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1 Insights from Microbial Transition State Theory on Monod's Affinity Constant

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9 **Abstract**

10 Microbial transition state theory (MTS) offers a theoretically explicit mathematical model for
11 substrate limited microbial growth. By considering a first order approximation of the MTS equation
12 one recovers the well-known Monod's expression for growth, which was regarded as a purely
13 empirical function. The harvest volume of a cell as defined in MTS theory can then be related to the
14 affinity concept, giving a new physical interpretation to it, and a new way to determine its value.
15 Consequences of such a relationship are discussed.

16

17 Since the success of Monod's expression (Equation 1) to model substrate-limited microbial growth¹,
18 many expressions have been proposed², accounting for a range of phenomena including substrate
19 inhibition and population density effects^{3,4}. All of these expressions rely on empirical rules,
20 differently to enzymology for which analogues of Monod and Haldane expressions have been
21 mathematically derived⁵). Microbial transition state theory⁶ recently introduced a new expression
22 for microbial growth based on the statistics of molecules distribution in medium inspired from
23 chemical transition state theory. In this communication we explore the physical meaning of the
24 affinity concept through the lens of MTS theory and particularly show how it may provide a novel
25 interpretation of Monod's growth function.

26 Equation 1

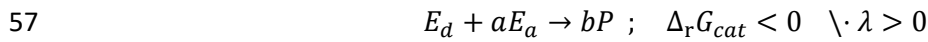
27
$$\mu_{max} \frac{s}{K_s + s}$$

28 Equation 1 represents the Monod growth function, where μ_{max} [1/day] is the maximal growth rate,
29 s [g/L] represents the substrate concentration of the medium and K_s [g/L] is known as the
30 “affinity constant”. Earlier works on kinetics ^{7,8} show the differences in reported literature values for
31 the affinity constant for the same species: these differences are explained by culture history, quality
32 of the experimental data, and posterior data analysis. However little to no consensus can be found in
33 the literature on its interpretation. Furthermore in a review of theoretical derivations of the Monod
34 growth function ⁹ the author concludes that no clear interpretation may be given to the affinity
35 constant. A revision of the affinity concept in Microbiology was made by Button ¹⁰, where fourteen
36 different expressions for affinity are documented. The concept is largely influenced by the Michaelis-
37 Menten model for enzyme kinetics interpretation of affinity from receptor and ligand binding sites,
38 since Monod’s expression for growth is mathematically equivalent to the Michaelis-Menten
39 expression. As stated by Monod himself, Monod’s growth function is purely empirical, while
40 Michaelis-Menten expression has a rigorous theoretical justification⁵, thus one might wonder if the
41 concept of affinity for representing cell growth has a solid conceptual ground.

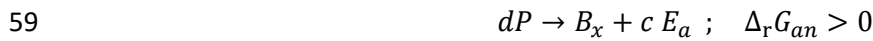
42 MTS theory relates the growth rate to the amount of energy available to perform cellular work. The
43 central idea of bioenergetics is that the energy consuming anabolism can only be thermodynamically
44 feasible if it is coupled with an energy yielding catabolism. The overall reaction resulting from the
45 coupling is known as metabolism ¹¹. The formulation and complexity of both catabolism and
46 anabolism vary greatly depending on the objective the modeller has in mind. On the one hand, when
47 describing the metabolic pathways within a specific microbial species, the formulation takes into
48 account ATP formation and intra cellular intermediates and quickly becomes a very complex web,
49 e.g. ¹². On the other hand if one is interested in observing the general metabolism of a culture at a
50 macroscopic level then the situation simplifies to just a couple of reactions ¹¹. We will focus on the
51 latter.

52 Let us consider a first reaction representing catabolism (Equation 2), a second reaction representing
 53 anabolism (Equation 3) then a linear combination of the two creates metabolism (Equation 4): by
 54 completing λ times the catabolism the energy requirements of the global metabolic reaction are
 55 fulfilled ¹³ (its negative free enthalpy constitutes the driving force for growth).

