

Thermodynamics of organisms in the context of dynamic energy budget theory

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We carry out a thermodynamic analysis to an organism. It is applicable to any type of organism because (1) it is based on a thermodynamic formalism applicable to all open thermodynamic systems and (2) uses a general model to describe the internal structure of the organism—the dynamic energy budget (DEB) model. Our results on the thermodynamics of DEB organisms are the following. (1) Thermodynamic constraints for the following types of organisms: (a) aerobic and exothermic, (b) anaerobic and exothermic, and (c) anaerobic and endothermic; showing that anaerobic organisms have a higher thermodynamic flexibility. (2) A way to compute the changes in the enthalpy and in the entropy of living biomass that accompany changes in growth rate solving the problem of evaluating the thermodynamic properties of biomass as a function of the amount of reserves. (3) Two expressions for Thornton’s coefficient that explain its experimental variability and theoretically underpin its use in metabolic studies. (4) A mechanism that organisms in non-steady-state use to rid themselves of internal entropy production: “dilution of entropy production by growth.” To demonstrate the practical applicability of DEB theory to quantify thermodynamic changes in organisms we use published data on *Klebsiella aerogenes* growing aerobically in a continuous culture. We obtain different values for molar entropies of the reserve and the structure of *Klebsiella aerogenes* proving that the reserve density concept of DEB theory is essential in discussions concerning (a) the relationship between organization and entropy and (b) the mechanism of storing entropy in new biomass. Additionally, our results suggest that the entropy of dead biomass is significantly different from the entropy of living biomass.

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I. INTRODUCTION

Many thermodynamic analyses of living organisms and cells have been made. Demirel *et al.* [1], for example, used thermodynamics to describe the coupled phenomena of transport and chemical reactions that take place inside living systems; Battley analyzed the entropy change accompanying the growth of *E. coli* [2] and the growth of *Saccharomyces cerevisiae* [3]; Stockar *et al.* [4,5] analyzed the internal entropy production in some micro-organisms; Esener *et al.* [6] studied the energetics of *Klebsiella aerogenes*; Duboc *et al.* [7] evaluated the thermodynamic efficiency of *Saccharomyces cerevisiae*; Qian and Beard [8] introduced a thermodynamic formalism to study metabolic biochemical reaction networks, etc.

The thermodynamic analyses mentioned use equations that describe chemical reactions that take place inside an organism as the model describing the organism’s behavior; examples of these aggregated chemical reactions are given in Battley [3, Table 2] for some micro-organisms. Because these models need a considerable amount of data they are not useful in thermodynamic analyses made of more complex organisms. Even for micro-organisms these models present problems because they do not give a mechanistic explanation of many of the energetic aspects of an organism’s growth process; this is patent in the *ad hoc* explanations given for the empirical results.

The thermodynamic analyses that have been made would benefit from a general theory underlying the description of the energetic fluxes in order to build up solid knowledge about an organism’s metabolism. The dynamic energy budget (DEB) theory is the most general non-species-specific theory of this kind [9–11]. It consists of a set of simple, mechanistically inspired rules that fully specify the uptake and use of mass and energy by an organism. The frequently applied classical models by Monod and Marr-Pirt on bacterial growth, and the well-known model by Droop for nutrient limited growth of unicellular algae are all special cases of DEB theory. DEB theory also considers phenomena of a complexity well beyond these simple models, including simultaneous nutrient limitation, adaptation, cometabolism, flocculated growth, product formation, aging, and syntrophy.

Here, we will carry out a thermodynamic analysis of an organism using (1) the most general framework of nonequilibrium thermodynamics applicable to all open thermodynamic systems (de Groot and Mazur [12], Bejan [13], and Moran *et al.* [14]) and (2) the knowledge of its internal dynamics given by DEB theory.

The paper is organized as follows. In Sec. II we begin by defining the DEB thermodynamic system, i.e., the mass and energy flows in the organism and the dynamics of its state variables. This is followed by a thermodynamic analysis of the organism in Sec. III. This analysis uses the mass, energy, and entropy balances together with DEB to obtain the thermodynamic constraints imposed on different types of organisms and to discuss the use of Thornton’s coefficient. In Sec. IV a thermodynamic characterization of *Klebsiella aero-*

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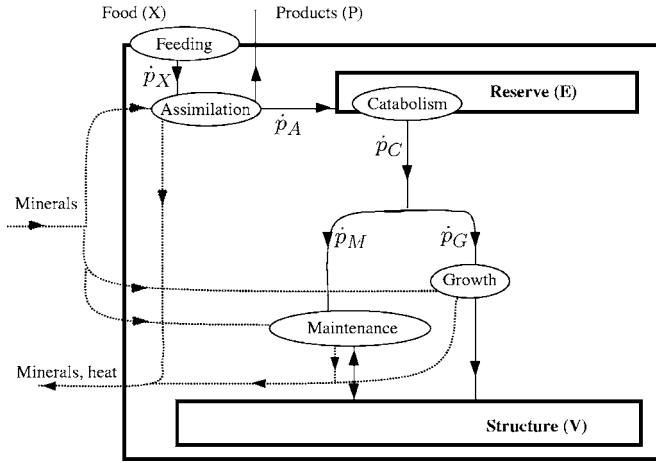


FIG. 1. Schematization of an asexually propagating heterotrophic organism following DEB theory. Boxes represent boundaries. Ovals represent chemical reactors. Lines represent mass and energy flows: \dot{p}_X has the chemical composition of food; \dot{p}_A , \dot{p}_C , \dot{p}_G , and \dot{p}_M have the chemical composition of reserve and the flow that goes from the growth reactor to the structure compartment has the chemical composition of structure.

genes is done using published data from chemostat experiments. Section V concludes.

II. THERMODYNAMIC SYSTEM

To simplify our analysis, we focus on a single-reserve, single-structure heterotrophic that lives on a single substrate. It is a V1 morph, i.e., an organism whose surface area is proportional to volume. Extensions to include more substrates, reserves, and/or structures have been formulated in the DEB theory to include autotrophs and nutritional details [9]. However, such extensions do not pose new thermodynamic problems. Appendix A presents an explanation of the notation used throughout the paper.

Figure 1 presents a scheme, according to DEB theory, for an asexually propagating heterotrophic organism [15]. The simplest DEB model delineates one reserve, E , with M_E C-moles, and one structure, V , with M_V C-moles; the chemical composition of each is assumed to be constant (the strong homeostasis assumption). This simplifying assumption combines nicely with the idea that the enzymes that catalyze chemical transformations in the organism do so in a constant chemical environment, which is essential for full control. Reserve has the dual role of providing both energy and essential compounds for growth, maintenance, development, and reproduction.

The incoming and outgoing fluxes through the outer surface of the system can be divided into organic (food, X and products, P), and mineral (CO_2 , H_2O , O_2 , and nitrogenous waste, N_{waste}) compounds. The energy flow of the food uptake per C-mol of structure \dot{p}_X , enters and is transformed into an assimilation energy flux per C-mol of structure, \dot{p}_A , that goes into the reserves and into products, P , that are excreted. The catabolic energy flux, \dot{p}_C , i.e., the rate per C-mol of structure, at which the organism mobilizes its reserve for

metabolic purposes [16], is partitioned between growth, \dot{p}_G , and maintenance, \dot{p}_M , i.e.,

$$\dot{p}_C = \dot{p}_M + \dot{p}_G. \quad (1)$$

Maintenance includes a variety of requirements, such as the turnover of chemical compounds of structure (e.g., proteins), the maintenance of concentration gradients across membranes, the maintenance of defense systems (e.g., the immune system), activity (including behavior), the heating of the body to a near constant temperature (endotherms only), and osmotic work (especially freshwater organisms) [9]. Growth is defined as the increase of structure; body weight has contributions from both reserve and structure.

Ellipses in Fig. 1 are idealized reactors where anabolic and catabolic processes take place. These transformations occur all over the organism, rather than at specific sites. Consequently, the idealized reactors are the transformations themselves. These processes are characterized by constant conversion efficiencies between mass flows, y_{*1*2} , i.e., the number of moles of *1 needed to produce one mol of *2. In the assimilation reactor food is converted into reserve, y_{XE} , and in the growth reactor reserve is converted into structure, y_{EV} .

The ratio $m_E \equiv M_E/M_V$ is the reserve density. For any constant food level, $X = X^* > 0$, there is a reserve density, m_E^* , that remains constant along the growth process. Furthermore, $\lim_{X^* \rightarrow \infty} m_E^* \equiv m_{Em}$ where m_{Em} is the maximum reserve density (the weak homeostasis assumption).

We now quantify the dynamics of the state variables structure and reserve. The amount of structure and reserve and the flows in the organism are measured in mass and Gibbs energy and the conversion between them is done using chemical potentials: the chemical potential of food, μ_X , converts the flow of food per C-mol of structure, j_X , to \dot{p}_X ; the chemical potential of reserve, μ_E , converts the flow of reserve that exits the assimilation reactor to \dot{p}_A , and the flow of reserve that exits the reserve compartment to $\dot{p}_C = \dot{p}_M + \dot{p}_G$.

The change in C-moles of reserve per C-mol of structure is

$$j_E \equiv \frac{1}{M_V} \frac{dM_E}{dt} = \frac{\dot{p}_A - \dot{p}_C}{\mu_E} \quad (2)$$

because the reserve is continuously used (catabolic power) and replenished (assimilation power). With Eq. (2), the reserve density dynamics is

$$\frac{dm_E}{dt} \equiv \frac{d(M_E/M_V)}{dt} = \frac{\dot{p}_A - \dot{p}_C}{\mu_E} - m_E \frac{1}{M_V} \frac{dM_V}{dt}, \quad (3)$$

where the last term on the right-hand side is the dilution by growth.

