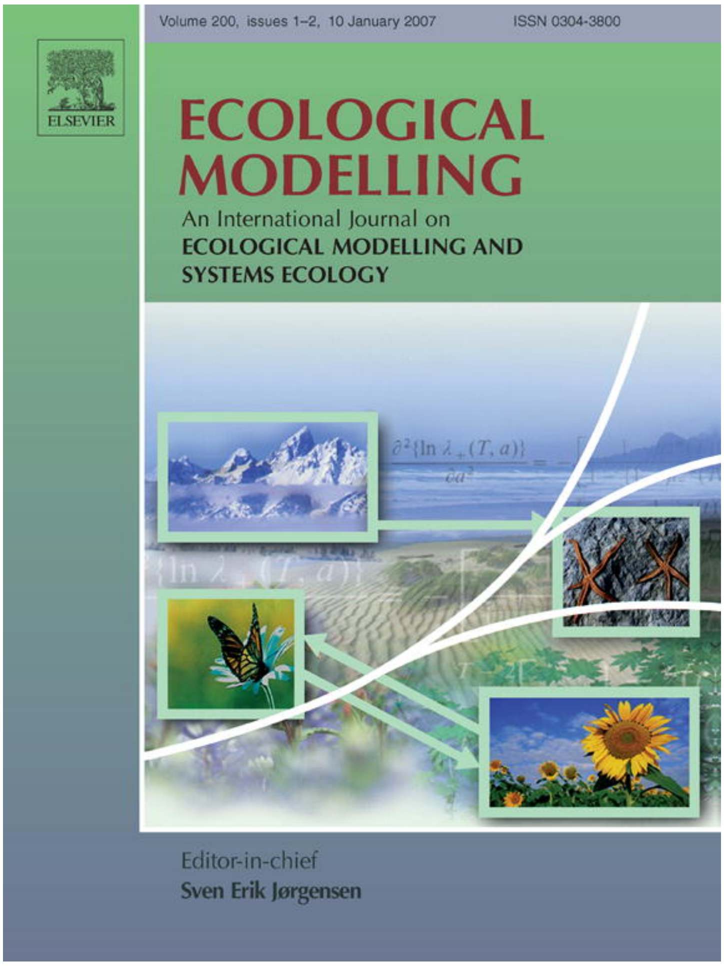
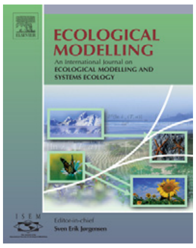

Volume 200, issues 1–2, 10 January 2007
ISSN 0304-3800
ELSEVIER
ECOLOGICAL MODELLING
An International Journal on
ECOLOGICAL MODELLING AND SYSTEMS ECOLOGY
Editor-in-chief
Sven Erik Jørgensen

ECOLOGICAL MODELLING 200 (2007) 160–170

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/ecolmodel



Variational model of microorganism polyculture development without re-supply of mutually irreplaceable resources

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ARTICLE INFO

Article history:
Received 8 December 2005
Received in revised form
28 June 2006
Accepted 5 July 2006
Published on line 11 September 2006

Keywords:
Variational modelling
Extremal principles
Limiting resources
Species composition
Community structure regulation

ABSTRACT

Stationary stage of accumulating cultures of *Pseudomonas aeruginosa* dissociants is described by variational model of consumption and growth. The "generalized entropy" as a goal function is used. Model's parameters are the dissociants requirements for basic nutrients: carbon, nitrogen and phosphorus. Using this model we calculate the limitation ranges for arbitrary combinations of environmental resource factors and the community population sizes at a stationary stage of growth as functions of the resources that constrain the growth. The requirements values obtained from experimental data allowed us to predict the limiting resources and dissociant's abundances. Our estimations match experimental results. The possibility of composition control is discussed in paper.

1. Introduction

Among the problems of modern biology, a central place belongs to the problem of adequate biological forecasting. Applied to synecology, in particular, to the ecology of microbiological communities, this problem may be re-formulated as the problem of rigorous quantitative prediction (calculation) of the sizes of species that form the community as functions whose arguments are the factors which determine the life of the organisms. One of the most important among such factors is an adequate supply of the individuals with environmental resources. One's ability to control the community structure, or, in other words, one's ability to maintain the necessary composition of the community, represented by groups of organisms, in certain proportions, may serve as an adequacy criterion for the calculation methods. An interest in such applied problems is caused by a strong dependence of biological process parameters and the yield of substances produced on the proportions of different organisms in the biomass of the community. A specific situation emerges in microbiological biotechnologies due to the microorganism dissociation phenomenon. In this case, the activity of the dissociants and their physiological characteristics, affecting the biologically active substance production, are different, and it is therefore necessary to control the composition of the emerging polyculture.

To study the possibility of describing microbiological cultures from the standpoint of forecasting and control

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doi:10.1016/j.ecolmodel.2006.07.029

problems, we are using a variational model of consumption and growth in ecological communities (Levich, 1993, 2000; Levich et al., 1994). By this model, using the requirements of physiologically different groups of organisms as parameters, it is possible to calculate the limitation ranges for arbitrary combinations of environmental resource factors as well as the community population sizes at a stationary stage of growth as functions of the resources that constrain the growth. The approach that underlies the variational model originates in classical statistical physics, where the canonical distribution (a stationary state of a perfect gas) is obtained by solving the maximum entropy problem for given mean energy of the gas. In the framework of the model used, a stationary state of the community of microorganisms is described by solving a conditional extremum problem for the generalized entropy function. (Some other goal functions and attempts to describe ecosystem species composition in terms of thermodynamics one can find in works (Wilhelm and Brüggemann, 2000; Ray et al., 2001; Marques and Jørgensen, 2002; Jørgensen et al., 2002a,b; Bossel, 2000; Fath et al., 2001; Eco Targets, Goal Functions, and Orientors, 1998).

