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# Boltzmann statistics as founding principle of 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. Historical data from Monod are used to illustrate the experimental accuracy of  
26 our law. In addition, two classes of microbial isotopic fractionation behavior are predicted and we  
27 show how their existence is supported by recent experimental reports. Our work opens the door to  
28 the modeling of microbial population dynamics through a thermodynamic state analysis of  
29 environmental systems. We anticipate that the predictive power of our law could be used to design  
30 and control biotechnological processes and applications. The contribution of microbial reactions to  
31 earth biogeochemical cycles could also be more accurately modeled. Importantly, our theory offers a  
32 new framework to mathematically assess ecological and evolutionary concepts.

33

35 “What is life?” was the question asked by Erwin Schrödinger in his famous book (Schrödinger 1944).  
 36 He opened the debate on how life could be envisioned from the thermodynamic standpoint. Ilya  
 37 Prigogine (Prigogine 1955) then made an important contribution by pioneering the application of  
 38 non-equilibrium thermodynamics to biology, underlying all modern developments of biological  
 39 flux-force models. Today, thermodynamic state functions are widely applied to living systems at  
 40 different organization levels (Jørgensen and Svirezhev 2004). The study of microbes, the simplest  
 41 form of life, however led to a deeper physical conceptualization of the problem (McCarty 1965, Roels  
 42 1980, Heijnen and Vandijken 1992, Rittmann and McCarty 2001, Kleerebezem and Van Loosdrecht  
 43 2010). In these contributions, microbial anabolism was linked to catabolism through energy  
 44 dissipation, sometimes expressed as a universal efficiency factor. A relation between dissipated  
 45 energy and growth stoichiometry was established, enabling the prediction and calculation of energy  
 46 and matter balances of microbial growth. However, the key question of the link between microbial  
 47 thermodynamics and growth kinetics remained unanswered.

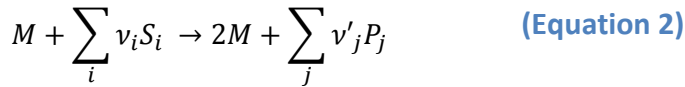
48 At the beginning of the XX<sup>th</sup> century, chemistry was facing a similar problem which finally resulted in  
 49 a thermochemical kinetic theory eighty years ago (Eyring 1935). The existence of a high energy  
 50 transition state, resulting from the collision of reactants, was postulated. Statistical physics was  
 51 invoked to estimate the probability for the colliding molecules to have enough energy to overcome  
 52 the transition state energy. A link between reaction kinetics and thermodynamic state of the system  
 53 was thus established. Along a similar path, let us be inspired by the visionary statement made by  
 54 (Lotka 1922): "The similarity of the [biological] units invites statistical treatment [...], the units in the  
 55 new statistical mechanics will be energy transformers subject to irreversible collisions of peculiar  
 56 type-collisions in which trigger action is a dominant feature..."

57 Let us consider the system constituted by a 3D physical space in which a clonal population of  $N$   
 58 microbes is consuming substrates and dividing. Each individual microbe has access to a volume  
 59 ( $V_{harv}$ ) in which it can harvest substrates. These elementary volumes define the statistical units of  
 60 our model. Let us assume that the total volume accessible to the microbes ( $N \cdot V_{harv}$ ) is small  
 61 compared to the rest of the system, the latter being considered as a reservoir. Then, let us also  
 62 consider a microstate consisting of a specific distribution of substrates molecules in the different  
 63 statistical units occupied by microbes. Each microstate can then be viewed as a part of a canonical  
 64 ensemble as defined in the framework of statistical physics. Therefore, the probability for a statistical  
 65 unit to contain a given number of molecules follows Boltzmann statistics (see supplementary  
 66 material for the detailed demonstration). Let us assume that the activation of a microbe for a division  
 67 event is triggered by threshold numbers ( $v_i$ ) of substrates molecules in its harvesting volume. Then,  
 68 the proportion of activated microbes is:

$$\frac{N^\ddagger}{N} = \prod_i \exp\left(-\frac{v_i}{V_{harv} \cdot [S_i]}\right) \quad \text{(Equation 1)}$$

69  $N^\ddagger$  being the number of activated microbes,  $S_i$  representing the different substrates.

