INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
I.C.T.P., P.O. BOX 586, 34100 TRIESTE, ITALY, CABLE: CENTRATOM TRIESTE

SMR.780 - 12

FOURTH AUTUMN COURSE ON MATHEMATICAL ECOLOGY

(24 October - 11 November 1994)

"The Transient Behaviour of Food Chains in Chemostats"

S.A.L.M. Kooijman
Department of Theoretical Biology
Vrije Universiteit
de Boelelaan 1087
1081 HV Amsterdam
The Netherlands

These are preliminary lecture notes, intended only for distribution to participants.

J. theor. Biol. (1994) **170**, 87-94

The Transient Behaviour of Food Chains in Chemostats

B. W. KOOI AND S. A. L. M. KOOIJMAN

Biological, Free University, De Boelelaan 1087, 1081 *HV Amsterdam, The Netherlands*

(*Received on* 1 *November* 1993, *Accepted in revised form on* 25 *March* 1994)

Classical chemostat models such as the Monod, Marr-Pirt and Droop models are formulated at the population level. These models are unstructured, which means that all individuals in the population are treated as being identical. Such models fail to describe the experimental data of Dent *et al.* (1976, *Arch. Microbiol.* **109**, 187-194) in detail. They grew vegetative myxamoebae of the cellular slime mould *Dictyostelium discoideum* in continuous culture with a bacterial food source, *Escherichia coli* B/r fed glucose.

In this paper a new structured model is proposed, based on dynamic energy budgets (DEB) for conspecific individuals which only interact via a common resource. The model fit for the time-course data of glucose, bacteria and myxamoebae is very good; it covers variations in mean cell volumes of both bacteria and myxamoebae. Comparison with curve fitting results for the classical models reveals the mechanisms that are responsible for the better performance of the DEB model. We show that elements from different models, specifically maintenance (Marr-Pirt, gives stability) and energy reserves (Droop, gives oscillations) must be combined to produce acceptable fits. Therefore we reject the assumption made by Bazin & Saunders (1978, *Nature, Lond.* **275**, 52-54) that additional intra-specific interactions must be postulated to explain the data.

Introduction

In Kooijman (1993) a new model for the growth of individuals is proposed on the basis of simple mechanistic assumptions for energy uptake and usage. It has two state variables: size, for which we take "length" as the cubit root of volume; and energy reserves. The governing equations form an autonomous system of two ordinary differential equations for the two state variables as functions of time. This paper focusses on organisms that propagate by binary fission, such as most bacteria, unicellular eukaryotes, planarians and some oligochaetes. In Kooi & Kooijman (1993, 1994), we derived an individual-based model for such populations which constitute a food chain in the chemostat. The equations resemble those for the classical models like the Monod model, but our parameters, unlike theirs, concern the individual level.

In this paper the new model is applied to experimental time-course data for the food chain glucose/bacteria/myxamoebae published by Dent *et al.* (1976). They measured the mean cell volumes (MCV) and the population densities using a Coulter Counter. These quantities combine to yield biovolumes as function of time.

We will show that the experimental MCV data can be reconstructed from the behaviour of the populations on the basis of the assumption that DNA replication starts when the individual reaches a species-specific fixed volume. The individual divides when the DNA replication, which takes a fixed amount of time while growth proceeds, is completed. Simplifying approximations with respect to the volume distribution of the individuals in the population enabled us to calculate the MCV as a function of time.

Because existing models, such as the Monod model, could not explain the data in any detail, Bazin &

0022-5193/94/170087+08 $08.00/0

Saunders (1978) and Saunders (1980) used catastrophe theory to evaluate the experimental results. The conclusions of that study were that catastrophe theory can describe the myxamoebial data qualitatively, conditional on smoothed data for the bacteria, and that this pointed strongly to interactions between the myxamoebae: the feeding rate per myxamoebae is proportional to the ratio of prey (bacterium, *Escherichia coli*) and predator (myxamoeba, *Dictyostelium discoideum*) densities, rather than being just proportional to prey density. Bazin and Saunders suggested that the interaction involved folic acid.

In this paper we show that the food chain in the chemostat can be described quantitatively by the dynamic energy budgets (DEB) theory, which has no species-specific elements. This means that the same model is used for each population and that only the values of the parameters may differ. The description includes glucose and bacteria as well as myxamoebae, rather than just myxamoebae conditionally under bacteria. This underlines the usefulness of the DEB theory. The mechanistic assumption on which it is based appears to be reasonable. This makes the species-specific approach proposed by Bazin & Saunders (1978) superfluous.

