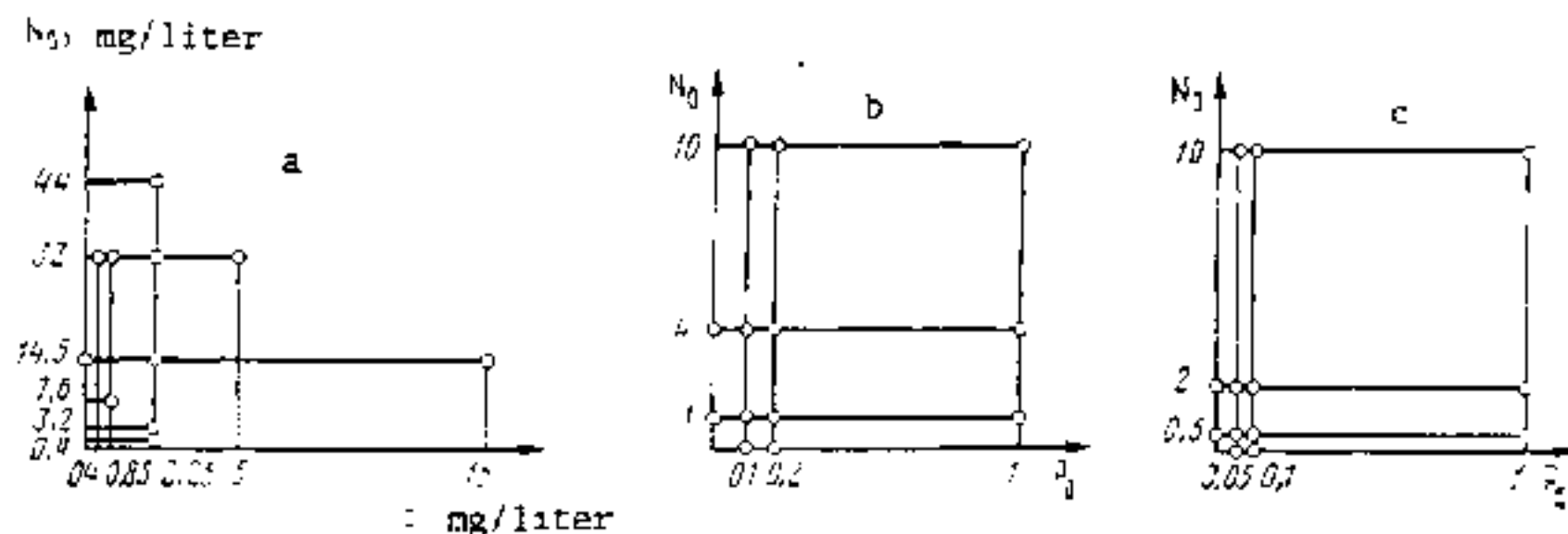


Fig. 2. Initial concentrations of nitrogen and phosphorus in culture medium: a) *Chl. ellipsoidea*; b) *Sc. quadricauda*; *Chl. vulgaris*; c) *Sc. obliquus*; *Ank. falcatus*.

In keeping with this concept of requirements, cell quotas of a number of plankton algae species were measured according to the proposed method.

4. MATERIALS AND METHODS

Monocultures of algae (two species of blue-green and five species of green) were grown in a cumulative regime on Dauta nutritive medium (Dauta, 1982) with various initial content of nitrogen and phosphorus.

Algae were grown in 0.5 liter flasks in a luminostat with 24-hour illumination with 2500 lx daylight lamps, temperature of 27-28° and initial pH of the medium at 7.5-7.8.

During all experiments, which lasted 1-2 months, numbers of algal cells were determined by direct count in a Nageotte chamber or nephelometrically. Nitrate concentrations in the medium were measured with ionoselective electrodes, phosphates, by the colorimetric method using ammonium molybdate and stannous chloride, twice a week on the average (more frequently at the beginning of the experiment, less at the end). The biogenic element assimilated by the algal cells was determined according to its decrease in the medium. Biomass was determined by weighing the raw mass. A specific volume of algal suspension with known numbers was filtered through membrane filters. The cell biomass was determined according to the difference between the filter mass with the cells precipitated on it and the mass of a pure filter through which distilled water was preliminarily passed, and per-cell computations were made. The relative error in computing biomass, determined in 10 repetitions, was 10%.