70 Threshold numbers of substrate molecules necessary for a division event are related to the  
 71 stoichiometry of growth:



72 where  $M$  represents a microbe,  $P_j$  metabolic products and  $v'_j$  are stoichiometric coefficients.

73 Let us consider the common situation where only one substrate ( $S_{lim}$ ) is limiting the division  
74 phenomenon. The nature of this limitation can either be due to the insufficient energy supply  
75 brought by  $S_{lim}$  or to an elemental stoichiometric restriction.

76 In the case of stoichiometric restriction, the threshold  $v_{lim}$  can be deduced from the elemental  
77 composition of the microbe  $M$  (see supplementary material) consequently determining the  
78 proportion of activated microbes.

79 In the case of energy limitation,  $v_{lim}$  depends on the energy allocation during growth, which has  
80 been extensively studied (for a review, see Kleerebezem et Van Loosdrecht; (Kleerebezem and Van  
81 Loosdrecht 2010)). The energy allocation can be expressed in terms of exergy balance for an  
82 elementary microbial division (see supplementary material and Figure 1), leading to the expression of  
83  $v_{lim}$  :

$$v_{lim} = \frac{E_M + E_{dis}}{E_{cat}}$$

84

85  $E_{cat}$  being the catabolic exergy of one molecule of energy limiting substrate,  $E_M$  the exergy stored in  
86 one microbe,  $E_{dis}$  the exergy dissipated during an elementary microbial division which can be  
87 calculated as explained in Kleerebezem et Van Loosdrecht; 2010.

88 This expression can thus be used together with (Equation 1) to infer the proportion of activated  
89 microbes as a function of catabolic exergy available. Let us now finally assume that each activated  
90 microbe has a probability  $\mu_{max}$  to divide by time unit. Then comes the expression of microbial  
91 growth rate as a function of microbial exergy balance:

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

92

93 The experimental validity of Equation 3 is illustrated using Monod's historical growth experiments. As  
94 shown on Figure 2-A, our law correctly transcribes microbial growth rate dependence on substrate  
95 concentration. It exhibits a typical sigmoid shape enabling the modeling of any microbial  
96 experimental growth data. Indeed, as stated by Monod: "En fait toute courbe d'allure sigmoïde  
97 pourrait être ajustée aux données expérimentales. Le choix ne sera donc guidé que par des  
98 considérations de commodité et de vraisemblance." (Monod 1942). Interestingly however, our law  
99 naturally accounts for the existence of an apparent threshold substrate concentration for growth  
100 (Figure 2-A-2), correcting a major flaw of previous empirical equations (Kovarova-Kovar and Egli  
101 1998). Although highly encouraging, the ability of our law to correctly represent data from microbial  
102 growth experiments is not *per se* sufficient to support the validity of our theory. We believe that the  
103 microbial isotope fractionation phenomenon is providing a more adequate opportunity to challenge  
104 the fundamental assumptions sustaining our theory.

105 According to our law, the microbial isotope fractionation phenomenon can be explained by the  
106 variation in catabolic exergy ( $E_{cat}$ ) due to the differences in thermodynamic properties of light or  
107 heavy isotopes, which results in slight differences in substrate consumption rates between  
108 isotopomers (see supplementary material). Thus, the kinetic fractionation factor  $\alpha_{S/P}$  of substrates  
109 towards products (Mariotti, Germon et al. 1981) can be expressed as:

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

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

113 It has long been considered that microbial isotopic fractionation was only dependent on the type of  
114 metabolism (Mariotti, Germon et al. 1981, Hayes 1993). Recent evidences however increasingly  
115 testify that it varies with environmental conditions (Conrad 1999, Conrad 2005). Our theory directly  
116 implies the dependence of  $\alpha_{S/P}$  to  $[S] \cdot E_{cat}^2$  (see Equation 4). This relationship is depicted on Figure 2-  
117 B-1, where increasing catabolic exergy density leads to the convergence of microbial fractionation  
118 towards an asymptotical value ( $\alpha_0$ ) whereas the behavior is divergent at low exergy density.  
119 Strikingly, the dependency of microbial fractionation on substrate concentration (Valentine,  
120 Chidthaisong et al. 2004, Kampara, Thullner et al. 2008, Goevert and Conrad 2009) and on Gibbs  
121 energy (Penning, Plugge et al. 2005) was recently evidenced. Moreover, our theory predicts two  
122 classes of fractionation behaviours (Figure 2-B-1, upper and lower curves) depending on the  
123 thermodynamic properties of light and heavy molecules considered (see supplementary material).  
124 Figure 2-B illustrates how experimental data obtained from previous reports (Valentine,  
125 Chidthaisong et al. 2004, Penning, Plugge et al. 2005, Kampara, Thullner et al. 2008, Goevert and  
126 Conrad 2009) are actually consistent with both predictions. These inferences, based on the  
127 mathematical expression deduced from our assumptions, strongly support our theory of microbial  
128 growth sustained by Boltzmann statistics of exergy distribution.

129 Equation 3 introduces a flux-force relationship between microbial growth rate and exergy. Our law  
130 applied to mixed cultures thus opens the door to the modeling of microbial population dynamics  
131 through a thermodynamic state analysis of environmental systems. Implemented into engineering  
132 models for environmental bioprocesses such as ADM1 for anaerobic digestion (Batstone, Keller et al.  
133 2002), it could naturally transcribe the well-known dependence of microbial activity to  
134 thermodynamic conditions, correcting a major flaw of current kinetic models (Kleerebezem and van  
135 Loosdrecht 2006, Rodriguez, Lema et al. 2006). At a larger scale, our law offers the possibility to  
136 integrate the various fluxes resulting from multiple biochemical reactions through the concept of  
137 exergy and to infer from it the growth and dynamics of microbial engines that drive earth's  
138 biogeochemical cycles (Falkowski, Fenchel et al. 2008). More fundamentally, our law offers a  
139 mathematical framework to consistently assess thermodynamic ecological principles such as the  
140 maximum power (Lotka 1922, Lotka 1922, DeLong 2008), maximum exergy (Jørgensen and Svirezhev  
141 2004) or minimum entropy production (Prigogine 1955) principles to go one step further in the  
142 fundamental scientific quest aiming to unveil "what is life".