The Model

Basic to the DEB theory are two quantities not present in the Monod model. The first quantity is the (dimensionless) reserve density e, which stands for the energy reserve as a fraction of the maximum reserves, i.e. the reserves an individual of that size would have if continuously exposed to abundant food (or substrate) so that $0 \leq e \leq 1$. Their role is apparent from dynamics; growth only depends on the internal state (reserves), not on external food density directly. The second quantity is the maintenance rate coefficient m_i, i.e. the ratio of the maintenance and growth costs. It is assumed that the individuals divide into two equal daughters when they reach a species-specific volume denoted as V_d. For a complete description of the model refer to the companion paper (Kooi & Kooijman, 1994).

Within the framework of the DEB theory (Kooijman, 1993), this model is based on the assumption that food uptake is proportional to the surface of the area of the individuals. For organisms propagating by binary fission the change in shape during their life cycle appeared to be negligible (see Kooi & Kooijman, 1994). For the sake of simplicity the area of the surface is taken to be proportional to the volume of the individual, which is realistic for filaments which grow in length only. We call this special case the DEBf model. In Kooi & Kooijman (1993) we showed that the total biovolume x is an appropriate statistic for the description of the population and that unstructured and structured population models become equivalent under this description.

The dynamics of densities of glucose (x_0), *E. coli* (x_1) and *D. discoideum* (x_2) and the energy reserve densities of *E. coli* (e_1) and *D. discoideum* (e_2) are given by

$$\frac{\mathrm{d}}{\mathrm{d}t}x_0 = D(x_r - x_0) - I_{m_{0,1}} f_{0,1} x_1, \tag{1}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}x_1 = (\mu_1 - D)x_1 - I_{m_{1,2}} f_{1,2} x_2, \tag{2}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}x_2 = (\mu_2 - D)x_2, \tag{3}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}e_1 = v_1(f_{0,1} - e_1), \tag{4}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}e_2 = v_2(f_{1,2} - e_2). \tag{5}$$

The parameters are described in Table 1. In these formula the overall population growth rate μ_i is given by

$$\mu_i = \frac{v_i e_i - m_i g_i}{e_i + g_i}. \tag{6}$$

The parameter g_i is proportional to energetic costs for growth and v_i is proportional to the assimilation rate of the individuals. The growth rate of all the individuals which constitute the population is equal to the overall population growth rate μ_i. The quantity $f_{i,i}$ is the Holling type-II functional response defined by

$$f_{i,i} = \frac{x_{i-1}}{k_{i,i} + x_{i-1}}, \tag{7}$$

for $i = 1, 2$ (the bacteria and myxamoebae, respectively). The parameter $k_{i,i}$ is the saturation constant. The initial values $x_0(0)$, $x_1(0)$, $x_2(0)$, $e_1(0)$ and $e_2(0)$ complete the mathematical formulation.

All parameters ($I_{m_{i,i}}$, v_i, g_i, m_i and $k_{i,i}$) of this model are defined at the individual level and the state variables (x_i and e_i) which are functions of the time t at the population level, where i denotes the population. The remaining parameters, the dilution rate D and the glucose concentration in the reservoir for the chemostat x_r, are control parameters. The first term on the right-hand side of eqn (1) represents the difference of the densities of glucose in the inflow (x_r) and outflow (x_0). The last term of eqns (1) and (2) represents the consumption per unit of time of glucose

by bacteria and bacteria by myxamoebae, respectively. The parameter $I_{m,\ i,x}$ is the maximum ingestion rate. The first terms on the right-hand side of eqns (2) and (3) are the growth rate minus outflow per unit of time. The last two equations (4) and (5) form the core of the model and show that there is a force driving to homeostasis.

This model reduces to the Droop model when the costs for maintenance are zero, ($m_i = 0$). It becomes the Marr–Pirt model when $g_i \to \infty$ and $v_i \to \infty$ such that v_i/g_i equals the maximum growth rate (i.e. $f_{i,x,i} = 1$ and $m_i = 0$). When $v_i \to \infty$ is substituted in eqns (4) and (5) we get $e_i = f_{i,x,i}$. This model degenerates to the first three equations [(1), (2) and (3)] and the overall population growth rate is given by

$$\mu_i = v_i/g_i f_{i,x,i} - m_i. \tag{8}$$

This model without maintenance costs, ($m_i = 0$), is the classical Monod model.