To further evaluate the reserve dynamics we need to quantify feeding and assimilation. The food uptake per C-mol of structure is given by $j_X = f j_{Xm}$ where f is the scaled functional response and j_{Xm} is the maximum food uptake per C-mol of structure, i.e., the food uptake that would occur at abundant food availability. The assimilation energy flow per C-mol of structure is

$$\dot{p}_A = \frac{j_X}{y_{XE}} \mu_E, \quad (4)$$

where j_X/y_{XE} is the reserve flow that exits the assimilation reactor per C-mol of structure.

The reserve dynamics can be further deduced considering (1) that the catabolic power is independent of the food availability, (2) that the mobilization of reserves does not depend on how they are partitioned among aggregates with different chemical compositions, and (3) the weak homeostasis assumption (for details on the derivation see Appendix B). The reserve dynamics is then

$$\frac{dm_E}{dt} = \dot{k}_E (f m_{Em} - m_E), \quad (5)$$

where \dot{k}_E is the maximum reserve turnover rate:

$$\dot{k}_E = \frac{j_{Xm}/y_{XE}}{m_{Em}}. \quad (6)$$

The parameter \dot{k}_E is a turnover rate because it is the ratio between the maximum flow of C-moles of reserve into the “reserve compartment,” $j_{Xm}/y_{XE}M_V$, and the maximum amount of C-moles of reserve kept in the “reserve compartment,” $m_{Em}M_V$.

By combining Eq. (3) with Eq. (5) the catabolic power simplifies to

$$\dot{p}_C = \mu_E m_E \left(\dot{k}_E - \frac{1}{M_V} \frac{dM_V}{dt} \right). \quad (7)$$

To evaluate the dynamics of the structure we need to quantify maintenance and growth. The maintenance energy flow per C-mol of structure is

$$\dot{p}_M = \dot{k}_M y_{EV} \mu_E, \quad (8)$$

where $\dot{k}_M y_{EV}$ is the reserve flow that exits the reserve for maintenance purposes per C-mol of structure and \dot{k}_M is the maintenance rate coefficient. The growth energy flow per C-mol of structure is

$$\dot{p}_G = j_V y_{EV} \mu_E, \quad (9)$$

where $j_V y_{EV}$ is the flow that exits the reserve for growth purposes per C-mol of structure and j_V is the molar flow of structure.

The dynamics of structure

$$j_V \equiv \frac{1}{M_V} \frac{dM_V}{dt} = \frac{\dot{p}_G}{y_{EV} \mu_E} = \frac{\left(\dot{k}_E \frac{m_E}{m_{Em}} - \dot{k}_M g \right)}{\left(\frac{m_E}{m_{Em}} + g \right)}, \quad (10)$$

is obtained by combining Eqs. (1), (8), (9), and (7), where the investment ratio, g , is a dimensionless quantity given by

$$g \equiv y_{EV}/m_{Em} \quad (11)$$

that stands for the ratio between the number of moles of reserve allocated to growth per mole of structure and the maximum number of moles of reserve allocated to mainte-

TABLE I. List of compounds.

Compound	
X	Food
P	Products
E	Reserve
V	Structure
W	Biomass
\mathcal{O}	Class of organic compounds (X, P, E, V)
\mathcal{M}	Class of mineral compounds ($\text{CO}_2, \text{H}_2\text{O}, \text{O}_2, N_{\text{waste}}$)

nance plus growth per mole of structure. At constant food the dynamics of structure simplifies to

$$j_V = (\dot{k}_E f - \dot{k}_M g)/(f + g) \quad (12)$$

because the weak homeostasis assumption implies that $dm_E/dt = 0$.

III. THERMODYNAMIC ANALYSIS

We now formalize the thermodynamic analysis. See Table I for a list of compounds. If the mole numbers of each compound, the total energy, and the total entropy of the organism are constant, the organism is in steady state. In this state there is still a continuous flow of matter, energy, and entropy through the system from and to external reservoirs. This is implied by maintenance requirements. Here, we will consider that the organism is not in steady state because our life cycle perspective of an individual comes with the necessity to consider changes in amounts of reserve and structure, both in mass and energy aspects.

The strong homeostasis assumption justifies one of the main simplifications used in the thermodynamic analysis: the molar chemical compositions, internal energies, enthalpies, and entropies of reserve and structure are taken to be constant, independent of the reserve density. In general, we consider that the chemical composition of biomass (structure plus reserve) can change; this implies that the molar thermodynamic properties of biomass can also change. Whenever the reserve density is constant, the chemical composition of biomass and its molar thermodynamic properties are also constant.

A. Mass balance equation

Most of the dry mass of biological systems consists of proteins, lipids, carbohydrates, and nucleic acids. The major chemical elements in the covalently bounded compounds are carbon, C, hydrogen, H, oxygen, O, nitrogen, N, phosphorus, and sulphur. The first four elements stand for more than 96% of the total dry mass [17], so we focus on these elements only. A “molecule” of structure is denoted by $\text{CH}_{n_{HV}} \text{O}_{n_{OV}} \text{N}_{n_{NV}}$, a “molecule” of reserve by $\text{CH}_{n_{HE}} \text{O}_{n_{OE}} \text{N}_{n_{NE}}$, where the chemical index n_{ij} is the number of atoms of element i per atom of carbon in compound j . The mass balance equation for the organism is written on a molar basis for each element because there is no conservation of compounds due

to the chemical transformations inside the organism. The mass balance equation is

$$0 = \mathbf{n}_M \dot{\mathbf{J}}_M + \mathbf{n}_O \dot{\mathbf{J}}_O \quad (13)$$

where $\dot{\mathbf{J}}_M$ is the vector with the molar fluxes of the minerals (\dot{J}_{CO_2} , $\dot{J}_{\text{H}_2\text{O}}$, \dot{J}_{O_2} , $\dot{J}_{N_{\text{waste}}}$), $\dot{\mathbf{J}}_O$ is the vector with the molar fluxes of the organics (\dot{J}_X , \dot{J}_P , $-\dot{J}_V$, $-\dot{J}_E$), \mathbf{n}_M is the matrix with the chemical composition of minerals, and \mathbf{n}_O is the matrix with the chemical composition of organic compounds. Each entry in these matrices, n_{*1*2} , is the number of atoms of element *1 in compound *2.

The fluxes $J_V \equiv d/dt M_V$ and $J_E \equiv d/dt M_E$ are the change in C-mols of structure and reserve per unit time in the organism. The other fluxes, \dot{J}_j , mole numbers per unit time, are positive if they represent a net input into the thermodynamically defined organism and negative otherwise. In a heterotrophic organism CO_2 is usually but not always an output, O_2 is an input, H_2O is an output formed metabolically from other compounds, nitrogenous waste is an output, food is an input, and products are an output. Equation (13) states that, for each element, the rate of mole numbers accumulation inside the organism (structure and reserve), equals the inputs minus the outputs (other organic and mineral flows).

B. Energy balance equation

The energy balance equation quantifies the organism's accumulation of energy as the result of inputs minus outputs of energy fluxes. The energy fluxes (Joule/s) are the net heat flux, the net work flux, and energy fluxes associated with input and output molar fluxes.

In this analysis mechanical work will be considered negligible. This is supported by Garby and Larsen *et al.* [18], who state that "the energy transfer as heat (in animals) is relatively large and directed outwards, while the energy transfer as work is small."

The temperature of structure, reserve, and outgoing products, T , is assumed constant and equal to the temperature of the body. We think this is a reasonable first approximation because chemical reactions inside the organism occur for a limited temperature range mainly due to enzymatic action [9]. Anyway this will probably be a better approximation for endotherms or for ectotherms in an environment where they are able to keep their temperature constant by moving.

This set of assumptions, i.e., a constant temperature of the organism, negligible mechanical work, and incoming fluxes with a temperature similar to the organism's imply that the net heat released by the organism equals the net heat produced in all chemical reactions inside the organism. Chemical reactions taking place are the degradation of food and reserve material in order to obtain energy (synthesize ATP from ADP) and release nutrients, and the building up of reserve and new structural material with the nutrients and energy obtained.

Here, we apply the energy balance equation to aerobic and to anaerobic organisms. The distinction between these types of organisms is useful because there are simplifications

applicable only to aerobic organisms (Secs. III B 2 and III C 2).

1. Energy balance equation: General organism

With the simplifications that were mentioned in Sec. III B, the energy balance of the entire thermodynamic system is

$$0 = \bar{\mathbf{h}}_M^T \dot{\mathbf{J}}_M + \bar{\mathbf{h}}_O^T \dot{\mathbf{J}}_O + \dot{P}_{T+}, \quad (14)$$

where \dot{P}_{T+} is the total released heat [19], $\bar{\mathbf{h}}_M^T = (\bar{h}_{\text{CO}_2} \bar{h}_{\text{H}_2\text{O}} \bar{h}_{\text{O}_2} \bar{h}_{N_{\text{waste}}})^T$ and $\bar{\mathbf{h}}_O^T = (\bar{h}_X \bar{h}_P \bar{h}_V \bar{h}_E)^T$ and \bar{h}_i is the molar enthalpy of compound i . The internal energy and the flow work linked to the input and output molar fluxes are lumped in the enthalpy.

Equation (14) is the energy balance for a non-steady-state organism defined according to DEB theory. This supports the use of direct calorimetry (the direct measurement of released heat) to assess enthalpy changes in organisms as referred by [4]: if the organism was completely burned then the net heat release plus the enthalpy of the combustion products would be equal to the organism's total enthalpy.

