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# Boltzmann statistics are underlying microbial growth

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17 **SUMMARY:** Microbes are the most abundant living forms on earth and major contributors to the  
18 biogeochemical cycles. However, our ability to model their dynamics only relies on empirical laws,  
19 fundamentally restricting our understanding and predictive capacity in many environmental systems.  
20 Preeminent physicists such as Schrödinger and Prigogine have introduced a thermodynamic  
21 interpretation of life, paving the way for a quantitative theory. From these seminal contributions  
22 have emerged microbial thermodynamics, which allows the prediction of microbial energy allocation.  
23 Nevertheless, the link between energy balances and growth dynamics is still not understood. Here  
24 we demonstrate a microbial growth equation relying on an explicit theoretical ground sustained by  
25 Boltzmann statistics, thus establishing a flux-force relationship between microbial growth rate and  
26 exergy. Historical data from Monod are used to illustrate the ability of our law to represent the  
27 classical sigmoidal relationship between growth rate and substrate concentration. More importantly,  
28 we derived a theoretical interpretation of microbial isotopic fractionation phenomenon and made  
29 associated predictions such as the existence of two classes of fractionation behavior depending on  
30 the thermodynamic properties of isotopomers. Using recent reports, we then show how these  
31 predictions are supported by experimental evidences. Our work opens the door to the modeling of  
32 microbial population dynamics through a thermodynamic state analysis of environmental systems.  
33 We anticipate that this flux-force relationship could improve the design and control of  
34 biotechnological processes and applications. The contribution of microbial reactions to earth  
35 biogeochemical cycles could also be more accurately modeled. More fundamentally, we believe that  
36 our theory offers a mathematical framework to assess the consistency of ecological and evolutionary  
37 concepts.

38

39 “What is life?” was the question asked by Erwin Schrödinger in his famous book [1]. He opened the  
 40 debate on how life could be envisioned from the thermodynamic standpoint. Ilya Prigogine [2] then  
 41 made an important contribution by pioneering the application of non-equilibrium thermodynamics  
 42 to biology, underlying all modern developments of biological flux-force models. Today,  
 43 thermodynamic state functions are widely applied to living systems at different organization levels  
 44 [3]. The study of microbes, the simplest form of life, however led to a deeper physical  
 45 conceptualization of the problem [4-8]. In these contributions, microbial anabolism was linked to  
 46 catabolism through energy dissipation, sometimes expressed as a universal efficiency factor. A  
 47 relation between dissipated energy and growth stoichiometry was established, enabling the  
 48 prediction and calculation of energy and matter balances of microbial growth. However, the key  
 49 question of the link between microbial thermodynamics and growth kinetics remained unanswered.

50 At the beginning of the XX<sup>th</sup> century, chemistry was facing a similar problem which finally resulted in  
 51 a thermochemical kinetic theory eighty years ago [9]. The existence of a high energy transition state,  
 52 resulting from the collision of reactants, was postulated. Statistical physics was invoked to estimate  
 53 the probability for the colliding molecules to have enough energy to overcome the transition state  
 54 energy. A link between reaction kinetics and thermodynamic state of the system was thus  
 55 established. Along a similar path, let us be inspired by the visionary statement made by [10]: "The  
 56 similarity of the [biological] units invites statistical treatment [...], the units in the new statistical  
 57 mechanics will be energy transformers subject to irreversible collisions of peculiar type-collisions in  
 58 which trigger action is a dominant feature..."

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



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

68 During the first step, each microbe has access to a volume ( $V_{harv}$ ) in which it can harvest the  
 69 chemical energy available in the form of substrate molecules (i.e catabolic exergy, see supplementary  
 70 material 1). Consequently, exergy levels can be attributed to each elementary volume  $V_{harv}$  and thus  
 71 to each microbe. Using statistical physics, we demonstrate that the occupancies of exergy levels by  
 72 microbes follow Boltzmann statistics (see supplementary material 1 for the detailed demonstration).  
 73 Let us denote  $E^\ddagger$  the threshold exergy level triggering division, the proportion of activated microbes  
 74 is then:

$$\frac{N^\ddagger}{N} = \exp\left(-\frac{E^\ddagger}{V_{harv} \cdot [S] \cdot E_{cat}}\right) \quad \text{Equation 2}$$

75  $N^\ddagger$  being the number of activated microbes,  $S$  representing the energy limiting substrate,  $E_{cat}$  being  
76 the catabolic exergy of one molecule of energy limiting substrate.