For bacteria the volume at division (V_d) depends on the food level: see Donachie (1968), Kooijman *et al.* (1991) and Kooijman (1993). DNA duplication is triggered upon exceeding a fixed cell size V_p and DNA duplication lasts a fixed time period t_D independent from the food density. We assume that this holds also for the myxamoebae, where t_D refers to the duplication time of the biggest chromosome. Observe that the growth rate μ_i given by eqn (6) is independent of the volume at division $V_{d,i}$, $i = 1, 2$. This allows us to use the model described above. In order to simplify the equations we assume that the growth rate μ_i given in eqn (6) is constant during the DNA duplication and equal to the value at the onset of the duplication ($V_i = V_{p,i}$). Thus it is easy to show that the following relationship between $V_{d,i}$ and $V_{p,i}$ holds

$$V_{d,i} = V_{p,i} \exp\{\mu_i t_{D,i}\}, \tag{9}$$

for $i = 1, 2$.

To use this relationship for the population level we have to make assumptions about the volume distribution of individuals. Experimental data for the distribution of the volumes were not reported in Dent *et al.* (1976). As a first approximation we assume that the volume distribution is proportional to V^{-2} which is the steady-state cell size distribution for exponential growth with fixed division size and division into two equal daughters. Then we have for the mean cell volume (MCV):

$$MCV_i = V_{d,i} \ln 2 = V_{p,i} \exp\{\mu_i t_{D,i}\} \ln 2. \tag{10}$$

Results and Discussion

A batch culture was inoculated by Dent *et al.* (1976) with bacteria and spores of myxamoebae and the experiment was started when the spores had germinated. The two control parameters are given in Dent *et al.* (1976); the throughput rate $D = 0{\cdot}064\ \mathrm{hr}^{-1}$ and the glucose concentration in the feed $x_r = 1\ \mathrm{mgl}^{-1}$. We assumed that $e_1(0) = 1$ and $e_2(0) = 1$, so the bacteria and myxamoebae were well fed at the start of the experiment.

The estimated parameters are shown in Table 2. These parameter values were obtained by weighted non-linear regression (Marquardt) of the numerical solutions of the system of five first-order ordinary differential equations (ODEs) [eqns (1–4)], solved numerically with a fourth-order Runge–Kutta

TABLE 1

State variables and parameters of the model for the chemostat; t = *time*, l_i = *cubic root of the volume of the individual*, l_r = *cubic root of the volume of the reactor*

Parameter	Dimension	Interpretation
t	t	Time
x_i	$l_i^3\ l_r^{-3}$	Biovolume of population i
e_i		Ratio of energy reserves and its maximum value
x_0	$l_r^3\ l_r^{-3}$	Glucose
$k_{x,i,x}$	$l_r^3\ l_r^{-3}$	Saturation constant
$f_{i,x,i}$		Functional response
$I_{m,i,x}$	t^{-1}	Maximum ingestion rate
v_i	t^{-1}	Energy conductance, $\propto$ assimilation rate
g_i		Energy investment ratio, $\propto$ costs for growth
m_i	t^{-1}	Maintenance rate coefficient
x_r	$l_r^3\ l_r^{-3}$	Glucose concentration in reservoir
D	t^{-1}	Dilution rate
V_i	l_i^3	Volume of individual
$V_{d,i}$	l_i^3	Volume at division
$V_{p,i}$	l_i^3	Volume at start of DNA replication
$t_{D,i}$	t	Time duration of DNA replication

TABLE 2

The parameter estimates for the different models and interpretation of the DEB model. The real values for $k_{0,1}$ are smaller than the given values

