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NOTATION

$(x, y, z) = \vec{r}$ = position variables, vector
t = time
A,L = macroscopic characteristic lengths
$\vec{v}$ = velocity vector, Darcy velocity vector
$\vec{U},\vec{w}$ = dimensionless velocity vector
W = characteristic scalar flow speed
C = concentration
$p_{(\vec{r},t)}$ = pressure function
p_i,p_o = inlet and outlet pressure
k = permeability
F = electrical formation resistivity factor
D = diffusion coefficient
$\mathcal{D}$ = effective dispersion coefficient (subscripted T or L for transverse or longitudinal)
$a_{1T},a_{1L};a_{2T}, \ldots$ = coefficients in expansion of $\mathcal{D}_T$ and $\mathcal{D}_L$
$\vec{G}$ = acceleration due to gravity, vector directed vertically upward

Greek Letters

ξ,η,ζ = dimensionless position variables
τ = dimensionless time
λ = microscopic characteristic length
ϕ = porosity
μ = fluid viscosity
ρ = fluid density
σ = interfacial tension
Σ = surface of volume element V

Superscript

$\hat{}$ = dimensionless variable, as in $\hat{p}$

LITERATURE CITED

1. Rapoport, L. A., and W. J. Leas, *Trans. Am. Inst. Mining, Met. Petrol, Engrs.*, **198**, 139-148 (1953).
2. Geertsma, J., G. A. Croes, and N. Schwarz, *Trans. Am. Inst. Mining, Met., Petrol. Engrs.*, **207**, 118-127. (1956) .
3. Miller, E. E., and R. D. Miller, *J. Appl. Phys.*, **27**, 324-32 (1956); also Elrick, D. E., J. H. Scandrett, and E. E. Miller, *Proc. Soil Science Soc. Am.*, **23**, 329-32 (1959).
4. Mattax, C. C., and J. R. Kyte, Paper presented at the 36th annual fall meeting of the SPE of *Am. Inst. Mining, Met. Petrol. Engrs.*, (SPE-187) (October 1961).
5. DeJong, G. Josselin, *Trans. Am. Geophys. U.*, **39**, 67-74; 1160-62 (1958).
6. Scheidegger, A. E., *J. Appl. Phys.*, **25**, 997-1001 (1954).
7. Saffman, P. G., *J. Fluid Mech.*, **6**, 321-349 (1959).
8. Aris, Rutherford, *Chem. Eng. Sci.*, **2**, 194-198 (1959).
9. Bear, Jacob, *J. Geophys. Res.*, **66**, 1185-1197 (1961).
10. Aronofsky, J. S., and J. P. Heller, *Petr. Trans. Am. Inst. Mining, Met. Petrol. Engrs.*, **210**, 345-349 (1957).
11. Blackwell, R. J., W. M. Terry, and J. R. Rayne, *ibid.*, **216**, 1-8 (1959).
12. Grane, F. E., and G. H. F. Gardner, *J. Chem. Eng. Data*, **6**, 283-287 (1961).

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Continuous Propagation of Microorganisms

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Microorganisms undergo a cycle of maturation during their life-span in a proliferating culture. This gives rise to a distribution of degrees of cellular maturation in the culture. The gross rate of any metabolic process carried on by the culture is therefore an average over all degrees of maturation present.

Equations embodying the life-cycle concept are developed for cultures of organisms which proliferate by binary fission and do not form spores. The equations are solved for the age distribution in the special case of a continuous, steady state fermentation. Expressions for calculating gross metabolic rates and cell size distributions are derived. Finally, the stability of the steady state fermentor is discussed.

Monod (*19*), Novick and Szilard (*22*), Maxon (*18*), Herbert et al. (*11*), and others have developed theories of microbial propagation which, in some cases, give accurate quantitative representation of certain aspects of growth phenomena. The theories mentioned are based on two postulates.

1. The growth rate of a culture is proportional to the rate of consumption of substrate.
2. The specific growth rate of a culture is some function of the substrate concentration.

These two postulates obviously apply only to cases where the availability of one substrate limits the growth rate.

The two postulates may be expressed mathematically as

$$R = -\beta R_s \tag{1}$$

$$R/C = F(C_s) \tag{2}$$

in which C is the microbial concentration, C_s is the substrate concentration, and β is the yield coefficient. It is usually assumed that the function $F(C_s)$ is of the Michaelis-Menten form [Monod (*19*)]

$$F(C_s) = \frac{\mu_m C_s}{K_s + C_s} \tag{3}$$

where μ_m and K_s are constants, the former being the maximum specific growth rate.

