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Utilization of N, P and organic C by heterotrophic bacteria. II. Comparison of experiments and a mathematical model

Ingrid Martinussen & T. Frede Thingstad

Department of Microbiology and Plant Physiology, University of Bergen, Allégt. 70, N-5000 Bergen, Norway

ABSTRACT: The consumption of ammonia as N-source, orthophosphate as P-source, and glucose as C-source was investigated in batch and chemostat cultures of *Pseudomonas putida* NCMB 1960. Consumption of nutrients and growth in cell numbers were compared to a general 'Droop' type mathematical model previously suggested. Important characteristics of the growth patterns in batch cultures such as depletion of more than one nutrient from the culture fluid, continued increase in cell numbers after depletion of one or more nutrients, and continued consumption of glucose after a stationary phase in cell numbers had been reached, could be explained by the model. The 3 nutrients were depleted from the cultures in various combinations dependent upon C:N:P-composition of the medium. In batch cultures, 4 different combinations of nutrient depletion were observed, while 3 additional combinations were observed in chemostat cultures. The observed patterns of nutrient depletion could be explained within the context of the proposed mathematical model. Three other bacterial strains of marine heterotrophic bacteria gave a similar qualitative pattern of substrate consumption in batch cultures, but different sets of numerical values were required for the parameters in order to fit the model to each strain. It is also demonstrated how the proposed model may be fitted to published experimental results describing the consumption of glucose and phosphate following the addition of phosphate to P-starved cultures of *Vibrio natriegens*.

INTRODUCTION

Heterotrophic bacteria have been suggested to play a key role in the transfer of dissolved organic material into particulates which, in turn, may enter the pelagic food chain (Azam et al. 1983). The efficiency of this transfer process has, however, been questioned (Ducklow et al. 1986). A deeper understanding of the process of carbon transfer through the bacteria, and of the possible variability in the efficiency by which carbon is transferred, would require an understanding of the biological and environmental factors influencing the process.

Since bacterial consumption of organic material will be coupled to a simultaneous consumption or excretion of mineral nutrients (Fenchel & Blackburn 1979), the processes of organic carbon transfer through the bacterial link of the food web will also be intimately related to the processes of mineral nutrient cycling. How these processes are coupled at the bacterial level, and whether bacteria will act as remineralizers or mineral nutrient consumers, will depend both on the C:N:P-ratios of the substrate, and on physiological properties of the bacteria such as the C:N:P-ratio of their biomass and the fraction of organic carbon lost by respiration. Bacteria growing on substrates poor in N and/or P have been suggested to compete with algae for dissolved inorganic forms of these nutrients (Mann 1982, Currie & Kalff 1984, Bratbak & Thingstad 1985). The potential success of bacteria in this competition will depend upon the kinetic properties of the uptake mechanisms for mineral nutrients. Not only will the C:N:P-composition of bacterial biomass influence the role of bacteria as remineralizers or consumers of mineral nutrients, it will also represent the composition of food available to bacterial predators and thereby influence the role of these organisms as remineralizers in the system (Güde 1985).

An understanding of the relevant aspects of bacterial physiology is thus a prerequisite for any detailed understanding of how heterotrophic bacteria interact with organisms at other trophic levels in the microbial

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food web. A model describing bacterial growth in an environment where both N, P, and organic-C could be potentially limiting substrates for bacterial growth has recently been suggested by Thingstad (1987). Based on the incorporation of a description of carbon metabolism into a mathematical formulation of the type proposed by Droop (1974) for algal growth, the utilization of the N-, the P-, and the C-source at steady state in chemostats could be explored as a function of medium composition and bacterial growth rate. The experiments presented have been designed specifically to allow a comparison of experimental results with some of the predictions of this model (Thingstad 1987).

MATERIALS AND METHODS

Culture conditions. Batch cultures of 1.5 l medium in 3 l Erlenmeyer flasks were used in the investigations of kinetics of nutrient consumption and growth in cell numbers as shown in Fig. 1. Cultures were incubated at 18 °C on a shaking platform. For mapping the final nutrient concentration after growth in batch cultures for a large range of medium compositions (Fig. 2 & 6), 100 ml medium in 250 ml Erlenmeyer flasks were incubated as above. For similar mapping of the results from steady-state chemostat cultures (Fig. 3), chemostats used were of the construction given by Pengerud et al. (1987).

Organisms. Most of the experiments were carried out on *Pseudomonas putida* NCMB 1960. For the experiments summarized in Fig. 6, bacteria were isolated from Bergen harbour natural seawater, using an enrichment procedure intended to select for bacteria efficient in storing nutrient reserves of C-, N-, or P-containing material. Enrichment cultures were contained in dialysis bags (Thomas 3787 D-32, 22 mm diam.), and dialyzed in an alternating sequence against media allowing storage of the nutrient, but no growth ('storage media'; Table 1), and media allowing growth exclusively for organisms with stores of the nutrient under consideration ('growth media'; Table 1). Between each transfer cultures were washed by dialyzing against seawater without nutrient additions. Residence time in each medium was 1 to 2 d, incubation temperature was 10 °C and the media were continuously mixed by magnetic stirrers. Isolation of pure cultures was made from colonies on a solid medium previously described (Pengerud et al. 1987). One of the isolated strains from each of the 3 sequences aimed at enrichment of C-, of N-, or of P-storing organisms were used for further experiments and denoted C1, N1, and P1, respectively.

