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Research Article

Mathematical Modeling of Growth Lag Phase Formulated on the Basis of Mass and Number of Axenic Microbial Cells in a Population

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Abstract

A simple algorithmic method for the study of the early growth phases of a microbial population is presented. Based on the growth of individual cell masses, this method simulates the evolution of the population to the exponential growth phase. Based on the well-established observation that the development of the inoculum on the culture medium (food source) depends on the history of this inoculum, we propose that the initial cells have random individual masses that obey a certain distribution function. We show that the empirical relation "lag phase x specific growth rate = constant" is mathematically justified in this context and to which parameters it can be related. We also identify the similarities of behaviors that are independent of the used distribution functions. Further development of the model should predict the specific maximum growth when the lag phase is known.

Keywords: *Predictive Biology; Lag-Phase to Maximum Specific Growth Rate Relationship; Food Control; Health Relevance; Prokaryotic Growth Cell Cycle*

Introduction

The first steps in the growth of a microbial population are likely less studied than other steps. In addition, the methods used for these studies are diverse and often inadequately or poorly documented.

The observation of a lag phase preceding the rapid (exponential) growth of a microbial population dates back to the end of the 19th century.

A remarkable fact in this significant and, for a long time, unexploited observation is the easily discernible difference between the observable and unobservable lag phase, depending on the method of biomass evaluation, even on the same culture. Previously, in 1961, Herbert [1] showed two distinctly different evolution profiles according to whether one measured the increase of the population as a function of time in terms of cell number per volume unit or in term of dry weight per volume unit. Using the latter method, no lag time appears, while it is clearly visible using the former method. The same observation was reported by Maaløe and Kjelgaard in 1966 [2]. The two profiles appear in figure 1. Pirt (1975 - [3]) previously gave a fairly detailed interpretation of the lag phase phenomenon, but he does not always distinguish how the biomass was calculated in each of in his explanations. This imprecision diminishes the general character of his theorization. Following this researcher's recommendations, we will most often use the method of Lodge and Hinshelwood (1943 [4] to calculate what Pirt calls "the true lag of growth" [3].

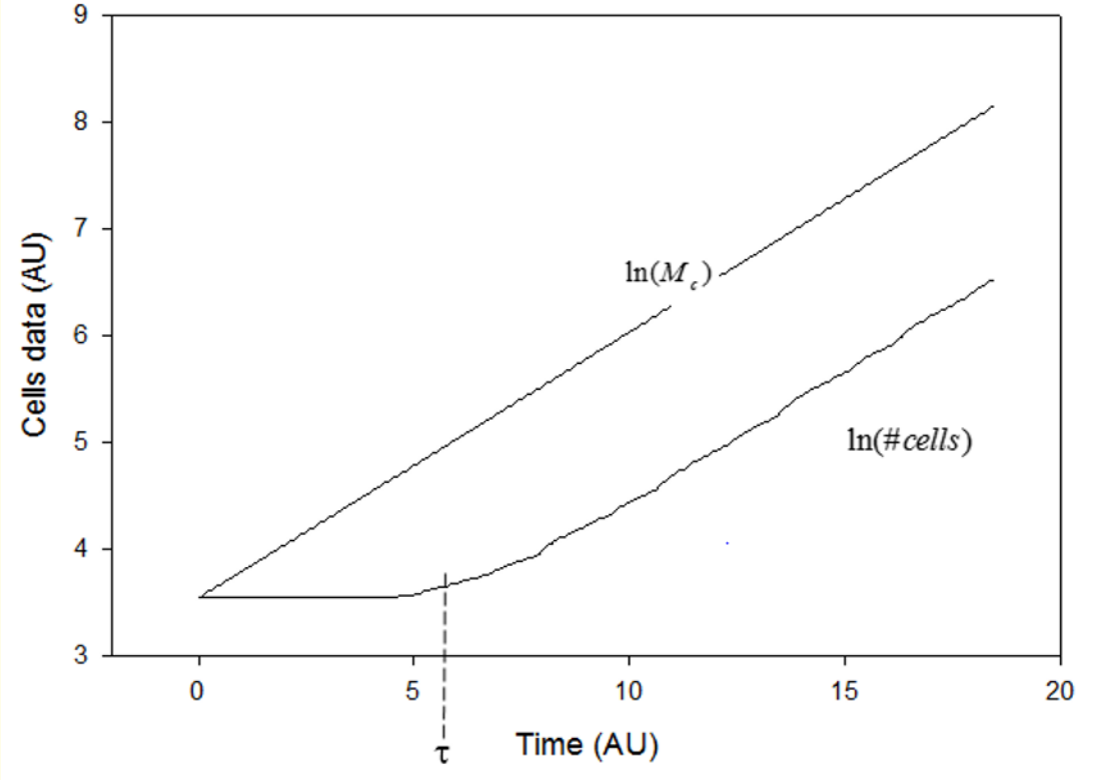

Figure 1: *Figure 1 shows the difference between temporal evolutions of the Naperian logarithm of cells number (ln(#cells)) and of total cell masses (ln(Mc)) in arbitrary units (AU). (Data are represented using reduced variables (as usual in literature) so that the origins of the curves are superimposed.) The approximate division lag phase,τ, is represented.*

After a peak of interest approximately 1930, the lag phase experienced a period of eclipse. However, the Third International Conference on Predictive Modelling in Food was held in Leuven in 2000, and according to Swinnen., *et al.* (2003) [5], "food safety is a growing concern in modern society, the scientific discipline of predictive food microbiology gains more and more interest worldwide". This observation also applies to the field of health [6,7] and thus gives renewed importance to the lag phase and predictive biology.

In this work, our goal is not to provide a basic explanation of the lag phase but rather to work on predictive biology by implementing simple models and concepts intended to pave the way for more powerful and more effective algorithms for studying this important but underestimated phase of cell growth.