It is not difficult to use Equations (1) and (2) to compute microbial concentration, substrate concentration, and productivity for a continuous, stirred-tank fermentor operating in the steady state. For unsteady state operation or for batch operation, both in stirred-tank fermentors, the problem is more complicated. However, it can always be solved by standard techniques of numerical analysis such as the Runge-Kutta method described by Lapidus (*15*), even when $F(C_s)$ is more complex than indicated by Equation (3). The theory and the numerical method of integration can also be applied to the design of fermentors of the tubular type, provided that one is justified in neglecting longitudinal mixing in the tube. If longitudinal mixing cannot be neglected, the residence time distribution discussed by Danckwerts (*6*) and others becomes important, and Reusser (*24*) has shown how empirical residence time data can be used for the design of tubular fermentors.

It is easy to see that the postulates expressed by Equations (1) and (2) are attempts to extend ideas of chemical kinetics to microbial cells. Thus, consider the simple autocatalytic chemical reaction which has stoichiometric equation

$$2B + Q \rightarrow 2Q \qquad (4)$$

Stoichiometry then requires

$$R_Q = -\tfrac{1}{2} R_B \qquad (1a)$$

that is, two molecules of B are consumed for each molecule of Q produced. Moreover, if the mechanism of the reaction is first-order in Q, but of complex order in B, then the mass action law yields

$$R_Q/C_Q = F(C_B) \qquad (2a)$$

where C_B and C_Q are the concentrations of B and Q, respectively.

Equation (1*a*) is an analog of Equation (1), with the stoichiometric factor ½ replacing the yield coefficient β; Equation (2*a*) is an analog of Equation (2). Thus, the theories developed in the papers cited above view the growth of a microbial culture as a sort of autocatalytic process.

The validity of this viewpoint is questionable. In the first place, a microbial culture is a population of individual organisms, each of which carries on a changing sequence of metabolic processes. Hence, in kinetic terminology, each cell is a batch reactor in which the rates and kinds of biochemical reactions change as the cell matures, even if conditions external to the cell are time-invariant, as in a continuous stirred-tank fermentor operating in the steady state. In other words, each microbial cell undergoes a cycle of maturation. Wherein does the theory of autocatalytic chemical reaction described above account for this very fundamental notion?

In the second place, a microbial culture is almost never synchronized* to any great extent. Therefore, in any culture, there is a distribution of metabolic rates which depends on the distribution of degrees of cell maturation, as well as on external conditions, such as culture medium pH, composition, oxidation-reduction potential, temperature, and (in the case of photosynthetic organisms) light intensity. Previous theories of microbial growth recognize no differences among the various cells of a culture, so that the required averaging over the distribution of degrees of maturation is not done in computation of gross metabolic rates of a culture.

In light of these objections to earlier theories, it is clear that a theory which accounts for the salient features of microbial life cycles and the resulting distribution of degrees of cell maturation is required. In this paper, degree of cell maturation will be synonymous with cell age, the time since the cell was formed from its parent.

Ping Shu (*23*) recognized the importance of the distribution of ages, and derived expressions for the distribution in various cases. No attempt was made to bring the life-cycle concept into the theory, however, and this results in a number of unsatisfactory features. For instance, expressions for the concentration of microbial mass having a given age are given, age taken as identical to degree of maturation, from which metabolic rates can be calculated by appropriate averaging. But this overlooks the fact that when a cell reproduces by fission, the resulting daughter cells do not have the same biological age as the parent cell; more specifically, the daughter cells will have age zero.* Hence, even though one can calculate the fraction of microbial mass which has been present in the culture for a given time, only a rough approximation of the distribution of degrees of cell maturation can be obtained.

This paper proposes a model of microbial growth embodying the life-cycle concept which can be used to find the distribution of degrees of cell maturation. One can then use this distribution function to obtain a quantitative description of metabolic processes in cultures of microorganisms which reproduce by binary fission and do not form spores.

GROWTH PHENOMENA IN BATCHWISE PROPAGATION

Each living organism undergoes a cycle of change and maturation during its life-span. This probably applies to microorganisms as it does to macroscopic animals and plants. Since the consequences of the cyclic scheme for the continuation of life are strongly manifested in batch cultures of microorganisms, a brief description of batchwise growth follows.

When a batch culture is inoculated, no apparent reproduction occurs until the end of the lag phase. It is noted, however, that cells increase in size [Henrici (*10*)] and that there is an increase in the amount of purine and pyrimidine moieties of nucleic acids in each cell [see, for example, Malmgren and Hedén (*17*)]. It appears that the accumulation of these compounds is due to acceleration of the rate of formation of ribonucleic acid (RNA). Since one function of RNA is to form new protein, one can therefore interpret the lag phase as the period in which the metabolic machinery of the cells is being formed.