The enthalpy can be substituted by $\bar{h} = \bar{u} + P\bar{v} = \bar{g} + T\bar{s} = \mu + T\bar{s}$, which is obtained using the definition of Gibbs energy $\bar{g} = \bar{u} - T\bar{s} + P\bar{v}$ and the equality between Gibbs energy and chemical potential $g = \mu$ for a single component:

$$0 = (\boldsymbol{\mu}_M + T\bar{\mathbf{s}}_M)^T \dot{\mathbf{J}}_M + (\boldsymbol{\mu}_O + T\bar{\mathbf{s}}_O)^T \dot{\mathbf{J}}_O + \dot{P}_{T+}, \quad (15)$$

where \bar{s}_E is the molar entropy of the reserve, \bar{s}_V is the molar entropy of the structure, $\bar{\boldsymbol{\mu}}_M$ and $\bar{\mathbf{s}}_M$ collect the values for the four minerals; and $\bar{\boldsymbol{\mu}}_O$ and $\bar{\mathbf{s}}_O$ do that for the organic compounds, as before.

2. Energy balance equation: Aerobic organism

We assume that reactors have negligible mass and are at pseudo steady state. An additional assumption that can also be made is based on Garby and Larsen [18] who consider, based on empirical knowledge, that for most important reactions in biological systems $T\Delta s$ is very small compared to Δh and therefore the enthalpy of the reaction $\Delta h_{\text{reaction}}$ is approximated using its Gibbs energy $\Delta g_{\text{reaction}}$, since at constant temperature $\Delta g = \Delta h - T\Delta s \approx \Delta h$. This assumption is valid only for aerobic reactions but it is less stringent than that of Kooijman [9], where entropy is set to zero.

We apply the simplification that $T\Delta s$ is very small to the set of all reactors to obtain

$$0 = \bar{\mathbf{s}}_M^T \dot{\mathbf{J}}_M + \bar{\mathbf{s}}_O^T \dot{\mathbf{J}}_O. \quad (16)$$

This equation can be disaggregated to

$$\bar{s}_V \dot{J}_V + \bar{s}_E \dot{J}_E = \bar{\mathbf{s}}_M^T \dot{\mathbf{J}}_M + \bar{s}_X \dot{J}_X + \bar{s}_P \dot{J}_P \quad (17)$$

to emphasize that the entropy variation of an aerobic organism (left-hand side) equals the net import of chemical entropy given by the right hand side of Eq. (17) [20]. With Eq. (17) we conclude that, for an aerobic organism, if weak homeostasis applies, i.e., reserve and structure are in constant proportions, then there is a positive net import of chemical

TABLE II. Net import of chemical entropy and Gibbs free energy in the overall metabolism for (1) exothermic aerobic organisms, (2) exothermic anaerobic organisms, and (3) endothermic anaerobic organisms. All organisms are at constant chemical composition (weak homeostasis). Organisms are either at constant, increasing, or decreasing biomass. There are two columns for the anaerobic and exothermic organisms because (1) they can either have a positive or negative net import of chemical entropy at constant biomass (first line) and (2) this is related to their behavior when they are increasing or decreasing their biomass (third and fifth lines).

		Aerobic and exothermic	Anaerobic and exothermic		Anaerobic and endothermic
Constant biomass	Chemical entropy	0	+	-	-
	Gibbs energy	+	+	+	+
Increasing biomass	Chemical entropy	+	+	+/-	+/-
	Gibbs energy	+	+	+	+
Decreasing biomass	Chemical entropy	-	+/-	-	-
	Gibbs energy	+/-	+/-	+/-	+/-

entropy during an increase in biomass, a negative net import of chemical entropy during a decrease in biomass, and a null net import at constant biomass [see the entries (1,1), (3,1), and (5,1) [21] in Table II]. If weak homeostasis does not apply and the structure and reserve molar entropies are different then an organism that is increasing its biomass could either have a positive or negative net import of chemical entropy. This is in accordance with Stockar and Liu [4] who concluded that microbial growth might result either from a positive or negative net import of chemical entropy.

The total entropy of an organism is easily computed if the molar entropy values and the chemical composition of biomass are known. Otherwise, Eq. (16) has to be integrated to obtain the total entropy of an organism as a function of total inputs and outputs from birth t_b until time t .

We apply the energy balance equation to the set of all reactors assuming that the reactors are in steady state but that the organism as a whole is not. We obtain [22]

$$0 = \dot{Q}_{reactions} + \bar{\mu}_O^T \dot{J}_O + \bar{\mu}_M^T \dot{J}_M, \quad (18)$$

where $\dot{Q}_{reactions}$ is the rate of the total heat release by all chemical reactions, since the work is null and the accumulation of energy is also null. Equation (18) is equivalent to the energy balance equation presented in [9, p. 153] but it is applicable only to an aerobic organism. It would only be applicable to other organisms if entropies were set equal to zero in Eq. (15). This is not a reasonable assumption because it is equivalent to assuming that the heat capacities of the various compounds including the minerals are null in the interval between the absolute temperatures 0 K and T . Equation (18) can be disaggregated to

$$\bar{\mu}_V \dot{J}_V + \bar{\mu}_E \dot{J}_E = \dot{Q}_{reactions} + \bar{\mu}_X \dot{J}_X + \bar{\mu}_P \dot{J}_P + \bar{\mu}_M^T \dot{J}_M. \quad (19)$$

C. Entropy balance equation

The entropy balance equation states that the change in entropy is equal to the entropy production inside the organism due to irreversible processes plus the net entropy flux

associated with heat and mass fluxes. The entropy balance equation is different from the other balance equations because the accumulation of entropy inside the organism depends on the internal processes, which control the value of the entropy production.

1. Entropy balance equation: General organism

The entropy balance for the organism is

$$0 = \dot{\sigma} + \frac{\dot{P}_{T+}}{T} + \bar{s}_M^T \dot{J}_M + \bar{s}_O^T \dot{J}_O, \quad (20)$$

which can be written as

$$\bar{s}_V \dot{J}_V + \bar{s}_E \dot{J}_E = \dot{\sigma} + \frac{\dot{P}_{T+}}{T} + \bar{s}_M^T \dot{J}_M + \bar{s}_X \dot{J}_X + \bar{s}_P \dot{J}_P, \quad (21)$$

where \dot{P}_{T+}/T is the entropy exchange coupled with heat fluxes.

With Eq. (21) we conclude that for an anaerobic and exothermic organism [23] at constant biomass, if weak homeostasis applies then the organism can have a (1) positive or (2) negative net import of chemical entropy [see the entries (1,2) and (1,3) in Table II]. In case 1 (case 2) $T\dot{\sigma} + \dot{P}_{T+} < 0$ ($T\dot{\sigma} + \dot{P}_{T+} > 0$). In case 1 (case 2), the organism will have a positive (positive or negative) net import of chemical entropy when its biomass is increasing and a positive or negative (negative) net import of chemical entropy when its biomass is decreasing [see the entries (3,2), (3,3) and (5,2), (5,3) in Table II]. For an aerobic and endothermic organism the net import of chemical entropy follows the same behavior as in case 2 because $T\dot{\sigma} + \dot{P}_{T+} > 0$ [see the entries (1,4), (3,4), and (5,4) in Table II].

Multiplying Eq. (20) by T and subtracting Eq. (15) we obtain

$$0 = \bar{g}_M^T \dot{J}_M + \bar{g}_O^T \dot{J}_O - \dot{\sigma} T, \quad (22)$$

where \bar{g}_i is the molar Gibbs energy per mole of compound i . This equation can be disaggregated to

$$\bar{g}_V \dot{J}_V + \bar{g}_E \dot{J}_E + \dot{\sigma} T = \bar{g}_M^T \dot{J}_M + \bar{g}_X \dot{J}_X + \bar{g}_P \dot{J}_P \quad (23)$$

to emphasize that the net import of Gibbs energy equals the rate of Gibbs energy change inside the organism plus an irreversibility production measure.

The net import of Gibbs energy by an anaerobic organism follows the same behavior for exothermic and endothermic organisms [see Eq. (23)]. When the organism is either increasing its biomass or at constant biomass the net input of Gibbs energy is positive [see the entries (2,2), (2,3), (2,4), (4,2), (4,3), and (4,4)]. When the organism is decreasing its biomass the net input of Gibbs energy is negative or positive [see the entries (6,2), (6,3), and (6,4)].

2. Entropy balance equation: Aerobic organism

The results of the previous section can be further narrowed for aerobic organisms. Subtracting Eq. (16) from Eq. (20), we obtain

$$\dot{\sigma} = - \frac{\dot{P}_{T+}}{T}, \quad (24)$$

i.e., the rate of heat released by the aerobic organism equals minus an irreversibility production measure.

Therefore since the second law tells us that entropy production is always positive, the total heat obtained from the organism is negative (released), which means that the sum of the processes of assimilation, dissipation, and growth must be exothermic for aerobic life. The result of Kooijman in [9], i.e., “the second law of thermodynamics implies that [each of] the processes of assimilation, dissipation and growth is exothermic,” is obtained only by considering that entropies are null.

The second law only forbids that processes as a whole are endothermic when the organism is aerobic and the heat released by the organism is equal to the heat released in all chemical reactions. For example, using direct calorimetry, Stockar *et al.* [5,24] showed the existence of a chemotroph whose overall metabolic process (assimilation plus dissipation plus growth) is endothermic. This rare type of overall metabolic process is called “enthalpy retarded growth.”