Materials and Methods

General

The primary features of the model are the following:

1. The significant quantity used for modeling is the cell mass (or m_i). This value is chosen to represent the individual mass of cell *i* and possibly over time. This choice is considered relevant by other authors [5,8], but in our approach, this value does not a significant magnitude and thus only the state variable is chosen to describe the system.

2. The cells are isogenic, and their viability is 100%.

3. The cells are in the planktonic part of the system and divide by scissiparity.

4. Each cell only gives birth to a daughter cell when it reaches a critical mass, $m_{i,crit}$. Once again, this critical value is not necessarily the cause of the division but is simply the mass observed during this division. At first, we state that the critical mass value is the same for all cells: $m_{i,crit} \approx m_{crit}; \forall i$.

5. The two daughter cells do not necessarily have the same mass. Nonetheless, the sum of the masses is conserved at the moment of division,

$$m_i(t-\varepsilon) \to \alpha m_i(t) + (1-\alpha) m_{i+1}(\mathrm{t}) \quad ; \alpha < 1; \varepsilon << 1 \qquad (1)$$

with $m_i(t-\varepsilon) = m_i(t) + m_{i+1}(t)$. (The very small value ε indicates the "almost zero" time interval before and after division.)

6. Each cell division, therefore, increases the number of cells, N_T , by one unit

$$N_T \xrightarrow{division} N_T + 1 \qquad (2)$$

7. The growth of the cell mass over time follows an exponential law

$$m_i(t) = m_i(0)\,\mathrm{e}^{kt} \qquad (3)$$

8. The interphase time is considered negligible compared to the total growth time, which therefore appears as a continuous phenomenon, with

$$m_i(0) \le m_i(t) \le m_{i,crit} \qquad (4)$$

9. To avoid ambiguities, we will call M_c the total cell mass and *X* the cell density, and with $X = \frac{M_c}{V}$, where *V* is the volume, this quantity is generally called biomass.

10. It will be necessary to distinguish between the two well-known possible meanings of the term "growth". Cell mass increase (CMI) or cell growth (CG) is the growth of a single cell, which is indicative of the increase in the mass of its intracellular components, while population mass increase (PMI) or population growth (PG) refers to the growth of the global population, which includes both the number of cells and the cell masses of each of them.

11. It is therefore clear that these quantities are related by

$$M_c(t) = N_T(t) \times m_i(t)\,; \qquad (5)$$

the explicit representation of time, *t*, indicates that the temporal variation in the number of cells N_T and the individual cell mass m_i is not necessarily isomorphic. Thus, for example, taking into account equation (2), the increase in the number of cells (PG) tends to be a discontinuous function, which is not the case for CG.

Algorithmic

The algorithm of the model is very simple. Starting from initial conditions $t = 0 \to m_i(0)$ defined for each cell, the CG is numerically integrated according to (3). When the mass of a cell reaches its critical value, it immediately divides into two cells, and the total number of cells is increased by one unit

$$\text{if } m_i(t) \geq m_{i,crit} \text{ then } N_T(t) = N_T(t) + 1 \quad (6)$$

Note: As most simulations use stochastic integrals, the proper integration method is Euler's method with a constant step (Prof. R. Lefever, ULB, personal communication).

The subsequent cell masses are later defined by (1), which does not imply that the daughter cells have identical masses (this is true only for $\alpha = 1/2$).

In this study, we investigate the lag phase, defined as the time period between cell inoculation and the first cells' division (or the beginning of the logarithmic growth state of the population). It is obvious according to (3) and its consequences that at this stage, this model does not include a steady [9] state of the population (nor cells, which is never the case except for starvation or dormancy).

In this preliminary approach, we will study three types of models:

- **Determinist (DET):** In this type of simulation, all kinetic constants and state variables are non-noisy (purely deterministic). This case is obviously a special one, but nonetheless, it is important. We will see that several conclusions apply to synchronous cultures which, although uncommon, are very real [1,10,11].

Many variables and constants can be noisy, either in isolation or in groups. The combinations of all of these possibilities are enormous, and we will limit ourselves presently to the initial conditions of the cell masses. The motivation of this choice is based on the simplifying approximation that according to their previous history of expansion (preceding the contact with the current substrate), the cells have acquired different masses that are distributed according to various distribution functions. This fact is clearly established in the literature [11-13], but unanimity on the form of the distribution function has not been attained. Therefore, we have chosen two functions of more or less rather arbitrary distributions to initiate the study:

- **Uniform (UNF):** This function is certainly unrealistic but has certain "pedagogical" aspects that validly illustrate certain features of the model.

- **Gaussian (GAU) or Normal:** This function is considered correct and adapted by some authors [14,15] but, as we said, is not unanimously considered correct [13].

Results

To facilitate the comparison of results between the three types of simulations, we chose the same parameters, such as integration constants and kinetics, for all of the cases. The sole difference will, therefore, concern the definition of the initial conditions of the cell masses. In some cases, we have kept the number of decimals unnecessarily high to allow the verifications of some results.

Definition of the general (GEN) constants common for the three types of simulations

(All values are in arbitrary units (AU)).

$k = 0.25$	cell growth rate ($= \mu_{\max}$); cf. (3)
$\alpha = 0.5$	partition coefficient of cell division; cf. (1)
$N_T(0) = 250$	number of initial cells of the inoculum
$m_{i,crit} = 1 \; ; \forall i$	critical cell mass

$\max_{cell} = 5000$ maximum number of cells for the simulation (since there is no steady state, a maximum number of cells is fixed for the end of the simulation)

$\Delta t = 0.001$ constant integration step; method: Euler.