During logarithmic growth, the mean lifetime of a generation remains constant. The term "mean lifetime" is used advisedly since the culture consists of cells of varying degrees of maturation. However, Henrici (*10*) found that the multiplication rate constant, K, is by no means an invariant property of the organisms used. If cells in the log growth phase are centrifuged from the medium and reinoculated into fresh medium (but at a higher initial count than the original culture), log growth will again occur, after a shorter lag phase than with the original culture, but with a smaller value of K. If the density of the new inoculum is sufficiently high, no log growth occurs and the population density of the culture will be constant.

When a batch culture has attained a certain population density, the rate of proliferation begins to decrease, finally reaches zero (stationary phase), and may even become negative (phase of decline). At least two explanations of these phenomena have been given.

1. Growth of the culture has depleted the medium of one or more essential nutrients.

* In a synchronous culture all cells have the same degree of maturation.

* However, Halvorson (*9*) has pointed out that when a cell reproduces by binary fission, only one of the daughter cells may have age zero.

2. Metabolic by-products excreted by cells have accumulated to a sufficient extent to be autotoxic.

The first explanation is inherent in the theory of autocatalysis described above; the second is not, and requires integral equations for its quantitative description.

Individual cells of a culture are passing through their life cycles during all of these growth phases of the culture. Thus, in the lag phase, the life cycle involves the adaptation of cells to their environment. During log growth, the mean period of the life cycle is shortest, and the greatest rate of proliferation occurs. In the later growth stages, the life cycle is slowed down, and may even be replaced by degradative processes (phase of decline). Clearly, these phenomena must be built into the theory which will be developed.

A MODEL OF MICROBIAL PROPAGATION

The metabolism of an organism is defined here as the totality of all chemical and physical processes occurring within the organism. From a macroscopic point of view, metabolism is the assimilation and utilization of nutrients with concomitant formation of new cellular material and release of metabolic products. From a microscopic point of view, metabolism is the occurrence within a cell of a large number of catenary reactions, catalyzed by enzymes, coupled with various transport processes, and interacting with the surrounding medium in a complex manner. If one knew the mechanisms of all reactions and transport processes occurring, the structure and geometry of the cell, the composition of the chemical environment, and the nature of the physical environment, it would be possible, in principle, to construct a kinetic theory of microbial propagation. Hinshelwood (*13*) has made the beginnings of such a theory; at present, however, the difficulties involved are so great that a simple, semi-empirical approach will be used here.

The approach to be taken may be stated as follows: consider a group of cells, all of which have just been formed by cell fission. All of these cells are not the same; that is, their structures may show variations, as may the amounts of various substances contained within them. Hence, as time progresses, all of the cells will not develop in the same way nor will the rates of processes in the various cells be the same. On the average, however, one expects these irregularities to be smoothed out, and all cells may be expected to develop in more or less the same way. Hence a statistical approach is needed.

Moreover, even though exactly what is happening in each cell as it develops is not known, one can measure, in principle, the statistical properties of the rate of any process of interest as a function of cell age, the time since a cell was formed by fission of its parent. Therefore, the cell age, τ, is regarded as an index of the degree of maturation of a cell and it is assumed that one can determine by appropriate experiments the probabilities of occurrence of various cellular events as functions of cell age.

Consider a unicellular organism which reproduces asexually by binary fission; that is, two daughter cells are formed on fission. It is assumed that sexual reproduction does not occur; this case though interesting must be left for future research.

A cell which has just been formed by fission has age $\tau = 0$. As its age increases, it absorbs and assimilates nutrients from the surrounding medium and excretes waste products into its environment. Also, there is an increase in the amount of RNA and similar substances necessary to bring about further cell division. These processes are called *growth* (assimilation of nutrients with increase in cell mass or volume) and *maturation* (development of capacity for cell division). The former should not be confused with the term "growth" applied to propagation in a culture.

When a cell reaches a particular degree of maturation, there is a probability that it will undergo binary fission. Let

$$\Gamma(t, \tau, N)\, dt \tag{5}$$

be the probability that a cell of age τ in a culture of population density N will undergo binary fission in time interval t to $t + dt$ (t is the chronological, or laboratory time). The function $\Gamma(t, \tau, N)$ may be determined by experiment most easily with synchronized cultures.* It is apparent that since cell fission is a random process, a given culture, even though synchronized at some time, will not remain so unless forced to do so by programming of the environmental conditions.

Γ is regarded as a function not only of age, τ, but also of population density and of laboratory time. This is necessary if the theory is to be in agreement with experimental observations discussed earlier. The reasons for the assumption that Γ is a function of t and N as well as of age may be made clearer by a mechanistic argument.