77 Threshold exergy  $E^\ddagger$  corresponds to both dissipated exergy  $E_{dis}$  and stored exergy  $E_M$  during growth  
78 (Figure 1a and supplementary material 1). These exergies can be evaluated using energy balances for  
79 growth established by different authors (see [5] for a review). Let us now finally assume that each  
80 activated microbe has a probability  $\mu_{max}$  to divide by time unit (see Supplementary Material 1). Then  
81 comes the expression of microbial growth rate as a function of microbial exergy balance:

$$\mu = \mu_{max} \cdot \exp\left(-\frac{E_M + E_{dis}}{V_{harv} \cdot [S] \cdot E_{cat}}\right) \quad \text{Equation 3}$$

82 Equation 3 introduces a flux-force relationship between microbial growth rate ( $\mu$ ) and catabolic  
83 exergy density ( $[S] \cdot E_{cat}$ ). It correctly transcribes the well-known microbial growth rate dependence on  
84 substrate concentration. It indeed exhibits a typical sigmoid shape enabling the modeling of any  
85 microbial experimental growth data as illustrated using Monod's historical experiments (Figure 1b).  
86 Additionally, our law naturally accounts for the existence of an apparent threshold substrate  
87 concentration for growth (Figure 1b, detail), correcting a major flaw of previous empirical equations  
88 [11]. However, the ability of an equation to correctly represent the experimental microbial growth  
89 rate dependence on substrate concentration is not *per se* sufficient to support its validity. Indeed, as  
90 stated by Monod: "En fait toute courbe d'allure sigmoïde pourrait être ajustée aux données  
91 expérimentales. Le choix ne sera donc guidé que par des considérations de commodité et de  
92 vraisemblance." [12]. The study of microbial isotopic fractionation in relation to the thermodynamic  
93 properties of isotopomers provides a more adequate opportunity to challenge the fundamental  
94 assumptions underlying our theory.

95 Indeed, according to our theory, the microbial isotope fractionation phenomenon can be explained  
96 by the variation in catabolic exergy ( $E_{cat}$ ) due to the differences in thermodynamic properties of light  
97 or heavy isotopes, which results in slight differences in substrate consumption rates between  
98 isotopomers. Thus, the widely used empirical kinetic fractionation factor  $\alpha_{S/P}$  of substrates towards  
99 products [13] can be expressed literally as follows (see supplementary material 1 for details):

$$\alpha_{S/P} = \alpha_0 \cdot \exp\left(-\frac{E_M + E_{dis}}{V_{harv} \cdot [S] \cdot E_{cat}^2} \cdot \Delta E_{cat}^{h-l}\right) \quad \text{Equation 4}$$

100  $\alpha_0$  being the residual biochemical fractionation factor of the catabolic reaction,  $\Delta E_{cat}^{h-l}$  represents the  
101 difference between exergy of the catabolic reaction involving one molecule of the heavy isotopomer  
102 minus the exergy of its light counterpart.

103 Our theory therefore directly implies the dependence of  $\alpha_{S/P}$  to  $[S] \cdot E_{cat}^2$  (see Equation 4). This  
104 relationship is depicted on Figure 2a, where increasing  $[S] \cdot E_{cat}^2$  leads to the convergence of  
105 microbial fractionation towards an asymptotical value ( $\alpha_0$ ) whereas the behavior is divergent at low  
106 values of  $[S] \cdot E_{cat}^2$ . In the literature, it has long been claimed that microbial isotopic fractionation was  
107 only dependent on the nature of the metabolism involved [13, 14]. Recent evidences however  
108 increasingly testify that, for a given metabolism, it also varies with environmental conditions [15, 16].  
109 Actually, the dependency of microbial fractionation on substrate concentration [17-19] and on Gibbs  
110 energy [20] was recently evidenced, thus confirming our theoretical prediction. Moreover, from our  
111 theory, two classes of fractionation behaviours are expected (Figure 2a, upper and lower curves)

112 depending on the thermodynamic properties of isotopomers considered (see supplementary  
113 material 1). The upper curve corresponds to the case  $\Delta E_{cat}^{h-l} < 0$  for which  $\alpha$  decreases when ( $[S] \cdot$   
114  $E_{cat}^2$ ) increases and could be named “microbial overfractionation”. The lower curve (i.e. “microbial  
115 underfractionation”) corresponds to the case  $\Delta E_{cat}^{h-l} > 0$  for which  $\alpha$  increases with ( $[S] \cdot E_{cat}^2$ ). Figure  
116 2b/c/d illustrates the fitting of Equation 4 on experimental data obtained from previous reports. Two  
117 sets of independently obtained data on  $^{13}\text{C}$  isotopic fractionation associated with hydrogenotrophic  
118 methanogenesis confirm the existence of microbial overfractionation as shown on Figure 2b [20]  
119 [19]. Conversely, experimental data on  $^2\text{D}/^1\text{H}$  fractionation obtained on aerobic toluene degradation  
120 [18] attest the existence of the microbial underfractionation phenomena (Figure 2c). Strikingly, in the  
121 case of microbial underfractionation, Equation 4 predicts that microbial isotopic fractionation can  
122 either enrich ( $\alpha < 1$ ) or deplete ( $\alpha > 1$ ) reaction products in heavy isotope depending on the value of  
123 ( $[S] \cdot E_{cat}^2$ ). The existence of such a phenomenon has never been clearly stated in the literature and  
124 would require further confirmation. Nevertheless, several sets of experimental data appear to  
125 support this prediction although alternative hypotheses were sometimes raised to account for these  
126 observations [17, 21-23]. An illustration of how Equation 4 could give a theoretical ground to such  
127 kind of observations is illustrated using data from [17]; on  $^{13}\text{C}$  isotopic fractionation associated with  
128 acetoclastic methanogenesis, for which experimental values of  $\alpha$  shows an apparent inversion of the  
129 isotopic fractionation phenomena (Figure 2d). Without denying the need for additional data on  
130 purposely designed and carefully controlled isotopic experiments, the overall consistency of the  
131 different mathematical predictions of our model with microbial isotopic fractionation data already  
132 strongly supports our theory of microbial growth sustained by Boltzmann statistics of exergy.