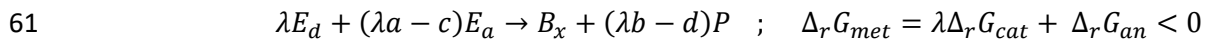
56 **Equation 2**



58 **Equation 3**



60 **Equation 4**



62 where E_d , E_a , and P stand for electron donor, electron acceptor, and products, respectively. B_x
 63 represents an equivalent biomass unit, for instance $B_x = CH_{1.8}O_{0.5}N_{0.2}$ is a generic composition of
 64 one C-mole of biomass ¹⁴. a, b, c, d are stoichiometric coefficients. Finally $\Delta_r G$ represents the Gibbs
 65 free energy variation for each reaction.

66 The reader should notice that λ is the inverse of the yield as usually expressed ($y_{x/s}$) in microbiology
 67 as shown in the equation 5.

68 **Equation 5**

$$69 \quad y_{x/s} = \frac{1}{y_{s/x}} = \frac{1}{\lambda}$$

70 $y_{x/s}$ represents how many moles of biomass are formed per mole of substrate consumed,
 71 conversely $y_{s/x} = \lambda$ represents how many moles of substrate are being consumed per mole of
 72 biomass formed. The methods reviewed by Kleerebezem et al. ¹¹ allow computing λ from mass
 73 balanced reactions with examples coming from a variety of biological process.

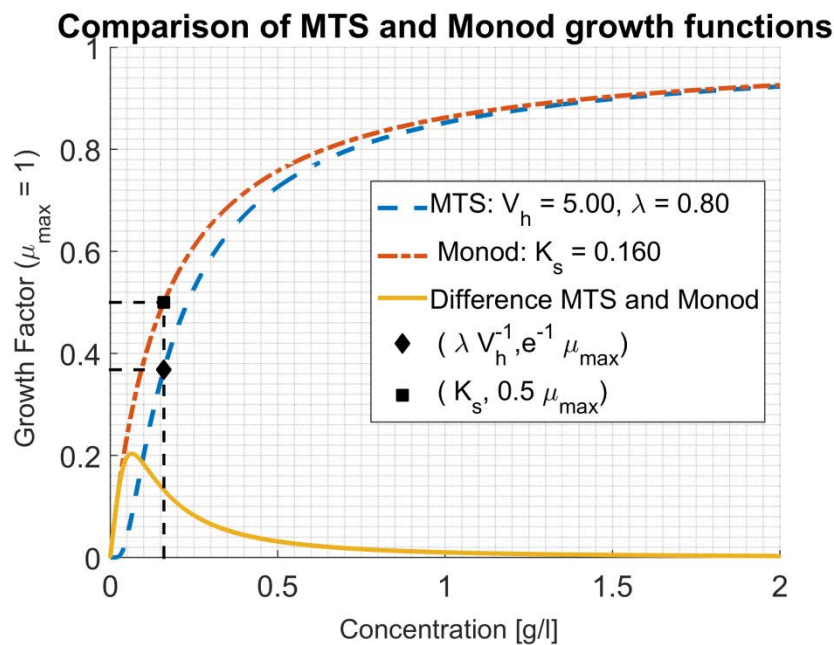
74 MTS theory demonstrates on a theoretically explicit ground a growth rate expression (μ) of a culture
 75 of bacteria limited by an electron donor in perfectly mixed conditions ⁶. More precisely, if we denote
 76 by s the concentration of the limiting electron donor and x the concentration of the species then
 77 these two concentrations are dynamically related by:

78 Equation 6

79
$$\dot{x} = \mu(s)x = \mu_{max} \exp\left(\frac{-\lambda}{V_h s}\right)x$$

80 where V_h , known as the harvest volume, represents the volume to which each microbe has access in
 81 order to harvest the substrate s during the time between two cell divisions. It is worth pointing out
 82 that the harvest volume is an average characteristic.