Suppose that cell division can only be accomplished when the amount of some substance, P, in the cell has reached a critical level. Substance P is formed from substrates in the medium by catenary enzymatic reactions of which a simplified scheme might be

$$A\ (\text{medium}) \longrightarrow A\ (\text{cell}) \qquad \text{(transport process)}$$

$$A + E\ (\text{enzyme}) \xrightarrow{k_1} X_1$$

$$X_1 \xrightarrow{k_2} X_2 + E$$

$$X_2 \xrightarrow[\text{fast}]{} \beta P$$

$$\beta P \longrightarrow \text{further transport processes and catenary reactions}$$

With the assumption that transport processes are not rate controlling, the usual pseudo steady state approximation indicates that the rate of formation of P in a cell of age τ is

$$\frac{\alpha \beta k_1 k_2 E_o [A]}{k_2 + k_1 [A]} \tag{6}$$

where $[A]$ is the concentration of A in the medium, E_o is the enzyme concentration in the cell, and α is the ratio of concentrations of A in the cell to A in the medium. This expression is the Michaelis-Menten form given in Equation (3). The amount of P in a cell of age τ at time t is then

$$[P(\tau)] = \int_{t-\tau}^{t} \frac{\alpha \beta k_1 k_2 E_o [A]}{k_2 + k_1 [A]}\, dt \tag{7}$$

and cell division will occur only when $[P(\tau)]$ exceeds some critical value.

The concentration of substrate in the medium will change with time and with population density. A material balance on a propagator of volume V fed with Q volumes per unit time of nutrient medium of substrate concentration A_o gives

$$Q([A]_o - [A]) - V\left(\frac{\alpha k_1 k_2 E_o [A]}{k_2 + k_1 [A]}\right)_{N_{\text{avg}}} = V\frac{d[A]}{dt} \tag{8}$$

* See, for example, Hotchkiss (*14*), Lark and Maaløe (*16*), Barner and Cohen (*2*), Campbell (*5*), Tamiya et al. (*27*), Nihei et al. (*21*), Morimura (*20*), and Sorokin (*26*). It should be noted, however, that synchronization is achieved by programming environmental circumstances so that data so obtained may not be applicable to the more customary methods of propagation.

This argument shows why the functional dependence of Γ was chosen as it was. A theory based on mechanistic arguments is seen to be very complicated, inasmuch as the past history of all cells is involved.

There is also a probability that a cell may die in the sense that it becomes nonviable and does not undergo fission. The probability that a cell of age τ in a culture of population density N will die in time interval t to $t + dt$ is defined to be

$$\Theta\ (t, \tau, N)\ dt \tag{9}$$

where the function $\Theta\ (t, \tau, N)$ may again be determined by appropriate experiments.

Finally a dead cell may be autolyzed (destroyed) by proteolytic enzymes present. The age, τ', of a dead cell is defined to be the length of time since it died. The probability of autolysis occurring to a dead cell of age τ' in a culture of population density N in time interval t to $t + dt$ is defined to be

$$\Lambda\ (t, \tau', N)\ dt \tag{10}$$

where the function $\Lambda\ (t, \tau', N)$ is again to be determined by experiment.

THE EQUATIONS OF CONSERVATION OF AGE GROUPS

An age group is a collection of cells with biological ages between specified limits. In particular, this paper will focus on groups with ages differing only by infinitesimal amounts. Thus, the age group τ to $\tau + d\tau$ here means that group of cells with ages between τ and $\tau + d\tau$. The equations of conservation of age groups are simply an accounting for all cells which enter, leave, accumulate, are created,* or are destroyed in a particular age group.

Consider a pure culture of microorganisms in which $N(t)$ organisms are present in unit volume of culture. Of these, a fraction $v(t)$ are viable and the fraction $(1 - v(t))$ are nonviable or dead. It is assumed that all parameters of the population, such as $N(t)$ and $v(t)$ are independent of position in the propagator. Chronological or laboratory time, t is measured from some arbitrary datum.

If $f(t, \tau)d\tau$ is the probability that a viable cell belongs to age group τ to $\tau + d\tau$ at time t, the number of viable cells of this age group present in unit volume of the culture at time t is

$$v(t)\ N(t) f(t, \tau) d\tau \tag{11}$$

Clearly,

$$\int_o^\infty v(t)\ N(t) f(t, \tau) d\tau = v(t) N(t)$$

so that

$$\int_o^\infty f(t, \tau) d\tau = 1 \tag{12}$$

Again, if $g(t, \tau')d\tau'$ is the probability that a nonviable cell belongs to age group τ' to $\tau' + d\tau'$ at time t, then the number of nonviable cells of this age group present in unit volume of the culture at time t is

$$[1 - v(t)]\ N(t) g(t, \tau') d\tau' \tag{13}$$

Herein, τ' is interpreted as the length of time a cell has been nonviable. As before

$$\int_o^\infty g(t, \tau') d\tau' = 1 \tag{14}$$

Hence, $f(t, \tau)$ and $g(t, \tau')$ are probability densities of the age distribution functions.