133 For clarity reasons, our approach was exposed for a single substrate in the case of energy limited  
134 microbial growth. However, we show that it can easily be extended to multiple substrates or to the  
135 cases of stoichiometric growth limitation (see supplementary materials 1). Again, we would like to  
136 stress that Equation 3 can be viewed as a flux-force relationship between microbial growth rate and  
137 exergy. Consequently, applied to mixed cultures, our law opens the door to the modeling of  
138 microbial population dynamics through a thermodynamic state analysis of environmental systems,  
139 which has wide practical and fundamental implications. For example, implemented into engineering  
140 models for environmental bioprocesses such as ADM1 for anaerobic digestion [24], it could thus  
141 naturally transcribe the well-known dependence of microbial activity to thermodynamic conditions,  
142 correcting a major flaw of current kinetic models [25, 26]. Moreover, at a larger scale, our law offers  
143 the possibility to integrate the various fluxes resulting from multiple biochemical reactions through  
144 the concept of exergy and to infer from it the growth and dynamics of microbial engines that drive  
145 earth’s biogeochemical cycles [27]. More fundamentally, we also believe that our law could be used  
146 as a mathematical framework to assess the consistency of thermodynamic ecological principles such  
147 as the maximum power [10, 28, 29], maximum exergy [3] or minimum entropy production [2]  
148 principles to go one step further in the fundamental scientific quest aiming to unveil “what is life”.

149

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219

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225



226 Figure 1 : (a) Graphical representation of microbial exergy levels along division coordinates.  
227 Microbial exergy ( $E_M$ ) is augmented by the catabolic exergy within the harvesting volume  
228 ( $E_{cat} \cdot [S] \cdot V_{harv}$ , symbolized as dots in a circle surrounding the microbe). Reaching the  
229 threshold catabolic exergy ( $E_M + E^\ddagger$ ), the microbe is activated (a state denoted  $X^\ddagger$ ), and an  
230 irreversible division process is triggered, associated with exergy dissipation ( $E_{dis} = E^\ddagger -$   
231  $E_M$ ), resulting in two microbes. (b) Growth rate of *E. coli* as a function of glucose  
232 concentrations under aerobic conditions [12]. The plain curve shows the fit of Equation 3 on  
233 the data. The dashed curve shows the fit of a Monod equation. A detail of the growth  
234 rate/concentration dependency at low substrate concentration is shown, illustrating that the  
235 mathematical expression of our law naturally accounts for the existence of an apparent  
236 substrate threshold concentration for growth.

237

238 Figure 2 : Modelling the isotopic microbial fractionation phenomenon dependency on  
239 energy and substrate concentrations as derived from our theory. (a) Isotopic microbial  
240 fractionation factor ( $\alpha$ ) as a function of substrate concentration times the square of the  
241 catabolic exergy (X-axis) as predicted from our microbial growth theory. (b) Experimental  
242 data from [20] (triangles); and [19] (circles); on  $^{13}\text{C}$  isotopic fractionation associated with  
243 hydrogenotrophic methanogenesis confirming the existence of microbial overfractionation.  
244 (c) Experimental data from [18] including fractionation factor obtained after 50% (triangles)  
245 or 70% (circles) of toluene degradation under aerobic conditions confirming the existence of  
246 microbial underfractionation. (d) Experimental data from [17]; on  $^{13}\text{C}$  isotopic fractionation  
247 associated with acetoclastic methanogenesis illustrating the switch between depletion ( $\alpha > 1$ )  
248 and enrichment ( $\alpha < 1$ ) of reaction products in heavy isotope depending on the value of  
249  $[S] \cdot E_{cat}^2$ .

250