Table 1. Concentrations of nutrients (µg-at C l^{-1}, µg-at N l^{-1}, µg-at P l^{-1}) in media used for enrichment

Medium	Nutrient	Enrichment for C storage	Enrichment for N storage	Enrichment for P storage
Storage	Glucose-C	900	0	0
	Ammonia-N	0	20	0
	Phosphate-P	0	0	1
Growth	Glucose-C	0	900	900
	Ammonia-N	20	0	20
	Phosphate-P	1	1	0

Media. The natural seawater medium described by Pengerud et al. (1987) was used in the batch culture experiments of Fig. 1, and during the enrichment procedures described above. No extra silicate was added; additions of glucose, ammonia and orthophosphate were made to final concentrations given in Tables 1 & 2. Synthetic medium used in other experiments was based on a commercial seawater salts mixture (Rila Marine Mix, Rila Products). Additions of glucose, ammonia and orthophosphate are given in 'Results'.

Table 2. Concentrations of nutrients (µg-at C l^{-1}, µg-at N l^{-1}, µg-at P l^{-1}) in media of batch cultures denoted M, N, and P

Nutrient	M	N	P
Glucose-C	2700	2700	2700
Ammonia-N	150	30	150
Phosphate-P	10	10	2

Measurements. Glucose, orthophosphate and cell numbers were determined as described previously (Pengerud et al. 1987). Ammonia was determined according to Strickland & Parsons (1972) modified to a sample volume of 5 ml.

RESULTS

Experiments with *Pseudomonas putida*

The experimental results obtained with *Pseudomonas putida* are summarized in Fig. 1, 2 & 3. Fig. 1 shows the changes over time in glucose, ammonia, orthophosphate, and cell numbers for 3 cultures differing with respect to initial concentrations of ammonia and orthophosphate, denoted M ('mixed limitation'), N ('nitrogen limitation') and P ('phosphate limitation'). In Cultures N and P, ammonia and orthophosphate respectively were the first of the substrates to be depleted (frames marked N and P in Fig. 1). Following depletion of the mineral nutrient, cell numbers continued to increase for about 1 d in these cultures. This

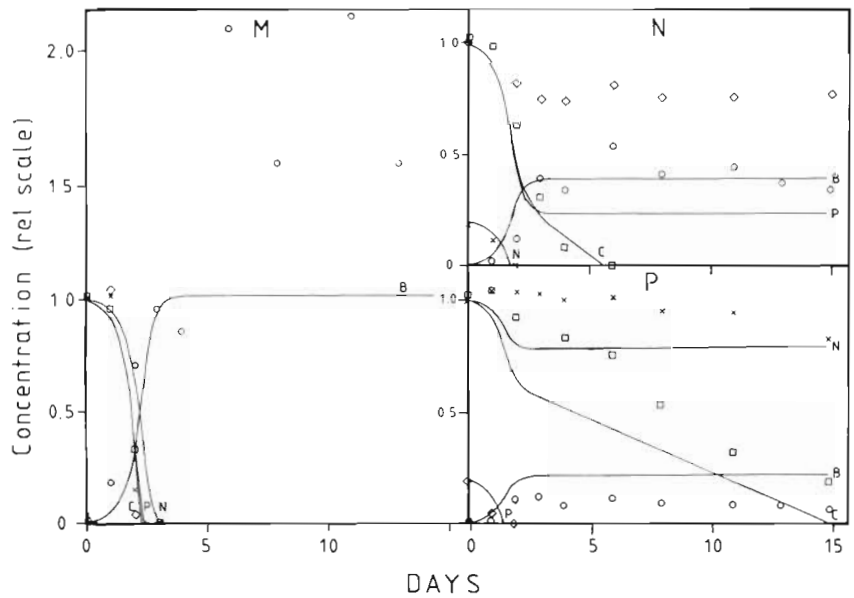

Fig. 1. Experimental results and model output. Growth curves for cell numbers (○) of *Pseudomonas putida*, and concentration in the culture fluid of glucose (□), ammonia (x), and orthophosphate (◇). The 3 frames denoted M, N, and P correspond to different initial media compositions as given in Table 2. Relative medium compositions of these cultures are also indicated in Fig. 2. Scale unit 1.0 corresponds to 2700 µg-at glucose-C l^{-1}, 150 µg-at ammonia-N l^{-1}, 10 µg-at orthophosphate-P l^{-1} and 10^8 cells ml^{-1}. Solid lines are simulated results obtained from numerical solutions of the equations given in Table 3 with parameter values given in Table 4

corresponded to about one additional doubling in cell numbers before a stationary phase was reached. Consumption of glucose, however, continued in both of these cultures, apparently as long as any glucose was left in the culture fluid. Eventually such cultures thus seem to reach a state of combined mineral nutrient and organic carbon depletion. In Culture M (Fig. 1), all 3 substrates were depleted more or less simultaneously. In this culture, the increase in cell number continued for about 3 d after nutrient depletion, and corresponded to approximately 2 doublings. An unexplained fluctuation in the measured cell density occurred for the rest of the 15 d period investigated.