Deterministic initial conditions (DET)

$m_i(0) = 0.1 \; ; \forall i$ initial conditions of cell masses

$M_c(0) = 250 \times 0.1 = 25$ initial condition of total cell mass (see (5))

Figure 2 shows all of the results of the simulation in DET conditions. Unlike figure 1, since the variables are not in reduced forms, the initial points of the two first curves (d1 and d2) are not merged but separately represent the values of the natural logarithms of the number of cells, N_T, and the total cell mass, M_c, initially ($t = 0$). The third (non-logarithmic) curve shows the ratio M_c / N_T, corresponding to the temporal evolution of a single cell mass (successive exponential "waves" corresponding to (3) can be distinguished).

Perhaps the most striking feature in figure 2 is the discontinuous aspect of the curve $\ln(N_T) = f(t)$, which appears as a discontinuous function. Obviously, cells starting from the same initial conditions and according to the same kinetics of growth all divide simultaneously. The cell culture is said to be synchronous. This situation can be experimentally induced [16,17]. It is apparent that the lag time,τ, (before the first division) is approximately 10 AU (arbitrary units). However, this value is very easy to calculate precisely by calculating the point of intersection of the two lines $d1 \cap d2$ as follows.

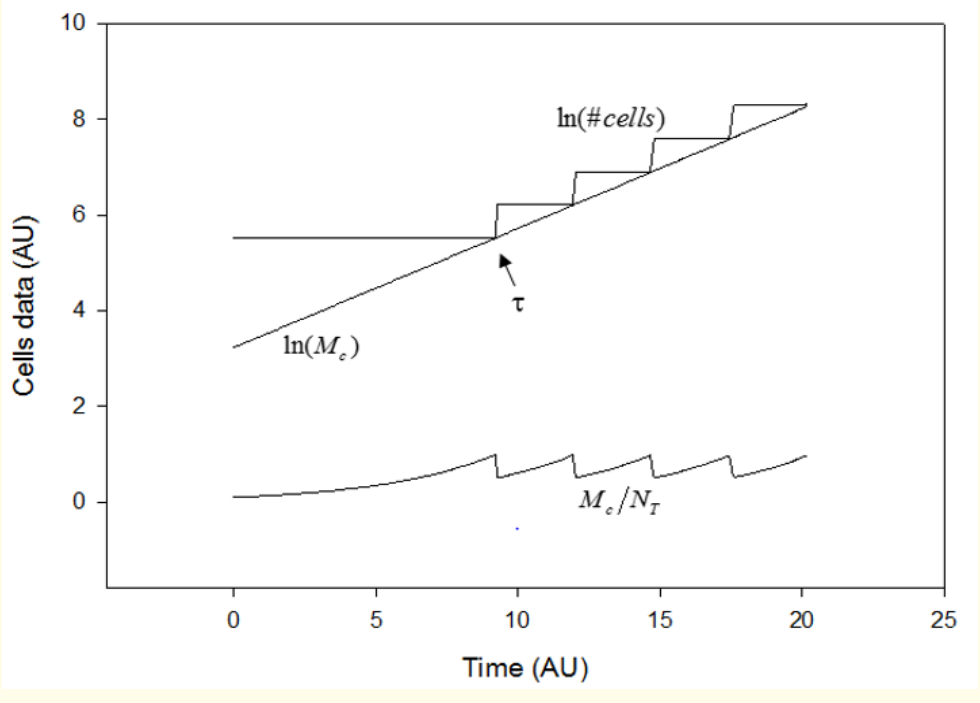

Figure 2: *Figure 2 clearly shows that the DET initial conditions induce synchronous cell growth. The latency time τ is defined without difficulty by the first cell division (first over four "generations"). The third (M_c / N_T - non-logarithmic) curve is an approximation of the growth of a single cell.*

The linear regression of *d*2 is considered comparable to that of *d*1. This approach yields the following:

$$d1 = \ln\left(M_c(0)\right) + at \qquad (7)$$

with $\ln\left(M_c(0)\right) = 3.2248 \pm 0.0003$; according to the initial conditions $\ln(250 \times 0.1) = 3.219$

and $a = 0.2499 \pm 0.0000$; we have defined that $k = 0.25$ and therefore $a \equiv k = \mu_{\max}$.

orrelation coefficient is $r^2 \approx 1.0$.

Using (7), the intersection point is given by ($t = \tau$)

$$\ln\left(N_T(0)\right) = \ln\left(M_c(0)\right) + \mu_{\max}\tau \qquad (8)$$

from which one obtains that

$$\tau = \frac{1}{\mu_{\max}} \ln\left(\frac{N_T(0)}{M_c(0)}\right) \qquad (9)$$

and using (5)

$$\tau = \frac{1}{\mu_{\max}} \ln\left(\frac{1}{m(0)}\right) \qquad (10)$$

In our numerical example, we obtain $\tau = 9.210$ AU.

Equation (10) indicates that the lag time is equal, in the DET case, to the inverse of the maximum specific growth rate multiplied by the logarithm of the inverse of the cell mass of the inoculum. Therefore, the lag time will be shorter as the specific growth rate will be high in logarithmic growth. In contrast, τ will be longer as the mass will be smaller. These two results seem consistent.

However, (10) is still of additional interest; put in the form

$$\mu_{\max}\tau = \ln\left(\frac{1}{m(0)}\right) = cte. \qquad (11)$$

it shows that the product of the lag time and the specific maximum rate is a constant. Not an absolute constant, but a constant depending on the experimental conditions ($\ln\left(\frac{1}{m(0)}\right) = 2.3$ here). In this case, the constant depends on the initial cell mass. This relationship was previously postulated empirically [18]. In this instance, the relationship finds a rigorous theoretical justification.

Although a statistical treatment is slightly superfluous when applied to a deterministic simulation, we will examine the distributions of cell masses over time. As a reminder, our model only studies the beginnings of growth kinetics and has no steady state. We will simply compare the starting situation ($t = 0$; $N_T(0)$) with the situation after growth, containing a larger number of cells ($t = f\left(\max_{cell}\right)$; $N_T = \max_{cell}$).