Parameter	Monod	Marr Pirt	Droop	DEB	Units	Interpretation
$x_0(0)$	0·45	0·55	0·0	0·58	mg ml^{-1}	Initial glucose concentration
$x_1(0)$	0·93	1·03	0·36	0·46	mm^3 ml^{-1}	Initial *E. coli* density
$x_2(0)$	0·044	0·0074	0·058	0·070	mm^3 ml^{-1}	Initial *D. discoideum* density
$e_1(0)$	0 (def)	0 (def)	1 (def)	1 (def)	-	Initial *E. coli* reserve density
$e_2(0)$	0 (def)	0 (def)	1 (def)	1 (def)		Initial *D. discoideum* reserve density
$k_{0,1}$	0·001	0·00061	0·0004	0·0004	mg ml^{-1}	Saturation constant *E. coli*
$k_{1,2}$	0·34	0·23	0·23	0·18	mm^3 ml^{-1}	Saturation constant *D. discoideum*
g_1	∞ (def)	∞ (def)	0·92	0·86		Investment ratio *E. coli*
g_2	∞ (def)	∞ (def)	1·72	4·43		Investment ratio *D. discoideum*
m_1	0 (def)	0·00004	0 (def)	0·0083	hr^{-1}	Maintenance rate coefficient *E. coli*
m_2	0 (def)	0·092	0 (def)	0·16	hr^{-1}	Maintenance rate coefficient *D. discoideum*
v_1	∞ (def)	∞ (def)	0·45	0·67	hr^{-1}	Specific energy conductance *E. coli*
v_2	∞ (def)	∞ (def)	0·69	2·05	hr^{-1}	Specific energy conductance *D. discoideum*
$I_{m,1}$	0·72	0·71	0·95	0·65	mg mm^{-3} hr^{-1}	Maximum ingestion rate *E. coli*
$I_{m,2}$	0·49	0·55	0·27	0·26	hr^{-1}	Maximum ingestion rate *D. discoideum*
v_1/g_1	0·43	0·54	0·59	0·80	hr^{-1}	Maximum growth rate *E. coli*
v_2/g_2	0·33	0·49	0·43	0·46	hr^{-1}	Maximum growth rate *D. discoideum*

technique. The measurement errors for the data points are unfortunately not reported in Dent *et al.* (1976). Within each data-set the weights were taken to be equal. The weights for all measurement points for the concentrations of the glucose were taken as 1/4 and for the bacterial biovolume 1/500 of that for

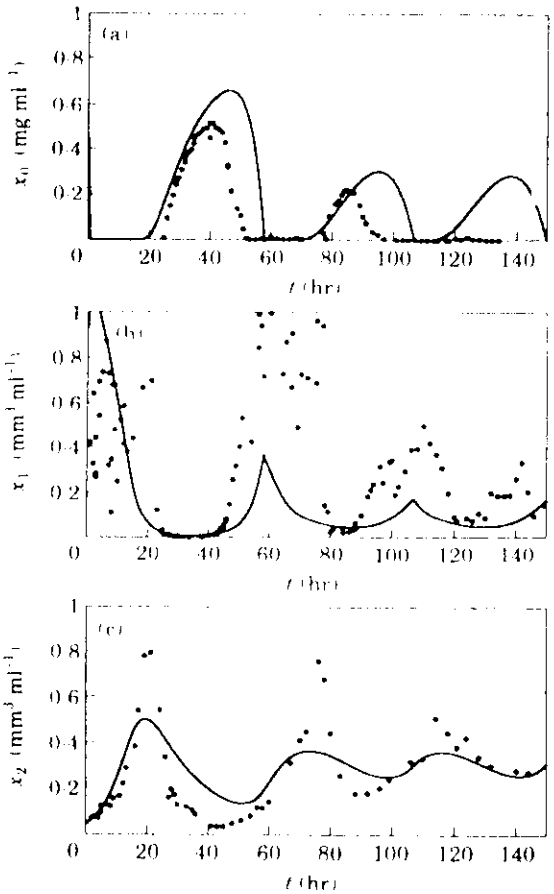

FIG. 1. Comparison of experimental data of Dent *et al.* (1976) and Monod model for (a) glucose; (b) *E. coli*; and (c) *D. discoideum*. Model predictions (——) superimposed on data (•).