The study consisted of the following main stages. We first obtained explicit formulae for calculating the boundaries of limitation ranges and the cellular group size in situations occurring in laboratory experiments. Then we analyzed the sensitivity of the model with respect to its parameter changes, which enabled us to judge on the agreement between theoretical and experimental results. Using the results of laboratory experiments with monocultures, we determined the model parameters which represent the demands of microorganisms for basic nutrients. Then, on the basis of experiments determining the resources constraining the community growth (the so-called experiments with additions) for both monocultures and mixed cultures, we checked the agreement between the limiting resources predicted by the stratification theorem and the experimental data. And lastly, for different variants of polycultures (for all binary mixtures and mixtures of three dissociants), we checked the adequacy of theoretical calculations of the community composition at the stage of stationary growth and the experimental results.

The represented mathematical model is applied to the description of bacterial culture for the first time. The received quantitative characteristics of dissociants (requirements for carbon, nitrogen and phosphorus) have not been discussed by other authors earlier. In general, it is possible to draw a conclusion that while defining the requirements, values appeared to be more exact for their ratio, rather than their absolute values. The performed research allows to make the following conclusions. The variational model where generalized entropy is used as a criterion function, gives an adequate description of results of accumulative cultivation of *Pseudomonas aeruginosa* dissociants. On the basis of modelling calculations and experimentally obtained data about values of dissociants requirements it is possible to predict: (a) what nutrients will be completely consumed by microorganisms; (b) what part of the whole number of culture cells will be related to each dissociant at a stationary stage of growth. It allows ones to give the recommendation on creation of such nutrient ratio in initial medium that a given resource was completely utilized or was established necessary parities between dissociants.

2. Objects and methods of the research

2.1. *Objects and methodology of the experiments*

The experimental objects of the study are R-, S- and M-dissociants of the bacteria *P. aeruginosa*. Dissociative transitions of bacteria, or phase variations make a bacterial population heterogeneous (Mil'ko and Yegorov, 1991). They result from genome reconstructions that occur with a high frequency, a few orders of magnitude higher than the random mutation frequency, and possess a pleiotropic action. This determines permanent and reversible changes of many morphological, physiological and biochemical properties of cells: their degradation ability, ability to synthesize substances valuable in practice, immunity to external impacts, growth rate (Mil'ko and Martynkina, 1996), nutrition demands. The dissociative forms are at present labelled by symbols according to certain morphological indications of their colonies: S (smooth), R (rough), M (mucoid). Representatives of the genus *Pseudomonas* are widely used in various biotechnological processes: in chemical production, in particular, for agriculture, in biohydrometallurgy, in polymer manufacturing, for increasing the yield of oil strata, in reclamation and contamination removal, including oil pollution (Kalakutskii et al., 1996; Mordukhova et al., 2000).

We carried out 152 experiments on monoculture growth and 77 on mixed culture growth (binary and triple combinations of dissociants) (Mil'ko and Il'inykh, 2001, 2004; Fursova et al., 2004a,b, 2005). The microorganisms were placed in a closed medium containing different nutrients necessary for their development. It is supposed that the resources are unable to replace one another since they perform different functions with respect to the growth. In the cultivation process, including cell fission and death, the resources and microorganisms were not added or removed in any artificial manner. Under such conditions, the community developed until a stationary phase was achieved. If the cultivation was continued, then, after some time spent in a stationary phase, the community entered into a natural degradation (extinction) phase.

Pseudomonas communities were grown in media with different initial concentrations of carbon, nitrogen and phosphorus using the accumulative cultivation method. These resources are necessary for the growth and are mutually irreplaceable. In the process of growth, the culture optical density, the acidity level were measured and, in some experiments, the chemical composition of the medium was monitored. In particular, we used the semi-quantitative express methods for glucose, nitrate and phosphate determination.

To determine the cell number in a culture at a stationary stage of the development, we obtained, for each dissociant, a coefficient connecting the optical density with the cell number.

The dissociant ratio in a population was determined according to the morphology of colonies by sowing on a dense substrate containing a beef-extract broth and wort in equal proportions.

To determine experimentally the resource that constrains the culture growth, we conducted the so-called experiments with additions. At the instant when a stationary phase was presumably achieved, the culture was divided into four test-tubes. Three of them were subject to adding glucose, nitrate or phosphate while the fourth one was left unchanged (for control). To test the possibility of multi-factor limitation, seven possible combinations of additions were inserted: each substance separately, their binary combinations and all three resources. The amount of a substance added was equal to its original content in the medium. The culture was left to grow in different experiments for 4–12 h, and after that the optical density was measured. If the addition of a nutrient component to the medium resulted in renewal of cell fission, we considered this resource to be limiting. In cases when the substance addition did not result in culture growth, we supposed that this factor was not limiting for the community growth.

If the cultivation and measurement conditions did not conform to the premises of the model used in this study, we excluded the corresponding experimental data from further consideration.

2.2. *Variational modelling of growth and consumption in a community of microorganisms*

A stationary stage of the development of a community consisting of w groups of microorganisms which consume m mutually irreplaceable resources may be described by a solution to the following conditional variational problem (Levich, 1982, 2000; Levich et al., 1994; Levich and Alexeyev, 1997):

$$\begin{cases} H(n_1,\ldots,n_w) = \left(\sum_{i=1}^{w} n_i\right) \ln \left(\sum_{i=1}^{w} n_i\right) - \sum_{i=1}^{w} n_i \ln n_i \rightarrow \text{extr}, \\ \sum_{i=1}^{w} q_i^k n_i \le L^k, \quad k = \overline{1,m}, \\ n_i \ge 0, \quad i = \overline{1,w}. \end{cases}$$

Here n_i is the sought-for size of the ith group of cells in the community at a stationary stage of growth; q_i^k is the amount of the kth resource consumed from the medium and necessary for fission of a cell from group i, calculated per cell (the requirement of an organism from group i for the resource k), and L^k is the content of the resource k in the medium at the beginning of cultivation ($L^k \ge 0$).

It is assumed that for sets of cellular requirements the equality $\text{rank}(q_i^k) = \min(m; w)$ is fulfilled. That enables one to distinguish the groups of cells.