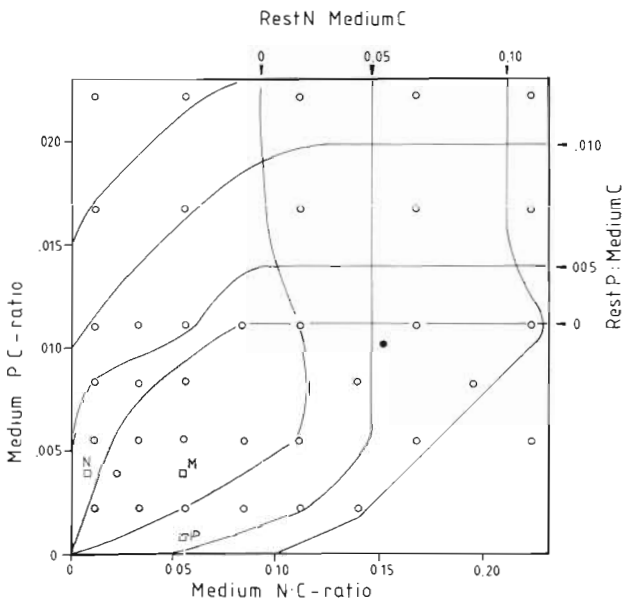

Fig. 2. Experimental results. Concentrations at stationary phase in batch cultures of *Pseudomonas putida*. Coordinates represent the initial N:C and P:C of the media, each point in the plane thus represent a specific medium composition. (○) Experimental batch cultures with an initial concentration of 900 µg-at glucose-C l^{-1}. (□) Position of the 3 cultures M, N, and P (initial concentration 2700 µg-at glucose-C l^{-1}), for which the growth curves are given in Fig. 1. Isolines connect points with equal concentration of ammonia and of orthophosphate at stationary phase. Isoline values are normalized by division by initial glucose-C concentration of the medium. Glucose was depleted from all cultures investigated. Within the shaded area, both ammonia and orthophosphate were depleted. (•) Substrate with Redfield ratio composition

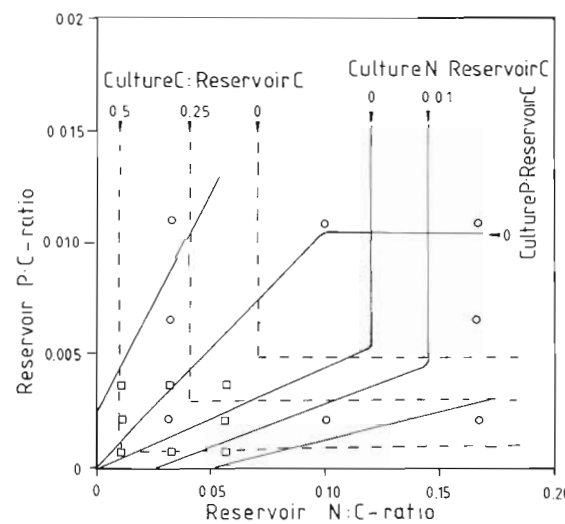

Fig. 3. Experimental results. Concentrations at steady state in chemostat cultures of *Pseudomonas putida*. Cultures with (□) 2700 and (○) 900 µg-at glucose-C l^{-1}. As opposed to the batch culture situation of Fig. 2, glucose was not depleted from cultures of low N:C and/or low P:C ratios. Shaded area is the estimated area for which all 3 nutrients would be depleted from the cultures. This figure is redrawn in Fig. 8 to emphasize the partitioning of the plane into 7 areas of different combinations of nutrient depletion

Table 3. Equations of model. See Table 4 for definitions of parameters. (From Thingstad 1987)

Biological processes

Specific growth rate for cell number B:

$$\mu = \mu^{max}\,(1-f) \tag{1a}$$

where

$$f = \text{maximum of } \frac{Q_C^g}{Q_C}\ \frac{Q_N^{min}}{Q_N}, \text{ or } \frac{Q_P^{min}}{Q_P} \text{ if } Q_C > Q_C^g, \text{ and } f = 1 \text{ if } Q_C < Q_C^g \tag{1b}$$

and

$$Q_C = \frac{B_C}{B} \tag{1c}$$

$$Q_N = \frac{B_N}{B} \tag{1d}$$

$$Q_P = \frac{B_P}{B} \tag{1e}$$

Specific uptake rates of C, N, and P:

$$V_C = v_C^{max}\ \left(\frac{Q_C^{max} - Q_C}{Q_C^{max} - Q_C^{min}}\right)\ \frac{C}{K_C + C} \tag{2a}$$

$$V_N = v_N^{max}\ \left(\frac{Q_N^{max} - Q_N}{Q_N^{max} - Q_N^{min}}\right) \cdot \frac{N}{K_N + N} \tag{2b}$$

$$V_P = v_P^{max}\ \left(\frac{Q_P^{max} - Q_P}{Q_P^{max} - Q_P^{min}}\right) \cdot \frac{P}{K_P + P} \tag{2c}$$