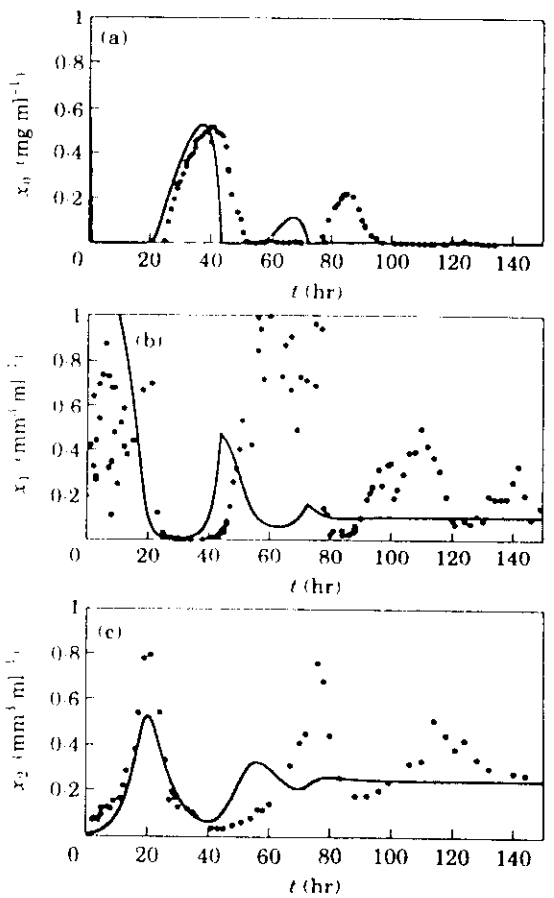

FIG. 2. Comparison of experimental data of Dent *et al.* (1976) and Marr Pirt model for (a) glucose; (b) *E. coli*; and (c) *D. discoideum*. Model predictions (——) superimposed on data (•).

4.

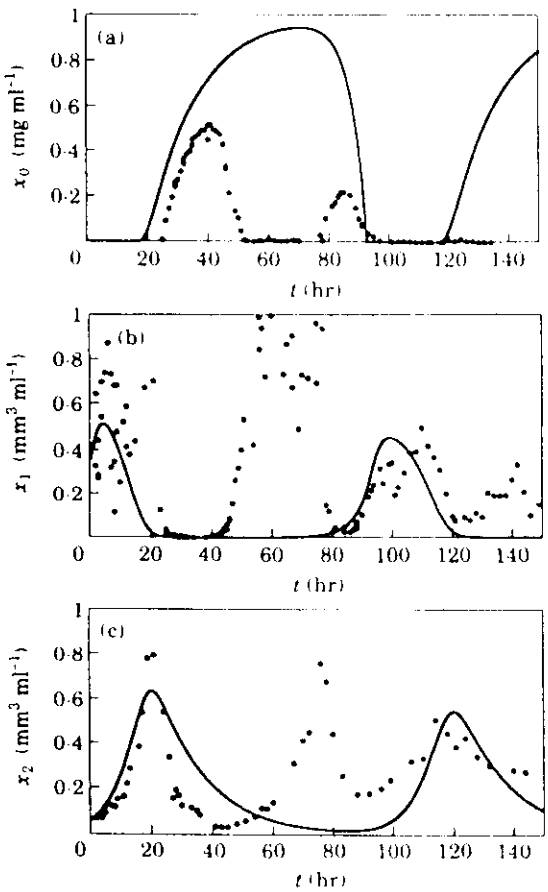

FIG. 3. Comparison of experimental data of Dent *et al.* (1976) and Droop model for (a) glucose; (b) *E. coli*; and (c) *D. discoideum*. Model predictions (—) superimposed on data (•).

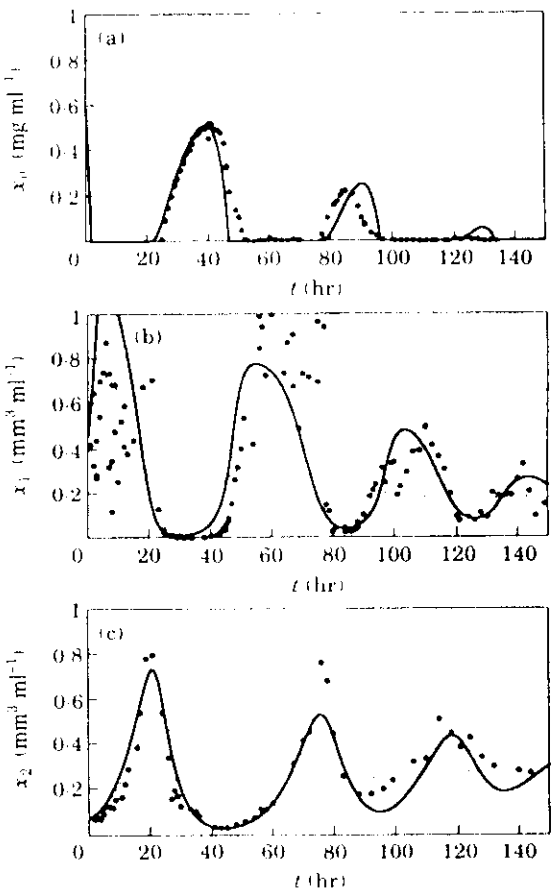

FIG. 4. Comparison of experimental data of Dent *et al.* (1976) and DEB model for (a) glucose; (b) *E. coli*; and (c) *D. discoideum*. Model predictions (—) and with enforced $e = f$ (· · · ·) superimposed on data (•).

myxamoebal biovolume, the measurements being expressed in the units reported in the figures. The large scatter in the data for the bacteria suggests that the error for the biovolumes of the bacteria is very large and therefore their weights are taken small. The results obtained depend of course on these weighting factors. (The model fit presented in Kooijman, 1993 differs slightly from the ones presented here by a different choice for the weight coefficients.) A good model should predict the prey and the glucose correctly when the prediction of the predator is good because they are connected by mass-balance equations.

