ON THE MASS DISTRIBUTION MODEL FOR MICROBIAL CELL POPULATIONS

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The full implications of a statistical model for growth of a microbial cell population using cell mass as the index of physiological state have been examined by solving the partial differential integral equations resulting from the model. Calculations reveal that a lag phase is predicted during the initial stages of batch growth although no specific cellular mechanism for the phenomenon of lag had been incorporated into the model. The model predicts several situations of batch and continuous growth in which the population density and biomass concentration show opposing trends due to significant variation in the cell mass distribution with time.

LIST OF NOTATIONS

c Concentration of biomass, gm./liter
c Concentration of substrate, gm./liter
c Concentration of substrate in feed, gm./liter
f(m, t) Density of mass distribution of cells at time t, gm.\(^{-1}\)
k\(_{\text{m}}\) Michaelis constant in growth expression for single cell, gm./liter
m, m'; m\(_{c}\) Cell mass, gm.; mean cell mass at division, gm.
N(t) Population density at time t, cells/liter
\( p(m, m') \) Mass distribution density for daughter cells from mother cell of mass \( m' \), \( \text{gm.}^{-1} \)
\( r(m, c_s) \) Rate of growth of single cells of mass \( m \) in environment with substrate concentration \( c_s \), \( \text{gm./hr.} \)
\( r'(m, c_s) \) Rate of mass uptake of cells of mass \( m \) in environment with substrate concentration \( c_s \), \( \text{gm./hr.} \)
\( r''(m) \) Rate of mass release of cells of mass \( m \), \( \text{gm./hr.} \)
\( R \) Radius of cell, cm.
\( t \) Time, hr.
\( W(m, t) \) Number density of cells of mass \( m \), \( \text{gm.}^{-1} \)
\( \beta(m) \) Fraction of substrate in mass uptake by cells of mass \( m \)
\( \gamma(m) \) Fraction of substrate in mass release by cells of mass \( m \)
\( I(m, c_s) \) Transition probability function for division of cells of mass \( m \) in environment with substrate concentration \( c_s \), \( \text{hr.}^{-1} \)
\( \epsilon \) Measure of spread of division mass about the mean \( m_c \)
\( \phi_m \) Maximum flux of mass across cell surface, \( \text{gm./cm.}^2 \text{hr.} \)
\( \mu_c \) Specific rate of mass release by cells, \( \text{hr.}^{-1} \)
\( \rho \) Density of cell, \( \text{gm./cm.}^3 \)
\( \theta \) Holding time, \( \text{hr.}^{-1} \)

**Introduction.** Eakman, Fredrickson and Tsuchiya (1966) have presented a statistical model for a microbial cell population in which the physiological state of the individual organism is assumed to depend only on its mass. Earlier, Koch and Schaechter (1962) had also suggested cell mass as an index of the physiological state. Starting from this premise Eakman et al. derived so-called population balance equations for the dynamics of microbial populations in batch and continuous cultures. The mathematical complexity involved in the solution of these equations prevented a detailed evaluation of the implications of this model and only certain simplified features could be extracted. Thus it was assumed, by these authors, for instance, that cells divided into two equal halves; this simplification permitted the solution of the steady state equations for a continuous culture. Solution of the transient equations even for the simplified model in batch and continuous cultures presented difficulties.

In this paper we shall present a detailed analysis of the mathematical model formulated by Eakman et al. Changes only of a very minor nature have been introduced into their model. The assumption of symmetric division is relaxed and the transient and steady state equations have been solved by a numerical method referred to as the method of weighted residuals.

**Mathematical Model.** Consider a well-stirred vessel containing a suspension
of microorganisms in a nutrient medium, which is continuously fed with a medium of constant concentration, while simultaneously the homogeneous culture is withdrawn from the vessel. The feed and withdrawal rates are both equal to $q$ and the volume of the culture in the vessel remains constant at $V$. In the following we shall assume that cells divide by binary fission. There is a single growth-limiting substrate in the nutrient medium. The growth of single cells is a known function of cell mass and the substrate concentration in the environment. Moreover no cell death is assumed.