Figure 3 shows, in red, that for t = 0, 100% of the cells (i.e. 250) have a mass of *CI* = 0.1 AU and a zero variance, whereas for 5000 cells (*t* = 20), the average mass is approximately 0.8 AU, with a variance of 0.235. (A fitting test showed that the distribution at *t* = 20 could be likened to a "bathtub curve", but the data are insufficient and likely offer little interest).

This simulation shows the following:

- The initial distribution function of the cell masses of the inoculum cannot be maintained over time;
- The average cell size, $\overline{m}$, increases;
- The variability (variance, σ^2) of this variable also increases.

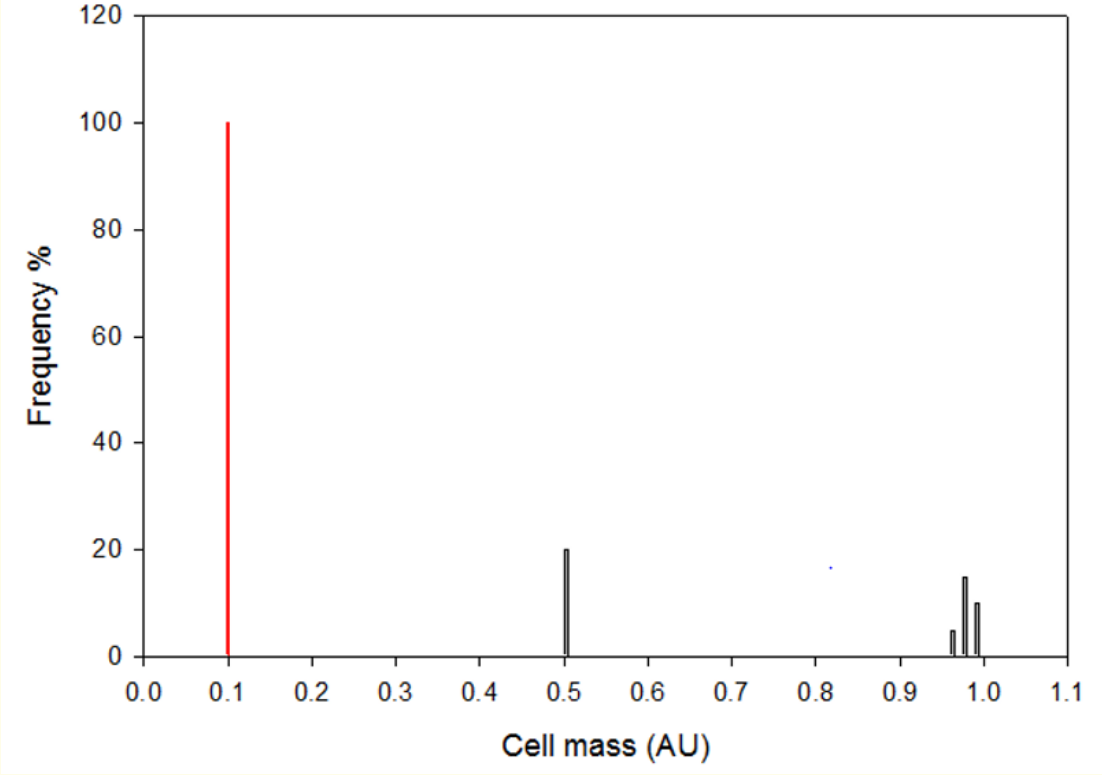

Figure 3: *This figure shows the histogram of the frequency distribution (%) of cell masses by bin. At t = 0 (in red), 100% of the cells have a mass of 0.1 AU and, obviously, zero dispersion; at t = 20 (5000 cells), the "distribution function" is completely modified and takes on the appearance of a "bathtub curve". The average increases drastically (from 0.10 to 0.79 AU) as well as dispersion (from 0.0 to 0.23). (For each histogram, the number of classes is computed by* $N_{CL} = \sqrt{cells\ number}$ *and the bin by:* $Et = (m_{\max} - m_{\min}) / N_{CL}$ *. For t = 0, the bin is, by exception, [0.1, 0.1] and there is only one class. The histogram representation for the deterministic case is questionable but allows comparison of different situations (UNF and GAU).)*

The DET case is certainly unrealistic but has already brought a large number of observations, which clearly remain to be established.

Uniformly distributed initial conditions (UNF)

An inoculum with a uniform cell mass distribution function is hardly more realistic than the deterministic situation. The UNF case, however, introduces the important notion of a nonzero variance of the initial masses. As a reminder, a uniform distribution over a range *a, b* is defined by $\frac{1}{b-a}$ for $a \le x \le b$ and 0 elsewhere [19] and mean and variance of

$$mean \equiv E(X) = \frac{b+a}{2} \quad (12)$$

$$variance \equiv E(X^2) - \left[E(X)\right]^2 = \frac{(b-a)^2}{12} \quad (13)$$

The initial conditions were defined by

$$m_i(0) = m_{moy} + \sigma_m \times R\left(U(0,\sigma_R)\right) \quad (14)$$

where m_{moy} is the average value of the initial cell mass, σ_m is the standard deviation of this mass and $R\left(U(0,\sigma_R)\right)$ is a random variable of zero mean and of standard deviation σ_R.

The random variable $R\left(U(0,\sigma_R)\right)$ was obtained by using the *RND* (1) function of Microsoft Basic Compiler 6.0, which returns a single precision variable of between [0,1] using the algorithm

$$R\left(U(0,\sigma_R)\right) = 2 \times RND(1) - 1 \quad (15)$$

The red histogram of figure 6 shows the result of a simulation as defined above, with an average value of 0.098 instead of 0.1 and a standard deviation of 0.06 (constants: $m_{moy} = 0.1$ AU and $\sigma_m = 0.1$).

