Review Article   OMICS International

A Self-Consistent Approach to the Lag Phase of Planktonic Microbial Cultures

Schiraldi A*

Department of Chemistry, Division of Physical Chemistry, University of Milan, Milano, Italy

Abstract

A two-parameter model presented in a previous paper [1] allows description of the growth of duplicating microbes in planktonic conditions. The model does not explicitly account for the preceding lag phase, the duration of which can nonetheless be empirically assessed by shifting the growth trend to comply with the predictive model. This work shows that the duration of the lag phase determined in this way is consistent with the model as it is related to the maximum specific growth rate and to the previous virtual history of the microbial population. The formal expressions presented are in line with the experimental evidence of the inverse correlation between the duration of the lag phase and the sharpness of the growth trend and the overall extent of the growth span.

Keywords: Lag phase; Cell density; Population density; Generation time; Planktonic conditions

Introduction

During the lag phase, no increase of cell density takes place. Once ready, the cells start duplication with a pace (generation time) that is progressively adjusted during the growth span. A suitable model [1] to describe this process implies the obvious assumption that the progress of the microbial population via duplication mechanism obeys the law.

$$N = N_0 2^{t/\tau(t)} \qquad (1)$$

where N_0 is the starting population (or population density) and the generation time, τ, is a function of the time, t, namely,

$$\tau = \frac{a}{t} + bt \qquad (2)$$

a and b being parameters to asses through a best fit of the plate counts. These parameters reflect the starting growth environment (pH, available volume and substrate, co-existence of other microorganisms, etc.) and its endogenous modifications during the growth span, in the absence of any external perturbation. It comes out that these two parameters may be given a physical meaning [1]: 1/b is the maximum achievable growth extent, namely, $N_{max}/N_0 = 2^{\wedge 1/b}$, 1/b being the average number of duplications for each generation line along the growth path from N_0 to N_{max}; the ratio (b/a) reflects the sharpness of the growth trend, the maximum specific growth rate, being attained at $t = t^* = (a/3b)^{1/2}$. The tangent to the growth trend at t^* crosses the end plateau at $t_{end} = 3t^*$.

The reduced quantity, $\xi(t) = b \log_2(N/N_0)$, with $0 \leq \xi(t) = \dfrac{t^2}{\left(\dfrac{a}{b}\right) + t^2} \leq 1$

can therefore describe the growth extent.

Such a behavior concerns any microbial species that grows via cell duplication and can be described with the reduced quantities: ξ and $t_R = (t-t_0)/(t^* - t_0)$. Reminding that $(t^* - t_0) = t^*_{id} = (a/3b)^{1/2}$, one easily obtains for ξ the expression:

$$\xi = \frac{t^2_R}{3 + t^2_R} \qquad (3)$$

that does not depend on the parameters a and b and allows one to gather all the growth trends of duplicating microbial species, at any temperature and environmental conditions, in a single master plot (Figure 1).
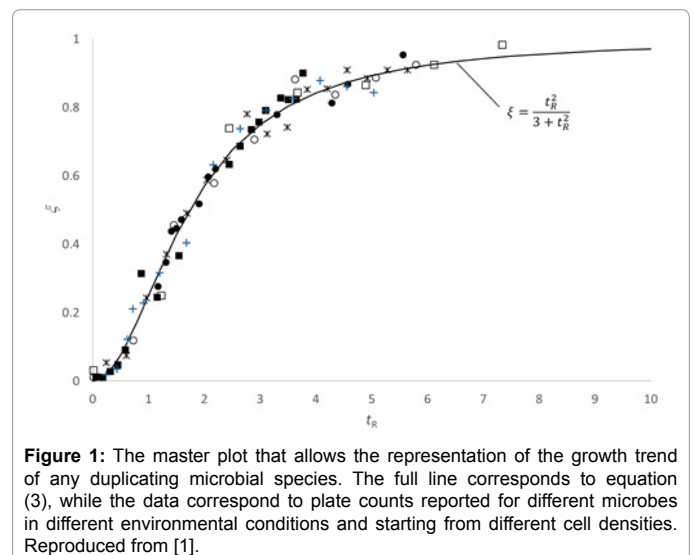
Figure 1: The master plot that allows the representation of the growth trend of any duplicating microbial species. The full line corresponds to equation (3), while the data correspond to plate counts reported for different microbes in different environmental conditions and starting from different cell densities. Reproduced from [1].

