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SHORT COMMUNICATION

A thermodynamic theory of microbial growth

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Our ability to model the growth of microbes only relies on empirical laws, fundamentally restricting our understanding and predictive capacity in many environmental systems. In particular, the link between energy balances and growth dynamics is still not understood. Here we demonstrate a microbial growth equation relying on an explicit theoretical ground sustained by Boltzmann statistics, thus establishing a relationship between microbial growth rate and available energy. The validity of our equation was then questioned by analyzing the microbial isotopic fractionation phenomenon, which can be viewed as a kinetic consequence of the differences in energy contents of isotopic isomers used for growth. We illustrate how the associated theoretical predictions are actually consistent with recent experimental evidences. Our work links microbial population dynamics to the thermodynamic driving forces of the ecosystem, which opens the door to many biotechnological and ecological developments.
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In his famous book 'What is life?', Erwin Schrödinger opened the debate on how life could be envisioned from the thermodynamic standpoint (Schrödinger, 1944). Ilyia Prigogine (Prigogine, 1955) then made an important contribution by pioneering the application of nonequilibrium thermodynamics to biology, underlying modern developments of biological flux-force models (Westerhoff *et al*., 1982, 1983). Today, thermodynamic state functions are widely applied to living systems at different organization levels (Jørgensen and Svirezhev, 2004). The study of microbes, the simplest form of life, however, led to a deeper physical conceptualization of the problem (McCarty, 1965; Roels, 1980; Heijnen and Vandijken, 1992; Rittmann and McCarty, 2001; Kleerebezem and Van Loosdrecht, 2010). In these contributions, microbial anabolism was linked to catabolism through energy dissipation, sometimes expressed as a universal efficiency factor. A relation between dissipated energy and growth stoichiometry was established, enabling the prediction and calculation of energy and matter balances of microbial growth. However, the key question of the link between microbial thermodynamics and growth kinetics remained unanswered.

At the beginning of the 20th century, chemistry was facing a similar problem that finally resulted in a thermochemical kinetic theory 80 years ago (Eyring, 1935). The existence of a high-energy transition state, resulting from the collision of reactants, was postulated. Statistical physics was invoked to estimate the probability of the colliding molecules to have enough energy to overcome the transition state energy. A link between reaction kinetics and thermodynamic state of the system was thus established. Lotka (1922a) suggested that a similar approach could be applied to biological units: 'The similarity of the [biological] units invites statistical treatment [...], the units in the new statistical mechanics will be energy transformers subject to irreversible collisions of peculiar type-collisions in which trigger action is a dominant feature…'

Let us treat statistically a clonal population of N microbes consuming substrates, transducing energy and dividing. For each individual, we propose that harvesting a threshold level of energy from the environment fundamentally triggers the microbial division process. Each division can thus be described as a succession of two steps: (i) the reversible capture of energy by the microbe and (ii) its irreversible transduction leading to division. We indeed assume that the second irreversible step is a slow kinetically limiting process and that the first step can thus be considered as close to equilibrium. The elementary microbial division act can thus be symbolized as follows:

$$M \leftrightarrow X^{\ddagger} \rightarrow 2M \qquad (1)$$

where M represents a microbe and $X^{\ddagger}$ the intermediate microbial activated state in which the microbe is able to divide (Figure 1a).

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During the first step, each microbe has access to a volume (V_{harv}) in which it can harvest the chemical energy in the form of substrate molecules. The part of the chemical energy that is available for growth depends on the thermodynamic state of the environment surrounding the microbe. It can thus be transcribed using the meaningful concept of chemical catabolic exergy, which represents the maximum work available when bringing the substrate molecules in thermodynamic equilibrium with the microbe's environment (see Supplementary Material). Consequently, exergy levels can be attributed to each elementary volume V_{harv} and thus to each microbe. Using statistical physics, we demonstrate that the occupancies of exergy levels by microbes follow Boltzmann statistics (see Supplementary Information for the detailed demonstration). Let us denote $E^{\ddagger}$ the threshold exergy level triggering division corresponding to both dissipated exergy E_{dis} and stored exergy E_M during growth (Figure 1a). These exergies can be evaluated using energy balances for growth established by different authors (see Supplementary Information and reference Kleerebezem and Van Loosdrecht, 2010 for a review). Let us now finally introduce the parameter μ_{max}, representing the division rate of an activated microbe (see Supplementary Information). Then comes the expression of microbial growth rate as a function of microbial exergy balance:

$$\mu = \mu_{max} \cdot \exp\left(- \frac{E_M + E_{dis}}{V_{harv} \cdot [S] \cdot E_{cat}} \right) \qquad (2)$$

S represents the energy-limiting substrate, E_{cat} being the catabolic exergy of one molecule of energy-limiting substrate.

Equation (2) introduces a flux-force relationship between microbial growth rate (μ) and catabolic exergy density ($[S] \cdot E_{cat}$). It correctly transcribes the well-known microbial growth rate dependence on substrate concentration enabling the modeling of any microbial experimental growth data as illustrated using Monod's historical experiments (Figure 1b; Monod, 1942). In addition, our theory naturally accounts for the existence of an apparent threshold substrate concentration for growth (Figure 1b, detail), correcting a flaw of previous empirical equations (Kovarova-Kovar and Egli, 1998). However, the correct transcription of microbial growth rate dependence on substrate concentration is not *per se* sufficient to support the validity of our theory. This can indeed be achieved with any empirical equation exhibiting a sigmoid shape as outlined by Monod himself (Monod, 1942). Moreover, the simple process of fitting growth rate equations with measurements do not provide a sufficiently precise framework to compare different growth models as already demonstrated (Senn *et al.*, 1994).

Hopefully, in our theory, the growth rate more exactly depends on the spatial distribution of exergy around microbes. This implies that, in addition to substrate concentration, the intrinsic thermodynamic properties of molecules involved in the metabolism also determine the growth rate. We thus realized that the study of microbial isotopic fractionation related to pure culture experiments could provide us with an adequate experimental opportunity to challenge the fundamental nature of the relationship between exergy and growth rate. Indeed, for a given microbe and in relation to a