Specific respiration:

$$R = \underbrace{\rho^g \cdot Q_C \cdot \mu}_{\text{'growth'}} + \rho^r \cdot (Q_C - Q_C^{min}) \cdot (1 + \delta\ \left(\frac{Q_P^{max} - Q_P}{Q_P^{max} - Q_P^{min}}\right)) \tag{3a}$$

State variables

B	10^9 cells l^{-1}	Bacterial density
B_C	µmol C l^{-1}	Bacterial C
B_N	µmol N l^{-1}	Bacterial N
B_P	µmol P l^{-1}	Bacterial P
C	µmol C l^{-1}	Conc. of C substrate in culture
N	µmol N l^{-1}	Conc. of N substrate in culture
P	µmol P l^{-1}	Conc. of P substrate in culture

Chemostat operation parameters

D	h^{-1}	Dilution rate
C_i	µmol l^{-1}	Conc. of C substrate in reservoir
N_i	µmol l^{-1}	Conc. of N substrate in reservoir
P_i	µmol l^{-1}	Conc. of P substrate in reservoir

Differential equations

$$\frac{dB}{dt} = \mu \cdot B - D \cdot B \tag{4a}$$ Growth – dilution

$$\frac{dB_C}{dt} = (V_C - R)B - D \cdot B_C \tag{4b}$$ Uptake – respiration – dilution

$$\frac{dB_N}{dt} = V_N \cdot B - D \cdot B_N \tag{4c}$$ Uptake – dilution

$$\frac{dB_P}{dt} = V_P \cdot B - D \cdot B_P \tag{4d}$$ Uptake – dilution

$$\frac{dC}{dt} = -V_C \cdot B + D \cdot (C_i - C) \tag{4e}$$ – Uptake + input – dilution

$$\frac{dN}{dt} = -V_N \cdot B + D \cdot (N_i - N) \tag{4f}$$ – Uptake + input – dilution

$$\frac{dP}{dt} = -V_P \cdot B + D \cdot (P_i - P) \tag{4g}$$ – Uptake + input – dilution

To map the outcome of batch culture experiments for a larger range of media compositions, a system of 36 cultures was used. Glucose was added to a concentration of 900 µg-at C l^{-1} in all cultures; ammonia and orthophosphate additions for each culture are indicated in Fig. 2. The graphical representation used in Fig. 2 shows the plane defined by the medium N:C and P:C additions. The concentrations of glucose, ammonia, and orthophosphate left in the culture fluid 7 d after inoculation are given as isolines connecting points of equal culture fluid concentrations in Fig. 2. For all medium compositions investigated, glucose was depleted from the culture fluid. For cultures with medium compositions within the shaded area of Fig. 2, both ammonia and orthophosphate were depleted. The plane described in Fig. 2 is thus divided into 4 areas, each characterized by depletion of the C-source, the C- and N-sources, the C- and P-sources, or the C-, N-, and P-sources, respectively.

In the chemostat experiments (Fig. 3) with a dilution rate of 0.043 h^{-1}, glucose was not depleted in the cultures if the N:C ratio of the reservoir medium was below approximately 0.07 and/or the P:C ratio was below approximately 0.05. Starting from the origin of Fig. 3, the area where both N and P are depleted is delimited by 2 lines (numbered 1 and 2 in Fig. 4) separated by a certain angle. The results suggest that this angle is smaller in the case of steady-state chemostat cultures (Fig. 3) than in the case of stationary-phase batch cultures (Fig. 2).

Comparison with mathematical model

As a conceptual framework for interpretation of these results, we have used the model proposed by Thingstad (1987). The equations of this model are summarized in Table 3. Numerical solution of the differential equations were obtained as described by Thingstad (1987), allowing a comparison between the model and the experimental results of Fig. 1, 2 & 3. The biological model contains a total of 17 parameters. We wanted to use only one set of numerical values for these parameters to represent the properties of *Pseudomonas putida* NCMB 1960. An initial guess of parameter values was based on the shape and position of the shaded area of Fig. 2, on the number of cells formed, and on the rate of glucose consumption following N and P depletion (Fig. 1). Some optimization was done through an iterative procedure with manual adjustment of parameters between each iteration. The set of parameters eventually chosen is given in Table 4. This set of numerical parameter values is not claimed to be optimal in any sense. Other sets may fit the data equally well or better.