Self-consistency of the duration of the lag phase

Another peculiarity of a given planktonic culture is the duration of the lag phase, t_0, which is not explicitly included in the above model, since it does not imply changes of the population density. Nonetheless, one can easily determine t_0 as the shift of the abscissa that allows the compliance with the constraints imposed by the model (Figure 1). The main one is $(t_{end} - t_0) = 3 (t^* - t_0)$, having replaced the variable t with $(t-t_0)$ in equations (1) and (2), which allows evaluation of t_0:

$$t_0 = \frac{3t^* - t_{end}}{2} \qquad (4)$$

*Corresponding author: Schiraldi A, Department of Chemistry, Division of Physical Chemistry, University of Milan, Milano, Italy, Tel: +39 02 503111; E-mail: alberto.schiraldi@unimi.it

Equation (4) makes the duration of the lag phase consistent with the model. The relationship between the intercept, γ, and the slope, s, of the tangent at t= t* in the (ξ, t) plan (Figure 2), namely,

$$s = \xi = \frac{3}{8}\sqrt{\frac{3b}{a}} = \frac{3}{8}\frac{1}{t_{id}^*} \ (where\ t_{id}^* = t^* - t_0),\ \text{or}\ t_0 = \frac{\gamma - 0.125}{s}$$

supports this conclusion.

Equation (5) shows that, for a given γ, a large s (sharp growth trend) corresponds to a small t_0, in agreement with most experimental evidences and the prediction of some other models [2]. Figure 2 reports an example of such correlation.

Now it is possible to draw a new plot (Figure 3) where the time is expressed as $(t - t_0)/(t^* - t_0) = t_R$.

Looking at Figure (3) and applying the relationships for similar triangles (heavy contours in Figure 4), one gets

$$|\gamma| : \left[t_0 + \left(\frac{t^* - t_0}{3} \right) \right] = \frac{1}{8} : \left(\frac{t^* - t_0}{3} \right), \text{ namely,}$$

$$t_0 = t^* \frac{8|\gamma| - 1}{8|\gamma| + 2} \quad \text{(accordingly, } t_0 \leq t^*) \quad (6)$$
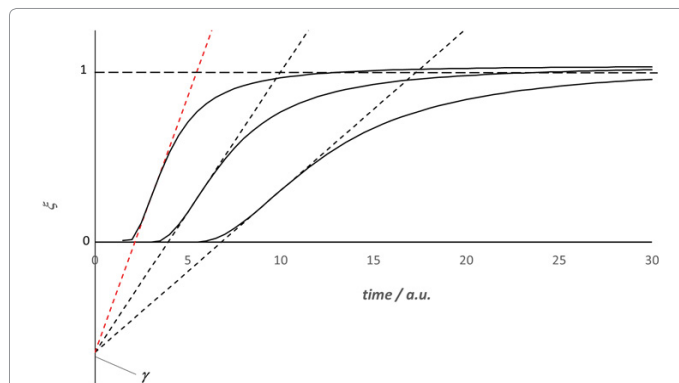

Figure 2: Growth trends with a common intercept γ. The used parameters are: a = 5(time units)², b = 0.1, 0.3 and 1. The larger b, the sharper the growth trend. The corresponding duration of the lag phase is t_0 = 5.44, 3.14 and 1.72 time units, respectively
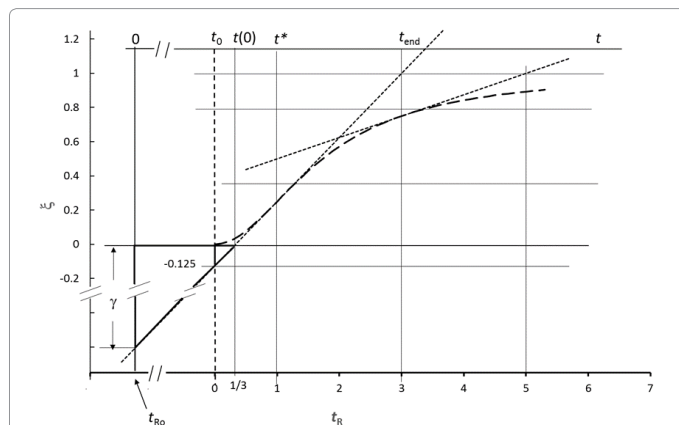
Figure 3: Master plot of any duplicating planktonic microbial culture (like in Figure 1) that includes the duration of the lag phase. ξ is the attained fraction of the $\log_2(N_{max}/N_0)$ gap. The "origin" of the true growth (dotted line) is identified thanks to the constraint that the tangent at t* must intercept the ξ axis at ξ = -0.125 [1]. Notice that |γ| ≥ 0.
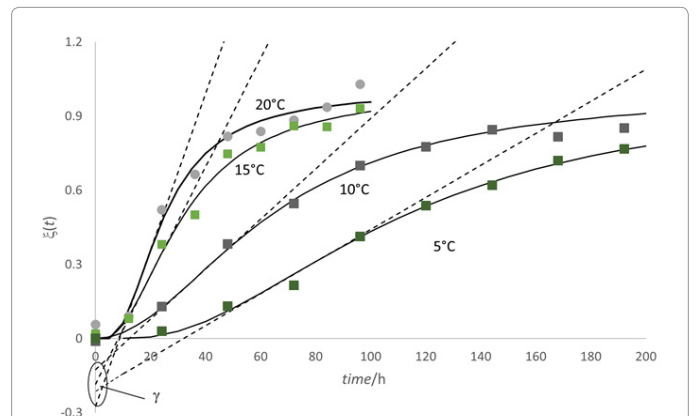
Figure 4: Growth trends of Pseudomonas fluorescens in a real system (after [3]), expressed in reduced units as ξ, at various temperatures.
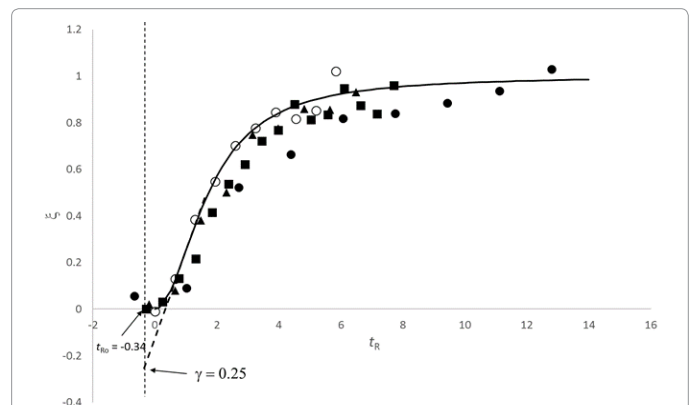
Figure 5: The same data as in Figure 6 reported using the reduced time t_R. Using -0.34 as the mean value of t_{Ro} = -t_0/(t*-t_0) (i.e., for t = 0), one can estimate 0.25 as a common value for γ