Initial concentrations of nitrogen and phosphorus were selected from this computation so that three concentrations of the other element would correspond to each fixed concentration. Thus, a pattern of concentrations was obtained from the nine combinations with different initial content of nitrogen and phosphorus (Fig. 2). This number of selected concentrations is required for checking reproducibility of results and provides opportunities for various limitations of both nitrogen and phosphorus. Calculation of limitation is necessary for analysis of quotas. With this selection of concentrations, we obtain nine different ratios of initial concentrations of nitrogen to phosphorus from 0.5 to 200; these give us some idea of the possible limiting by one or the other element even before the experiment. If this ratio is high, for example, 100 or 200, then it is most probable that growth will be limited by lack of phosphorus and, on the other hand, a very low ratio, let us say, 0.5 or 5 will most likely be due to limiting of growth by nitrogen.

In addition to these nine concentrations, to determine initial cell quota, it is necessary to study zero initial concentrations of nitrogen and phosphorus. For this purpose, at a concentration of one of the elements equal to zero, two concentrations of the other element were investigated.

In the experiment, a total of 13 various media were used that differed in initial content of nitrogen, phosphorus and their ratios, but were similar in other chemical elements.

The cultures were grown with these concentrations to the stationary phase. When algal growth stopped, nitrogen or phosphorus was added in all nine cases to determine which of the factors, nitrogen or phosphorus, might stop growth, and would, therefore, be the limiting factors. For example, if the culture, having stopped growing, began to grow after phosphorus was added, and addition of nitrogen had no effect on growth, it would mean that in this case phosphorus limited development of the algae.

TABLE 1. Algal Cell Quotas of Nitrogen and Phosphorus with Various Initial Contents of Biogens in the Medium for Chl. ellipsoidea

N_0 , mg/ liter	P, mg/ liter	σ_{max}^P	σ_{div}^P	σ_{end}^P	N_{max}	N_{div}	N_{end}	N/P	Limiting factor
14.5	2.25	2.8	0.14	0.18	5.1	1.7	1.14	6	Nitrogen
32.1	5.0	3.4	0.19	0.25	26.4	1.08	1.6	6	
7.6	0.85	1.13	0.5	0.07	13.25	1.26	0.66	1	
14.6	13.25	Excretion of phosphorus			6.5	2.39	1.17	1	
3.2	2.25	1	0.46	0.64	3.4	1.67	0.91	1.4	
14.9	0	—	—	—					
0.9	2.25	Excretion of phosphorus			1.5	No stage	1.1	0.5	
						R			
32.1	0.4	1	No stage	0.03	30.43	0.91	2.38	80	Phosphorus
32.1	0.75	1.25	No stage	0.05	32.1	1.11	2.25	15	
			B						
32.1	2.25	4.1	0.09	0.14	31.5	2.1	2.05	11	Light
44.1	2.25	4.05	0.12	0.11	56.25	1.17	2.17	19	
					36.25	3.34			

TABLE 2. Cell Quotas of Nitrogen and Phosphorus (10^{-9} mg/kl) for Various Algae

Species of algae	Quota of nitrogen			Quota of phosphorus		
	maximum	division	minimum	maximum	division	minimum
<i>Chlorella ellipsoidea</i>	15—56.5	1.6±0.11	1.5±0.19	1—4	0.25±0.07	0.04±0.01
<i>Chl. sp.</i>	2—15	2.8±0.5	20.6	0.6—2.6	1.1±0.16	20.02
<i>Scenedesmus quadricauda</i>	5—6	3.3±0.36	1.2	0.2—0.5	1.2±0.3	20.02
<i>Sc. obliquus</i>	—	1.9±0.2	0.46±0.04	1—3.8	0.2±0.04	0.025
<i>Ankistrodesmus jalcatus</i>	—	3.0±0.6	0.4±0.08	1.25	0.2±0.1	20.03
<i>Anacystis nidulans</i>	—	0.1±0.03	0.05±0.02	0.01—0.2	0.02±0.01	0.0025±0.0003
<i>Anabaena variabilis</i>	0.4—2.3	2.1±0.27	1.95±0.63	0.2—0.5	0.2±0.1	0.09±0.1