For comparison with experimental data, the changes over time in simulated batch cultures are included in Fig. 1. The summarized results of simulations corresponding to Fig. 2 & 3 are given in Fig. 4 & 5 respectively. Due to the storage capacity for nutrients, the model provides an explanation of the continued cell growth after depletion of a nutrient. With this flexibil-

Table 4. Parameter values used to simulate experiments with *Pseudomonas putida*

μ^{max}	0.15	h^{-1}	Maximum specific growth rate
ρ^{g}	0.6	(Dimensionless)	Proportionality factor between carbon incorporated and respired for growth
ρ^{r}	0.01	(Dimensionless)	Fraction of surplus carbon respired h^{-1}
δ	0.001	(Dimensionless)	Proportionality constant of decoupling. Value corresponds to increase in growth-independent respiration when $Q_P = Q_P^{min}$
υ_C^{max}	50	µmol C l^{-1} h^{-1} $(10^9 cells)^{-1}$	Maximum specific uptake rate C
υ_N^{max}	4	µmol N l^{-1} h^{-1} $(10^9 cells)^{-1}$	Maximum specific uptake rate N
υ_P^{max}	0.5	µmol P l^{-1} h^{-1} $(10^9 cells)^{-1}$	Maximum specific uptake rate P
K_C	1.0	µmol C l^{-1}	Half-saturation const. uptake C
K_N	0.1	µmol N l^{-1}	Half-saturation const. uptake N
K_P	0.01	µmol P l^{-1}	Half-saturation const. uptake P
Q_C^{min}	5.0	µmol C $(10^9 cells)^{-1}$	Minimum cell quota C
Q_N^{min}	0.8	µmol N $(10^9 cells)^{-1}$	Minimum cell quota N
Q_P^{min}	0.1	µmol P $(10^9 cells)^{-1}$	Minimum cell quota P
Q_C^{max}	30	µmol C $(10^9 cells)^{-1}$	Maximum cell quota C
Q_N^{max}	1.6	µmol N $(10^9 cells)^{-1}$	Maximum cell quota N
Q_P^{max}	0.2	µmol P $(10^9 cells)^{-1}$	Maximum cell quota P
Q_C^{g}	5.1	µmol C $(10^9 cells)^{-1}$	Cell quota of C at which growth stops

ity in biomass composition, the model also allows for the observed depletion of both mineral nutrients (Frame M, Fig. 1). Due to the assumed consumption of organic carbon for maintenance purposes, the model also has the property of allowing a continued consumption of glucose in stationary phase. As a consequence, there will be an eventual depletion of glucose from all batch cultures as indicated by experimental

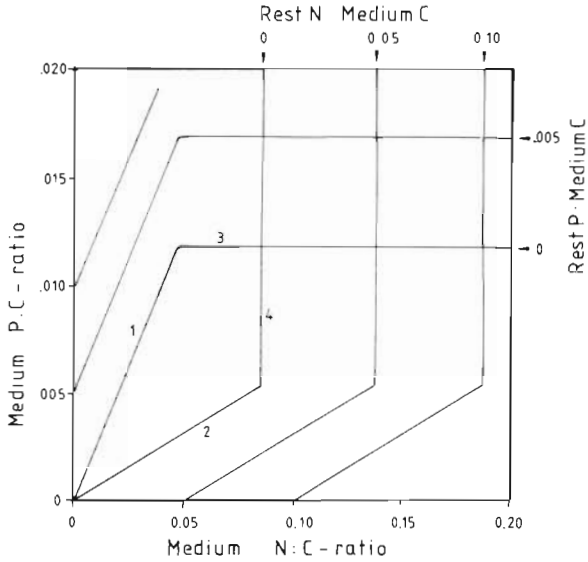

Fig. 4. Model output. Results of simulated batch cultures corresponding to the experimental results of Fig. 2. Model and values of model parameters are given in Tables 3 & 4 respectively

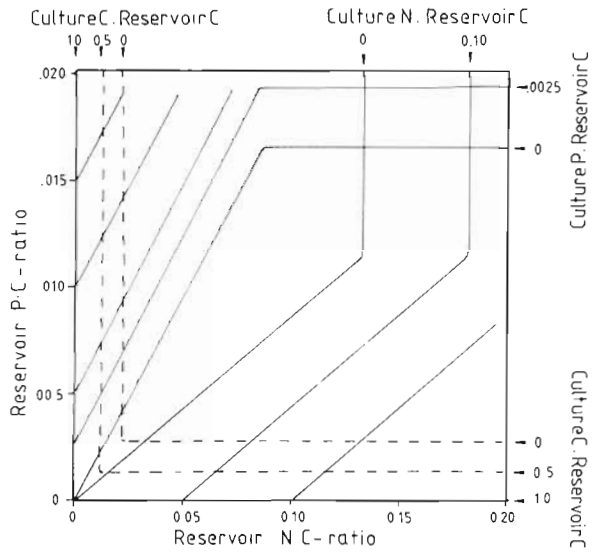

Fig. 5. Model output. Results of simulated chemostat experiments corresponding to the experimental results of Fig. 3. Model and values of model parameters are given in Tables 3 & 4 respectively