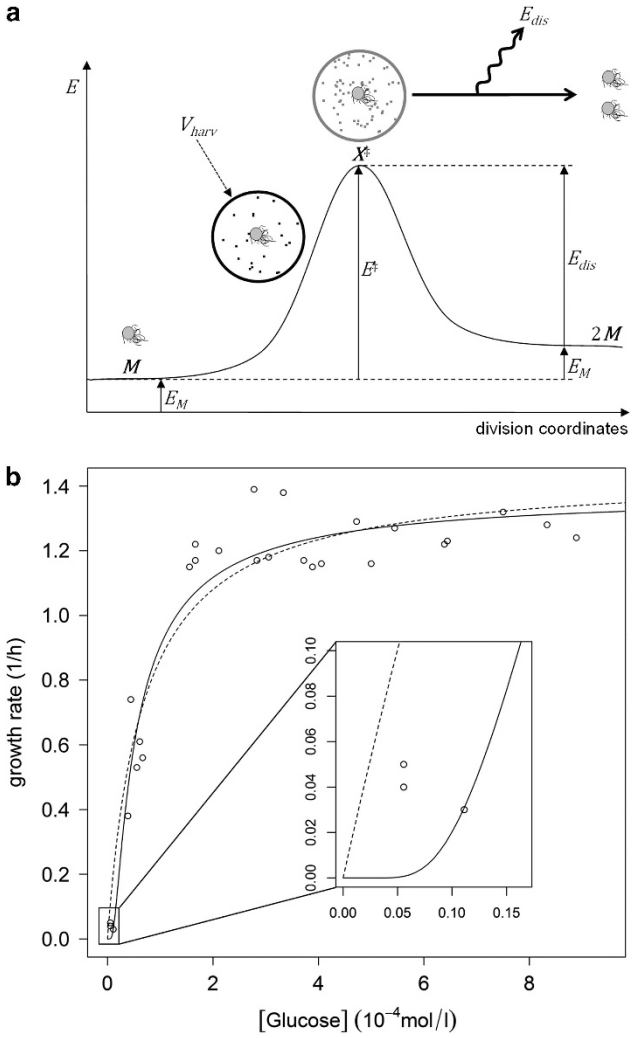

Figure 1 (**a**) Graphical representation of microbial exergy levels along division coordinates. Microbial exergy (E_M) is augmented by the catabolic exergy within the harvesting volume ($E_{cat} \cdot [S] \cdot V_{harv}$, symbolized as dots in a circle surrounding the microbe). Reaching the threshold catabolic exergy ($E_M + E^{\ddagger}$), the microbe is activated (a state denoted $X^{\ddagger}$), and an irreversible division process is triggered, associated with exergy dissipation ($E_{dis} = E^{\ddagger} - E_M$), resulting in two microbes. (**b**) Growth rate of *E. coli* as a function of glucose concentrations under aerobic conditions (Monod, 1942). The plain curve shows the fit of Equation (2) on the data. The dashed curve shows the fit of a Monod equation. A detail of the growth rate/concentration dependency at low substrate concentration is shown, illustrating that the mathematical expression of our law naturally accounts for the existence of an apparent substrate threshold concentration for growth.

well-defined metabolic reaction (that is, μ_{max} and V_{harv} are fixed), our theory predicts that the variation in catabolic exergy (E_{cat}) due to the differences in thermodynamic properties of isotopic isomers (isotopomers) would induce slight differences in substrate consumption rates between heavy or light molecules, thus giving a theoretical ground to the well-known microbial isotopic fractionation phenomenon.

Using our theory, we therefore derived a literal expression of the widely employed empirical kinetic fractionation factor $\alpha_{S/P}$ of substrates toward products (Mariotti *et al.*, 1981; see Supplementary Information for details):

$$\alpha_{S/P} = \alpha_0 \cdot \exp\left(- \frac{E_M + E_{dis}}{V_{harv} \cdot [S] \cdot E_{cat}^2} \cdot \Delta E_{cat}^{h-l} \right) \quad (3)$$

α_0 being the residual biochemical fractionation factor of the catabolic reaction, ΔE_{cat}^{h-l} represents the difference between exergy of the catabolic reaction involving one molecule of the heavy isotopomer minus the exergy of its light counterpart.