Let us define:

- $W(m, t)\, dm =$ Number of cells per unit volume of culture at time $t$ that have mass between $m$ and $m + dm$.
- $\Gamma(m, c_s)\, dt =$ Transition probability that a cell of mass $m$ at time $t$ in environment with substrate concentration $c_s$ will divide in the time between $t$ and $t + dt$.
- $p(m, m')\, dm =$ Probability that a daughter cell formed from a mother cell of mass $m'$ has a mass between $m$ and $m + dm$.
- $r(m, c_s) =$ Growth rate or rate of mass increase of a cell of mass $m$ in environment with substrate concentration $c_s$.

Assuming that individual cells behave independently a number balance can be made on cells with mass between $m$ and $m + dm$. For the complete derivation of the equation we refer to Eakman et al. (1966). The population balance yields

$$\frac{\partial W}{\partial t} + \frac{\partial}{\partial m} [r(m, c_s)W] = 2 \int_m^\infty \Gamma(m, c_s)p(m, m')W(m', t) \, dm' - \left[ \Gamma(m, c_s) + \frac{1}{\theta} \right] W. \tag{1}$$

Since the behavior of the population depends on and alters the substrate concentration in the environment (1) must be considered in conjunction with the following substrate equation:

$$\frac{dc_s}{dt} = \frac{c^0_s - c_s}{\theta} - \int_m^\infty [\beta(m)r'(m, c_s) - r''(m)\gamma(m)]W(m, t) \, dm. \tag{2}$$

In (2), $c^0_s$ represents the concentration of the substrate in the feed, $r'(m, c_s)$ the rate of mass uptake and $r''(m)$ the rate of mass release by cells of mass $m$; $\beta(m)$ and $\gamma(m)$ represent the fractions of mass as substrate taken up and released respectively by cells of mass $m$. Clearly

$$r(m, c_s) = r'(m, c_s) - r''(m). \tag{3}$$
Equations (1) and (2) are subject to the initial conditions

\[ W(m, 0) = N_0 g(m); \]

\[ c_s(0) = c_s; \]

where \( N_0 \) is the initial population density, \( g(m) \) the initial distribution of mass and \( c_s \) is the initial substrate concentration.

By assuming that the distribution of cell mass at division is Gaussian, Eakman et al. (1966) derived the following expression for the transition probability function \( \Gamma(m, c_s) \):

\[ \Gamma(m, c_s) = \frac{2}{e^{\sqrt{\pi}}} \frac{e^{-\frac{(m-m_c)^2}{\epsilon}}}{\text{erf}(\frac{m-m_c}{\epsilon})} \cdot r(m, c_s). \]  

A Gaussian distribution was also assumed for the probability density \( p(m, m') \) but we shall assume here a simpler relation of the form

\[ p(m, m') = 30 \frac{m^2(m'-m)^2}{m'^3}. \]  

Expression (6) satisfies the following requirements:

\[ p(m, m') = p(m'-m, m') \]  

and

\[ \int_0^{m'} p(m, m') \, dm = 1. \]

The growth rate function \( r(m, c_s) \) remains to be specified. We will assume that the cells are rod-shaped organisms and use the expression employed by Eakman et al. (1966) following von Bertalanffy's (1942) reasoning that the rate of mass uptake by the cell is proportional to the cell surface area while the rate of mass release should be proportional to the cell mass. Thus:

\[ r'(m, c_s) = \frac{2}{R \rho} \frac{\phi_m c_s m}{(k_0 + c_s)} \]  

and

\[ r''(m) = \mu_0 m. \]

In (9) the quantity \( \phi_m \) represents the maximum flux of the mass uptake across the cell surface, \( R \) is the radius of the cell and \( k_0 \) is a constant. It is clear from (9) and (10) that the individual cell will grow at the same rate for a given mass and substrate concentration regardless of the phase of growth. Thus a cell for example should grow at the same rate be it in the lag phase or in the exponential
phase of a batch culture, as long as the cell has the same mass and substrate concentration is the same.

