Communication to the Editor

The Monod Equation and Mass Transfer

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An alternative interpretation of the growth rate–substrate concentration dependence is presented. This is based on the assumption that the main factors affecting growth rate are transfer of substrate from the medium and the maximum growth velocity, which is that observed when no substrate limitations occur. This approach allows the approximate prediction of one of the two kinetic constants required, and may be of great use, especially for continuous cultures. It is the first attempt to provide a phenomenological explanation for the large variations observed in the values of the Monod constant, K_S, reported in the literature. Key words: mass transfer • Monod equation • growth rate • kinetics

INTRODUCTION

It is a well-accepted fact that, although the well-known Monod equation is used extensively for practical reasons, it corresponds to an oversimplification and has no mechanistic basis. Originally[5] it was proposed for convenience as hyperbolic equations find broad use to develop simple models in phenomena such as adsorption.

Indeed, Michaelis–Menten kinetics applied to enzymatic reactions gives mechanistic meaning to the constants. The constant v_{max} is the rate of an elementary reaction of product formation by dissociation of the enzyme–substrate complex, and K_m is either the equilibrium constant for the enzyme–substrate system (rapid equilibrium assumption), or the combination of the constants corresponding to the elementary reactions of reversible formation of the complex and its dissociation (quasi-steady-state assumption[2]). None of those meanings can be applied readily to a substrate–cell system. The present communication, on the other hand, proposes to interpret the growth rate–substrate concentration dependence on the basis of mass transfer considerations, allowing an approximate prediction of the Monod constant.

THEORETICAL MODEL

Following the argument stated in the Introduction, the substrate limitation phenomenon will be considered here as a pure mass transfer effect. If h_S is the overall mass transfer coefficient for the limiting substrate being consumed, its flux from the bulk of the liquid into the cell can be given as

$$N_S = h_S(S - S_c) \tag{1}$$

where S and S_c are the substrate concentration in the bulk and on the surface of the cell, respectively. It is assumed here that h_S is an overall mass transfer coefficient, which will allow for the transport of the substrate from the bulk of the liquid to the cell surface.

In the present approach we assume that the specific metabolic rate of the main limiting nutrient such as a carbon source is constant, independently of its concentration. However, the rate of transport of this nutrient from the bulk of the broth to the cell is concentration dependent, as expressed in Eq. (1). Because both steps occur in series, the sum of the resistances of the two consecutive steps will give the inverse of the overall growth rate

$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{1}{\mu_t} \tag{2}$$

where μ is the overall growth rate, and μ_{max} and μ_t are the specific rates of metabolic consumption and transport of the nutrient, respectively. It is possible to use Eq. (1) to find the appropriate expression of μ_t. Assuming without loss of generality that the cells can be taken as spherical, the area-to-volume ratio of the cell is $(6/d_c)$, where d_c is the characteristic cell diameter, the area of cells per unit reaction volume is expressed as

$$\frac{A_c}{V} = \frac{6X}{\rho_c d_c} \tag{3}$$

where X is the biomass concentration, V is the volume of the culture, and ρ_c the density of the cell. For cell forms different from a sphere, the area-to-volume ratio will change accordingly. In the case of rodlike microorganisms, the area-to-volume ratio for a cylinder with spherical caps and a total length of three diameters is $1/(0.22 * d_c)$. This is almost independent of the cell length, because an infinite rod would give a ratio of $1/(0.25 * d_c)$. Eq. (1) can be now converted into the limiting rate of S uptake, $(-r_S)$, which depends on S

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$$(-r_S) = N_S \frac{A_c}{V} = \frac{6\,h_S}{\rho_c d_c} X\,(S - S_c) \tag{4}$$

Using the definition of biomass yield on substrate, Y_{XS}, the rate at which cell growth will take place when completely controlled by the rate of substrate flux toward the cell will be

$$\mu_t = \frac{6 Y_{XS} h_S}{\rho_c d_c}\,(S - S_c) \tag{5}$$

where Y_{XS} indicates the yield of biomass on substrate S. When the substrate concentration is high, this rate will be much higher than the potential metabolic rate of the cell at the given conditions (temperature, pH, nature of the substrate, etc.), μ_{max}. Being the mass transfer and the bioreaction in the cell in series, the influence of μ_t on the overall rate is negligible in this phase, and $\mu = \mu_{max}$. As the substrate concentration diminishes, so does μ_t, until it becomes rate controlling. This is shown graphically in Figure 1, which was calculated for the growth of *S. cerevisiae* using Eq. (5) and the data shown in Table I.