results presented in Fig. 2. For some observations, however, the fit of the numerical output of the model (with the set of parameters chosen), is less satisfactory. The observed large increase and fluctuation in cell number in the situation of 'mixed' (M) limitation is not reproduced by the model. Also, the simulated consumption of orthophosphate in the nitrogen-limited (N) situation and ammonia consumption in the phosphate-limited (P) situation are both somewhat too large compared to experimental results. A change in the model parameters to decrease the consumption of N and P would produce a less satisfactory fit between Fig. 4 & 2. With the parameters used, both the general form and the position of the shaded area in Fig. 2 where all 3 nutrients are depleted is to a large extent reproduced by the model (Fig. 4).

Simulating the chemostat cultures, glucose remained in the cultures at steady state for low N:C and/or P:C ratios (Fig. 5), as was observed experimentally (Fig. 3). The model predicts a narrower angle between lines 1 and 2 in the chemostat situation (Fig. 5) than in the batch culture situation (Fig. 4), a feature suggested by the experimental results (Fig. 3 & 2 respectively). The model, however, also predicts an extension of the shaded area by movement of lines 3 and 4 towards higher P:C and N:C values when going from the batch (Fig. 4) to the chemostat (Fig. 5) situation. This was not observed experimentally (Fig. 2 & 3).

Batch culture experiments with other strains

The nutrient concentrations in 7 d old batch cultures of the 3 organisms C1, N1, and P1 are shown in Fig. 6.

For all 3 organisms, an area (shaded in Fig. 6) of initial media compositions was found for which all 3 nutrients were depleted. The position and extension of this area does, however, vary between organisms.

Estimated values defining the borderlines of the shaded area are given in Table 5. In terms of the model, this area is delimited by 4 lines as indicated by numbers 1 through 4 in Fig. 4. The slope of line 1 (r_1, Table 5) corresponds to the ratio between the maximum storage capacity for P and the minimum storage capacity for N (Q_P^{max}/Q_N^{min}). Conversely, the slope of line 2 (r_2, Table 5) is given by Q_P^{min}/Q_N^{max}. The positions of the horizontal line 3 (r_3) and the vertical line 4 (r_4) are determined by the ratio between Q_P^{max} or Q_N^{max} respectively, and the consumption of carbon for biomass production and respiration purposes during growth to a carbon-limited situation. Making the approximation that this carbon consumption is independent of whether it is the N- or the P-source that is depleted from the medium along with the C-source, some algebra gives the storage capacities:

$$Q_P^{max}/Q_P^{min} = r_1\, r_4/r_3,$$

and $$Q_N^{max}/Q_N^{min} = r_3/r_2\, r_4$$

The computed estimates for these are included in Table 5.

All organisms isolated from the different enrichment cultures seem from this to be able to change their P content per cell by a factor of about 3, while only *Pseudomonas putida* and the organism isolated after enrichment for N storage gave indications of being able to store N.

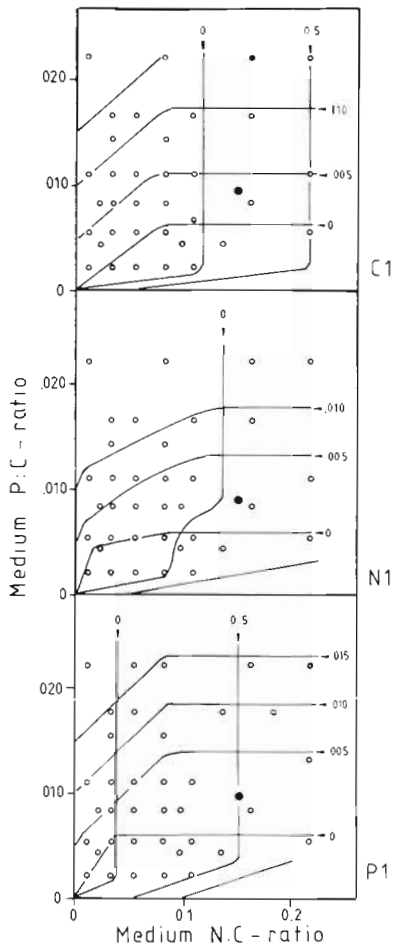

Fig. 6. Experimental results. Concentrations of ammonia and orthophosphate at stationary phase in cultures of the 3 strains C1, N1, and P1 isolated according to the description in text. Storage capacities for nitrogen and phosphorus estimated from these figures are given in Table 5. (•) Substrate with Redfield ratio composition

Transient uptake of phosphorus

Nissen et al. (unpubl.) added phosphate to cultures of *Vibrio natriegens* that had been cultured in phosphate-limited chemostats and then further starved for phosphorus by incubation for 1 h without nutrient supply before phosphate addition. Fig. 7 gives the experimental results of Nissen et al. for the period following phosphate addition.