In order to make numerical computations it was assumed that the function $\beta(m)$ was a constant and $\gamma(m)$ was identically zero. The latter assumption implies that the mass released by the cell contains no substrate. Table I lists the numerical values used for the various constants in the model.

<table>
<thead>
<tr>
<th>$m_c$</th>
<th>$k_s$</th>
<th>$\mu_c$</th>
<th>$\phi_m$</th>
<th>$\beta$</th>
<th>$\epsilon$</th>
<th>$\rho$</th>
<th>$c_0^0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$3 \times 10^{-12}$ gm</td>
<td>0.02 gm/liter</td>
<td>1 hr$^{-1}$</td>
<td>$6 \times 10^{-6}$ gm/cm$^2$hr</td>
<td>0.75</td>
<td>$3\sqrt{2} \times 10^{-13}$ gm</td>
<td>1.01 gm/cm$^3$</td>
<td>2.5 gm/liter</td>
</tr>
</tbody>
</table>

Solution of Equations. A detailed analysis of the method used for the simultaneous solution of (1) and (2) is avoided here since this has been discussed by Subramanian and Ramkrishna (1970). Briefly, the method consists in assuming that the unknown function $W(m, t)$ can be expanded as a linear combination of basic functions (preferably from a complete set) in the semi-infinite domain. The coefficients of this expansion are evaluated so that the residual obtained by substituting the trial solution into (1) can be approximated as closely to zero as possible for all values of $m$ in the semi-infinite domain. This is done by stipulating that the weighted average of the residual is zero over the domain. The weighting functions belong to a complete set in the semi-infinite domain. Thus a set of equations in the undetermined coefficients of expansion of the trial solution (algebraic for the steady state case and differential for the unsteady state case) are obtained and can be readily solved. This method is known as the method of weighted residuals. The trial or basic functions used were the Laguerre functions $d^n[e^{-x}x^n]/dx^n$ and the weighting functions belonged to the class $\{x^n e^{-\lambda x}\}$, $\lambda > 0$.

RESULTS OF COMPUTATION

Continuous Culture:

(a) Steady state: The steady state equations are obtained by putting the time derivatives equal to zero in (1) and (2). The equations are

$$
\frac{d}{dm} [r(m, \tilde{\epsilon}_s) \bar{W}] = 2 \int_{m}^{\infty} \Gamma(m', \tilde{\epsilon}_s)p(m, m') \bar{W}(m') \, dm' - \left[ \Gamma(m, \tilde{\epsilon}_s) + \frac{1}{\theta} \right] \bar{W}(m)
$$

(11)
and
\[
\frac{1}{\tilde{\vartheta}} (c^0_s - \tilde{c}_s) = \int_{m}^{\infty} \beta r'(m, \tilde{c}_s) \tilde{W}(m) \, dm. \tag{12}
\]

Before we present the solutions of these equations we pause to examine the nature of \( \Gamma(m, c_s) \), the transition probability function for division, and the function \( p(m, m') \). Figure 1 shows a plot of \( \Gamma(m, c_s) \) versus \( m \) for a fixed substrate concentration \( c_s \). It is seen that the probability of division is practically zero until the cell mass is greater than \( 2 \times 10^{-12} \) gms and increases steeply when the cell mass is around \( 4 \times 10^{-12} \) gms. Figure 2 shows a plot of \( p(m, m') \) versus \( m \) for a fixed value of \( m' \). An "even split" is the most probable result, as well as the average result.

Figure 3 shows the steady state distribution of cell masses for two different holding times. Clearly the number of cells in a steady state for the larger holding time is seen to be less than that for the smaller holding time. This is
Figure 2. Distribution of daughter cell mass.