We choose as the goal function H the so-called generalized entropy functional (Alexeyev and Levich, 1997; Levich and Alexeyev, 1997), while the constraints are represented by conservation laws. It is important to note that the functional H is not postulated but is derived on the basis of the category-functor method of mathematical structure comparison (Levich, 1982; Levich and Solov'yov, 1999). The community itself is described by a mathematical structure of sets of n elements divided into w non-intersecting classes of size n_i $\left(n = \sum_{i=1}^{w} n_i\right)$. Admissible transformations for these objects are injective and surjective ones, which are not everywhere defined and are not functional correspondences (this kind of transformations adequately reflects the conditions of experiments on accumulative bacteria cultivation). The logarithm of the specific number of admissible transformations of a given state of the system, termed generalized entropy, is a measure of structuredness of system states (Levich, 1995a). The extremal principle, formalized as a variational problem, postulates that dynamical systems pass from a given state to a state with an extremal structure (under the restrictions determined by the resources available) (Levich, 2000).

It is essential that the constraints of the variational problem are written in the form of inequalities. This takes into account the fact that the resources cannot replace one another and therefore, to stop the community growth, it is sufficient that at least one of the resources be exhausted, and the occurrence of a stationary stage does not at all mean that all the nutrients have been consumed completely.

The variational problem formulated above has a solution which has been termed "the community structure formula":

$$n_i(\vec{L}) = n \exp\left\{-\sum_{k=1}^{m} \lambda^k q_i^k\right\}.$$

The Lagrange multipliers λ^k and the full number of cells n are found as functions of the consumed resources $\vec{L}$ from the algebraic equations

$$\begin{cases} \sum_{i=1}^{w} \exp\left\{-\sum_{k=1}^{m} \lambda^k q_i^k\right\} = 1; \\ \lambda^k \left(n \sum_{i=1}^{w} q_i^k \exp\left\{-\sum_{k=1}^{m} \lambda^k q_i^k\right\} - L^k\right) = 0, \quad k = \overline{1,m}. \\ \lambda^k \ge 0, \quad k = \overline{1,m}. \end{cases}$$

It has been shown that this solution always exists, is unique and provides a maximum of the functional H (Levich et al., 1994). A stratification theorem has been proved, making it possible to determine, for any given set of resources L^k, the components which will bound the community growth at a stationary stage (Levich et al., 1994; Alexeyev and Levich, 1997; Levich, 2000).

The authors have obtained all relations necessary for determining the boundaries of the strata in the cases under study, i.e., for monocultures and for mixtures of two and three groups of cells consuming two or three nutrients (Fursova, 2003a). For instance, let there be a community consisting of two groups of cells consuming two resources L^1 and L^2. Suppose the requirements of the microorganisms for the resources $\{q_i^k\}$, $k = 1, 2$; $i = 1, 2$ are known (the index k numbers the resources while the index i numbers the groups). According to the stratification theorem, the resource space splits into three domains (Fig. 1). In one of them, both resources turn out to be limiting (domain I) while in two others only one resource (L^1 in domain II and L^2 in domain III). The slope coefficients of the straight lines that bound the limitation domains are specified in the following way:

$$v = v(q_i^k) = \frac{q_1^1 x_0^{q_1^1} + q_2^1 x_0^{q_2^1}}{q_1^2 x_0^{q_1^1} + q_2^2 x_0^{q_2^1}}, \quad \eta = \eta(q_i^k) = \frac{q_1^1 y_0^{q_1^2} + q_2^1 y_0^{q_2^2}}{q_1^2 y_0^{q_1^2} + q_2^2 y_0^{q_2^2}},$$

where x_0 is a root of the equation $x^{q_1^1} + x^{q_2^1} = 1$, and y_0 is a root of the equation $y^{q_1^2} + y^{q_2^2} = 1$.

Depending on the limitation conditions, using the community structure formula, one calculates which part of the whole

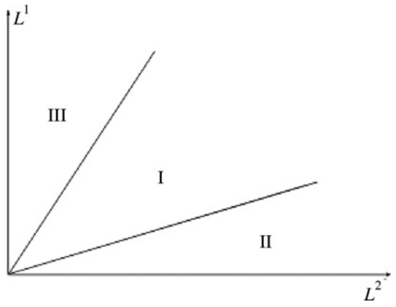

Fig. 1 – Stratification of the space of two resources consumed. Both factors are limiting in domain I, L^1 in domain II and L^2 in domain III.

community size will fall on each of the two or three groups of constituent cells. Thus, in the domain of limitation by a single resource L^k, $k = 1, 2, 3$, the dissociants' relative sizes at the stationary stage of growth are given by the expressions $n_1/n = x_0^{q_1^k}$, $n_2/n = x_0^{q_2^k}$, $n_3/n = x_0^{q_2^k}$, where x_0 is a root of the equation $x_0^{q_1^k} + x_0^{q_2^k} + x_0^{q_3^k} = 1$. Analogously, for a culture consisting of two groups of organisms, the proportions will be given by the expressions $n_1/n = x_0^{q_1^k}$, $n_2/n = x_0^{q_2^k}$, such that $x_0^{q_1^k} + x_0^{q_2^k} = 1$.

An analysis of the variational model sensitivity to its parameter changes has been performed (Fursova, 2003b). Each value of the requirements (of cells belonging to each group for each kind of substance) was separately changed by 10, 25, 50 and 100% under invariable values of all other parameters. As an indicator for estimating the model sensitivity to its parameter changes, we used the relative sizes of each group at a stationary phase of growth, the whole size of the community, and the quantities characterizing the boundaries of the limitation domains. Our analysis of the results makes it possible to reach the following conclusions. The greatest changes in the sensitivity estimation factors occur in the single-factor limitation domains. The relative values of the deflections of group abundances do not exceed the relative changes of the parameters. Exceptions are the cases when a requirement change leads to a change in the organisms' requirement rank with respect to a given resource (i.e., a change in the order of groups in the sequence from the highest requirement for a given resource to the lowest one).