Figures 1, 2, 3 and 4 give the weighted least-squares fit for the Monod, Marr–Pirt, Droop and DEB models, respectively. The Monod model predicts the global behaviour of the myxamoebae rather well. It fails, however, to describe the behaviour of the bacteria and glucose. After one oscillation the system is in a limit cycle, while the data suggest oscillations with a diminishing amplitude and period. The Jacobian of the linearized system in the corresponding equilibrium possesses a pair of complex conjugate eigenvalues with positive real parts. The prediction of the rate of decrease of the myxamoebae in the decrease phase is much too small. Accurate examination of that phase indicates that the myxamoebal biovolume density declines at a rate greater than the dilution rate D.

In Fig. 2 maintenance is introduced as in the Marr–Pirt model. The least-square sum is larger than for the Monod model and this implies that the minimum is a local minimum. The results for the myxamoebae show that the model describes the first oscillation much better than the Monod model. This shows that the introduction of maintenance explains why the rate of decrease of the myxamoebae can be so sharp. This is also clear from the equations. When m_2 has a substantial value, the term $m_2 g_2$ can become larger than $v_2 f_{12}$ (Marr–Pirt model) or $v_2 e_2$ (DEB model). For the individual level this means that the energy reserves are deficient in the supply of energy

5.

for maintenance and this implies that the individuals will shrink.

In Kooi & Kooijman (1994) we showed that the introduction of maintenance implies a much larger region in the "operation diagram" where the food chain is stable. The calculated time evolution of the biovolumes supports this finding. This shows that maintenance forces the oscillations to become very heavily damped. The real parts of the eigenvalues of the Jacobian of the linearized system in the equilibrium are all strongly negative.

The Marr Pirt model fails to describe the distinct second and third oscillation. In particular, the peak in the response of the bacteria is much too sharp. When they increase in number, the myxamoebae do so as well, and this implies that the food density diminishes sharply while the predator becomes apparently extinct. This enforces a collapse of the biovolume of the bacteria. The experimental data do not show this dynamic behaviour. Note that a better fit can be obtained when unreal, negative values for the maintenance rate coefficient for the bacteria are allowed.

In Fig. 3 energy reserves are introduced but without costs for maintenance as in the Droop model. Because of the energy reserves the peaks of the oscillations of the bacteria are much broader. The predictions suggest, however, that the food chain is in a limit cycle almost from the start of the experiment as in the case of the Monod model. The rate of decrease of the biovolume of the myxamoebae is too small, clearly because there is no maintenance. Therefore the model misses the second distinct oscillation but predicts the third oscillation well. The least-square sum is, however, larger than for the Monod model, so the Droop model is even less adequate than the Monod model. This is explained as follows. The Marquardt technique yields in this case a local minimum for which the results are given in Fig. 3. The best fit for the Monod model is also the global minimum for the least-square sum for the Droop model with $g_i \to \infty$ and $v_i \to \infty$ where $v_i g_i$ is the maximum growth rate and by virtue of eqns (4) and (5), $e_i = f_{i,1,t}$.

The DEBf model predictions are given in Fig. 4. The maintenance for the myxamoebae yields a good fit for the sharp decrease of the biovolumes in the phases of decrease. Because of the energy reserves the bacteria can withstand the sudden disappearance of their food. The effects of these reserves stem from two contributions.

First, the relationship between the growth rate and the energy reserves is hyperbolic. In the Marr Pirt model the overall population growth rate is given by eqn (8) instead of eqn (6) of the DEBf model. This means that, even if all physiological parameters of both models are the same and if $e_i = f_{i,1,t}$, the DEB growth rate is always smaller than in the Monod model. Figure 4 gives also the results of the simulation of the system with enforced $e_i = f_{i,1,t}$, and all the parameters with values of the DEBf. As in the Droop model case, the best fit for the constrained situation is the degenerated model, which now represents the Marr Pirt model.