TABLE 3. Quotas of Nitrogen and Phosphorus per Unit Biomass (mg/g biomass) for Various Algae

Species of algae	Cell mass 10^{-10} g	Quota of nitrogen		Quota of phosphorus	
		division	minimum	division	minimum
<i>Chlorella ellipsoidea</i>	0.4	40	37.5	5.5	1
<i>Chl. sp.</i>	0.87	42	27	12.6	<0.2
<i>Scenedesmus quadricauda</i>	0.94	35	21.3	11	<0.2
<i>Sc. obliquus</i>	1.27	15	4	1.1	0.2
<i>Ankistrodesmus jalcatus</i>	5.0	8.3	1	0.6	<0.1
<i>Anacystis nidulans</i>	0.06	166	83	3.1	1
<i>Anabaena variabilis</i>	0.4	50	50	5	2

In cases where the final number of cells was high, the possibility of growth limitation by light due a possible self-shading of the algal cells was also checked. For this, a culture that had stopped growing was diluted by three volumes of medium without nitrogen and phosphorus, and growth was checked subsequently, if there was no growth, it meant that light had not limited development. In our experiments, light limited algal growth.

5. DETERMINING CELL QUOTAS FOR ALGAL CULTURES

In this section, the method of determining quotas will be illustrated with analytical data from an experiment with a Chl. ellipsoidea culture. Quotas found for all species studied are presented in Tables 1 and 2.

For Chl. ellipsoidea, we selected a pattern of concentrations with a range of ratios of initial concentrations of nitrogen to phosphorus from 0.4 to 80. For the other algae,