3. Results and discussion

3.1. *Determination of the variational model parameters: the microorganisms' requirements for basic nutrients*

The requirement of an organism is understood as the resource amount (per cell) necessary for growth of the species. Thus, one can obtain the corresponding values of the dissociants' requirements for nutrients from the relation $q_i^L = \Delta L/\Delta n_i$, where ΔL is the substance amount consumed from the medium, L is the nutrient component, Δn_i is the number of newly formed cells for the same period of time, and the index i characterizes the dissociant.

Table 1 – Mean values of R-, S-, M-dissociants' requirements for carbon, nitrogen and phosphoruse ($\times 10^{-12}$ mg/cell) with 95% confident intervals

Dissociant	Resource		
	Carbon	Nitrogen	Phosphorus
R	129 ± 22	7.0 ± 2.5	1.0 ± 0.5
S	409 ± 55	17.5 ± 3.0	4.0 ± 1.5
M	525 ± 114	31 ± 3.0	6.0 ± 2.5

The requirements of R-, S- and M-dissociants of *P. aeruginosa* bacteria were determined by analyzing the experimental data obtained with monocultures (Fursova et al., 2004a). To calculate the dissociants' requirements from the experiments where the chemical composition of the medium was determined, it was sufficient to assure that the cell number changes and changes of the nutrient content were fixed for the same time interval. The requirements were calculated on the basis of the corresponding measurements of ΔL and Δn_i. In the majority of experiments, a chemical determination of nutrient concentrations was not conducted in the cultivation dynamics. In this case, it was first necessary to check whether or not a stationary stage had been achieved (and whether the number of cells was known at that moment); whether or not the growth termination was indeed caused by depletion of the nutrients under consideration (other reasons could be violation of the cultivation temperature regime, medium acidulation, insufficient airing, a too large culture density for the test-tube in use, etc.). It was then necessary to find out which nutrients could have been entirely depleted in each experiment, using different indirect indications. Knowledge of the limiting resource, i.e., the one completely consumed from the medium, makes it possible to calculate the demand value: if the growth had stopped due to depletion of a certain nutrient, then the amount of this nutrient consumed by the culture by the end of its growth may be identified with its content in the medium at cultivation beginning. To correctly calculate the requirement, it is necessary to use the number of cells which had grown for the same period of time, i.e., by the instant when the stationary phase was achieved.

All experimental data were analyzed from the standpoint of answering the question: which resources could stop the culture growth in each of the experiments. As a result, we chose the data from those experiments in which the requirements might be calculated. The mean values are given in Table 1.

3.2. *Limiting resource determination*

By the stratification theorem, the space of m resources consumed by the community splits into $2^m - 1$ limitation domains (Levich et al., 1994). For each set of nutrients determining the initial experimental medium, one can determine the limitation domain it belongs to and consequently indicate the resources whose depletion will stop the culture growth. It is the stratification theorem that gives an univalent algorithm for calculating the boundaries of limitation domains for a community with a given set of requirements q_i^k. Stratification of spaces of two and three resources for mono- and mixed cultures is presented in Figs. 1–3.

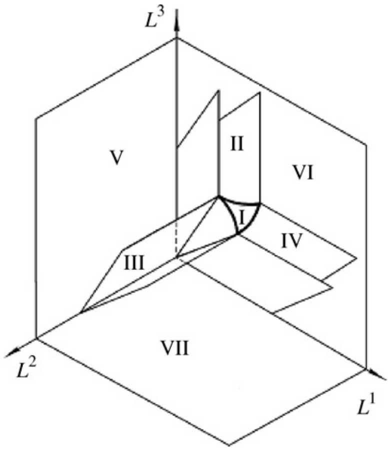

Fig. 2 – Stratification of the space of three resources consumed. All three resources are limiting in domain I; in domain II, L^1 and L^2 are limiting; in III, L^1 and L^3; in IV, L^2 and L^3; in V, L^1; in VI, L^2; in VII, L^3.

To check the adequacy of variational model predictions concerning the factor that restricts the growth of a microbiological community, we compared the calculated and experimental data. The latter were obtained from experiments with additions, which realized a method of experimental determination of a limiting resource (Fursova et al., 2004b).

Using for the calculations the requirement values of R-, S- and M-dissociants (Table 1), we have obtained the boundaries of limitation domains for these bacteria. For the initial conditions of the experiments with additions, we determined the limitation domain which contains each medium under study on the basis of calculations corresponding to every culture. A comparison of the model and experimental data has shown that, in 75% of the experiments, the growth resumed after adding the substance that had been determined by the model as a limiting one.

The creation of initial conditions for cultivation, such that the growth be limited by factors prescribed by the researcher, may be considered as one of the aspects of community structure control. To investigate this opportunity, using as a basis the calculated requirement values, we composed certain media which, from the variational model viewpoint, are limited by carbon, nitrogen and phosphorus. Then, using the data from experiments with additions, we checked the occurrence of limitation for mono- and mixed culture growth. The results of experiments with additions in a medium limited by carbon have entirely confirmed the prescribed character of limitation: in all cases, the growth resumed after adding glucose or a combination of substances containing it. When all cultures were grown in a medium limited by nitrogen, poorness of the media led to acidulation which probably caused death of the bacteria since neither combination of the resources resulted in growth resuming. In a similar way, we did not succeed in observing any growth limitation by phosphorus. Although the relative content of phosphates decreased, a full depletion of this resource was not observed. Analyzing the data on dissociant community cultivation in such media, we have formulated a hypothesis on repeated use of phosphorus by cells of *P. aeruginosa* dissociants. (A similar idea was previously expressed on the basis of biochemical studies of microorganisms' phosphorus metabolism (Kulayev, 1975).) It may happen that, for this phenomenon, it is important that the media used for culture growth at this stage are poor (in earlier studies, which used richer media, phosphorus depletion was indeed detected as well as growth resuming after its addition, and this allowed one to calculate the dissociants' requirement for this resource).