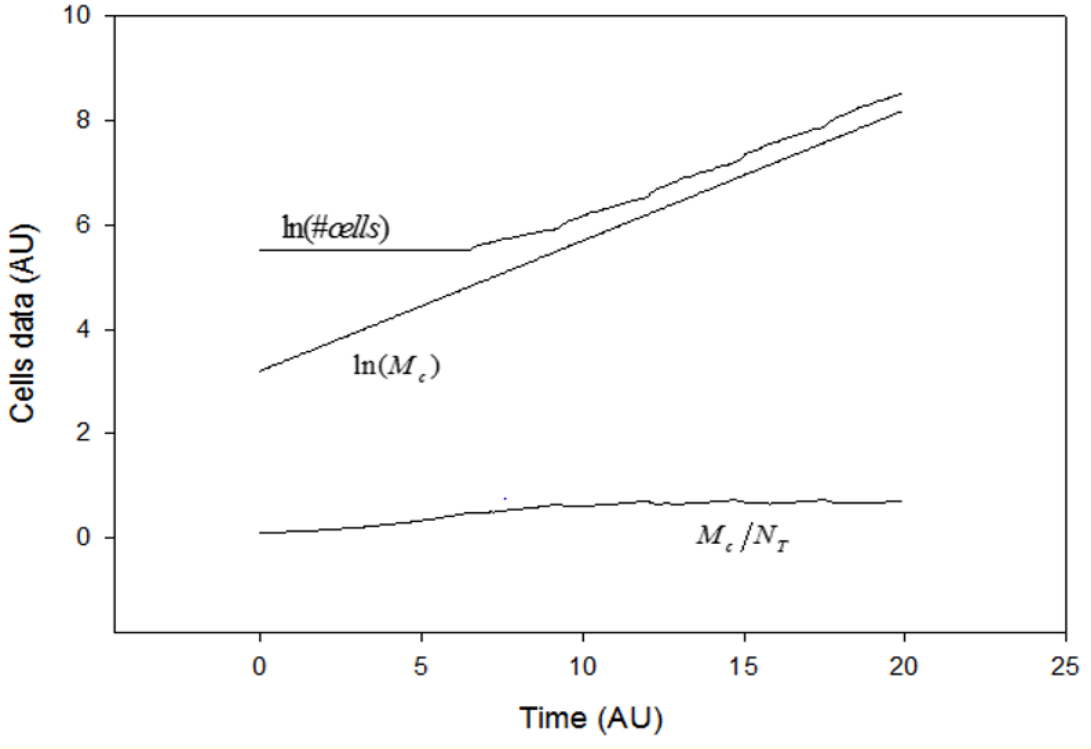

Figure 4: *Legend of figure 4 is identical to that of Figure 2, but this time, cell growth is no longer synchronous and curves are smoother than before. The two logarithmic curves are separate and the determination of the lag-time must be performed using the fitting of the strictly logarithmic part of the number of cells growth curve.*

Figure 4 again shows the temporal evolution of cellular data. This time, the growth of cells has lost its synchronous character and is similar to the figures found in the literature [1-3]. The logarithmic curve of the number of cells shows a lag time, a pre-logarithmic phase and a logarithmic phase. The latency is approximately 7 - 8 AU, but there the point of intersection between the two logarithmic curves has disappeared. The lag time is later determined by the (virtual) intersection of the regression line constructed on the exponential growth phase (see figure 5) and the horizontal line of ordinate.