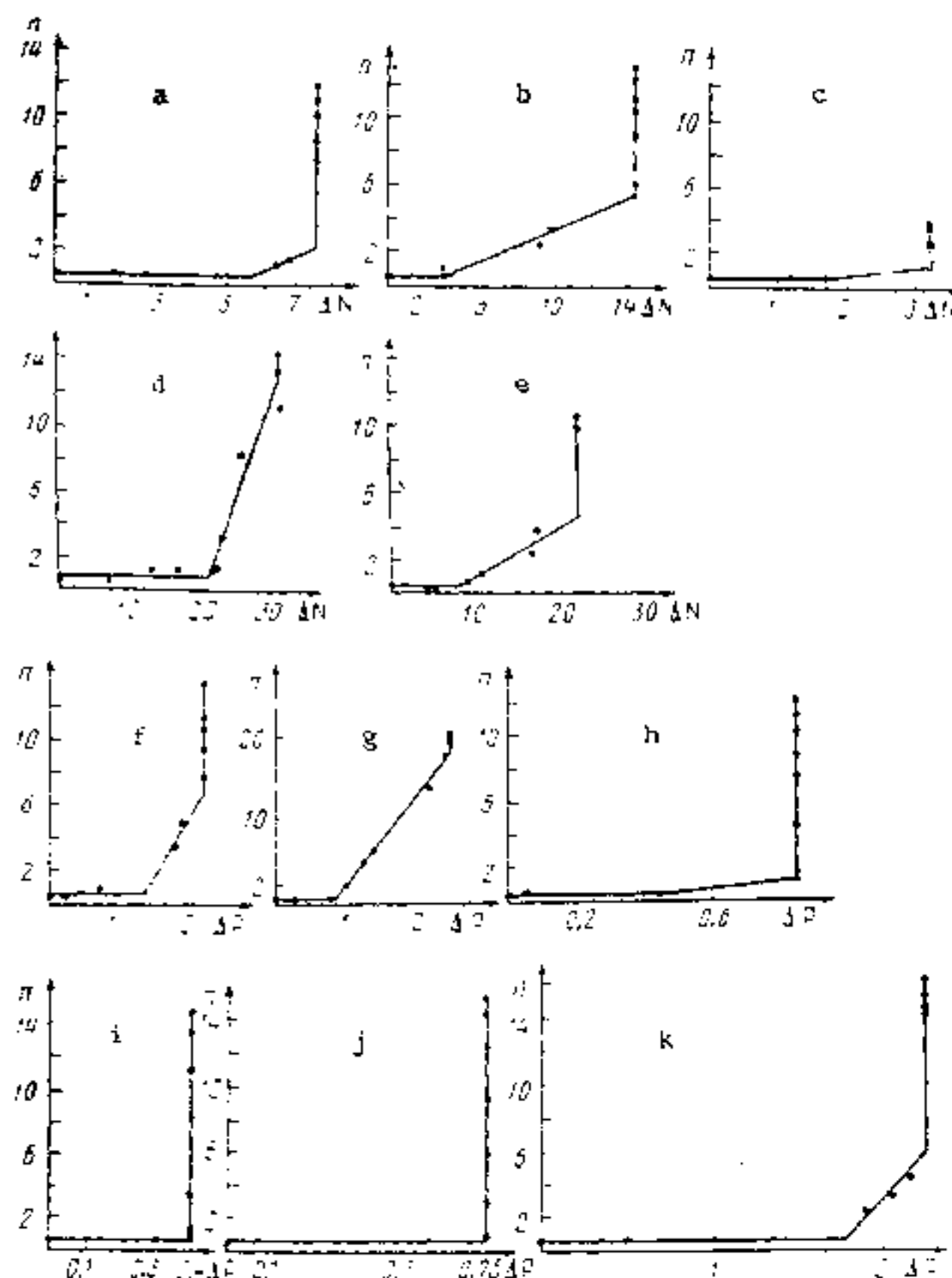


Fig. 3. Variants of growth curves for *Chl. ellipsoidea* (mg/liter): a) $N_0 = 7.6$; $P_0 = 0.85$; b) $N_0 = 14.6$; $P_0 = 13.25$; c) $N_0 = 3.2$; $P_0 = 2.25$; d) $N_0 = 32.1$; $P_0 = 0.4$; e) $N_0 = 44.3$; $P_0 = 2.25$; f) $N_0 = 14.5$; $P_0 = 2.25$; g) $N_0 = 32.1$; $P_0 = 5$; h) $N_0 = 7.6$; $P_0 = 0.85$; i) $N_0 = 32.1$; $P_0 = 0.4$; j) $N_0 = 32.1$; $P_0 = 0.75$; k) $N_0 = 32.1$; $P_0 = 2.25$.

a range of these ratios of nitrogen and phosphorus concentrations from 0.5 to 200 was studied subsequently and is presented in Fig. 2a.

On the basis of data obtained on amounts and concentrations of nitrogen and phosphorus in the dynamics of culture development, curves were constructed that describe the relation of growth to assimilated substance separately for nitrogen and phosphorus (growth curves).

Variants of growth curves for *Chl. ellipsoidea* are presented in Fig. 3. In most cases, these are typical curves with three stages of culture development: A, B and C. In some cases stage A or B is absent. For all algae that we studied, stage A is present in 68 of 136 cases and stage B, in 78.

Cell quotas, maximum quotas, q_{max} , division quotas q_{div} , and minimum quotas q_{min} , corresponding to the three stages of development of the cultures, we computed on the basis of data obtained on numbers and substance assimilated separately for nitrogen and phosphorus; the quotas are presented in Table 3.

For both phosphorus and nitrogen, q_{max} varies within broad limits: q_{max}^P from 1 to ∞ , q_{max}^N from 1.5 to $50.0 \cdot 10^{-9}$ mg/kg. This value is not a stable characteristic of the species and may depend on various factors, specifically, initial state of the culture, medium, growing conditions, etc.