* Since cells may appear in an age group by cell fission or may disappear by death or autolysis, they are not conserved. The terminology is used in the same sense as in continuum mechanics where one speaks of conservation of momentum, etc.

In a microbial culture of population density N at time t, focus attention on a single viable cell of age τ to $\tau + d\tau$. During the time interval t to $t + dt$, one of the following events may occur:

1. The cell ages to an older age group.
2. The cell undergoes fission.
3. The cell becomes nonviable (dies).
4. The cell is washed out of the culture.

Clearly, these events are mutually exclusive, so that their joint probability (which is the probability that the age graup τ to $\tau + d\tau$ will be depleted) is the sum of the individual probabilities of events 1 to 4. One must of course calculate the individual probabilities of each of the events (1 to 4) on the basis that the other three do not occur. The individual probabilities are calculated as follows:

At time t, the number of viable cells of age group τ to $\tau - d\tau$ present in the culture of volume V is

$$Vv(t)\ N(t) f(t, \tau - d\tau) d\tau \tag{15}$$

and the number of viable cells of age group τ to $\tau + d\tau$ present at time t is

$$Vv(t)\ N(t) f(t, \tau) d\tau \tag{16}$$

During time interval $dt = d\tau$, all cells of age group τ to $\tau - d\tau$ will become cells of age group τ to $\tau + d\tau$, and all cells of age group τ to $\tau + d\tau$ become members of an older age group. Here, it is assumed that events 2 to 4 do not occur.

Hence, the net rate of depletion in age group τ to $\tau + d\tau$ is

$$\frac{Vv(t)\ N(t) f(t, \tau) dt - Vv(t)\ N(t) f(t, \tau - d\tau) dt}{dt} =$$

$$Vv(t)\ N(t)\ \frac{\partial f(t, \tau)}{\partial \tau} d\tau \tag{17}$$

The probability of event 1 in time t to $t + dt$ if events 2 to 4 do not occur is then

$$Vv(t)\ N(t) \frac{\partial f(t, \tau)}{\partial \tau} d\tau\ dt \tag{18}$$

The probability of event 2, that the cell undergoes fission in time t to $t + dt$, is obtained from expression (5) as

$$Vv(t)\ N(t)\ \Gamma(t, \tau, N) f(t, \tau) d\tau\ dt \tag{19}$$

and the probability of event 3, a cell dies in time interval t to $t + dt$, is derived from expression (9):

$$Vv(t)\ N(t)\ \Theta(t, \tau, N) f(t, \tau) d\tau\ dt \tag{20}$$

Finally, the probability of event 4, a cell is washed out of the culture in time t to $t + dt$, is

$$V(Q/V) v(t)\ N(t) f(t, \tau) d\tau dt \tag{21}$$

where Q is the volumetric flow rate of nutrient medium to the culture. Expression (21) assumes that the culture is perfectly mixed, so that the probability of washout of any cell is independent of its age and location in the propagator.

The joint probability of events 1 to 4 is the probability of depletion of age group τ to $\tau + d\tau$ in time t to $t + dt$; that is

$$Vv(t)\ N(t) \frac{\partial f(t, \tau)}{\partial \tau} d\tau\ dt +$$

$$Vv(t)\ N(t)\ \Gamma(t, \tau, N) f(t, \tau) d\tau\ dt +$$

$$Vv(t)\ N(t)\ \Theta(t, \tau, N) f(t, \tau) d\tau\ dt +$$

$$Qv(t)\,N(t)f(t,\tau)\,d\tau dt = -V\frac{d}{dt}\,[v(t)\,N(t)f(t,\tau)\,d\tau]dt \tag{22}$$

Simplification of this equation yields the conservation equation for viable cells of age group τ to $\tau + d\tau$

$$\frac{\partial f(t,\tau)}{\partial t} + \frac{\partial f(t,\tau)}{\partial \tau} + \left(\frac{1}{\theta} + \Gamma(t,\tau,N) + \Theta(t,\tau,N) + \frac{d}{dt}\ln\,[v(t)\,N(t)]\right) f(t,\tau) = 0 \tag{23}$$

where

$$\theta = V/Q \tag{24}$$

is the mean residence time in the propagator of a molecule of feed solution. Equation (23) is valid for $\tau > 0$; it is not valid for $\tau = 0$ since it contains no term for the probability that cells of age zero may be formed by cell fission. This eventuality will be accounted for by a boundary condition to be derived later.