Unexpectedly, two classes of isotopic fractiona-

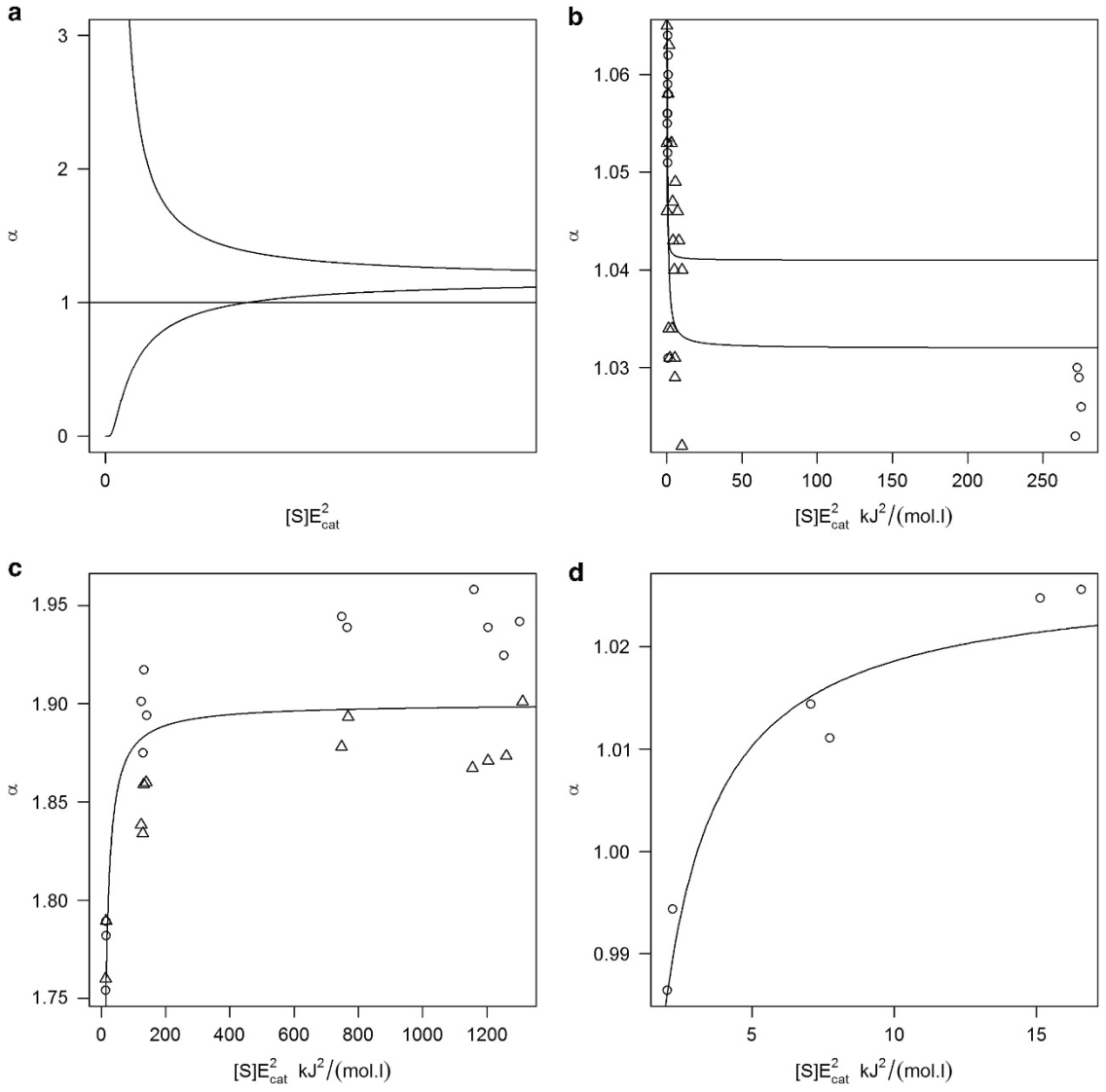

Figure 2 Modeling the isotopic microbial fractionation phenomenon dependency on energy and substrate concentrations as derived from our theory. (**a**) Isotopic microbial fractionation factor (α) as a function of substrate concentration times the square of the catabolic exergy (x axis) as predicted from our microbial growth theory (Equation (3)). (**b**) Experimental data from Penning *et al.* (2005) (triangles) obtained with *Methanobacterium bryantii*; and Valentine *et al.* (2004) (circles) obtained with *Methanothermobacter marburgensis*; on ^{13}C isotopic fractionation associated with hydrogenotrophic methanogenesis confirming the existence of microbial overfractionation. (**c**) Experimental data from Kampara *et al.* (2008) including fractionation factor obtained after 50% (triangles) or 70% (circles) of toluene degradation by *Pseudomonas putida* under aerobic conditions confirming the existence of microbial underfractionation. (**d**) Experimental data from Goevert and Conrad (2009); on ^{13}C isotopic fractionation associated with aceticlastic methanogenesis by pure cultures of *Methanosarcina barkeri* and *M. acetivorans*. Interestingly, in this case, it illustrates the switch between depletion ($\alpha > 1$) and enrichment ($\alpha < 1$) of reaction products in heavy isotope depending on the value of $[S] \cdot E_{cat}^2$ as predicted by our model (see Supplementary Material).

tion behaviors are predicted from Equation (3) depending on the sign of ΔE_{cat}^{h-l}, in relation to the thermodynamic properties of isotopomers (see Supplementary Material): if $\Delta E_{cat}^{h-l}<0$, then α should decrease when $([S]\cdot E_{cat}^{2})$ increases and this case could be named 'microbial overfractionation' (Figure 2a, upper curve).