In a preceding work (Artyukhova et al., 1988) an assessment was made of linear regression of correlation of cell requirements and nitrogen concentration (in a range of 10 to 60

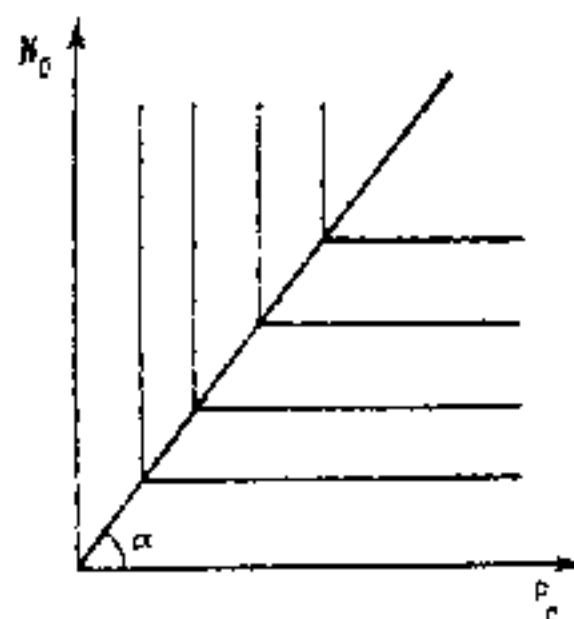


Fig. 4. Lines of level of response surface $n(N_0, P_0)$. Tangent of the angle $\alpha = q_{\min}^N / q_{\min}^P$.

mg/liter), phosphorus concentration (in a range of 0 to 5 mg/liter), and illumination (100, 500 and 2000 lx). It developed that variability of requirements is evidently determined not so much by changes in levels of factors as by a change in stage of development of the culture. There is no relation between division quota or minimum quota and initial concentrations of biogens within limits of experimental error, and it is insignificant with respect to light.

Of all the values of end quotas, we selected as minimum only those that corresponded to the limiting quotas according to the given element and only after that did we average the selected values.

Phosphorus limited development in only two cases with ratios of initial content of nitrogen to phosphorus equal to 80 and 43. Actually, in these cases, the cell quotas for phosphorus had the lowest values 0.03 and 0.05 ($\cdot 10^{-3}$ mg/kl). The values of q_{end}^N according to nitrogen in these cases, however, were the highest of all q_{end}^N , which once again confirms limitation of growth by phosphorus in these two variants. Thus we determine that $q_{\min}^P = 0.04 \cdot 10^{-3}$ mg/kl.

In most cases, nitrogen was the limiting factor in the development of *Chl. ellipsoidea*. For these cases, we found the average value of minimum cell quotas for nitrogen $q_{\min}^N = 1.5 \cdot 10^{-3}$ mg/kl.

In two variants with $N_0 = 32$ and 44 mg/liter and $P_0 = 2.25$ mg/liter, development was limited by light. Actually, the values of cell quotas at stage C in these cases were not minimum for either nitrogen or phosphorus.

Maximum cell quotas were not determined for all algae due to absence of stage A in some cases, that is, when the algae began to grow immediately without storing the biogen. These were not specific characteristics of species and are presented to illustrate the orders of the values.

In preliminary methodological experiments (10 repetitions) we established that at a confidence level of 90%, errors in determining numbers were 8%, biomass, 10%, nitrates, 7% and phosphates, 10%. Consequently, errors in determining cell quotas due to errors in the original data are approximately 20-30%. Errors in cell quotas equal to the error of averaging of variants of the experiment in which, according to analysis of experimental conditions, this quota was produced are presented in Table 1.

6. RESPONSE SURFACE AND THE PRINCIPLE OF LIMITATION

The relation of the maximum numbers in the culture growing on given medium and initial amounts of biogen in the medium, the response surface $n(N_0, P_0)$, has the form of a "hut," that is, it is formed by the intersection of two planes: One of the planes intersects the border (n, P_0) parallel to the axis P_0 at a distance equal to $q_{\text{init}}^N n_{\text{init}}$ from the axis; the second plane similarly intersects the border (n, N_0) at a distance $q_{\text{init}}^P n_{\text{init}}$ from it; these planes intersect along a straight line from the beginning of the coordinates in such a way that the projection on the plane (N, P_0) forms an angle with the P_0 axis with a tangent equal to q_{\min}^N / q_{\min}^P . The line of the plane of the response surface described is presented in Fig. 4 and answers the principle of a minimum in the formulation specified by Liebig's law. Specifically, the growth of a culture is limited by a factor that is inadequate in amount with respect to species requirements of the given substance:

$$n_{max} = \min \left\{ \frac{N_0 + q_{init}^N n_{init}}{q_{min}^N}; \frac{P_0 + q_{init}^P n_{init}}{q_{min}^P} \right\}. \quad (4)$$

This principle and its generalization to polycultures with growth limited by a random complement of substrate-energy factors (Levich and Lebed', 1987) confirm the importance of measuring ecological associations' requirements for components of environmental resources.