83



84

85 Figure 1. Example of plots of equations 1 and 6, with the values chosen such that $K_s = \frac{\lambda}{V_h}$. The measurement of the
 86 harvest volume from growth experiments can be obtained in an analogous fashion to the determination of the affinity
 87 constant: by noting s^* the value of substrate concentration at which the growth rate is $e^{-1}\mu_{max}$ (represented by the
 88 black diamond) one obtains V_h by the formula $V_h = \frac{1}{y_{x/s} s^*}$, similarly to the K_s value identified as the concentration for
 89 which the specific growth rate μ is equal to $\frac{\mu_{max}}{2}$ in the Monod expression, (represented by the black square).

90 If one considers a first order approximation of the exponential function near zero (see
 91 supplementary material) then one recovers Monod's expression of growth:

92 Equation 7

93
$$\mu_{max} e^{-\frac{\lambda}{V_h s}} \approx \mu_{max} \frac{s}{s + \frac{\lambda}{V_h}} = \mu_{max} \frac{s}{s + \frac{1}{y_{x/s} V_h}}$$

94 The approximation holds true for high substrate concentrations. More precisely, it can be shown
 95 that the two curves differ by less than 10% for $s \geq 1.92 K_s$, (see supplementary material). In
 96 Figure 1 the graphical comparison of both growth functions can be seen for a given set of
 97 parameters. The MTS growth function is approximated very well by the Monod growth function,
 98 which is reassuring from a practical point of view: in a re-examination of the kinetics of *Escherichia*
 99 *coli*¹⁵ different empirical substrate limiting expressions- all of them with a Monod-like shape- were
 100 compared and no difference was found in the identifiability of their parameters.

101 Note also that in equation 7, $\frac{1}{y_{x/s} V_h}$ replaces the K_s parameter of Monod's expression. In that sense
 102 the affinity constant can be interpreted as a decreasing function of the harvest volume of the cell and
 103 its yield per mole of substrate. On one hand, associating low K_s values to large harvest volumes is
 104 well in line with our understanding of the affinity concept, since a cell that can harvest substrate
 105 molecules in a more extended region should be less substrate limited. On the other hand, the fact
 106 that a low K_s value could be due to a higher conversion yield of substrate to biomass sheds a new
 107 light on the affinity concept. The order of magnitude of V_h can be seen from Table 1 for some
 108 literature references for E. Coli ML 30. In the cases where no yield was reported the energy
 109 dissipation method¹¹ can be used as illustrated in table 1 and supplementary material. For
 110 computing the yield a unique biomass formula was used ($CH_{1.8}O_{0.5}N_{0.2}$). However, for each case,
 111 the biomass composition could be different and, consequently, the yield, thus contributing
 112 to the explanation of the observed variability of K_s .

K_s reported [$\mu g/L$]	λ (gS/gX)	V_h [L/gX]	V_h [$\mu m^3/cell$]	Radius [μm]
for E. Coli ML 30	*Estimated by Energy dissipation Method (Supplementary Material) **Measured during experiment		(cell dry weight: $2.8 \cdot 10^{-13}$ gr/cell) (Ref : ¹⁶ BNID: 103904 Neidhart et al.)	of a sphere of volume V_h .