The global behaviour of the dynamics is about the same as that predicted by the Marr–Pirt model in Fig. 2 but the introduction of energy reserves has damped the large fluctuations of the bacterial dynamics predicted by the Marr–Pirt model. The growth rate is damped when there is a lot of glucose, $e_i = 1$, and this occurs when the biovolume of the bacteria is small. When the biovolume increases, the glucose concentration diminishes sharply and so do the energy reserves e_i. Consequently the growth rate decreases less sharply than in the Marr–Pirt model.

Observe that the introduction of the energy reserves always decreases the growth rate, the effect being larger when e is large. This is because e is a density. When an individual grows it has to ingest food which is used partly to maintain the energy density through homeostasis. It works as extra energy costs for growth. This explains why the estimated value of the maximum growth rate v_1/g_1 is larger under the DEBf model than under the Marr–Pirt model. In order to have the same fast dynamic response the individuals must have a better assimilation rate or the energetic costs for growth must be smaller.

Second, there is an effect of the inertia of the energy reserves just like the mass inertia in mechanical systems obeying Newtons law which is a second-order ODE equivalent to a system of two first-order ODEs. In Fig. 5 both functions $f_{i,1,t}(t)$ and $e_i(t)$ for $i = 1, 2$ are shown. The energy reserves follow the functional response with a delay which is large for the bacteria and very small for the myxamoebae. This is clear from the eqns (4) and (5) and the values for v_i in Table 2. The bent in the response of the biovolume of the bacteria at the moment the glucose becomes exhausted disappeared in the prediction of the DEBf model. Growth is now governed by a system of two first-order ODEs which smooths out the dynamics.

Figure 6 shows that the relationship between mean cell size and growth rate agrees with the DEB theory. The chosen values for the two parameters V_p and t_D were: $V_{p_1} = 0{\cdot}39\ \mu m^3$, $V_{p_2} = 281{\cdot}4\ \mu m^3$, $t_{D_1} = 4$ hr and $t_{D_2} = 3{\cdot}48$ hr. Dent *et al.* (1976) state that "during the declining phases in the number density of the bacteria

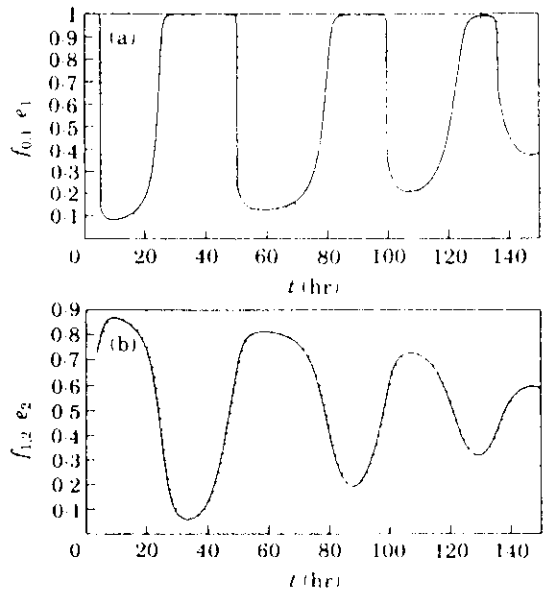

FIG. 5. The calculated functional response $f_{i,1}$ (— · —) and the bioenergy e_i (· · · ·) for the bacteria ($i = 1$) and myxamoebae ($i = 2$) as functions of time t for the DEBf models for (a) *E. coli*; and (b) *D. discoideum*.

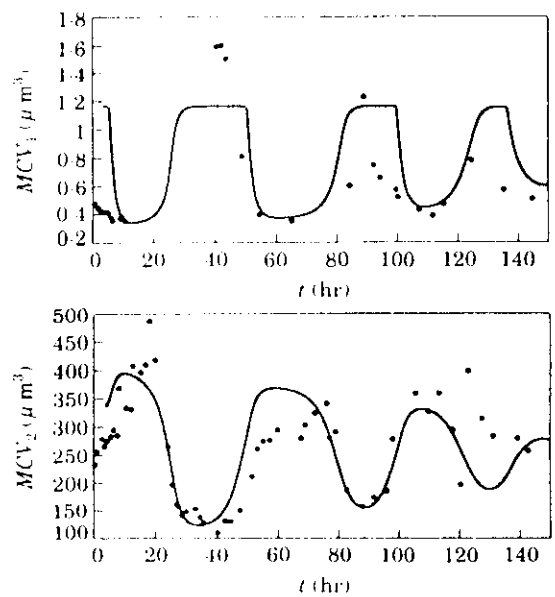

FIG. 6. Comparison of experimental data for the MCV of myxamoebae after Dent *et al.* (1976) and DEBf model for (a) *E. coli*, and (b) *D. discoideum* (— · —) superimposed on data (•).

cell counts were so low and the size so small that it was difficult to determine bacterial MCV". Therefore we did not use a regression technique to estimate the four parameters. Observe that the data at the start of the experiment suggest that the energy reserves were not maximal ($e_i < 1$). Therefore the results presented in Fig. 6 were calculated with $e_2(0) = 0{\cdot}75$ instead of $e_2(0) = 1$. The effects of the choice for the initial values in the dynamic behaviour of the whole system are very small; only the large change in the energy reserves of the myxamoebae shortly after the start disappeared.