Replacing Eq. (5) in Eq. (2)

$$\mu = \frac{\mu_{max}\left[\dfrac{6 Y_{XS} h_S}{\rho_c d_c}\right](S - S_c)}{\mu_{max} + \left[\dfrac{6 Y_{XS} h_S}{\rho_c d_c}\right](S - S_c)} \tag{6}$$

The concentration of substrate at the cell boundary, S_c, is unknown. If we assume that it is much smaller than at the bulk, $(S \gg S_c)$, then Eq. (6) becomes equivalent to the Monod equation

$$\mu = \frac{\mu_{max}\,S}{K_S + S} \tag{7}$$

with

$$K_S = \frac{\mu_{max}}{\left[\dfrac{6 Y_{XS} h_S}{\rho_c d_c}\right]} \tag{8}$$

In the range of substrate limitation μ_t becomes smaller than μ_{max}. The substrate concentration that leads to this situation is:

$$(S - S_c) \ll K_S \tag{9}$$

or

$$S < S_c + \frac{\mu_{max}}{\left[\dfrac{6 Y_{XS} h_S}{\rho_c d_c}\right]} \tag{10}$$

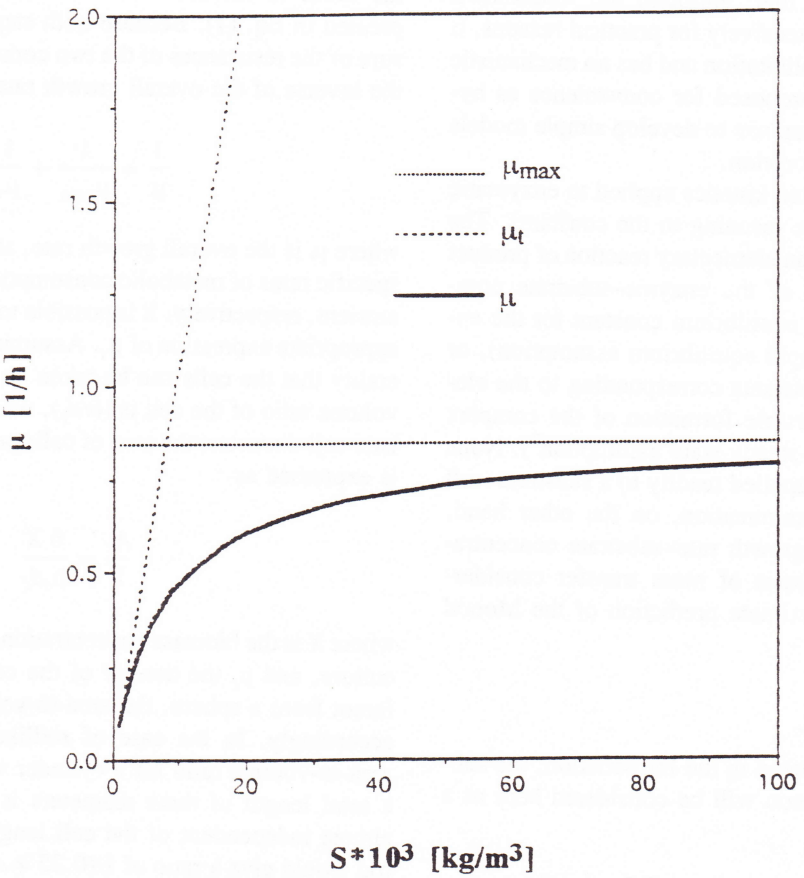

Figure 1. Relationship between μ_{max}, μ_t, and μ. The values taken for the calculation correspond to the case of *S. cerevisiae* shown in Table I.

Table I. Comparison with published data.

Culture	Substrate	Ref.	μ_{max} (h^{-1})	$h_S * 10^5$ (ms^{-1})	$d_c * 10^6$ (m)	Y_{XS} (−)	$K_S * 10^3$ (kg m^{-3})	$(K_S * 10^3)_{calc.}$ (kg m^{-3})
K. aerogenes	Glycerol	7	0.85	1	0.86 (r)	0.55	9	8.1
K. pneumoniae	Glucose	7	0.50	0.85	0.86 (r)	0.435	31	7.15
A. aerogenes	Glucose	7	1.33	0.85	0.6 (r)	0.435	8	10.2
E. coli	Glucose	5	0.95	0.85	0.6 (r)	0.435	11.4	9.4
S. cerevisiae	Glucose	4	0.39	0.85	8 (s)	0.15 (e)	42.7	86
C. tropicalis	Glucose	4	0.39	0.85	6 (s)	0.16 (e)	198	79.5

(r) rod, (s) sphere, (e) experimental.