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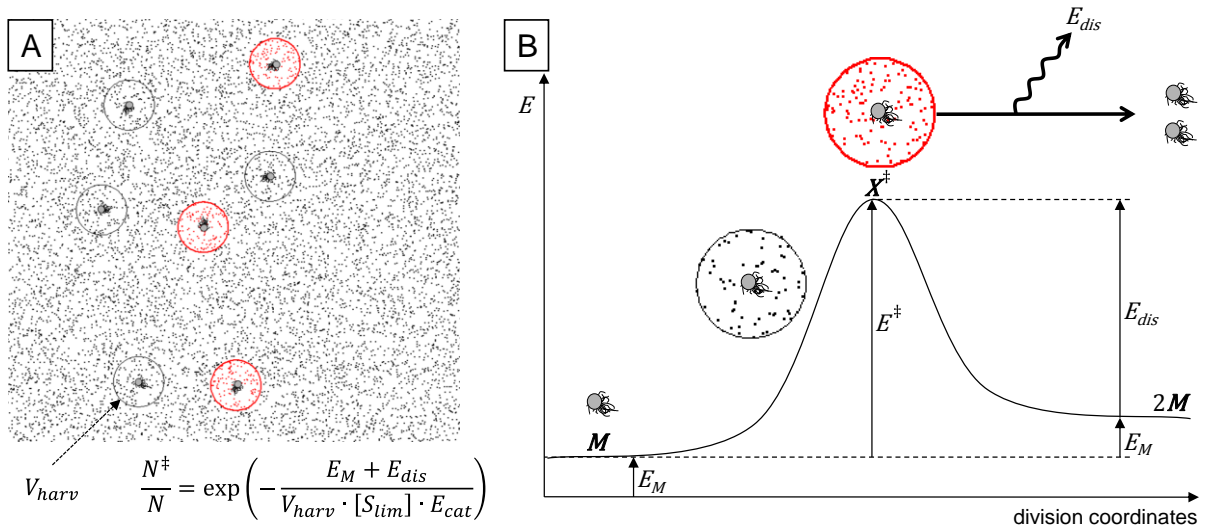
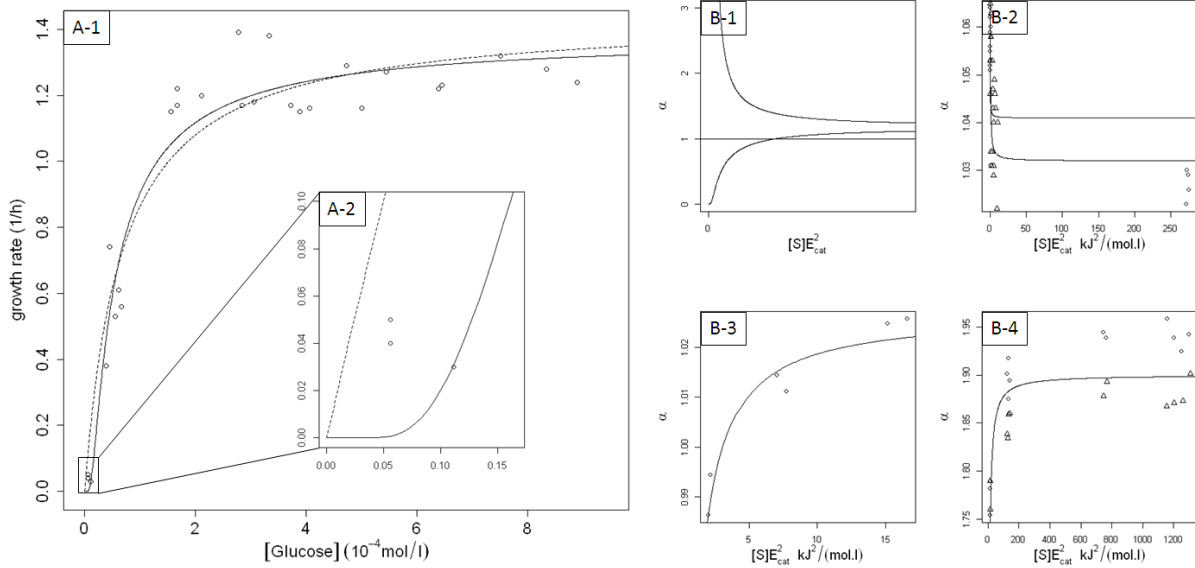


Figure 1 : General principle of an elementary microbial division as sustained by Boltzmann statistics. (A) Representation of substrate molecules (dots) distribution depicting those falling within microbial harvesting volumes (circles) and highlighting activated microbes (red) for which enough substrate molecules are available to trigger an elementary division act. (B) Graphical representation of microbial exergy levels along division coordinates. Microbial exergy ( $E_M$ ) is augmented by the catabolic exergy within the harvesting volume ( $E_{cat} \cdot [S_{lim}] \cdot V_{harv}$ ). 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.