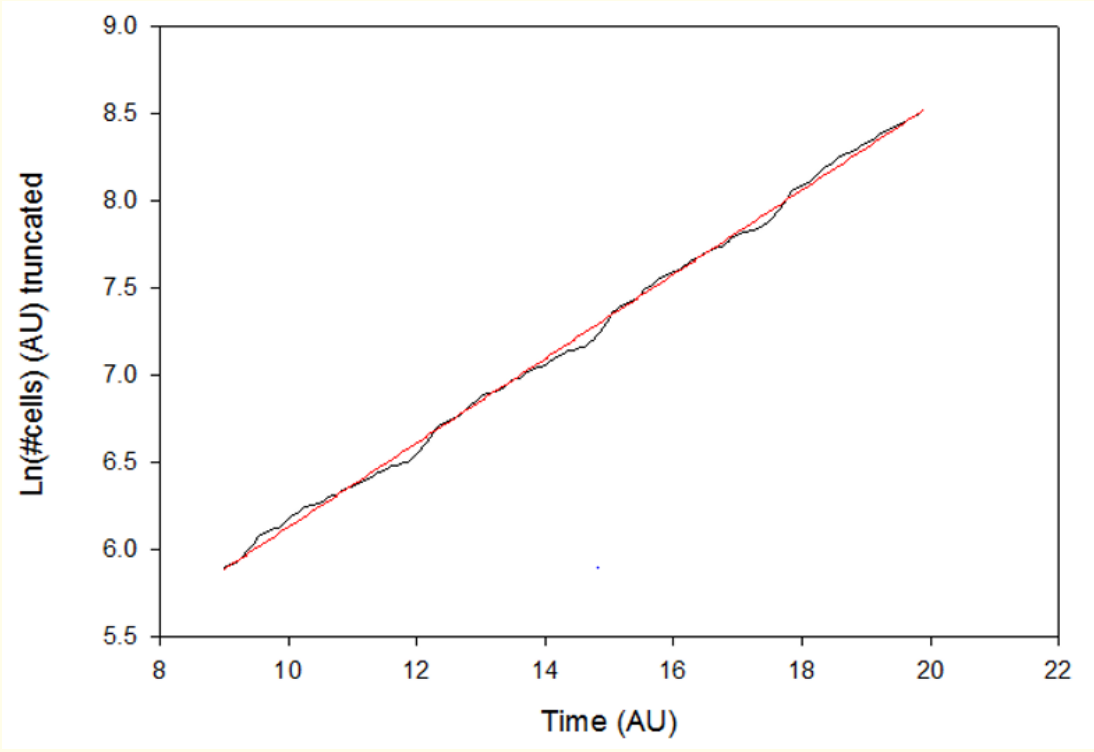

Figure 5: *Showing the linear regression (in red) of the strictly logarithmic part of the number of cells (in black). The fitting is excellent and gives a correlation coefficient of very close to 1 and* $\ln(N_T(t)) = 3.7 + 0.24t$ *. Just introduce the value* $\ln(N_T(t)) = \ln(N_T(0))$ *in this equation and solve it in relation to time to find* $\tau \approx 7.6$ *AU (see text). This value is somewhat skewed because* $\mu_{max} = 0.24$ t^{-1} *is slightly lower than its nominal value (0.25* t^{-1}*).*

The results of the fitting are as follows:

$$\ln(N_T) = a + k\,t \qquad (16)$$

$$a = 3.70\ ;\ k = 0.24\ ;\ r^2 \approx 1.0$$

The lag time $t = \tau$ is then given by

$$\tau = \frac{\ln(N_T(0)) - a}{k} \approx 7.6 \text{ AU} \qquad (17)$$

The latency time is therefore diminished compared to the deterministic case.

It should be noted that the relationship (using $k = \mu_{max}$)

$$\mu_{max}\tau = cte \qquad (18)$$

is still valid, but the constant is now more complex and no longer only depends on the initial cell mass, as in (11). (It is smaller and is 1.8 instead of 2.3. A discussion of the variation of this constant is outside the scope of this work).

Figure 6 shows, in red the histogram of initial cell masses (250 cells at $t = 0$) with an average mass close to 0.1 and a standard deviation on the order of 0.06. For 5000 cells ($t \approx 20$) the average mass increased considerably (0.72 > < 0.1 AU), as well as the standard deviation (0.14 > < 0.06). We also note that the distribution function is not preserved over time. A priori, it would not seem familiar and may even present a certain character of multimodality and asymmetry to the right (all of this remains to be confirmed).

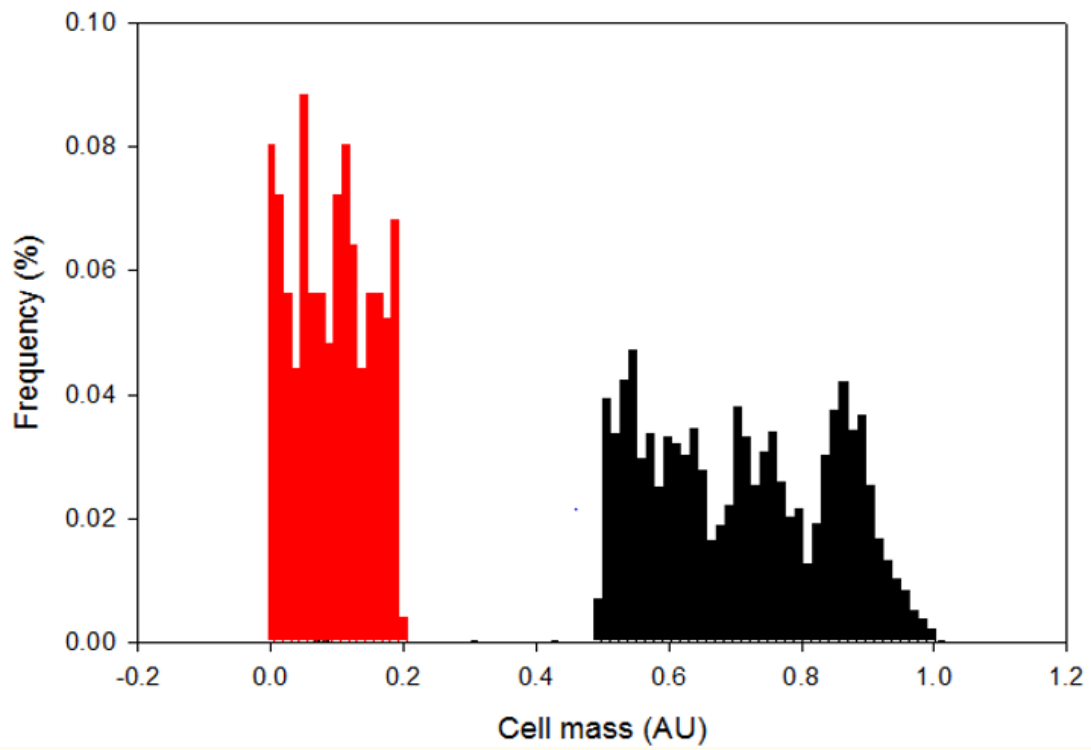

Figure 6: *Histogram of frequencies distribution (%) of cell masses by bin. At t = 0 (in red), the simulation of the uniform distribution is shown, with an average of 0.098 AU and a standard deviation of 0.059; for t = 20 (5000 cells) we see that the uniform distribution is not conserved (and could be multimodal?). The average of the cell masses is 0.72 AU and the standard deviation also increases up to 0.14. On the whole, the behavior is the same as for the deterministic case. (The method of constructing histograms is the same as in Figure 3).*

Initial conditions normally distributed; Gaussian (GAU)

While the two previous cases (DET and UNF) may seem unrealistic (or exceptional), the same is not true the hypothesis of an initial Gaussian distribution (GAU), which has been observed previously by others [14,15].

As a reminder, a Gaussian distribution function has the general form [20]

$$f(x)=\frac{1}{\sigma\sqrt{2\pi}}\exp\left(\frac{-1}{2}\left(\frac{x-\overline{x}}{\sigma}\right)^2\right) \quad (19)$$

where $\overline{x}$ is the mean and σ the standard deviation.