With appropriate parameters, a Droop-type model of the kind suggested here will predict a rapid initial uptake of phosphate as a response to phosphate addition. The specific rate of uptake will then decrease as cells fill up their internal store of P. The growth

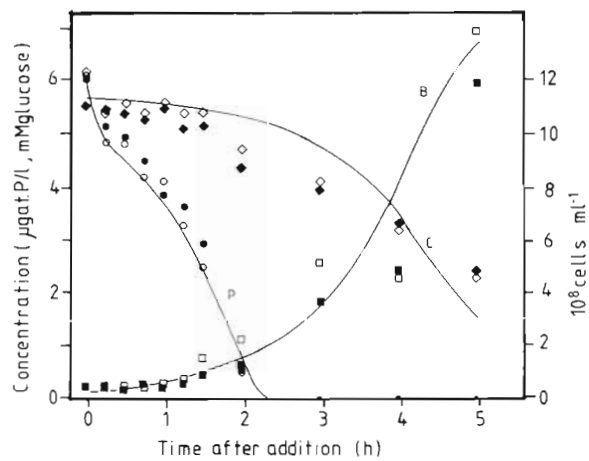

Fig. 7. Experimental results and model output. Addition of orthophosphate to P-starved cells of *Vibrio natriegens*. Data from Fig. 1 of Nissen et al. (1987) showing concentration in the culture fluid of glucose (○, •), orthophosphate (○, •) and cell density (□, ■) in the period following addition. Open and filled symbols represent independent experiments. Initial conditions in the model based on the assumption that cells at time = 0 had maximum cell quotas of C and N, and a minimum cell quota of P. Numerical values of model parameters are given in Table 6

Table 5. Numerical estimates of the slopes and positions of lines delimiting the shaded areas in Fig. 6. Storage capacities for N and P derived from these. For explanation see text

Strain	r_1	r_2	r_3	r_4	$\frac{Q_N^{max}}{Q_N^{min}}$	$\frac{Q_P^{max}}{Q_P^{min}}$
C1	0.067	0.02	0.006	0.1	1.1	3.0
N1	0.25	0.025	0.006	0.08	3.3	3.0
P1	0.17	0.05	0.006	0.04	1.1	3.0
Pseudomonas putida	0.25	0.063	0.011	0.11	2.5	1.6

Table 6. Parameter values used to simulate experiments with *Vibrio natriegens*. See Table 4 for definitions of paramters

μ^{max}	1.8	h^{-1}
ρ^g	1.0	(Dimensionless)
ρ^r	0.002	(Dimensionless)
δ	0.001	(Dimensionless)
υ_C^{max}	50	µmol C l^{-1} h^{-1} $(10^9 cells)^{-1}$
υ_N^{max}	4	µmol N l^{-1} h^{-1} $(10^9 cells)^{-1}$
υ_P^{max}	3.5	µmol P l^{-1} h^{-1} $(10^9 cells)^{-1}$
K_C	1.0	µmol C l^{-1}
K_N	0.1	µmol N l^{-1}
K_P	0.001	µmol P l^{-1}
Q_C^{min}	2.5	µmol C $(10^9 cells)^{-1}$
Q_N^{min}	0.4	µmol N $(10^9 cells)^{-1}$
Q_P^{min}	0.004	µmol P $(10^9 cells)^{-1}$
Q_C^{max}	11.0	µmol C $(10^9 cells)^{-1}$
Q_N^{max}	0.8	µmol N $(10^9 cells)^{-1}$
Q_P^{max}	0.4	µmol P $(10^9 cells)^{-1}$
Q_C^g	2.505	µmol C $(10^9 cells)^{-1}$

response in cell numbers is not explicitly coupled to nutrient uptake, and follows at a later stage. Due to the increase in cell number, the total rate of P-uptake then increases again. These effects correspond to the observed pattern as can be seen in Fig. 7 where the results are given for a simulation where cells are started at t = 0 filled with C and N ($Q_c = Q_c^{max}$, $Q_N = Q_N^{max}$), but completely starved for P ($Q_p = Q_p^{min}$). The set of parameters used in this simulation are given in Table 6.

DISCUSSION

Whether the fit between a numerical model and biological observations is judged as 'good' or 'bad' will always contain an element of subjectivity, and will depend upon whether emphasis is put on the features explained, or on the points where misfits exist between model and observation. For some purposes, a model with a great explanatory power may even be felt as more satisfactory than a purely empirical model with a fit to data which is 'good' in the statistical sense of the word.

The proposed model contains a total of 17 parameters. With this relatively large number of parameters, a reasonable fit to experimental data is not unexpected, and a good fit is not necessarily a demonstration of the validity of the biological assumptions underlying the formulation of the model. This is particularly true in a case like the present where only a subset of model variables is compared to experimental data. Under the circumstances, it was not regarded as a fruitful exercise to put extensive efforts into an optimization of the set of numerical values for the parameters.