33 (Ref: ¹⁷)	$\lambda^* = 1.89$	$4.91 \cdot 10^4$	$1.6 \cdot 10^7$	156
33 (Ref: ¹⁷)	$\lambda^* = 1.88$	$4.85 \cdot 10^4$	$1.6 \cdot 10^7$	156
53 (Ref: ¹⁵)	$\lambda^* = 1.88$	$3.02 \cdot 10^4$	$9.94 \cdot 10^6$	133
72 (Ref: ¹⁵)	$\lambda^* = 1.88$	$2.22 \cdot 10^4$	$7.32 \cdot 10^6$	120
76 (Ref: ¹⁸)	$\lambda^{**} = 2.22$	$2.92 \cdot 10^4$	$8.19 \cdot 10^6$	125
90 (Ref: ¹⁸)	$\lambda^{**} = 2.22$	$2.47 \cdot 10^4$	$6.91 \cdot 10^6$	118
100 (Ref: ¹⁸)	$\lambda^{**} = 2.22$	$2.22 \cdot 10^4$	$6.22 \cdot 10^6$	114
132 (Ref: ¹⁸)	$\lambda^{**} = 2.22$	$1.68 \cdot 10^4$	$4.71 \cdot 10^6$	104
125 (Ref: ¹⁸)	$\lambda^{**} = 2.22$	$1.77 \cdot 10^4$	$4.98 \cdot 10^6$	105

113

114 **Table 1. Literature values (Table 2 ⁸) of K_s and calculation of $V_h = \frac{\lambda}{K_s}$, for different chemostat experiments using hexoses**
115 **as substrates.**

116

117 On a more conceptual ground, the MTS approach proposes a way to revisit our current perception of
118 the "affinity-concept" of a microbial culture for a given substrate. It offers an alternative view of the
119 microbial affinity notion than its enzymatic analogue related to Michaelis-Menten theory. It unravels
120 a contribution that is related to the yield (mole of biomass formed per mole of substrate consumed)
121 from another that represents the capacity of the microbial culture to explore a fraction of its
122 surroundings in order to harvest substrate (V_h term). To this extent, it allows to compute the affinity
123 constant from the knowledge of the yield and the harvest volume, which is a completely new
124 approach to determining this constant.

125 This analysis thus plants a seed towards a more physically grounded view of affinity than earlier
126 proposals made from attempts to theoretically derive Monod's equation⁹. The physical interpretation
127 of the affinity concept raises new opportunities to analyse and experimentally challenge the meaning
128 of the V_h parameter. Particularly interesting would be to assess to which extent V_h constitutes an
129 intrinsic trait of the microbial culture, or if extrinsic attributes associated to the culture conditions

130 (such as agitation, viscosity or ionic force) could also significantly influence its value. Such questions
131 remain open and obviously await further studies.

132 **Conflict of Interest**

133 The authors declare no conflict of interest.

134 **Acknowledgements**

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180 several other sugars in carbon-limited chemostat culture. *Can. J. Microbiol.* **46**, 72–80 (2000).
- 181

182 **Author Contributions**

183 P.U. wrote the main manuscript text. T.B and E.D. conceived the harvest volume calculations. A.R.
184 and P.U. derived the mathematical expressions. J.H. supervised the work. All authors participated in
185 the online meetings and corrected the manuscript.

Supplementary Material

February 25, 2020

Approximation

Consider the first order approximation of the exponential function:

$$\exp(x) = 1 + x + o(x) \quad (1)$$

and the first order approximation:

$$\frac{1}{1+x} = 1 - x + o(x) \quad (2)$$

Then rewrite MTS expression as follows:

$$\mu_{max} \exp\left(\frac{-\lambda}{V_h s}\right) \approx \mu_{max} \left(1 - \frac{\lambda}{V_h s}\right) \quad (3)$$

$$\approx \mu_{max} \frac{1}{1 + \frac{\lambda}{V_h s}} \quad (4)$$

$$= \mu_{max} \frac{s}{s + \frac{\lambda}{V_h}} \quad (5)$$

Comparison of both expressions

The substrate limitation range can be studied through the ratio of both growth functions, shown in expressions (6) and (7), respectively.