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

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



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156 **Figure 2 : Experimental evidences supporting Boltzmann sustained microbial growth. (A-1) Growth rate of *E. coli* as a**  
 157 **function of glucose concentrations under aerobic conditions (Monod 1942). The plain curve shows the fit of Equation 3**  
 158 **on the data. The dashed curve shows the fit of a Monod equation. (A-2) Detail of the growth rate/concentration**  
 159 **dependency at low substrate concentration showing that the mathematical expression of our law naturally accounts for**  
 160 **the existence of an apparent substrate threshold concentration for growth. (B-1) Isotopic microbial fractionation factor**  
 161 **( $\alpha$ ) as a function of substrate concentration times the square of the catabolic exergy ( $X$ -axis) as predicted from our**  
 162 **microbial growth theory. Two classes of behaviour are predicted depending on thermodynamic properties of the**  
 163 **isotopomers considered. The upper curve corresponds to the case  $\Delta E_{cat}^{h-l} < 0$  for which  $\alpha$  decreases when ( $[S] \cdot E_{cat}^2$ )**  
 164 **increases and could be named “microbial overfractionation”. The lower curve (i.e. “microbial underfractionation”)**  
 165 **corresponds to the case  $\Delta E_{cat}^{h-l} > 0$  for which  $\alpha$  increases with ( $[S] \cdot E_{cat}^2$ ). Surprisingly, in this case, our law predicts that**  
 166 **microbial isotopic fractionation can either enrich ( $\alpha < 1$ ) or deplete ( $\alpha > 1$ ) reaction products in heavy isotope depending**  
 167 **on ( $[S] \cdot E_{cat}^2$ ). (B-2) Experimental data from (Penning, Plugge et al. 2005) (triangles); and (Valentine, Chidthaisong et al.**  
 168 **2004) (circles); on <sup>13</sup>C isotopic fractionation associated with hydrogenotrophic methanogenesis confirming the existence**  
 169 **of microbial overfractionation. (B-3) Experimental data from (Goevert and Conrad 2009); on <sup>13</sup>C isotopic fractionation**  
 170 **associated with acetoclastic methanogenesis confirming the existence of microbial underfractionation. The inversion of**  
 171 **isotopic enrichment factor as predicted from our theory appears to be supported by this set of experimental data. (B-4)**  
 172 **Experimental data from (Kampara, Thullner et al. 2008) including fractionation factor obtained after 50% (triangles) or**  
 173 **70% (circles) of toluene degradation under aerobic conditions. This graph illustrates the consistency of the microbial**  
 174 **underfractionation prediction with another type of element (hydrogen/deuterium fractionation) further supporting the**  
 175 **strong dependency of biological fractionation on the thermodynamic properties of isotopomers. See supplementary**  
 176 **material 2 for detailed calculations.**