With Eqs. (19) and (24) we conclude that for an aerobic organism, if weak homeostasis applies then there is a positive net import of Gibbs energy at constant and at increasing biomass [see the entries (2,1) and (4,1) in Table II]. When the organism’s biomass is decreasing the net import of Gibbs energy is positive or negative [see the entry (6,1) in Table II].

D. Constraints imposed by the second law and DEB theory

In the previous sections we made predictions on the sign of the net input into the organism of Gibbs free energy and chemical entropy. These results are synthesized in Table II. These predictions are made for aerobic exothermic organisms, anaerobic exothermic organisms, and anaerobic endothermic organisms in a constant environment, for three situations: (1) steady state (constant biomass), (2) non-steady state with increasing biomass, and (3) non-steady state with

decreasing biomass. The imposition of a constant environment is a sufficient condition for weak homeostasis, i.e., constant biomass molar entropy.

Table II highlights the differences between aerobic and anaerobic organisms in a constant environment: (1) only anaerobic organisms can be endothermic, (2) only anaerobic organisms can have a net negative import of chemical entropy while increasing their biomass, and (3) only anaerobic organisms can have a net positive import of chemical entropy while decreasing their biomass. Apparently, anaerobic organisms have a higher thermodynamic flexibility.

For steady state, the internal entropy production ($\dot{\sigma} > 0$) implies a relation between the heat and the chemical entropy exchange with the environment [see Eq. (21)]. Based on this, Stockar and Liu [4] distinguish different overall metabolisms: “entropy neutral growth” (first column in Table II), “entropy driven growth” (third and fourth columns in Table II) and “entropy retarded growth” (second column in Table II) respectively, for a null, negative, or positive net import of chemical entropy.

However, the classification of Stockar and Liu [4] developed for the steady state should not be used for the non-steady state because it is misleading. An example is provided by aerobic organisms increasing their biomass. In this case they would be classified as “entropy retarded” when the mechanism used to get rid of entropy production is heat dissipation only [see Eq. (24)].

In non-steady states, organisms can get rid of internal entropy production by using an additional mechanism: accumulation of chemical entropy in new biomass. This mechanism can be called “dilution of entropy production by growth.” The importance of the mechanism of accumulation of chemical entropy in biomass is fully dependent on the distinction introduced by DEB theory between reserve and structure. For an organism that is not in steady state but has a constant chemical composition (weak homeostasis) the capacity of this mechanism remains constant per C-mol of biomass increase (decrease) because the additional C-mol has the same entropy. In contrast, when the reserve density changes, the chemical composition of biomass and its entropy also change. Thus the additional C-mol of biomass increase (decrease) has a different capacity to accumulate chemical entropy.

E. Calorimetry

1. Indirect calorimetry

Indirect calorimetry is an empirical method of estimating heat production based on the measurements of gaseous exchanges and the nitrogenous waste flux [25] using multiple linear regression; see [26] or [27] for a good overview.

A theoretical underpinning for indirect calorimetry was provided by Kooijman [9]. Here, we obtained the linearity between the dissipated heat flux and the fluxes in a simpler and more direct way by using only a subset of DEB theory: the existence of strongly homeostatic reserve and structure.

We obtain the linear dependence between the mineral fluxes and the dissipated heat by combining Eq. (13) with Eq. (14),

$$\dot{P}_{T+} = (\bar{h}_{\mathcal{O}}^T \mathbf{n}_{\mathcal{O}}^{-1} \mathbf{n}_{\mathcal{M}} - \bar{h}_{\mathcal{M}}^T) \dot{\mathbf{J}}_{\mathcal{M}}. \quad (25)$$

The coefficients that are obtained by linear regression are given by the expression in parentheses and can be computed without knowing any biochemical details. Only the chemical composition and the enthalpies of the reserve, the structure, and the input and output products are needed. This result is a theoretical basis for the linear dependence between the mineral fluxes and the dissipated heat because it equates the total dissipating heat to a weighted sum of consumed dioxygen, produced carbon dioxide, nitrogen waste, and water.

2. Thornton's rule

In the literature, Thornton's rule [28] is used to estimate heat production in aerobic organisms. This rule establishes a constant proportionality between the heat released in the combustion of organic compounds and the consumed oxygen: 444 kJ per mol of O₂ consumed. Recently the usefulness of this rule has been questioned by an experimental study made by Walsberg and Hoffman [29] because significant variations were obtained experimentally in the amount of heat released per mol of O₂ in a Kangaroo rat and a dove.

We now use DEB theory to obtain the conditions that keep constant the proportionality coefficient, h_{OT} , in the amount of heat released,

$$\dot{P}_{T+} = h_{OT} \dot{J}_{O_2}. \quad (26)$$

If the heat and the oxygen flows in Eq. (26) are written as functions of the organic fluxes using Eqs. (13) and Eq. (25), then the proportionality coefficient is given by

$$h_{OT} = \frac{(\bar{h}_{\mathcal{O}}^T - \bar{h}_{\mathcal{M}}^T \mathbf{n}_{\mathcal{M}}^{-1} \mathbf{n}_{\mathcal{O}}) \dot{\mathbf{J}}_{\mathcal{O}}}{\mathbf{n}_{\mathcal{M}}^{-1}(3,:) \mathbf{n}_{\mathcal{O}} \dot{\mathbf{J}}_{\mathcal{O}}} = \frac{\sum_{i=1}^{i=3} [h_{\mathcal{O}}(i) - \bar{h}_{\mathcal{M}}^T \mathbf{n}_{\mathcal{M}}^{-1} \mathbf{n}_{\mathcal{O}}(:,i)] \dot{J}_{\mathcal{O}}(i)}{\sum_{i=1}^{i=3} \mathbf{n}_{\mathcal{M}}^{-1}(3,:) \mathbf{n}_{\mathcal{O}}(:,i) \dot{J}_{\mathcal{O}}(i)}, \quad (27)$$

where $i=1$ is food, X , $i=2$ is reserve, E , $i=3$ is structure, V , $\mathbf{n}_{\mathcal{O}}(:,i)$ is column i of matrix $\mathbf{n}_{\mathcal{O}}$, and $\mathbf{n}_{\mathcal{M}}^{-1}(3,:)$ is the third line of matrix $\mathbf{n}_{\mathcal{M}}^{-1}$; it is the third line that appears because the third column of matrix $\mathbf{n}_{\mathcal{M}}$ has the chemical composition of O₂. Equation (27) can be written as

$$h_{OT} = \frac{\sum_{i=1}^{i=3} \dot{P}_{Ti+} \dot{J}_{\mathcal{O}}(i)}{\sum_{i=1}^{i=3} \dot{J}_{O_2}(i) \dot{J}_{\mathcal{O}}(i)} = \frac{\sum_{i=1}^{i=3} h_{OT_i} \dot{J}_{O_2}(i) \dot{J}_{\mathcal{O}}(i)}{\sum_{i=1}^{i=3} \dot{J}_{O_2}(i) \dot{J}_{\mathcal{O}}(i)}, \quad (28)$$

where \dot{P}_{Ti+} is the heat released in the complete combustion of one C-mol of the organic compound i and $\mathbf{n}_{\mathcal{M}}^{-1}(3,:) \mathbf{n}_{\mathcal{O}}(:,i)$ is the number of O₂ moles consumed, $\dot{J}_{O_2}(i)$, in the complete combustion of a C-mol of the organic compound i . Because the heat released in the complete combustion of an organic

compound is constant it can be written as the product of a constant h_{OT_i} and $\dot{J}_{O_2}(i)$.

With Eq. (28) the coefficient h_{OT} can be interpreted as a mean of the heats released per each mol of O₂ that would be spent in the complete combustion of each organic compound weighted by its net flow. For h_{OT} to be constant it has to be independent of the values of the organic flows $\dot{J}_{\mathcal{O}}(i)$. For this to occur, the coefficients h_{OT_i} must be equal, i.e., the heat released per mole of O₂ for each organic compound must be the same, which is usually not the case.

In the remainder of this section, we use DEB theory to obtain an expression that establishes the link between the coefficient of proportionality between the heat released and the oxygen flow and the internal energetics of the organism. In the literature this coefficient has already been used to assess the metabolic pathways in aerobic organisms. For example, Hansen *et al.* [30] obtained an expression that explains the difference between the mean accepted value for Thornton's coefficient and the observed proportionality coefficient by the existence of anaerobic reactions with an enthalpy change different from zero.

Equations (4) and (9) establish a connection between the organic flows of food, j_X , and structure, j_V with the assimilation, \dot{p}_A , and growth powers, \dot{p}_G . The flow of reserve can be written as a function of the three powers using Eqs. (2) and (1):

$$j_E = (\dot{p}_A - \dot{p}_M - \dot{p}_G) / \mu_E. \quad (29)$$

The flows j_X , j_V , and j_E multiplied by M_V can be assembled as

$$\dot{\mathbf{J}}_{\mathcal{O}} = \boldsymbol{\eta}_{\mathcal{O}} \dot{\mathbf{p}} M_V, \quad (30)$$

where $\dot{\mathbf{p}}$ is the vector with the three powers of assimilation, growth, and maintenance per C-mol of structure and $\boldsymbol{\eta}_{\mathcal{O}}$ is the matrix with the coefficients that link each organic flow with \dot{p}_A , \dot{p}_M , and \dot{p}_G . With Eqs. (30) and (28) the coefficient of proportionality h_{OT} can be written as

$$h_{OT} = \frac{\sum_{i=1}^{i=3} h_{OT_i} \dot{J}_{O_2}(i) \boldsymbol{\eta}_{\mathcal{O}}(i,:) \dot{\mathbf{p}}}{\sum_{i=1}^{i=3} \dot{J}_{O_2}(i) \boldsymbol{\eta}_{\mathcal{O}}(i,:) \dot{\mathbf{p}}}. \quad (31)$$

Equation (31) establishes a link between the proportionality coefficient and the organism's internal energetic flows: assimilation, maintenance, and growth. Again, if the coefficients h_{OT_i} are the same, h_{OT} is independent of the relative amounts of energy spent in each internal process; otherwise its change can be used to assess the internal allocation of energy in the organism between assimilation, growth, and maintenance.