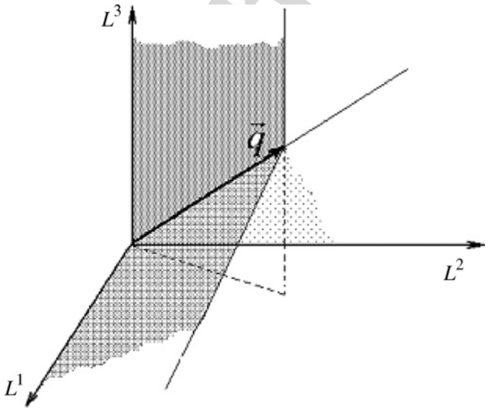

Fig. 3 – Splitting of three resources space into limitation domains of monoculture growth.

Having accepted the hypothesis of repeated phosphorus metabolism, we have to regard this resource being present in the medium in a sufficient amount. As a result, we revised all experimental data from the viewpoint of two factors able to constrain the growth of the dissociants, namely, carbon and nitrogen. Altogether, 71 experiments turned out to be suitable for analyzing the experiments with additions (37 experiments with monocultures and 34 with mixed cultures). In 59 of them, the growth resumed after adding the resource predicted by the model as a limiting one (Tables 2 and 3, Figs. 4 and 5). Thus, by the results of two experimental stages and taking into account the hypothesis on repeated phosphorus metabolism, the theoretical and experimental data coincide in 83% of the cases. Disagreements between the predicted and revealed limitation characters may be explained by errors in the requirements values used as parameters in the calculations of strata boundaries and by faults in preparing the initial media for cultivation. It seems to be of interest that an experimental confirmation was obtained for joint culture growth limitation by carbon and nitrogen. (The tables present the results of all the experiments. Figs. 4 and 5 give, as clear illustrations, diagrams characterizing the experimental data for a mixed culture consisting of three dissociants.)

3.3. *Mixed culture composition at a stationary stage of the development*

In the framework of the variational model of consumption and growth, the organisms' requirements data make it possible to determine the resources that will restrict the culture growth depending on the medium initial composition. The knowledge of a limiting factor, in turn, allows one to calculate the community composition (the proportion of each group of constituent cells) at a stationary stage of development. The authors have

Table 2 – Effect of additions on monocultures (%)

No.	Limiting resource by the model	Addition	R	S	M
1	C	C	**121**	**220**	**110**
		N	91	102	96
2	N	C			101
		N			**131**
3	C	C			117
		N			119
4	C	C	**220**	**140**	**217**
		N	97	80	74
5	C	C		**227**	
		N		73	
6	N	C	93		
		N	93		
7	C	C	94	62	100
		N	95	100	68
8	N	C	95	100	90
		N	**143**	**112**	100
9	C	C		**122**	**160**
		N		100	90
10	C	C	212		97
		N	162		66
11	C	C	**129**	**129**	**143**
		N	86	100	86
12	C	C	**131**	**101**	**119**
		N	76	68	81
13	C	C			**112**
		N			91
14	C	C	**246**	**171**	**184**
		N	103	76	92
		CN			
		C1			
		N1			
		CN1	**195**	**140**	**163**
15	C	C	**213**	**213**	**222**
		N	104	100	110
		CN	**204**	**178**	**208**
		C1	223	209	212
		N1	100	120	120
		CN1	**223**	**219**	**194**
16	CN	C	105		
		N	105		
		CN	86		
		C1	104		
		N1	82		
		CN1	**168**		
17	CN	C	135	149	178
		N	91	102	104
		CN	**214**	**202**	**216**
		C1	135	153	158
		N1	100	104	92
		CN1	**207**	**196**	**218**

100% is, by definition, the optical density of a culture without additions (the figure of 1 in the notation of resources inserted means the additional presence of phosphorus). The bold values indicates that signs of growth resuming were observed as a result of inserting the nutrient predicted by the model as a limiting one.

Table 3 – Effect of additions on mixed cultures (%)

No.	Limiting resource by the model	Addition	RS	RM	SM	RSM
1	C	C		82	89	87
		N		59	84	98
2	N	C	92	96	104	96
		N	**104**	**104**	**116**	**128**
3	C	C	**142**	**160**	**160**	**150**
		N	75	100	90	71
4	C	C			**121**	
		N			79	
5	C	C	**120**	**120**		**113**
		N	100	93		75
6	C	C	**103**	**143**		
		N	81	102		
7	C	C	**103**	**109**		**129**
		N	88	81		91
8	C	C	**220**	**200**	**194**	**254**
		N	98	94	98	114
		CN	**202**	**198**	**214**	**237**
		C1	**204**	**227**	**276**	**254**
		N1	110	114	94	116
		CN1	**212**	**227**	**270**	**237**
9	C	C		**233**		
		N		102		
		CN		**246**		
		C1		**200**		
		N1				
		CN1		**173**		
10	C	C				**222**
		N				100
		CN				**222**
		C1				**256**
		N1				113
		CN1				**220**
11	CN	C	102	99	77	126
		N	116	81	87	93
		CN	116	**157**	**107**	**137**
		C1	111	104	103	86
		N1	96	68	91	89
		CN1	113	**157**	**125**	**200**
12	CN	C	100	108	116	142
		N	90	92	110	102
		CN	104	**125**	**127**	**205**
		C1	90	98	112	142
		N1	92	92	102	98
		CN1	**170**	**163**	**163**	**193**

100% is, by definition, the optical density of a culture without additions (the Fig. 1 in the notation of resources inserted means the additional presence of phosphorus). The bold values indicates that signs of growth resuming were observed as a result of inserting the nutrient predicted by the model as a limiting one.

obtained the formulae which enable one to determine which part of the total cell number will fall at each of two or three groups of cells that form the community, for all possible limitation conditions (Fursova, 2003b). On the basis of the obtained requirement values of *P. aeruginosa* dissociants and boundaries of limitation domains, we have calculated the compositions of