The authors express sincere appreciation to the scientific director of the work, Professor V. N. Maksimov and his colleagues who presented data for analysis on a number of species, G. V. Vereshchagin (Sc. bijugatus), A. A. Khudoyan and N. S. Bulgakov (A. variabilis).

LITERATURE CITED

- Artyukhova, V. I., Bykova, N. I., Goryunova, S. V., Martashvili, G. Sh., and Strelkova, L. G. "Kinetics of growth, and requirements and assimilation of nitrogen and phosphorus of four species of plankton algae," *Vestn. Mosk. Gos. Univ.*, No. 1, 47 (1988).
- Eraginskii, L. P., "The relation between composition of pond phytoplankton and manifestation of its "requirements" of biogenic elements," in: *Primary Production of Seas and Inland Waters* [in Russian], Minsk (1961), p. 407.
- Dauta, A., Brunel, L., and Guerri, M. M., "Determination experimentale des Paramètres liés à l'assimilation de l'azote et du phosphore par Scenedesmus crassus," *Annales Limnol.*, 18, (1), 33 (1982).
- Fundamentals of Aqueous Ecosystem Research [in Russian], Leningrad (1979), p. 228.
- Gutelmacher, B. L., and Petrova, N. A., "Phosphorus and carbon assimilation rates of the individual species of algae in Ladoga Lake," *Int. Revue der Gesamten Hydrobiologie*, 67, No. 6, 837 (1982).
- Jørgensen, S. E., *Handbook on Environmental Data and Ecological Parameters*, Pergamon Press, New York (1979).
- Jørgensen, S. E., *Controlling Lake Systems* [in Russian], Agropromizdat, Moscow (1988).
- Kuchai, L. A., "The use of the concept of cell quotas in models of phytoplankton dynamics," *VINITI*, Dep. 8567, B85 (1985).
- Levich, A. P., and Lebed' A. B., "Requirements of biological species for nutritive components and assimilation of factors in the environment by an ecological association," in: *Problems of Ecological Monitoring and Ecosystem Modeling*, Vol. 10 [in Russian], (1987), p. 268.
- Levich, A. P., Lyubimova, E. D., and Martashvili, G. Sh., "Species structure and assimilation of substrate-energy factors in laboratory algocenoses," in: *Ecological Prognosis* [in Russian], Izd-vo Mosk. Un-ta, Moscow (1986a), p. 69.
- Levich, A. P., Revkova, N. V., and Bulgakov, N. G., "The 'assimilation-growth' process in microalgae cultures and the requirements of cells for mineral nutritive components," in: *Ecological Prognosis* [in Russian], Moscow (1986b), p. 132.
- Levich, A. P., "Requirements of phytoplankton for environmental substrate factors and ways of controlling the structure of algocenoses," *Zh. Obshch. Biol.*, No. 4, 316 (1980).
- Maksimova, M. P., "Mineral nutrition and the problem of providing phytoplankton with nutritive salts," *Obzornaya Informatsiya TsNII TEIRKh*, No. 1, 39 (1977).
- Revkova, N. V., "Comparison of algal growth in mono- and polycultures on media with various phosphorus content," *Nauch. Dokl. Vyssh. Shk. Biol. Nauki*, 70 (1987).
- Sverdrup, H. V., Johnson, N. W., and Fleming, R. H., *The Oceans. Their Physics, Chemistry and General Biology*, Prentice Hall, N. Y. (1942).
- Tilman, D., *Resource, Competition and Community Structure*, Princeton, (1982).