IV. EMPIRICAL APPLICATION

To demonstrate the practical applicability of the well-tested DEB theory to quantify thermodynamic changes in organisms we use published data on *Klebsiella aerogenes*

growing in a continuous culture at different steady states obtained from Esener *et al.* [31,32]. *Klebsiella aerogenes* is growing aerobically on glycerol as the sole carbon and energy source, using ammonia as the N source, at a temperature of 35 °C and a pH of 6.8 [31]. The products are only biomass (structure plus reserve), carbon dioxide, and water [33]. The synthetic medium used by Esener *et al.* [33] is described by Evans *et al.* in [34]. More details about the experimental setup are given by Esener *et al.* [31].

Measurements include 26 measures of the specific CO₂ production rate (mol of CO₂ per C-mol of biomass inside the chemostat), 26 measures of the specific O₂ consumption rate, 26 measures of the yield of biomass on substrate Y_{WX} (C-mol biomass produced per C-mol of substrate consumed), and nine measurements of the biomass composition at different steady states [31,32]. Each steady state is characterized by a different throughput or dilution rate, d . The dilution rate is equal to the growth rate, j_V , because otherwise the biomass concentration in the chemostat would increase or decrease. The maximum growth rate was also measured by [31,32].

A. DEB parameters

We used the measurements to estimate the following essential DEB parameters: \dot{k}_E , y_{XE} , y_{EV} , \dot{k}_M , g and the chemical compositions of a C-mol of structure $n_{CV} \equiv 1$, n_{HV} , n_{OV} , n_{NV} and a C-mol of reserve $n_{CE} \equiv 1$, n_{HE} , n_{OE} , n_{NE} .

The method is as follows. (1) We depart from a first guess of 13 DEB parameters. (2) For each steady state we compute DEB variables such as the functional response and the reserve density using the DEB parameters. (3) For each steady state we calculate the predicted values of CO₂, O₂, Y_{WX} , and biomass composition. (4) We compute the difference between the predicted values and the 105 measurements and make another estimation of the DEB parameters using the Newton-Raphson method. Steps 2–4 are repeated in order to minimize the sum of the squared errors. A detailed description of steps 1–3 is given below.

1. Estimation method

We depart from a first estimation of parameters \dot{k}_E , y_{XE} , y_{EV} , \dot{k}_M , and g and the chemical compositions of structure n_{CV} , n_{HV} , n_{OV} , n_{NV} and reserve n_{CE} , n_{HE} , n_{OE} , n_{NE} . Equation (12) is then used to compute the functional response f for each steady state. This equation is also used to compute the maximum growth rate, r_m , that occurs when f is equal to one.

The value for m_{Em} is computed from the definition of investment ratio g given by Eq. (11). For each steady state Eq. (5) is used to compute the reserve density m_E because $dm_E/dt=0$.

With the reserve density m_E and the parameters n_{CV} , n_{HV} , n_{OV} , n_{NV} , n_{CE} , n_{HE} , n_{OE} , and n_{NE} , the chemical composition of a C-mol of biomass is computed using

$$n_{HW} = (n_{HV} + m_E n_{HE}) / (1 + m_E), \quad (32)$$

$$n_{NW} = (n_{NV} + m_E n_{NE}) / (1 + m_E), \quad (33)$$

and

TABLE III. DEB parameters for *Klebsiella aerogenes*.

Parameter	Value	Units
\dot{k}_M	0.021	h^{-1}
\dot{k}_E	2.11	h^{-1}
y_{VE}	0.904	—
y_{XE}	1.345	—
g	1	—

$$n_{OW} = (n_{OV} + m_E n_{OE}) / (1 + m_E). \quad (34)$$

The yield of biomass produced $j_V(1+m_E)$, on substrate consumed fj_{Xm} , is computed from

$$y_{WX} = j_V(1 + m_E) / (fj_{Xm}). \quad (35)$$

The substrate consumption rate per mole of structure, j_X , the structure production rate per mole of structure, j_V , and the reserve production rate per mole of structure, j_E , are

$$j_X = fj_{Xm}, \quad (36)$$

$$j_V = j_V, \quad (37)$$

$$j_E = j_V m_E. \quad (38)$$

The flows in Eqs. (36)–(38) are divided by $(1+m_E)$ to be converted to flows per C-mol of biomass. Then, these flows are used together with the mass balance, Eq. (13), applied to each steady state of the *Klebsiella aerogenes* culture to compute the CO₂, H₂O, O₂, and NH₃ flows. In Eq. (13), \mathbf{n}_M is the matrix with the chemical composition of minerals (CO₂, H₂O, O₂, and NH₃) and \mathbf{n}_O is the matrix with the chemical composition of organic compounds (X, E, V).

2. Results

The elemental composition of structure and reserve are CH_{1.64}O_{0.379}N_{0.198} and CH_{1.66}O_{0.422}N_{0.312}, respectively. The values obtained for the other parameters are listed in Table III. The maximum growth rate measured is 1.052 h⁻¹ and the value obtained with the DEB model is 1.044 h⁻¹. The comparison between the other measurements and the DEB model results is presented in Figs. 2 and 3. The root mean square errors for O₂ and CO₂ are 0.0088 and 0.0086 mol C⁻¹ h⁻¹, respectively, and the root mean square error for Y_{WX} is 0.0249 mol C⁻¹ mol⁻¹. The root mean square error for n_{HW} , n_{OW} , and n_{NW} are 0.009, 0.0191, and 0.0113 mol C⁻¹ mol⁻¹, respectively. Since the fits are very good, and the DEB model obeys mass balances, we have an automatic check on the empirical mass balances, i.e., the measurements obey the mass balance. The change in the chemical composition of biomass (see Fig. 3) is not very significant because the chemical compositions of a C-mol of structure and a C-mol of reserve are similar with the exception of the amount of nitrogen.

The values obtained for the reserve density for each dilution rate are in Fig. 4. We also obtained the flows per unit of biomass of assimilation, maintenance, and growth [Eqs. (4),

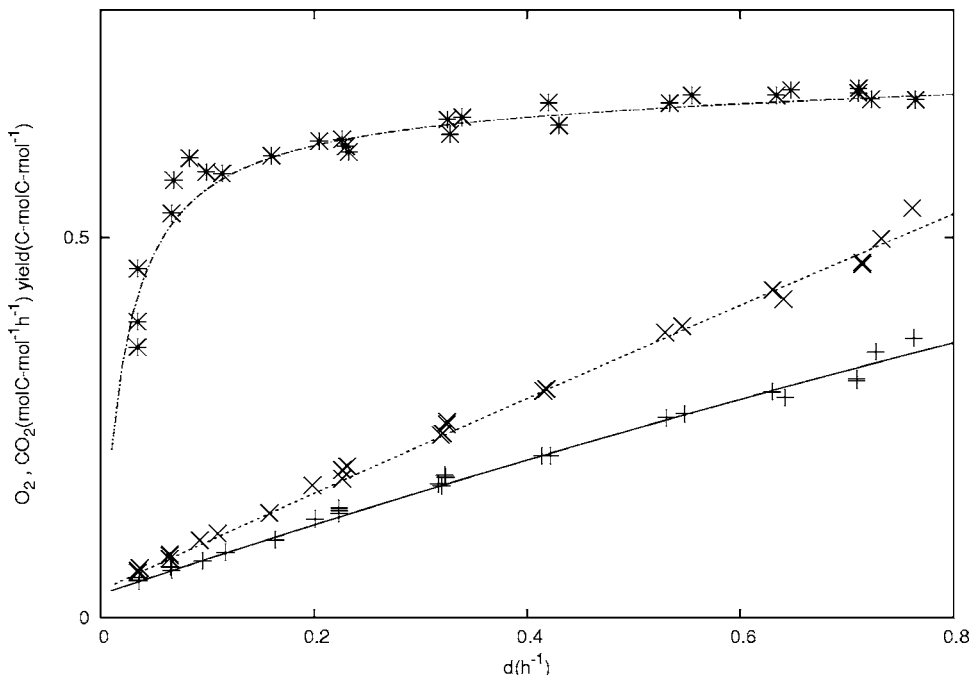


FIG. 2. Measurements (points) and DEB model results (lines). Specific rate of consumption of O₂ (×), specific rate of production of CO₂ (+), and yield (*) vs dilution rates. Units are mol C-mol⁻¹ h⁻¹ for O₂ and CO₂ and C-mol C-mol⁻¹ for the yield.

(8), and (9) shown in Fig. 5. The ratio of the assimilation flow to the food flow,

$$\dot{p}_A/\dot{p}_X = (1/y_{XE})(\mu_E/\mu_X), \quad (39)$$

is obtained with $\dot{p}_X = j_X \mu_X$ and Eq. (4). This ratio is constant because Eq. (39) is a function of parameters only. The assimilation flow increases with the throughput rate because the flow of food, X , also increases. Although the maintenance flow per C-mol of structure is constant because it is a function of parameters only [Eq. (8)], the maintenance flow per C-mol of biomass decreases with the dilution rate because the reserve density increases. This occurs because it is the

structure that is costly in terms of maintenance and not the reserve. The growth flow per C-mol of biomass increases with the dilution rate because it is proportional to the specific growth rate, j_V [see Eq. (9)] and the specific growth rate (equal to the dilution rate) increases more than the reserve density (see Fig. 4).