Conclusions

The classical models for microbial food chains in chemostats, which are special cases of the DEB model, fit time-course data of glucose, bacteria and myxamoebae in a continuous culture substantially less well than the DEB model. The introduction of both maintenance and reserves in the Monod model are necessary to grasp the dynamics. The introduction of reserves alone makes the fit even worse.

Generally, the introduction of new parameters in a model improves the fit to experimental data, independent from their realism. In this paper we emphasized the basic mechanisms behind our extensions that have a firm biological basis.

The maximum rate of decrease in biovolume of the myxamoebae exceeds the outflow rate, which means that volume is also lost by shrinking of the individuals; this is consistent with the model.

The model predicts the mean cell volumes well. The predictions for the myxamoebae are better than those for the bacteria, but, the experimental determination of the mean cell volumes of the bacteria was difficult (see Dent *et al.*, 1976). That the predictions are good shows that it is advantageous to use an individual-based population model, even when the structured model equals the unstructured one. Parameters for individual can be estimated and this gives more insight into the mechanisms that govern the growth of populations.

The advantage of the present analysis above Bazin and Saunders' explanation is that it is not species-specific (no specific interactions within the myxamoebae are required to understand the results) and that our analysis explains the simultaneous behaviour of glucose, bacteria and myxamoebae, rather than just that of the myxamoebae on the basis of an empirical description for the bacteria.

We thank Wim van der Steen and Martin Boer for corrections to the manuscript and helpful discussions.

REFERENCES

BAZIN, M. J. & SAUNDERS, P. T. (1978). Determination of critical variables in a microbial predator prey system by catastrophe theory. *Nature, Lond.* **275**, 52–54.

7.

DENT, V. E., BAZIN, M. J. & SAUNDERS, P. T. (1976). Behaviour of *Dictyostelium discoideum* amoeba and *Escherichia coli* grown together in chemostat culture. *Arch. Microbiol.* **109,** 187–194.

DONACHIE, W. D. (1968). Relationship between cell size and time of initiation of DNA replication. *Nature, Lond.* **264,** 1077–1079.

DROOP, M. R. (1973). Some thoughts in nutrient limitation in Algae. *J. Phycol.* **9,** 264–272.

FREDRICKSON, A. G. (1991). Segregated, structured, distributed models and their role in microbial ecology: a case study based on work done on the filter-feeding ciliate *Tetrahymena pyriformis*. *Microb. Ecol.* **22,** 139–159.

KOOI, B. W. & KOOIJMAN, S. A. L. M. (1993). Many limiting behaviour in microbial food chains. In *Conference Proceedings of the 3rd International Conference on Mathematical Population Dynamics* (Arino, O., Axelrod, D. eds) Wuerz Biological Systems.

KOOI, B. W. & KOOIJMAN, S. A. L. M. (1994). Existence and stability of microbial prey predator systems. *J. theor. Biol.* **170,** 75–85.

KOOIJMAN, S. A. L. M., MULLER, E. B. & STOUTHAMER, A. H. (1991). Microbial dynamics on the basis of individual budgets. *Antonie van Leeuwenhoek* **60,** 159–174.

KOOIJMAN, S. A. L. M. (1993). *Dynamics Energy Budgets in Biological Systems; Theory and Applications in Ecotoxicology*. Cambridge: Cambridge University Press.

METZ, J. A. J. & DIEKMANN, O. (1986). *The Dynamics of Physiologically Structured Populations. Lecture Notes in Biomathematics*, Vol. 68. Berlin: Springer-Verlag.

MONOD, J. (1942). *Recherches sur la Croissance Bactériennes*. Paris: Hermann.

PIRT, S. J. (1965). The maintenance energy of bacteria in growing cultures. *Proc. R. Soc. Lond.* **B163,** 224–231.

SAUNDERS, P. T. (1980). *An Introduction to Catastrophe Theory*. Cambridge: Cambridge University Press.