DISCUSSION

Eq. (6) is equivalent to the Monod equation, but has the advantage that, whereas K_S in the Monod equation is an empirical constant, all the components of Eq. (8) have a known meaning, including h_S. This simplifies considerably the task of obtaining a kinetic expression (when only a fair approximation is needed), because only a value of μ_{max} must be experimentally obtained. An approximate value for K_S, which is the constant most difficult to obtain from simple batch experiments, and the influence of changes in physical characteristics and operational variables can thus be predicted using Eq. (8).

Comparison between experimental data and calculated values of K_S are shown in Table I. In the calculations, the value of the transfer coefficient of 10^{-5} m/s was adopted for glycerol, which is based on experimental data by Kushalkar and Pangarkar.[3] This takes into account all the resistances to mass transfer, including that through slime capsules around the cell if they exist, and through the cell walls when this step is gradient driven. Alternatively, it could be considered as an adjustable parameter in the model.

It was assumed that the mass transfer coefficient can be considered proportional to the square root of the diffusivity of the substrate

$$h_S \propto \sqrt{D_S} \tag{11}$$

Diffusivities needed for adjustments in h_S were taken from Schwartzemberg and Chao.[8]

Yield coefficients, unless experimental values were available, were taken from Roels.[7] The yield in terms of the single carbon formulas, Y_b^c, was calculated from

$$Y_b^c = 0.13\ \nu \tag{12}$$

where ν is the reductance of the substrate. The unit carbon formula weights (UCFW) of the substrate and biomass (the latter taken as 25.14 g/UCFW) were used to convert Y_b^c into Y_{XS}

$$Y_{XS} = Y_b^c \frac{(\text{UCFW})_b}{(\text{UCFW})_S} \tag{13}$$

It should be noticed that, in a few instances, exceptionally large values of K_S have been obtained. In the case of *M. tuberculosis*, mentioned by Monod[5] as an example of this uncommonly large K_S, the present approach allows a qualitative prediction that a larger value is to be expected, because the effective area-to-volume ratio of the micellum will be much smaller than for single cells. This consideration may apply also to the case of *Candida tropicalis* growing on glucose,[4] shown in Table I. Thus, the present approach allows prediction of the trend of those "anomalies" in K_S.

The interaction of mass transfer with biokinetics has been considered before. Powell[6] and Atkinson[1] have presented studies of different kinetic forms representing microbial growth, including the step of substrate diffusion. However, in their approach, the Monod form at the surface of the biomass was maintained, in addition to the mass transfer mechanism. Therefore, the result is the addition of a third parameter to the model, or the retention of the two conventional parameters if the mass transfer parameters are known a priori. Here, on the contrary, we propose that the Monod form is a consequence of the finite transfer rate of the substrate, and consequently, one of the two parameters in the Monod equation is eliminated.

CONCLUSION

An alternative interpretation of the growth rate–substrate concentration dependence is presented. This approach constitutes only an approximation, but the simplicity of the procedure may justify its use in some cases. Although an accurate value of K_S is not really needed for usual batch operation, it is much more important for continuous and fed-batch operation when substantial depletion of the limiting nutrient is observed. The question in this case is how much can the substrate be depleted without substantial decrease of the bioreaction rate, which depends on K_S. In most cases, K_S will be very low, and the substrate concentration assuring robust operation will be several times this value. Considering this fact, the proposed approximation appears to be satisfactory for most practical cases.

It can be seen in Table I that some predictions are very accurate, such as for *K. aerogenes*, *A. aerogenes*, and *E. coli*, and the discrepancy for the yeasts and *K. pneumoniae* is somewhat larger but the predicted values are of the same order of magnitude. The present approach is the first attempt to provide a phenomenological explanation for the

extremely large variations observed in the values of K_S reported in the literature.

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NOMENCLATURE

A_c area of a cell (m^2)
D diffusivity coefficient ($m^2\ s^{-2}$)
d_c cell diameter (m)
h_S mass transfer coefficient for the substrate (ms^{-1})
K_m Michaelis–Menten constant ($kg\ m^{-3}$)
K_S Monod constant ($kg\ m^{-3}$)
N_S flux of substrate ($kg\ m^{-2}\ s^{-1}$)
r reaction rate ($kg\ s^{-1}\ m^{-3}$)
S substrate concentration ($kg\ m^{-3}$)
V cell volume (m^3)
X biomass concentration ($kg\ m^{-3}$)
Y_{XS} biomass yield on substrate (kg biomass per kg substrate)
Y_b^c biomass yield on substrate (UCFW biomass per UCFW substrate)

Greek letters

μ specific growth rate (h^{-1})
μ_{max} maximal specific growth rate (h^{-1})
μ_t specific transport rate of substrate (h^{-1})
ρ_c cell density (kg/m^3)
ν reductance degree (av. electrons)

Subscripts

b biomass
c cell
s substrate
t transport

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