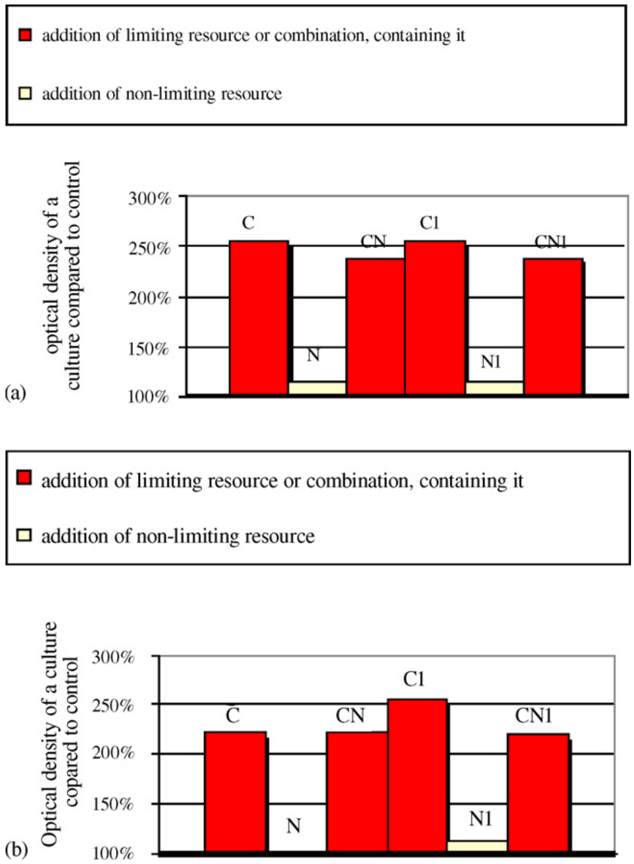

Fig. 4 – Effect of additions on the optical density of a mixed culture consisting of R-, S- and M-dissociants, limited by carbon. By definition, 100% is the optical density of a culture without additions (control); (a) corresponds to experiment No. 8 from Table 3; (b) corresponds to experiment No. 10 from Table 3.

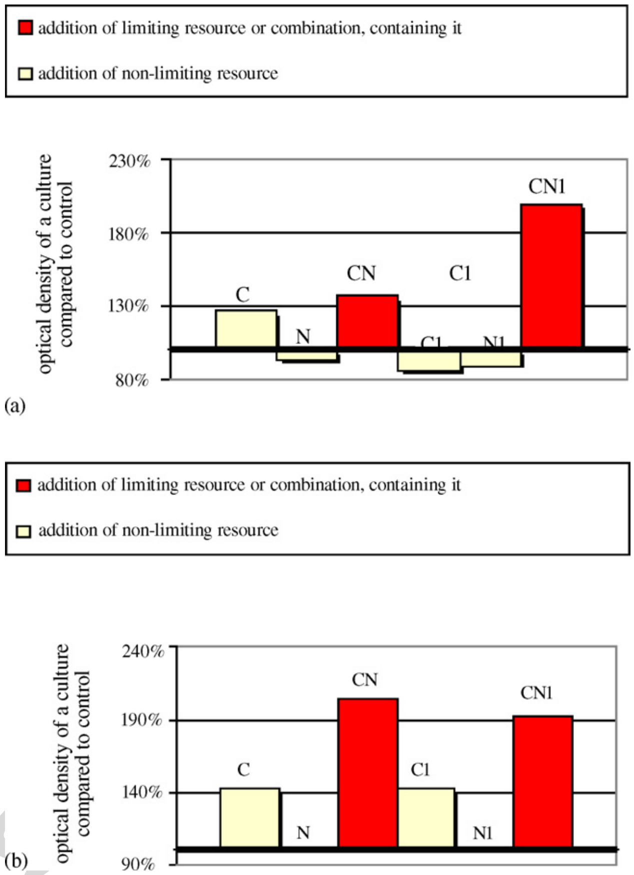

Fig. 5 – Effect of additions on the optical density of a mixed culture consisting of R-, S- and M-dissociants, in cases of joint limitation by carbon and nitrogen. By definition, 100% is the optical density of a culture without additions (control); (a) corresponds to experiment No. 11 from Table 3; (b) corresponds to experiment No. 12 from Table 3.

mixed bacterial cultures at their stationary stages of growth. The adequacy of the model predictions was verified by comparison with experimental results (Fursova et al., 2005).

The experimental data are formed from the results of growing four types of mixed cultures of *P. aeruginosa* dissociants. For theoretical calculations of the stationary community composition for each experimental medium, we first determined the resources restricting the community growth. It turned out that all sets of resources, determined by the initial medium composition, belong to strata with single factor limitation (the results obtained well agree with the results of experiments with additions).

The relative sizes of dissociant groups obtained from model calculations were compared with experimental data. It is necessary to remark on the comparison of theoretical and experimental data. An experiment gives, by sifter results, the proportions of units that form colonies of dissociants, whereas the model allows calculations of relative cell numbers. In this work, we accept the following hypothesis: at a stationary stage of growth, the cells of bacteria are in the same physiological state, and the proportion of germinated cells among all cells is the same for all dissociants.

Thus, we have obtained that, in 33 of 36 data sets, the polyculture composition predicted by the model differs from the experimental data by less than 15%. The largest difference between the calculated and experimental data comprises 17%. An analysis of the model sensitivity with respect to parameter variations shows that the disagreements obtained may be explained by errors in the requirement values used in the calculations. Besides, in the results of mixed culture composition determination in replication, the scatter reaches 15%. After the experiments on media with prescribed limitation conditions and a subsequent transition to two relevant resources (see the previous section), a comparison of the calculated and experimental culture compositions over all experiments conducted gave the following results. The data are represented in Table 4. (The table contains the results of all experiments. Figs. 6–8 give, as clear illustrations, the proportion of the R-dissociant in different mixed cultures.) In 7 of the 45 experiments, the difference in dissociant proportions in the whole size of the community is 15% or more (24, 32, and less than 20% in the remaining cases). In the remaining 38 cases (i.e., in 85% of the experiments), the difference between the dissociant proportions predicted by the model and those obtained in the