The conservation equation for nonviable cells of age group τ' to $\tau' + d\tau'$ may be obtained by methods analogous to those described above; the result is

$$\frac{\partial g(t,\tau')}{\partial t} + \frac{\partial g(t,\tau')}{\partial \tau'} + \left(\frac{1}{\theta} + \Lambda(t,\tau',N) + \frac{d}{dt}\ln\,[(1-v(t))\,N(t)]\right) g(t,\tau') = 0 \tag{25}$$

Again, this equation does not hold for $\tau' = 0$; a boundary condition accounting for formation of dead cells of age $\tau' = 0$ must therefore be obtained.

The probability that a viable cell of age zero will be formed in time t to $t + dt$ is found from expression (19) to be

$$2Vv(t)\,N(t)dt\int_0^\infty \Gamma\,(t,\tau,N)\,f(t,\tau)\,d\tau \tag{26}$$

The factor of two accounts for the fact that fissions are binary. Similarly, the probability of formation of dead cells of age $\tau' = 0$ in time t to $t + dt$ results from expression (20) and is

$$Vv(t)\,N\,(t)dt\int_0^\infty \Theta\,(t,\tau,N)\,f\,(t,\tau)\,d\tau \tag{27}$$

The probability of depletion in time t to $t + dt$ for viable cells of age 0 to $d\tau$ is the sum of the probabilities of events 1 to 4 above less the probability given by expression (26). However, expression (18) cannot be used for calculating the probability of event 1 since no cells of age zero are formed by aging of younger cells. Rather, for cells of age group 0 to $d\tau$, the probability of event 1 is

$$Vv(t)\,N\,(t)\,f\,(t,0)\,d\tau \tag{28}$$

Hence, the probability of depletion in age group 0 to $d\tau$ is

$$Vv(t)N(t)f(t,0)\,dt + Vv(t)N(t)\Gamma(t,0,N)f(t,0)\,d\tau dt + Vv(t)N(t)\Theta(t,0,N)f(t,0)d\tau\,dt + Qv(t)N(t)f(t,0)d\tau dt - 2Vv(t)N(t)dt\int_0^\infty \Gamma\,(t,\tau,N)f(t,\tau)\,d\tau = -V\frac{d}{dt}\,[v(t)N(t)f(t,0)\,d\tau]dt \tag{29}$$

or, when differentials are neglected and the expression is rearranged

$$f(t,0) = 2\int_0^\infty \Gamma\,(t,\tau,N)\,f\,(t,\tau)\,d\tau \tag{30}$$

Equation (30) is the required boundary condition which solutions of Equation (23) must satisfy. The analogous boundary condition for Equation (25) is readily found to be

$$g(t,0) = \frac{v(t)}{1-v(t)}\int_0^\infty \Theta\,(t,\tau,N)\,f\,(t,\tau)\,d\tau \tag{31}$$

A conservation equation may also be written for the total number of cells in unit volume of culture. It is

$$v(t)\int_0^\infty \Gamma\,(t,\tau,N)\,f\,(t,\tau)\,d\tau - [1-v(t)]\int_0^\infty \Lambda(t,\tau',N)\,g(t,\tau')\,d\tau' - 1/\theta = \frac{d}{dt}\ln N(t) \tag{32}$$

It is to be noted that Equation (32) is not independent, but may be derived from Equations (12), (14), (23), and (25).

Expressions somewhat similar to (23) and (25) were developed by Ping Shu (23) for the age distribution of microbial mass; Rudd (25) also obtained equations of this type for a related problem.

Finally, note that the equations for a batch culture may be obtained simply by setting $1/\theta$ equal to zero (infinite residence time).

METABOLIC RATES IN MICROBIAL CULTURES

The metabolism of an organism is defined here as the totality of all chemical and physical transformations occurring within the organism. Biochemistry has revealed many of the pathways of metabolism, but such information is not yet sufficient to construct a quantitative theory of metabolic rates. Hence, as in treating cell division and death, metabolic rates are regarded as capable of experimental measurement, and it is supposed that the variation of these rates with cell age is known.

Even in cells of the same age group, there will be variations in the rates at which metabolic processes occur. In principle, one could investigate the statistics of metabolic rates by experiments with single cells; in practice, the mean rates obtainable from experiments with cells from synchronized cultures are all that are needed for calculation of metabolic rates of the culture.