Conversely, if $\Delta E_{cat}^{h-l}>0$, then α should increase when $([S]\cdot E_{cat}^{2})$ increases and this case could be named 'microbial underfractionation' (Figure 2a, lower curve).

For both cases, α should vary with substrate concentration and catabolic exergy, increasing $[S]\cdot E_{cat}^{2}$ values leading to the convergence of microbial fractionation toward an asymptotical value (α_0). These theoretical predictions were then questioned with experimental microbial isotopic fractionation data from the litterature.

Although it has long been considered that microbial isotopic fractionation was only dependent on the type of metabolism (Mariotti *et al.*, 1981; Hayes, 1993), recent evidences have suggested that it could vary with environmental conditions (Conrad, 1999, 2005). Strikingly, the dependency of microbial fractionation on substrate concentration (Valentine *et al.*, 2004; Kampara *et al.*, 2008; Goevert and Conrad, 2009) and on Gibbs energy (Penning *et al.*, 2005) was recently proven. Figures 2b–d illustrate how these experimental data are actually in agreement with the predictions obtained from our model and thus supports the dependency of microbial kinetics to the spatial distribution of exergy. Moreover, it demonstrates how the reanalysis of these data under a consistent theoretical framework (see Supplementary Information) show the existence of over- and underfractionation as predicted from our theory, which had never been explicitly claimed in the literature to our knowledge. The fact that these two fractionation classes are related to the thermodynamic properties of molecules (the sign of ΔE_{cat}^{h-l}) is a strong argument in favor of a threshold microbial exergy level triggering division, as considered in our theory. Without denying the need for additional data on purposely designed and carefully controlled isotopic experiments, the overall consistency of the different mathematical predictions of our model with microbial isotopic fractionation data already strongly supports our theory of microbial growth sustained by Boltzmann statistics of exergy distribution.

For clarity, our approach was exposed for a single substrate in the case of energy-limited microbial growth. However, we also demonstrate how it can easily be extended to multiple substrates or to the cases of stoichiometric growth limitation (see Supplementary Information). Equation (2) applied to mixed cultures links microbial population dynamics to the thermodynamic driving forces of the ecosystem, which has wide practical and fundamental implications. For example, implemented into engineering models for environmental bioprocesses such as ADM1 for anaerobic digestion (Batstone *et al.*, 2002), it could thus naturally transcribe the well-known dependence of microbial activity to thermodynamic conditions (Jin and Bethke, 2007), correcting a major flaw of current kinetic equations (Kleerebezem and van Loosdrecht, 2006; Rodriguez *et al.*, 2006). Moreover, our model offers the possibility to couple multiple biochemical reactions through the concept of exergy and to infer the fluxes generated by the 'microbial engines that drive earth's biogeochemical cycles' (Falkowski *et al.*, 2008). On a more fundamental point of view, our theory could constitute a mathematical framework to evaluate how microbial communities would evolve considering various thermodynamic goal functions such as the maximum power (Lotka, 1922a, b; DeLong, 2008), maximum exergy (Jørgensen and Svirezhev, 2004) or minimum entropy production (Prigogine, 1955). Finally, and more generally, we also believe that the study of microbes, the simplest form of life, constitutes a fertile thinking ground for a deeper interlinking between physical and biological concepts.

Conflict of Interest

The authors declare no conflict of interest.

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