$$\mu_{max} \frac{s}{s + \frac{\lambda}{V_h}} \quad (6)$$

$$\mu_{max} \exp\left(\frac{-\lambda}{V_h s}\right) \quad (7)$$

Noting $K_s := \frac{\lambda}{V_h}$ One then considers the ratio:

$$R(s) = \frac{\exp\left(\frac{-K_s}{s}\right)}{s + K_s} \quad (8)$$

Note that ratio (8) does not depend on μ_{max} . It can be shown that $R(s) \in (0, 1)$, by using the well known inequality $\exp(x) < \frac{1}{1-x}$ for $x < 1$. Which is valid since the term inside the exponential is negative:

$$R(s) \leq \frac{1}{1 - \frac{-K_s}{s}} \frac{K_s + s}{s} = 1 \quad (9)$$

The change of variables $u = \frac{s}{K_s}$ is used to analyse the expression $R(s)$, which gives equation (10).

$$F(u) = \exp\left(-\frac{1}{u}\right) \frac{u+1}{u} \quad (10)$$

$$F'(u) = \exp\left(-\frac{1}{u}\right) \frac{1}{u^2} \frac{u}{u+1} + \exp\left(-\frac{1}{u}\right) \frac{1}{(u+1)^2} > 0 \quad (11)$$

Since F is monotonic, one gets that there exists a unique u^* such that $\exp\left(-\frac{1}{u^*}\right) \frac{u^*+1}{u^*} = 0.9$, implying a unique $s^* := u^*K_s$, such that $R(s^*) = 0.9$. From the former it can be seen that for each K_s MTS expression approximates to 90 % of the Monod expression whenever $s \geq u^*K_s$.

The curve $s \mapsto R(s)$ is shown in figure 1, for different K_s values of table 1 of the manuscript. One can see that $u^* \approx 1.92$ therefore $s \geq 1.92K_s$ then $R(s) \geq 0.9$.

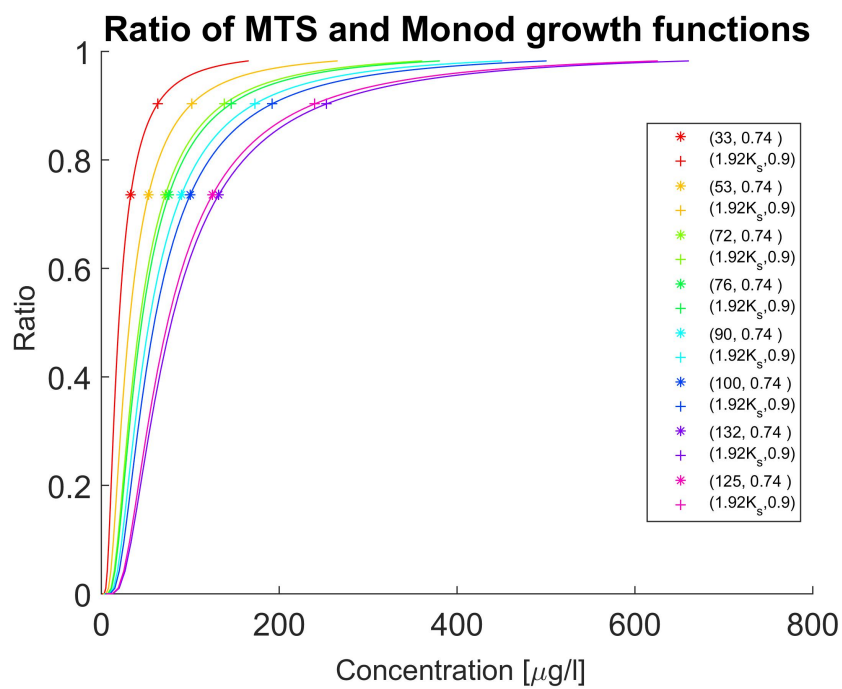


Figure 1: $R(s)$ curve for several K_s values obtained for E. Coli ML 30. The asterisk * represents K_s values evaluated in expression $R(s)$, note that $R(K_s) \approx 0.74$. The plus sign + represents the point where the $R(s) = 0.9$.