Figure 3. Steady state cell mass distribution for different holding times.

\[ \phi(x) = e^{x^2 - L(x)} \]

\[ n(x) = e^{2x} \]

N = 13 FNS

\[ \theta = \text{HR}. \]

\[ \theta = 2 \text{ HR}. \]
because larger holding times will lead to low steady state substrate concentrations, reducing the growth rate of the individual cell to such an extent that it offsets the effect of slower washout. The result is that for larger holding times the individual cells do not grow rapidly enough to reach masses for which the probability of division is high. This increase in population density with decrease in holding time will not hold for holding times only slightly larger than the minimum holding time (i.e., that holding time which results in a washout of the population). The distribution for both holding times is seen to attain a peak for a cell mass of nearly $2 \times 10^{-12}$ gms. This is what one might expect from an examination of Figures 1 and 2; i.e., cells of mass around $4 \times 10^{12}$ gms have a high probability of division and on dividing yield daughter cells the majority of which have a mass about $2 \times 10^{-12}$ gms. Moreover, cells of a mass $2 \times 10^{-12}$ gms have a negligible probability of division.

\[
W(m,\omega) = \frac{m}{\omega} e^{-\frac{m}{\omega}} \times 10^{24}
\]

\[
C(\omega) = 0.36 \text{ gm/l}
\]

\[
C_s(\omega) = 0.50 \text{ gm/l}
\]

\[\theta = 2 \text{ hrs.}\]

Figure 4. Transient continuous propagator; cell mass distribution for initial condition (13)
(b) **Transient state**: Transient calculations were made by solving (1) and (2) subject to various choices of the initial conditions given by (4). Three different initial distributions were considered. In the first it was assumed that a large fraction of the initial population was of small mass (smaller than $2 \times 10^{-12}$ gms), the algebraic expression for the initial distribution being given by

$$W(m, 0) = \frac{m}{\epsilon} e^{-m/\epsilon 10^{24}}. \quad (13)$$

Figure 4 shows the evolution of this initial condition to the steady state distribution. The smaller cells are seen to be readily washed out initially, while cells with larger mass gradually accumulate with time. Figure 5 shows a plot of the population density, biomass concentration (which is the first moment of

![Figure 5. Transient continuous propagator: variation of population density, biomass and substrate concentrations for initial condition (13)](image-url)
$W(m, t)$ and the substrate concentration against time. The population density first shows a decrease while the biomass concentration increases. The decrease of the population density is obvious because cells capable of division formed only a small fraction at the outset.

Figure 6 shows the result of transient calculations for a continuous culture when the initial population consists of a larger proportion of cells of larger mass. Figure 7 shows a plot of the population density, biomass and substrate concentrations versus time for this initial condition. The algebraic expression for the initial condition was

$$W(m, 0) = 0.01 \left( \frac{m}{\epsilon} \right)^5 e^{-m/\epsilon} 10^{24}. \quad (14)$$

Figure 7 shows a steeper rise of the population density than that of the biomass concentration during the initial stages.
A third initial condition was assumed to be

\[ W(m, 0) = 0.1 \left( \frac{m}{\epsilon} \right) e^{-m/\epsilon} 10^{24}. \]  

Expression (15) implies the same initial distribution as in (13) except that the total number of organisms has been reduced by a factor of 10. Figure 8 shows how the initial distribution in (15) evolves to the steady state distribution. Figure 9 shows the corresponding time courses of population density, biomass concentration and substrate concentration. A noteworthy fact is that with
further decrease in the size of the initial population for the initial distribution in (15) the cells would have been washed out of the culture vessel. Of course this would not be the case if for the same size initial population we had an initial distribution closer to that in (14). Thus we see that washout of the culture may occur even when the holding time is above the minimum holding time. Results of this kind have been predicted by the nonsegregated models of Ramkrishna, Fredrickson and Tsuchiya (1966, 1967). It is interesting to note that while the mass distribution model can be shown to yield the nonsegregated model of Monod with a correction for endogenous metabolism the former is capable of making predictions quite outside the scope of the latter. This strengthens the case for segregated models in the theoretical development of the
behavior of microbial populations. We now proceed to a discussion of the prediction of the mass distribution model for batch cultures.