As before, the initial conditions were defined by

$$m_i(0)=m_{moy}+\sigma_m\times R\left(\mathrm{G}(0,\sigma_R)\right) \quad (20)$$

where $R\left(\mathrm{G}(0,\sigma_R)\right)$ is a Gaussian random variable of zero mean and σ_R standard deviation. This variable was obtained by the MULLER-BOX polar form method [21]

$$\begin{aligned} v1&=2\times RND(1)-1\\ v2&=2\times RND(1)-1\\ \vartheta&=v1^2+v2^2\\ R_i(G(*))&=vi\left(\sqrt{-2(\ln(\vartheta)/\vartheta}\right)\ ;\ i=1,2 \end{aligned} \quad (21)$$

$RND(1)$ is defined as before.

Figure 7 shows the temporal evolution of cellular data. These data are highly similar to the UNF case, but the noisy nature of the curves is attenuated.

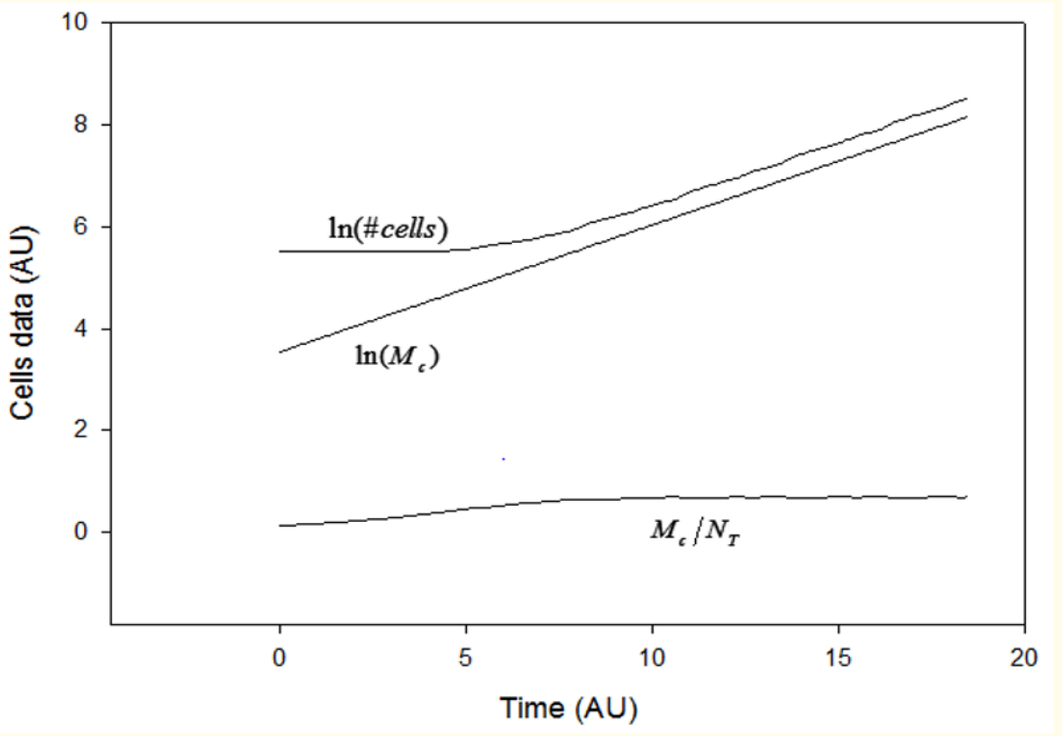

Figure 7: *See legend in figure 2. The smooth and noiseless appearance of the curves is currently still accentuated and takes a more realistic look compared to that of the literature. Qualitatively, we now see a pre-logarithmic stage just after the latency. The regression of the logarithmic curve straight line part is* $\ln\left(N_T(t)\right)=3.98+0.25t$ *and has a correlation coefficient almost equal to 1. The lag time, greatly reduced, is now* $\tau=6.17$ *AU.*

The latency determination is carried out as before. The fitting of the logarithmic growth of the cells yields the following:

$$\ln(N_T) = a + k\,t$$
$$a = 3.98\ ;\ k = 0.25\ ;\ r^2 \approx 1.0 \qquad (22)$$

Using (17), it is determined that $\tau = 6.17$ AU. This latency is smaller than that of the UNF case.

Again, the distribution function is not preserved during growth. Using the same constants as before for (20), $m_{moy} = 0.1$ AU and $\sigma_m = 0.1$, figure 8 clearly shows that the initial Gaussian distribution (250 cells for *t* = 0) disappears at 5000 cells (*t* = 8). The average cell mass increases from 0.14 to 0.70 AU and the standard deviation rises from 0.08 to 0.14.In addition, note that the time taken to reach 5000 cells decreases drastically, from 20 AU previously (DET and UNF) to only 8 AU, while the cell growth rate is unchanged.