Let R_i be the rate per cell at which the ith metabolic process occurs. If

$$G_i\,(\tau, N, R_i)\,dR_i \tag{33}$$

is the probability that the rate of the ith process will be between R_i and $R_i + dR_i$ in cells of age τ, then it is easy to show that the total rate of the ith process per unit volume of culture is

$$\overline{R_i} = v(t)\,N(t)\int_0^\infty <R_i>\,f(t,\tau)\,d\tau \tag{34}$$

where

$$<R_i> = \int_{-\infty}^{\infty} R_i G_i(\tau, N, R_i)\,dR_i \tag{35}$$

is the mean rate of the i^{th} metabolic process in cells of age τ. In Equation (34) it is assumed that the i^{th} metabolic process does not occur in nonviable cells. If the metabolic reaction of interest also occurs in nonviable cells, as may be the case in certain applications, Equation (34) must be generalized.

Thus, in order to calculate the rate of a metabolic process in a culture, one must know the population density, the probability density of the age distribution function and the mean rate of the process as a function of cell age and population density.

THE SIZE DISTRIBUTION

As an example of the use of the above equations, we derive a relation between the size distribution of cells, the age distribution, and the mean growth rate per cell. The usefulness of this relation is greatly enhanced by the recent appearance of electronic devices for direct experimental measurement of cell size distribution functions.

The stochastic variables of interest here are the growth rate per cell, R_G, with probability density $G_G(\tau, N, R_G)$, and the size of cells of age zero, with probability density $D(s_o,N)$. Herein, s_o is the size of a cell of age zero; s_o may be measured in either volume or mass units, whichever is convenient. Obviously, if s_o is in volume (mass) units, then R_G must be in volume (mass) units.

Consider the two following events:

(a). A cell of age τ has grown an amount between $R_G\tau$ and $(R_G + dR_G)\tau$ since it was formed by cell division.

(b). A cell of age zero has size between s_o and $s_o + ds_o$. The joint probability of these two events will give the probability that a cell has a certain size when it reaches the age τ.

From expression (33) the probability of event (a) is

$$R_G dR_G \int_o^\tau G_G(\zeta, N, R_G)\,d\zeta \tag{36}$$

and the probability of event (b) is of course

$$D(s_o, N)\,ds_o \tag{37}$$

If we assume that events (a) and (b) are independent, then their joint probability is

$$R_G D(s_o, N)\,ds_o dR_G \int_o^\tau G_G(\zeta, N, R_G)\,d\zeta \tag{38}$$

Introduce a transformation of variables

$$s = s_o + R_G\tau \tag{39}$$

$$w = R_G\tau \tag{40}$$

with Jacobian J given by

$$J = \begin{vmatrix} 1 & -1 \\ 0 & 1/\tau \end{vmatrix} = 1/\tau \tag{41}$$

The new variable s is the size of a cell of age τ; the variable w is the amount a cell of age τ has grown.

With these new variables, expression (38) is transformed into

$$\frac{w}{\tau^2} D(s-w, N)\,ds dw \int_o^\tau G_G\left(\zeta, N, \frac{w}{\tau}\right) d\zeta \tag{42}$$

and this may be interpreted as the joint probability of the two events: a cell of age τ has size between s and $s + ds$ and has grown by an extent w to $w + dw$.
Hence, since events (a') and (b')

(a'). A cell of age τ has size between s and $s + ds$ and has grown by an extent w to $w + dw$.

(b'). A cell of age τ has size between s and $s + ds$ and has grown by an extent w' to $w' + dw'$.

are mutually exclusive, one finds from (42) that the probability $U(s, \tau)ds$ that a cell of age τ has size between s and $s + ds$ is

$$\frac{ds}{\tau^2} \int_o^s D(s-w, N)\,dw \int_o^\tau G_G\left(\zeta, N, \frac{w}{\tau}\right) d\zeta = U(s,\tau)\,ds \tag{43}$$

$U(s, \tau)$ is the probability density of the size distribution for viable cells of age τ.

The probability density $U(s, \tau)$ cannot be determined experimentally; however, one can determine the probability density $W(t, s)$ defined by $W(t, s)ds$ = probability, at time t, that a cell will have size between s and $s + ds$. This can be calculated from $U(s, \tau)$ if one knows the fraction of cells which are viable and the distribution of ages of nonviable cells when they died. The latter is calculated as follows.

The probability, at time t, that a nonviable cell has been dead a time τ' to $\tau' + d\tau'$ is by definition

$$g(t, \tau')\,d\tau'$$

Such a cell died in the time interval $t - (\tau' + d\tau')$ to $t - \tau'$. The number of cells, per unit volume, of age group τ to $\tau + d\tau$ which died in that time interval is

$$v(t-\tau')N(t-\tau')\Theta(t-\tau', \tau, N(t-\tau'))\, f(t-\tau', \tau)\,d\tau\,d\tau' \tag{44}$$