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178 **References**

- 179 Batstone, D. J., J. Keller, I. Angelidaki, S. V. Kalyuzhnyi, S. G. Pavlostathis, A. Rozzi, W. T. M. Sanders,  
180 H. Siegrist and V. A. Vavilin (2002). "The IWA Anaerobic Digestion Model No 1 (ADM1)." Water  
181 Science and Technology **45**(10): 65-73.
- 182 Conrad, R. (1999). "Contribution of hydrogen to methane production and control of hydrogen  
183 concentrations in methanogenic soils and sediments." Fems Microbiology Ecology **28**(3): 193-202.
- 184 Conrad, R. (2005). "Quantification of methanogenic pathways using stable carbon isotopic signatures:  
185 a review and a proposal." Organic Geochemistry **36**(5): 739-752.
- 186 DeLong, J. P. (2008). "The maximum power principle predicts the outcomes of two-species  
187 competition experiments." Oikos **117**(9): 1329-1336.
- 188 Eyring, H. (1935). "The Activated Complex in Chemical Reactions." Journal of Chemical Physics **3**: 107-  
189 115.
- 190 Falkowski, P. G., T. Fenchel and E. F. DeLong (2008). "The microbial engines that drive Earth's  
191 biogeochemical cycles." Science **320**(5879): 1034-1039.
- 192 Goever, D. and R. Conrad (2009). "Effect of Substrate Concentration on Carbon Isotope  
193 Fractionation during Acetoclastic Methanogenesis by *Methanosarcina barkeri* and *M. acetivorans*  
194 and in Rice Field Soil." Applied and Environmental Microbiology **75**(9): 2605-2612.
- 195 Hayes, J. M. (1993). "Factors Controlling C-13 Contents of Sedimentary Organic-Compounds -  
196 Principles and Evidence." Marine Geology **113**(1-2): 111-125.
- 197 Heijnen, J. J. and J. P. Vandijken (1992). "In Search of a Thermodynamic Description of Biomass Yields  
198 for the Chemotropic Growth of Microorganisms." Biotechnology and Bioengineering **39**(8): 833-858.
- 199 Jørgensen, S. E. and I. M. Svirezhev (2004). Towards a thermodynamic theory for ecological systems.  
200 Amsterdam; Boston, Elsevier.
- 201 Kampara, M., M. Thullner, H. H. Richnow, H. Harms and L. Y. Wick (2008). "Impact of bioavailability  
202 restrictions on microbially induced stable isotope fractionation. 2. Experimental evidence."  
203 Environmental Science & Technology **42**(17): 6552-6558.
- 204 Kleerebezem, R. and M. C. M. van Loosdrecht (2006). "Critical analysis of some concepts proposed in  
205 ADM1." Water Science and Technology **54**(4): 51-57.
- 206 Kleerebezem, R. and M. C. M. Van Loosdrecht (2010). "A Generalized Method for Thermodynamic  
207 State Analysis of Environmental Systems." Critical Reviews in Environmental Science and Technology  
208 **40**(1): 1-54.
- 209 Kovarova-Kovar, K. and T. Egli (1998). "Growth kinetics of suspended microbial cells: From single-  
210 substrate-controlled growth to mixed-substrate kinetics." Microbiology and Molecular Biology  
211 Reviews **62**(3): 646-+.
- 212 Lotka, A. J. (1922). "Contribution to the Energetics of Evolution." Proceedings of the National  
213 Academy of Sciences of the United States of America **8**(6).
- 214 Lotka, A. J. (1922). "Natural Selection as a Physical Principle." Proceedings of the National Academy  
215 of Sciences of the United States of America **8**(6).
- 216 Mariotti, A., J. C. Germon, P. Hubert, P. Kaiser, R. Letolle, A. Tardieux and P. Tardieux (1981).  
217 "Experimental-Determination of Nitrogen Kinetic Isotope Fractionation - Some Principles - Illustration  
218 for the Denitrification and Nitrification Processes." Plant and Soil **62**(3): 413-430.
- 219 McCarty, P. L. (1965). "Thermodynamics of biological synthesis and growth." Air and water pollution  
220 **9**(10).
- 221 Monod, J. (1942). Recherches sur la croissance des cultures bactériennes. Paris.
- 222 Penning, H., C. M. Plugge, P. E. Galand and R. Conrad (2005). "Variation of carbon isotope  
223 fractionation in hydrogenotrophic methanogenic microbial cultures and environmental samples at  
224 different energy status." Global Change Biology **11**(12): 2103-2113.
- 225 Prigogine, I. (1955). Introduction to thermodynamics of irreversible processes. Springfield, Ill.,  
226 Thomas.
- 227 Rittmann, B. E. and P. L. McCarty (2001). Environmental biotechnology: principles and applications.  
228 Boston, McGraw-Hill.

229 Rodriguez, J., J. M. Lema, M. C. M. van Loosdrecht and R. Kleerebezem (2006). "Variable  
230 stoichiometry with thermodynamic control in ADM1." Water Science and Technology **54**(4): 101-110.  
231 Roels, J. A. (1980). "Application of Macroscopic Principles to Microbial-Metabolism." Biotechnology  
232 and Bioengineering **22**(12): 2457-2514.  
233 Schrödinger, E. (1944). What is life? : the physical aspect of the living cell. Cambridge, The University  
234 Press.  
235 Valentine, D. L., A. Chidthaisong, A. Rice, W. S. Reeburgh and S. C. Tyler (2004). "Carbon and  
236 hydrogen isotope fractionation by moderately thermophilic methanogens." Geochimica Et  
237 Cosmochimica Acta **68**(7): 1571-1590.

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