Table 4 – Mixed culture composition at a stationary stage of growth

No.	Culture	Composition by experiment (%)	Composition by model calculations (%)
1	RS	67:33	69:31
	SM	66:34	54:46
2	RS	62:38	65:35
	RM	71:29	74:26
	SM	50:50	60:40
	RSM	43:41:16	60:29:11
3	RS	70:30	69:31
	SM	50:50	54:46
	RSM	67:11:22	62:23:15
	RSM	58:18:24	62:23:15
4	RM	60:40	73:27
	SM	42:58	54:46
5	RS	61:39	69:31
	RM	62:38	73:27
	SM	50:50	54:46
	RSM	62:32:6	62:23:15
6	RM	78:22	73:27
	SM	43:57	54:46
	RSM	68:17:15	62:23:15
7	RS	79:21	69:31
	RM	87:13	73:27
	SM	52:48	54:46
	RSM	75:12:13	62:23:15
8	RSM	68:19:13	62:23:15
9	RS	60:40	67:33
10	RS	70:30	65:35
	RM	80:20	74:26
	RSM	45:31:24	60:29:11
	RSM	65:19:16	60:29:11
11	RM	75:25	74:26
12	RS	57:43	69:31
	RM	74:26	73:27
	RM	66:34	73:27
	SM	72:28	54:46
	RSM	54:34:12	62:23:15
	RSM	38:23:39	62:23:15
	RSM	61:22:17	62:23:15
13	RS	35:65	67:33
	RM	54:46	73:27
	SM	65:35	57:43
	RSM	56:24:20	61:26:13
14	RS	67:33	67:33
	RM		73:27
	SM	62:38	57:43
	RSM	76:9:15	61:26:13

experiment was less than 15% of the total size. The causes of the discrepancies are similar to those described above.

The requirement values obtained and the calculated dissociant sizes in each of the limitation domains allowed us to analyze the possible ways of regulating the culture composition by changing the ratios of resources stored in the medium (Fursova and Levich, 2004). The composition control is only possible within the limits prescribed by the model. It turned out that, for the dissociants of *P. aeruginosa* under study with their requirement values, the community structure cannot be changed essentially. This is connected, firstly, with the narrowness of intervals that specify the multi-factor limitation domains compared to the accuracy at which the initial media may be prepared, and, secondly, with tiny distinctions between the community compositions in different strata compared to the dissociant proportion determination error. Nevertheless, for "suitable" values of the requirements, it is possible to obtain different group distributions by ranks (from the least to the most numerous one) at the stationary stage of growth by varying the nutrient ratios in the medium. To demonstrate this opportunity, we present a calculation of the community composition for a community of four *Proto-*

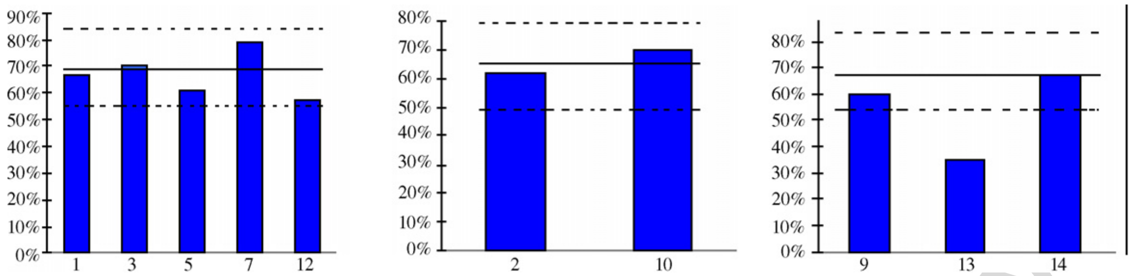

Fig. 6 – Proportions of the R-dissociant in a mixed culture consisting of R- and S-dissociants. *Notations*: (▦) experiment, (—) model prediction, (- - -) admissible error limits according to the results of determining the mixed culture composition in replication. The experiment number on the abscissa axis corresponds to its number in Table 4.

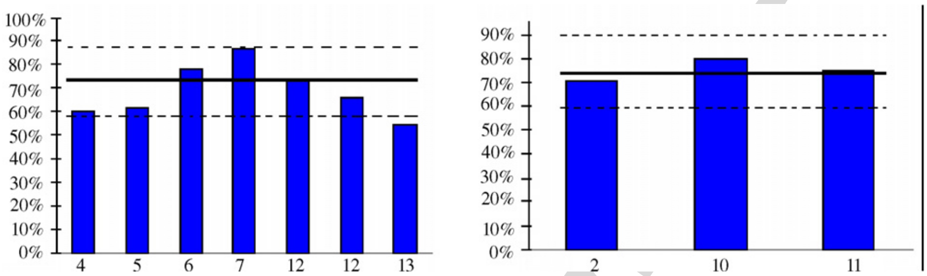

Fig. 7 – Proportions of the R-dissociant in a mixed culture consisting of R- and M-dissociants. *Notations*: (▦) experiment, (—) model prediction, (- - -) admissible error limits according to the results of determining the mixed culture composition in replication. The experiment number on the abscissa axis corresponds to its number in Table 4.

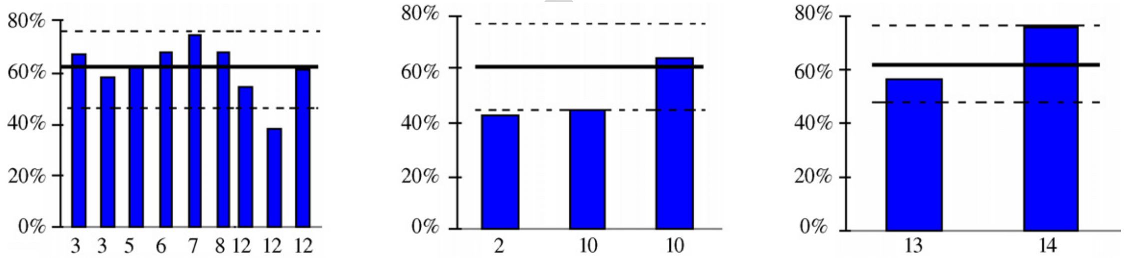

Fig. 8 – Proportions of the R-dissociant in a mixed culture consisting of R-, S- and M-dissociants. *Notations*: (▦) experiment, (—) model prediction, (- - -) admissible error limits according to the results of determining the mixed culture composition in replication. The experiment number on the abscissa axis corresponds to its number in Table 4.

coccale algae that consume nitrogen (N) and phosphorus (P) (Levich et al., 1997; Levich, 2000). Fig. 9 presents the relative sizes of the species p_i, $i = \overline{1,4}$, on the basis of the following experimental values of the requirements (related to 10^{-9} mg/cell):

$$q_1^N = 3.94;\quad q_2^N = 0.91;\quad q_3^N = 1.69;\quad q_4^N = 0.41;$$
$$q_1^P = 0.79;\quad q_2^P = 0.28;\quad q_3^P = 0.22;\quad q_4^P = 0.21.$$

By the results of laboratory experiments, replacement of a dominating species may be followed by observing, as an example, the cultivation of two species from the *Protococcale* order: *Ankistrodesmus falcatus* and *Scenedesmus quadricauda* (Levich et al., 1997). The results of a study of the community growth on media with different initial nitrogen to phosphorus ratios (N/P = 1.3; 57) are illustrated in Fig. 10.