The ratio of energy spent on maintenance to energy spent on growth, given by \dot{k}_M/r , increases with a decreasing dilution rate, i.e., growth per C-mole of biomass becomes more expensive with decreasing dilution rate. The energetic explanation rooted in DEB theory for this behavior is a decrease in the dilution rate translates into a lower catabolic power (defined in Fig. 1) and because maintenance has priority over

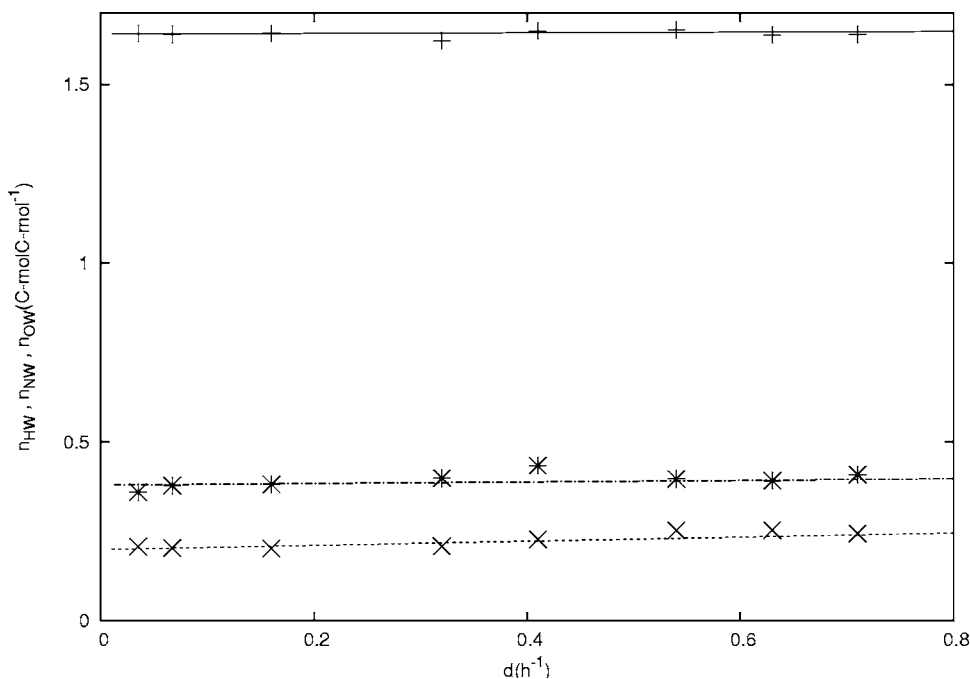


FIG. 3. Measurements (points) and DEB model results (lines). The variable chemical composition of biomass, n_{HW} (+), n_{NW} (×), and n_{OW} (*) vs dilution rates.

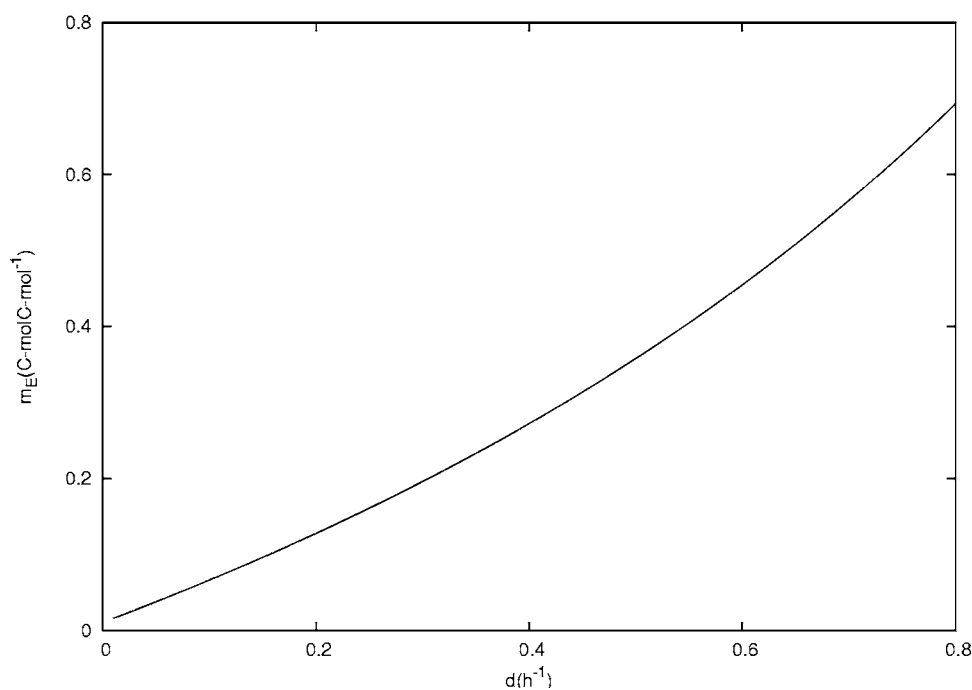


FIG. 4. Reserve density vs dilution rates.

growth a higher fraction of the catabolic power is spent on maintenance and a lower one on growth.

B. Structure and reserve specific enthalpies and entropies

1. The enthalpy balance

The enthalpy balance applied to the chemostat is given by Eq. (14). For each steady state \dot{J}_M and \dot{J}_O are computed using the DEB model with the parameters estimated in the previous section. Formation enthalpies of CO_2 , O_2 , H_2O taken from [35] and formation enthalpy of glycerol taken from [3] were corrected for the temperature of the experi-

ment using the specific heats at constant pressure taken from [35]. The formation enthalpy for NH_3 aq. is for 37 °C and was taken from [36]. Values used are in Table IV. The enthalpies of structure and reserve are unknown but constant for all steady states (strong homeostasis assumption). The dissipating heat depends on the steady state and is unknown.

Equation (14) is applied to different steady states and solved for the dissipating heats and the enthalpies of structure and the reserve. This system of equations involves two extra unknowns. The two missing constraints were taken from Roels [37] (i.e., dissipating heats for two steady states).

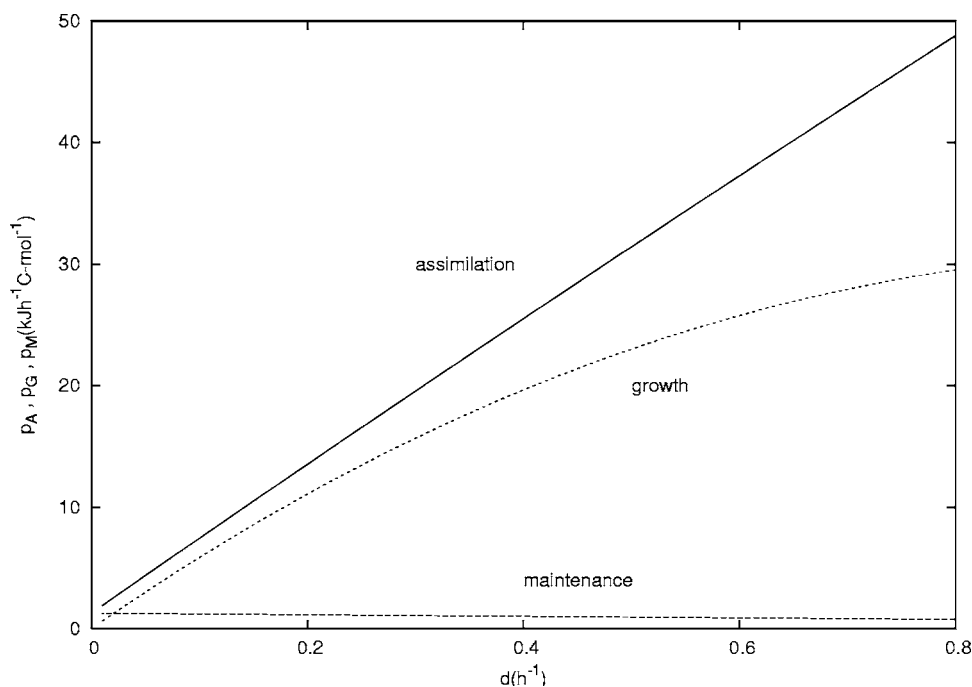


FIG. 5. The assimilation, growth, and maintenance energy flows per C-mol of biomass vs dilution rates.

TABLE IV. Enthalpies and entropies at 35 °C.

Formula	State	Enthalpy (kJ/mol)	Entropy (J/mol K)
CO ₂	<i>g</i>	-393.14	214.70
H ₂ O	<i>l</i>	-285.83	72.331
O ₂	<i>g</i>	0	205.80
NH ₃	aq.	-132.5	112.34
C mol glycerol	aq. ^a	-225.52	69.743

^aThe entropy is for the liquid state.

Released heats are presented in Fig. 6. Specific released heat ranges from -20 to -253 kJ/C-mol (see Fig. 6) for increasing throughput rates. The values obtained for the released heats are of the same magnitude as the released heats presented by [4] for aerobic growth of different micro-organisms on various substrates. The heat flow for each dilution rate is much higher than any of the assimilation, growth, and maintenance flows (Fig. 6). Its magnitude is four to five times higher than the magnitude of the assimilation flow. Therefore the energy dissipated as heat, which in an aerobic organism is a measure of the production of entropy, is a very significant energy drain.