Reminding that the model replaces the real system with a virtual one where all the generation lines are synchronous and have no truncated branches [1], $N_0 2^{-\gamma/b}$ would correspond to the virtual population density, N_{start}, (at $t = 0$) that would reach the actual starting population density, N_0, i.e., $\xi = 0$, at $t = (t^*-t_0)/3$ and the maximum population density, N_{max}, at $t = t_{end}$, via a duplication progress with the maximum specific growth rate [(d ξ /dt)* = 3/8 (3b/a)^{1/2} in reduced units, see Figure 3]. Accordingly $\log_2 \left(\frac{N_{max}}{N_{start}} \right) = \frac{|\gamma| + 1}{b}$

If N_{start} = 1 CFU/mL is tentatively assumed, then

$$\log_2 \left(N_{max} / CFUmL^{-1} \right) = \frac{|\gamma| + 1}{b} \ and \ \log_2 \left(N_0 / CFUmL^{-1} \right) = \frac{|\gamma|}{b} \quad (7)$$

Figure 3 and equations (6) and (7) provide a consistent description of the correlation between the duration of the lag phase and the intercept γ, which reflects a correlation between the relevant biological meanings. Equations (6) and (7) link the values of t_0 and γ to the values of N_0, N_{max}, and the relevant $\log(N_{max}/N_0)$. In particular, these equations lead to the prediction that a small (N_{max}/N_0) implies a long lag phase.

Using the literature data [3] relevant to *Pseudomonas fluorescens* in

a real system, one indeed finds a substantial agreement with the above picture (Figure 4)

These data support the reliability of the assumption that slope and duration of lag-phase practically counterbalance each other, small discrepancies being due to the scarcity of the available data and the related uncertainty of estimating ξ , as well as to the fact that the relevant growth process occurred in non-planktonic conditions [3]. Reporting the same data in the (t_R, ξ) plan and averaging the value of t_{Ro} (-0.34), one can estimate a common mean value of γ (-0.25). Since $N_0 \sim 300$ CFU [3] and $b \sim 0.031$, a rough estimation of N_{start} leads to a value close to 1 CFU mL^{-1} (Figure 5).

Conclusion

Although not explicitly included in the growth model proposed for duplicating species in planktonic conditions [1], the duration of the lag phase, t_0, can be easily assessed as the time shift that allows compliance with the growth trend. Once the plate count data are converted in reduced quantities, namely the fraction of the $\log_2 (N_{max}/N_0)$ gap, ξ, the slope of the growth trend at t* (where it reached its maximum value) is correlated with t_0 and the two quantities almost counterbalance each other. Any experimental growth trend may be supposed to start from a virtual population density, $N_{starting} < N_0$, defined by the intercept of the tangent at t*, γ , which does not change too much on changing the culture conditions.

Acknowledgements

References

1. Schiraldi A (2017) Microbial growth in planktonic conditions. Cell & Dev Biol 6: 185.

2. Vadasz P, Vadasz AS (2002) The neoclassical theory of population dynamics in spatially homogeneous environments. Physica A 309: 329-359.

3. Tyrer H, Ainsworth P, Ibanoglu S, Bozkurt H (2004) Modelling the growth of *Pseudomonas fluorescens* and Candida sake in ready-to-eat meals. J Food Eng 65: 137-143.