Let us point out one more property of the variational problem, related to the control (or regulation) of the com-

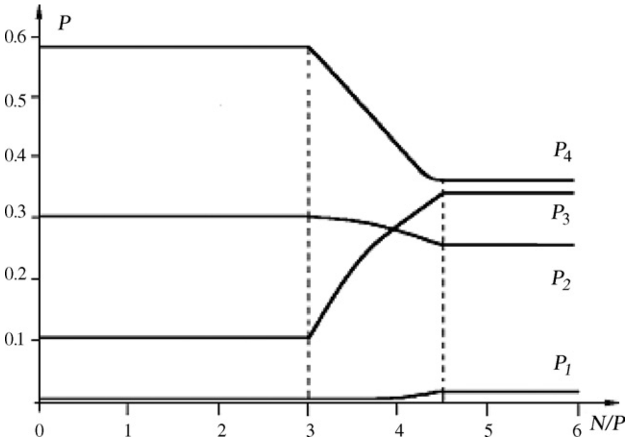

Fig. 9 – Relative size of p_i, $i = \overline{1,4}$ for experimental requirement values as functions of N/P (Levich et al., 1997).

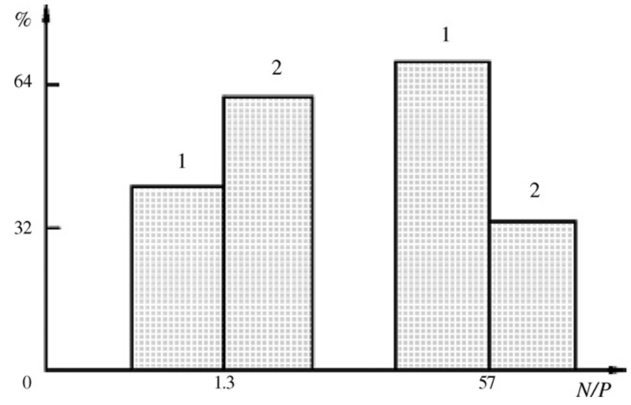

Fig. 10 – Relative size of the species vs. nitrogen to phosphorus ratio in laboratory experiments with two-species cultures: 1—*S. quadricauda*, 2—*A. falcatus* (Levich et al., 1997).

munity composition: the relative size of a given species takes on its maximum value when the resource ratios in the medium take on the values equal to the resource requirements ratio of the same species (Levich et al., 1993; Levich, 2000). The experimental data confirm this theoretical fact. Thus, for instance, the experimental value of the nitrogen to phosphorus requirement ratio for *A. falcatus* is equal to 7 and for *S. quadricauda* to 25. Consequently, on media with the resource ratios equal to 1.3 and 57, the *Ankistrodesmus* and *Scenedesmus*, respectively, must predominate. And this is precisely what is observed in the experiment (Fig. 10). (A more detailed description of this experiment as well as other experiments is contained in a book devoted to phytoplankton community structure control (Levich and Bulgakov, 1992; Zamolodchikov and Levich, 1992; Levich, 1995b, 1996, 2000; Bulgakov and Levich, 1999, 1999; Levich et al., 1997).)

Thus, the property of relative abundances in a community formulated above may be used for creating a community of unicellular organisms with required properties by changing the nutrient ratios in the medium. The results obtained enable one to speak of perspectives of solving the problems related to biological utilization of multi-component contaminations, the problems of creating forage reserves for herbivorous fish in fish-farms and the problems of reservoir eutrophication suppressing.

4. Conclusions

To conclude, let us once again point out some peculiarities of the methods used in the present work. The modelling methods, widely used in mathematical biology, employ the means of (ordinary or partial) differential equations. It allows one to study the dynamics of processes occurring in a biological system and to predict the system's state at any moment of time. The present approach only gives a stationary state eventually achieved by the system. If knowing of the stationary state is the aim, one can avoid a number of difficulties of studying the dynamical models by using variational modelling. One of them is uncertainty of the values of numerous parameters. Thus, for instance, for our experimental system when modelling a community of three species which consume three resources with variational methods, to find the cell numbers, we have to solve a set of three algebraic equations with three unknowns. In doing so, we have the following parameters: 9 requirement values and 3 values of the initial nutrients deposits (12 parameters altogether). For a realistic description of the development of a similar community with ordinary differential equations, one has to write down 15 equations (for the biomass dynamics, the dynamics of intracellular content of three substances for each species and the dynamics of three substances in the medium). These equations will contain 42 parameters (the minimal and maximal content of each substance in a cell, the semi-saturation constants and the substance consumption rates). And it is important that these parameters will be obtained from experiment, which means with errors. So if we have fewer parameters, we can get more accurate information.

Variational modelling has found its application in different branches of biology, but, despite a number of advantages, it is still a modelling method of little relevance. The main reason for that is the difficulty of choosing the goal function. The variational model used in the present work rests on the maximum generalized entropy principle, which develops **Janes**'s formalism. The results of this study may be used for the description of various biological objects satisfying the requirements inherent to the model. They may also be used in other areas of natural science dealing with similar extremal problems.

Acknowledgement

The study was supported by the Russian Basic Research Foundation (Grant Nos. 05-04-49238 and 05-06-80062).

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