In the present work, however, objections given above are felt to be counterbalanced by the broadness of situations to which the model seems to be applicable. The model was shown by Thingstad (1987) to allow for a general and consistent concept of maintenance, and thereby for a description of energy consumption in situations of both carbon and of mineral-nutrient-limited growth. For the organisms investigated here, the model was found to give a good qualitative explanation for observed features in steady-state, as well as in transient situations. This explanatory power of the model is felt to be more important than the actual numerical fit between model and data, although the numerical fit also was found to be reasonable in most cases.

A rather complicated pattern for steady-state chemostats with 7 different areas of reservoir medium composition, characterized by different combinations of nutrient depletion, was derived theoretically by Thingstad (Fig. 3 in Thingstad 1987). These areas may also be found in the experimental results of Fig. 3 (this paper) by regarding the isolines of zero culture concentrations of glucose, ammonia, and phosphate. To enhance this feature, and to allow a direct comparison with Fig. 3 of Thingstad (1987), the isolines of zero concentrations in Fig. 3 may be redrawn as shown in Fig. 8.

The adaption of the conceptual framework underlying a Droop-type formulation for bacterial activity would have consequences for our comprehension of the stoichiometry of carbon versus mineral nutrient fluxes in microbial ecosystems. With the flexibility demonstrated in Fig. 2 & 3, the stoichiometry of the link between C, N and P transport through the bacteria will not be rigid. Specifically, the efficiency by which

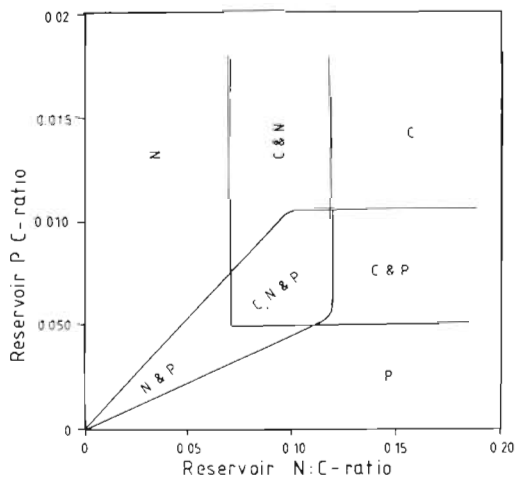

Fig. 8. Experimental results. Isolines of zero culture concentration in chemostats redrawn from Fig. 3 to emphasize the partitioning of the plane into 7 areas of different combinations of nutrient depletion. Nutrients depleted are indicated in each area

carbon is transferred from dissolved organic substances via bacteria and bacterial predators into higher trophic levels of the food chain will depend upon the growth conditions of bacteria. The experimental data presented here do not, however, give explicit information on the partitioning of the consumed glucose between biomass, respiration, and excretory products. In terms of the proposed model, organic carbon may be respired away by the bacteria without any net production of biomass. The condition leading to this would be extreme mineral-nutrient limitation. Such a relation between mineral-nutrient limitation and bacterial respiration has been suggested (Azam et al. 1983) with reference to experimental results of Koop et al. (1982).

Thingstad (1987) used the framework of this mathematical model to discuss the remineralization/consumption of mineral nutrients by bacteria growing on a substrate with a Redfield ratio of C:N:P = 106:16:1 (Redfield et al. 1963). The position of a substrate with this composition is indicated in Fig. 2 & 6. For all 4 bacterial strains investigated, such a substrate would be outside the area where both the N and the P source are depleted, suggesting that these bacteria might function as remineralizers during degradation of such a substrate.

An acceptance of this type of model would also have effects on our concepts concerning bacteria/bacteria and bacteria/algae competition for mineral nutrients. In environments with fluctuations in the levels of available mineral nutrients, the ability for rapid uptake and storage may be of greater competitive advantage than having a high affinity for the substrate. Such effects have already been demonstrated for algal/algal competition (Sakshaug & Olsen 1986). As shown experimentally by others (Harold 1966, Nissen et al. unpubl.), a rapid uptake and storage system for P exists also among bacteria. Fig. 7 demonstrates that a Droop-type mathematical formulation, as used here, may provide a valid framework for description of important aspects of such mechanisms. Our results (Table 5) indicate that a storage system may also exist for N.

Not unexpectedly, the experimental results indicate that parameters of the model have to be varied extensively to fit different bacterial strains. A large variability between species in uptake kinetics, biomass composition, and respiration coefficients would provide additional flexibility at the community level to the flexibility at species level investigated here.

The potential future value of the proposed type of model formulation is felt to be based upon its apparent dual capabilities as a both a conceptual and a practical tool. On the theoretical side it seems to provide a framework sufficiently general to allow a unified and consistent description of a range of physiological properties of bacteria. On the practical side it seems sufficiently specific to be used in the design of experiments as well as in the interpretation of experimental results.

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