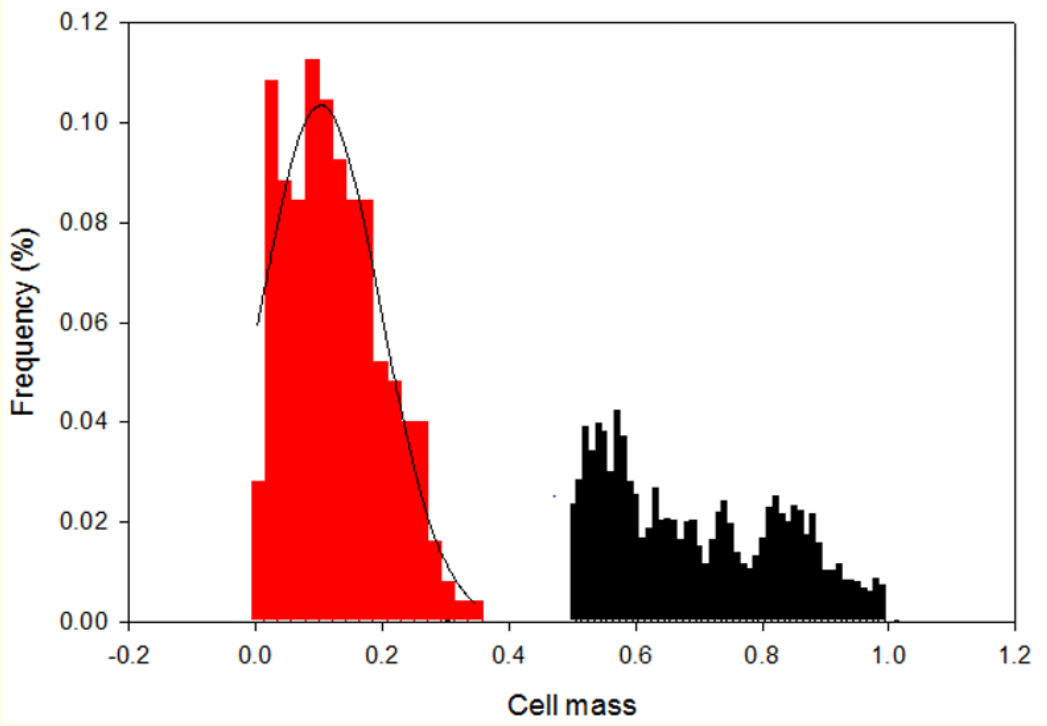

Figure 8: *The Gaussian distribution function (in red) appears at t = 0 (250 cells). The regression curve (in black) presents an average value equal to 0.10 AU and a standard deviation of 0.095 (the correlation coefficient is 0.94), well corresponding to the definition (19) with* $m_{moy} = 0.1$ *AU and* $\sigma_m = 0.1$. *(The Kolmogorov-Smirnov normality test is passed: P = 0.3206). At 5000 cells (t = 8 – black bars), the mean and the standard deviation both increase from 0.14 to 0.70 AU and from 0.08 to 0.34 respectively (values calculated during the simulation and not resulting from the fitting).*

Again, the average and dispersion of cell masses increase.

In addition, note that the time taken to reach 5000 cells decreases drastically, from 20 AU previously (DET and UNF) to only 8 AU, while the cell growth rate is unchanged.

Discussion

In this preliminary study, we choose the inoculum as the only stochastic state variable. It is obvious that this is appreciably simplistic; however, this choice is guided by the observation that growing populations of microorganisms have statistically distributed characteristics (masses and sizes) [22-24]. There is currently no consensus on the form of distribution functions [13], and we have doubts that this form can be common to all strains and/or all growing situations. However, since the inoculum is a growing cell population fraction, it seems clear that this inoculum could not be a uniform singular sample of the total population. The stochastic hypothesis for the inoculum therefore seems justified.

However, we describe at least four additional parameters for the description of the lag phase process (see Results, § 2). It is highly unlikely that these parameters are non-noisy constants. For example, it is almost inconceivable that the critical mass of division, $m_{i,crit}$, is a non-noisy value (the same is true for the inoculum size, in a natural system, or the growth rate, *k,* or the partition coefficient, α). The combinations of these potentially random values are very large, and it would be interesting (and profitable) to make a sensitivity analysis (possibly extended to other values) to optimize the simulation by highlighting those parameters whose statistical variability could be preponderant.

To conclude, it appears that our simple stochastic representation model enables us to understand phenomena as complex as synchronous growth and to validate a previously empirical relationship, linking the lag time and the maximum growth rate.

Synchronous growth is characteristic of the deterministic case (DET), which resembles an unnatural situation. Moreover, in real cases, the synchronicity disappears progressively over time, which is not the case in our model. It may be assumed that in the true case, the initial deterministic character is gradually giving way to greater statistical variability (not related to that of the inoculum). This last observation confirms that although rich and promising, this model is still too simple to grasp the complexity of the phenomenon. What we could hope for is that notwithstanding this simplicity, the model could be sufficiently effective to help practitioners in the fields of agri-food, medicine or any other applied field of activity involved in the initial phase of the "cycle" of cell growth.

Conclusion

Given the complexity of a more realistic model, we plan to attempt to apply the computing techniques of population balance [23,25]. This technique of distribution calculations, which is associated with several state variables and constants, seems particularly well-suited to our problem. However, it will be necessary to check whether it is possible to preserve a sufficient "level of simplicity and applicability", which does not always seem to be easy (or even possible) for all biological representations.

Annex

Demonstration that $M_c(t)$ is isomorphic to $m_i(t)$.

In the model described above, we have that for each cell

$$m_i = m_i(0).\mathrm{e}^{kt} \qquad \text{(A.1)}$$

with *k,* a constant and identical specific growth rate for each cell. This is obviously an approximation since we know that

$$k_i = k + \varepsilon_i \qquad \text{(A.2)}$$

where one simply admits that $\varepsilon_i << 1, \forall i.$

The form (A.1) must, however, be compatible with the equally exponential form of the total cell mass, M_c. The demonstration that this is true is as follows. By definition

$$M_c = \sum_{i=1}^{N_T} m_i \qquad \text{(A.3)}$$

After differentiating, we obtain the following:

$$\frac{dM_c}{dt} = \sum_i \frac{dm_i}{dt}$$

and, using (A.1)

$$\frac{dM_c}{dt} = \sum_i km_i = k\sum_i m_i$$

which gives via (A.3)

$$\frac{dM_c}{dt} = kM_c$$

and by integrating

$$M_c(t) = M_c(0)\,\mathrm{e}^{kt} \qquad \text{(A.4)}$$

which shows that (A.1) is consistent with the experimental observation that the form of the total cell mass growth is exponential.

Conflict of Interest

There is no conflict of interest associated with this work.

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