We obtained a molar enthalpy of formation of -33 kJ/C-mol for the reserve and a molar enthalpy of formation of -107 kJ/C-mol for the structure. Thus the formation of 1 C-mol of structure and 1 C-mol of reserve from their components at a reference state are both exothermic reactions, with the former being more exothermic. The steady-state enthalpy of the biomass, $(\bar{h}_V + \bar{h}_E m_E)/(1 + m_E)$, decreases with the dilution rate from -76 to -105 kJ/C-mol. In the literature we found no formation enthalpy values for *Klebsiella aerogenes*. Some formation enthalpy values referred in the literature are -95.68 kJ/C-mol for *Escherichia coli* growing

aerobically on succinic acid [2], -97.8 kJ/C-mol for the same micro-organism [38], and -133.09 kJ/C-mol for *Saccharomyces cerevisiae*.

2. The entropy balance

The entropy balance applied to the chemostat is given by Eq. (16). For each steady state \dot{J}_M and \dot{J}_O are computed using the DEB model with the parameters estimated in Sec. IV A. Absolute entropies were taken from Dean [35] and corrected for temperature (see Table IV). The entropies of structure and reserve are unknown but constant for all steady states (strong homeostasis assumption).

Equation (16) is applied to different steady states and solved for the entropies of structure and the reserve with nonlinear regression. We obtained a molar entropy of 74.8 J/C-mol K for the reserve and a molar entropy of 52.0 J/C-mol K for the structure. To test the reliability of these specific entropy values we computed the left-hand side of Eq. (16) for many steady states. It is very close to zero, i.e., it is at maximum 0.04% of any other term in the equation. The first important remark is that these entropies are not null and are different from the entropies of the inputs and outputs. The steady-state entropy of the biomass, $(\bar{s}_V + \bar{s}_E m_E)/(1 + m_E)$, increases from 52.4 to 61.4 J/C-mol K with increasing dilution rate (see Fig. 7). The molar biomass entropy increases with the increasing dilution rate because the reserve density increases. In the literature we found no absolute entropy values for *Klebsiella aerogenes*. Other absolute entropy values comprise: 94.4 J/C-mol K for dried *Escherichia coli* growing on succinic acid [2] and 34.17 J/C-mol K for *Saccharomyces cerevisiae* [39].

We also compare the molar biomass entropy obtained with DEB with the entropy given by the empirical rule proposed by Battley [40] for organic substances (see Fig. 7). The entropy of the biomass computed by DEB theory in-

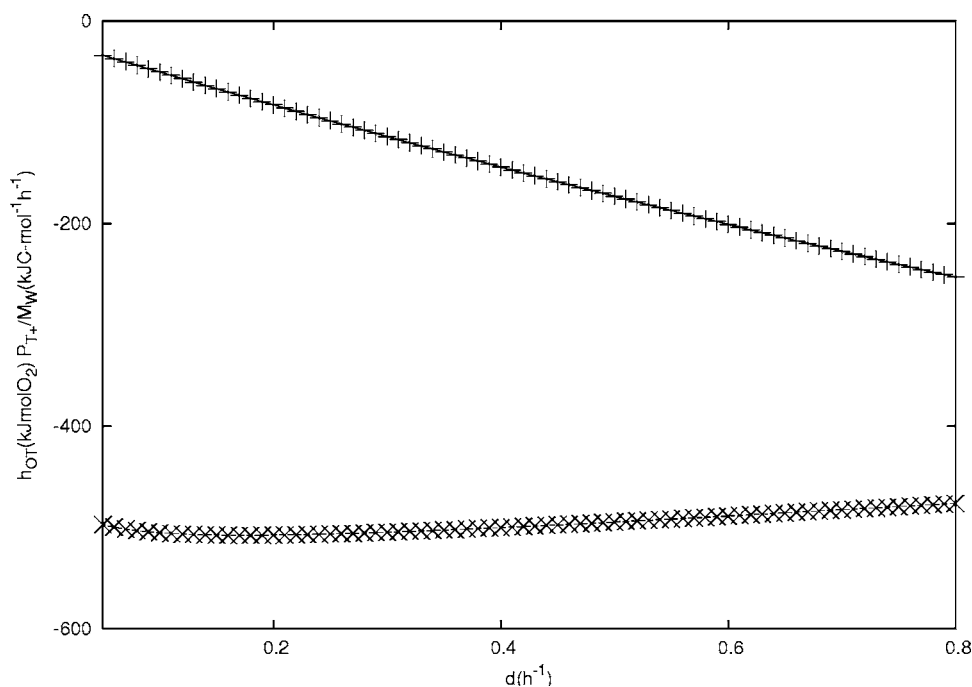


FIG. 6. The heat production rate per C-mol of chemostat biomass per hour (+) and heat production per mol of O₂ consumed (×) (Thornton's rule) vs dilution rates. Units are (×) in kJ mol O₂ and (+) in kJ C-mol⁻¹ h⁻¹.

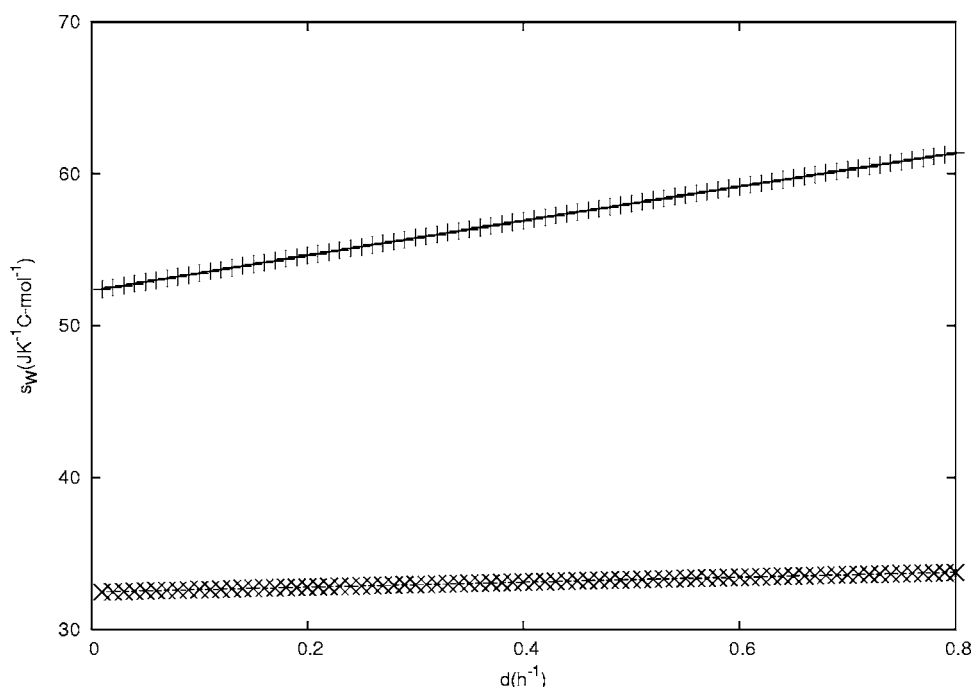


FIG. 7. Entropy of biomass computed with DEB model (+) and entropy of biomass given by Battley's rule (×) vs dilution rates.

creases more with the dilution rate and is significantly higher. However, the application of Battley's rule to dead biomass of *Saccharomyces cerevisiae* [40] gave a very similar result to the entropy obtained experimentally in [39]. Therefore (1) the entropy of dead biomass is different from the entropy of living biomass and (2) Battley's rule should not be applied for living biomass. The entropies of structure and reserve should be computed for other organisms in order to evaluate the generality of these results.

In the literature [38] the entropy of biomass has been compared to the entropy of the substrate. In our case, the biomass entropy ranges from 3.03 to 2.69 J/g K while the entropy of glycerol is 2.03 J/g K. The fact that 1 g of biomass has a higher entropy than 1 g of substrate is in accordance with results obtained by Battley [38] for *Escherichia coli* and succinic acid. Battley argues that this result points to the fact that specific entropy is not related with complexity, otherwise, how could the lower entropy value for the substrate be explained? We disagree because we think that 1 g is an arbitrary quantity: why not compare 1 C-mol? In that case the entropy of a C-mol of structure is lower than the entropy of a C-mol of glycerol.

C. Calorimetry

The heats released in the complete combustion of compound i per mol of consumed O_2 are -472 kJ for the food, -485 kJ for the reserve, and -447 kJ for the structure of *Klebsiella aerogenes*. These values are more or less in agreement with the values obtained theoretically by Gnaiger and Kemp [41] for other organic compounds. Although the heats of combustion of X , E , and V are similar, the net flow of food is positive while the net flows of structure and reserve are negative, suggesting that the coefficient h_{OT} is not bounded by the values of the various h_{OT_i} . This is indeed the case for the dilution rates considered, where the ratio of the heat flow

to the oxygen flow varies between -476 and -507 kJ per mol of O_2 consumed (see Fig. 6).

V. CONCLUDING REMARKS

The thermodynamic analysis made in this paper is applicable to any organism because (1) it is based on a thermodynamic formalism applicable to any open thermodynamic system and (2) uses a general model to describe the internal structure of the organism—the dynamic energy budget model.

We obtain the thermodynamic constraints for organisms with constant food availability, i.e., organisms with a constant chemical composition (DEB's weak homeostasis assumption). These constraints are that only anaerobic organisms can (1) be endothermic, (2) have a net negative import of chemical entropy while increasing their biomass, and (3) have a net positive import of chemical entropy while decreasing their biomass (see Table II). Apparently, anaerobic organisms have a higher thermodynamic flexibility.