**Batch Culture.** Equations (1) and (2) represent growth in a batch culture when the terms containing the holding time $\theta$ are deleted. The batch transients were calculated using the initial condition (13). Thus the inoculum consisted mainly of cells of small mass. Figure 10 shows the results of the computation,
Figure 10. Batch growth, variation of cell mass distribution for initial condition (13) as plots of $W(m, t)$ versus $m$ at various instants of time. Figure 11 shows the variation of population density, biomass concentration, and the substrate concentration during batch growth. Until a time of about 0.8 hours after the onset of batch growth the proportion of small-sized cells diminishes steadily while that of large-sized cells rises. The population density does not change significantly, as seen from Figure 11, during this period of growth. The biomass concentra-
Figure 11. Batch growth, variation of population density, biomass and substrate concentrations for initial condition (13)

tion, although not increasing rapidly, shows a slightly faster rise than the population density. Beyond 0.8 hours and until about 1.2 hours Figure 10 shows that the profiles of $W(m, t)$ versus $m$ for each $t$ would be "similar." In other words the distribution of mass represented by

$$f(m, t) = \frac{W(m, t)}{N(t)},$$

where $N(t)$ represents the population density, becomes independent of time during this time interval. Figure 11 shows that during this time interval growth is exponential. Beyond 1.2 hours the mass distribution curve starts shifting to the left (i.e., it is time-dependent); the substrate concentration drops to low levels and exponential growth ceases. The population density thereafter remains constant since no death has been assumed in the model. However, the cells show consistently a loss of mass due to the rate of mass release given by (10), which is the reason for the shift to the left of the distribution curves. The biomass concentration also drops for the same reason beyond the exponential phase of batch growth. A phase of decline could well have been predicted by
this model if a death probability had been included. Since the mass distribution beyond the phase of exponential growth is strongly biased to the left, inoculation from this population into a fresh batch culture would lead to an initial lag phase. The period of lag would lengthen as the transfer of the inoculum is made from the parent culture in more advanced stages of post-exponential phase. On the other hand, a transfer from an exponentially growing culture would produce no lag phase in the new culture since the initial distribution for the latter is the same as the time-independent distribution attained during exponential growth. The general experimental findings are in accord with these observations (for example, Penfold (1914)).

Although a lag phase is predicted by the mass distribution model, care must be taken to interpret this lag phase in terms of the observed lag phase in experiments. The lag phase predicted by the model is a period during which the initial left-biased distribution changes to the time-independent distribution during exponential growth. There are no changes in the response of the individual organism to the environment, according to the model. Thus if one were to select out the larger cells from a population in a culture at a stage of its post-exponential phase and inoculate them into a fresh medium, the model would not predict any lag. If the inoculum to a fresh medium consists of cells entirely in the mass range for which the probability of division is high, one may even get a situation of increased growth rate at the beginning, gradually tapering to the exponential growth rate. While it is not certain that these predictions may be true, the above experiment appears to be an extremely interesting one. If the observed lag phase is mainly a period during which the individual organism is "adapting" itself to the new environment, then the lag phase should not be sensitive to the initial mass distribution of the population. If on the other hand, the observed lag is mainly due to adjustment of the mass distribution then the length of the lag phase should be subject to considerable variation if the mass distribution of the inoculum is varied by some such process as filtration. In all likelihood, the observed lag phase may include both kinds of lag. It has been tacitly assumed in the above discussion that transfer of inoculum from one culture to another involved cultures of the same chemical media.

As pointed out by Eakman et al. (1966), a single parameter such as cell mass or cell age cannot adequately represent the physiological state of the organism if it is desired to take into account the fact that the response of a cell to the external environment depends on the cell's origin. Thus the state of the individual cell may require a vector description, such as that of Fredrickson et al. (1967). In order to obtain a mathematically tractable model the dimension of this vector must be as small as is reasonable.
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LITERATURE


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