We obtain Thornton's coefficient as a function of either (1) the flows of organic compounds [see Eq. (28)], or (2) assimilation, maintenance, and growth [see Eq. (31)] using DEB theory. These relationships are useful in providing new insights into the discrepancies obtained between Thornton's constant and experimental values.

We use experimental data on the aerobic growth of *Klebsiella aerogenes* to obtain molar enthalpies and entropies for the reserve and structure. The knowledge that these properties are constant (DEB's strong homeostasis assumption) is sufficient to compute changes in the enthalpy and in the entropy of living biomass that are known to accompany changes in the reserve density. The importance of being able to compute thermodynamic properties as a function of the amount of reserves has been acknowledged in the literature, e.g., Battley [38] computes the enthalpy of a C-mol of *E. coli*

under conditions that impose that “no storage materials are produced.” Previously, the entropy of living organisms was obtained either by (1) experimental methods or by (2) Battley’s empirical rule [39]. The use of DEB theory for these computations is better than the methods referred because (1) experimental methods are destructive and (2) Battley’s rule does not give results similar to the results we obtained. This last point suggests that the entropy of living biomass is different from the entropy of dead biomass because Battley’s rule has been validated with good results for dead biomass and organic compounds.

We introduce the mechanism of “dilution of entropy production by growth” for organisms that are not in steady state. The capacity of this mechanism to store entropy in new biomass changes with DEB’s reserve density because the molar entropy of the reserve is different from the molar entropy of the structure. We proved this for *Klebsiella aerogenes* where the entropies obtained are different from zero and the structure’s molar entropy is significantly lower than the reserve’s. Additionally, this result suggests that the reserve density concept of DEB theory is essential in discussions concerning the relationship between organization and entropy because the entropy of the organism is a function of the reserve density but the entropy of the structure, which can be related with the organization of the organism, is not.

The development of the generic thermodynamic analysis carried out in this paper can contribute to enlighten the discussions mentioned here and others including thermodynamic measures of biological organization, the explanation of evolutionary increase in size, and evolutionary strategies of energy allocation.

ACKNOWLEDGMENTS

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APPENDIX A

In this Appendix we briefly explain the notation used throughout the paper. A list of compounds is in Table I.

Mass of compound $*$, M_* , is measured in moles for P (product) or X (food) and in C-moles for E (reserve), V (structure), and W (biomass). The ratio $m_E \equiv M_E/M_V$ is the reserve density of the organism and m_{Em} is the maximum reserve density.

Mass flows of compound $*$, j_* , are measured in moles (or C-moles) per C-mol of structure per time. There is one exception to this rule, j_{Xm} , which is the flow of X measured in moles per C-mol of structure per unit time at the maximum ingestion rate. If mass flows are measured in moles (or C-moles) per unit time they are represented as $J_* \equiv j_* M_V$.

Coefficients that relate two mass flows are y_{*1*2} . They represent the number of moles of $*1$ needed to produce one mol of $*2$. In the assimilation reactor food is converted into reserve, y_{XE} , and in the growth reactor reserve is converted into structure, y_{EV} .

Energy flows, \dot{p}_* , are measured in Gibbs energy per C-mol of structure per unit time. The $*$ = X, A, C, M, G , stands for the process which the energy flow is associated with: X (feeding), A (assimilation), C (catabolism), M (maintenance), and G (growth). Chemical potentials convert mass flows to energy flows: μ_X converts the flow of food to \dot{p}_X ; μ_E converts the flow of reserve that exits the assimilation reactor into \dot{p}_A , the flow of reserve that exits the reserve compartment into $\dot{p}_C = \dot{p}_M + \dot{p}_G$.

The energy flow \dot{p}_X is associated with the adimensional functional response $f(X) \in [0, 1]$ that is equal to 1 at abundant food ($X \rightarrow \infty$) and 0 at no food availability ($X=0$). Other DEB parameters include: the reserve turnover rate \dot{k}_E and the maintenance rate coefficient \dot{k}_M , both parameters’ dimensions are per time. The first is related with the velocity of use of the reserve and the second with the velocity of degradation of the structure. Also related with the structure there is the adimensional investment ratio g , a measure of the relative cost of building structure.

Thermodynamic properties have the usual notation: \bar{g}_* is the molar Gibbs energy, \bar{h}_* is the molar enthalpy, \bar{s}_* is the molar entropy, \bar{u}_* is the molar internal energy, μ_* is the chemical potential of compound $*$, T is the temperature, \dot{P}_{T+} is the rate of heat release by the organism, and $\dot{Q}_{reactions}$ is the rate of heat release by all chemical reactions inside the organism.

Vectors and matrices are in bold. The transpose of a vector is indicated by a superscript T and the inverse of a matrix is indicated by a superscript -1 . An overbar means that it is a molar quantity.

The matrix with the chemical composition is \mathbf{n} : \mathbf{n}_O is the matrix with the chemical composition of the organic compounds (X, P, E, V) and \mathbf{n}_M is the matrix with the chemical composition of the minerals ($\text{CO}_2, \text{O}_2, \text{H}_2\text{O}, N_{waste}$). Each entry in these matrices, n_{*1*2} , is the number of atoms of element $*1$ in compound $*2$.

APPENDIX B

The dynamics of the reserve density

$$\frac{dm_E}{dt} = fm_{Em}\dot{k}_E - \frac{\dot{p}_C}{\mu_E} - m_E \frac{1}{M_V} \frac{dM_V}{dt} \quad (\text{B1})$$

is obtained by combining Eq. (3) with Eq. (4), Eq. (6), and $j_X = fj_{Xm}$. One of the assumptions of DEB theory is that the mobilization of reserves, i.e., the catabolic power, cannot depend on food availability, which means that it can only depend on the state variables, the reserve density, m_E , and the amount of structure, M_V . Under this assumption, the last two terms in Eq. (B1) are a function only of m_E and M_V . So, Eq. (B1) can be written as

$$\frac{dm_E}{dt} = fm_{Em}\dot{k}_E - \Phi(m_E, M_V). \quad (\text{B2})$$

At constant food, the weak homeostasis assumption implies that the reserve density is constant $m_E = m_E^*$. Thus

$$fm_{Em}\dot{k}_E = \Phi(m_E^*, M_V). \quad (\text{B3})$$

However, the weak homeostasis assumption also implies that m_E^* is dependent on food level but not on the amount of structure M_V because the organism can grow with a constant reserve density. Thus

$$\Phi(m_E^*, M_V) = H(m_E^*) \quad (\text{B4})$$

because $fm_{Em}\dot{k}_E$ does not depend on M_V . The function $\Phi(m_E, M_V)$ can be generalized out of steady state as $\Phi(m_E, M_V) = H(m_E) + (m_E^* - m_E)G(m_E, M_V)$. With this specification for $\Phi(m_E, M_V)$ Eq. (B2) becomes

$$\frac{dm_E}{dt} = fm_{Em}\dot{k}_E - H(m_E) - (m_E^* - m_E)G(m_E, M_V). \quad (\text{B5})$$

With Eqs. (B1) and (B5) the catabolic flux per C-mol of structure is

$$\dot{p}_C = \mu_E H(m_E) + \mu_E (m_E^* - m_E)G(m_E, M_V) - \mu_E m_E \frac{1}{M_V} \frac{dM_V}{dt}. \quad (\text{B6})$$

Additionally, $G(m_E, M_V) = 0$ because m_E^* is a function of food availability and according to DEB theory the catabolic power cannot depend on food availability. Thus,

$$\dot{p}_C = \mu_E H(m_E) - \mu_E m_E \frac{1}{M_V} \frac{dM_V}{dt}. \quad (\text{B7})$$

Equation (B7) can be written as a function of m_E , M_V , and parameters using Eq. (10):

$$\dot{p}_C = \mu_E H(m_E) - \mu_E m_E \left(\frac{\dot{k}_E \frac{m_E}{m_{Em}} - \dot{k}_M g}{\frac{m_E}{m_{Em}} + g} \right). \quad (\text{B8})$$

To proceed with the derivation we need another of DEB's assumptions: the partition ability of reserves. If the organism's reserve is partitioned among different aggregates then the catabolic power that is mobilized from each aggregate must be proportional to the amount of energy embodied in it:

$$M_V \dot{p}_C(\lambda m_E, M_V, \lambda g) = \lambda M_V \dot{p}_C(m_E, M_V, g). \quad (\text{B9})$$

Also, the number of moles allocated to growth per mole of structure, from each aggregate, must be proportional to the amount of energy embodied in it.

The imposition that the catabolic power given by Eq. (B8) must obey Eq. (B9) implies that $\lambda H(m_E) = H(\lambda m_E)$. Therefore $H(m_E) = \gamma m_E$ and Eq. (B7) simplifies to

$$\dot{p}_C = \mu_E m_E \gamma - \mu_E m_E \frac{1}{M_V} \frac{dM_V}{dt}. \quad (\text{B10})$$

With Eq. (B10) the reserve density dynamics given by Eq. (B1) simplifies to

$$\frac{dm_E}{dt} = fm_{Em}\dot{k}_E - m_E \gamma. \quad (\text{B11})$$

At abundant food availability, (1) the ingestion rate $j_X = j_{Xm}$ implying that $f = 1$ and (2) the steady-state reserve density is $m_E^* = m_{Em}$. With conditions (1) and (2) $\gamma = \dot{k}_E$ and

$$\frac{dm_E}{dt} = \dot{k}_E (fm_{Em} - m_E). \quad (\text{B12})$$

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[21] Entry is written as (line,column).
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