Thus, the total number of cells, per unit volume, which died in that time interval is

$$v(t-\tau')\,N(t-\tau')d\tau' \int_o^\infty \Theta\,(t-\tau', \tau, N(t-\tau'))\,\times f(t-\tau', \tau)\,d\tau = [1 - v(t-\tau')]\,\times N(t-\tau')g(t-\tau', 0)\,d\tau' \tag{45}$$

because of Equation (31). Hence, if it is assumed that the probabilities of washout and autolysis are independent of the age of a cell when it died, then: the probability, at time t, that a cell which has been dead a time τ' had age τ to $\tau + d\tau$ when it died is

$$\frac{v(t-\tau')}{1-v(t-\tau')} \cdot \frac{f(t-\tau', \tau)}{g(t-\tau', 0)} \Theta\,(t-\tau', \tau, N)\,d\tau \tag{46}$$

Hence, the probability, at time t, that a nonviable cell had age to $\tau + d\tau$ when it died is

$$d\tau \int_{-\infty}^t g(t, \tau') \frac{v(t-\tau')}{1-v(t-\tau')} \cdot \frac{f(t-\tau', \tau)}{g(t-\tau', 0)} \Theta\,(t-\tau', \tau, N)\,d\tau' \tag{47}$$

The probability density of the size distribution function can now be calculated; it is just

$$W(t, s) = v(t) \int_o^\infty f(t, \tau)U(s, \tau)\,d\tau + [1 - v(t)] \int_o^\infty U(s, \tau)\,d\tau \int_{-\infty}^t g(t, \tau') \frac{v(t-\tau')}{1-v(t-\tau')} \cdot \frac{f(t-\tau', \tau)}{g(t-\tau', 0)} \Theta\,(t-\tau', \tau, N)\,d\tau' \tag{48}$$

Thus, the size distribution is a complicated function of the age distributions of both viable and nonviable cells at all earlier times.

The mean cell size is of course

$$\langle s \rangle = \int_o^\infty s\,W(t, s)\,ds \tag{49}$$

so that the total amount of cellular material present in unit volume of the culture is

$$N(t)\,\langle s \rangle \tag{50}$$

Since $<s>$ depends on N, in general, there is a nonlinear relation between population density and total cell mass or volume per unit volume of culture. This shows that various empirical methods of expressing cell concentration, such as packed cell volume (obtained by centrifugation), cannot be simply related to the population density but must be experimentally related.

In general, each of the integrals in (48) will have a graph somewhat resembling the Poisson distribution. Hence, the sum of the two integrals will yield a bimodal probability density for the size distribution. This phenomenon would be observed only when conditions are such that a large fraction of the cells are dead. Hence, another explanation of a bimodal probability density for the size distribution is provided.

CONTINUOUS PROPAGATION OF MICROORGANISMS: THE STEADY STATE

Now a population of microorganisms propagated in a continuous fermentor will be considered. If external conditions are maintained constant* for a sufficient length of time, a steady state will eventually be reached. The required equations for the steady state may be obtained from (12), (14), (23), (25), (30), (31), and (32) by suppressing the time dependence; thus, we obtain

$$\frac{df(\tau)}{d\tau} + \left(\frac{1}{\theta} + \Gamma(\tau, N) + \Theta(\tau, N)\right) f(\tau) = 0 \qquad (23a)$$

$$\frac{dg(\tau')}{d\tau'} + \left(\frac{1}{\theta} + \Lambda(\tau', N)\right) g(\tau') = 0 \qquad (25a)$$

$$f(0) = 2 \int_0^\infty \Gamma(\tau, N)\, f(\tau)\, d\tau \qquad (30a)$$

$$g(0) = \frac{v}{1-v} \int_0^\infty \Theta(\tau, N)\, f(\tau)\, d\tau \qquad (31a)$$

$$\int_0^\infty f(\tau)\, d\tau = 1 \qquad (12a)$$

$$\int_0^\infty g(\tau')\, d\tau' = 1 \qquad (14a)$$

and

$$\tfrac{1}{2} v f(0) - (1-v) \int_0^\infty \Lambda(\tau', N)\, g(\tau')\, d\tau' = \frac{1}{\theta} \qquad (32a)$$

Integration of Equation (23a) gives

$$f(\tau) = f(0) \exp\left[-\frac{\tau}{\theta} - \int_0^\tau [\Gamma(\zeta, N) + \Theta(\zeta, N)]\, d\zeta\right] \qquad (51)$$

where $f(0)$ is determined from Equation (12a)

$$f(0) = 1 \Big/ \int_0^\infty \exp\left[-\frac{\tau}{\theta} - \int_0^\tau [\Gamma(\zeta, N) + \Theta(\zeta, N)]\, d\zeta\right] d\tau \qquad (52)$$

Similarly, integration of Equation (25a) gives

$$g(\tau') = g(0) \exp\left[-\frac{\tau}{\theta} - \int_0^\tau \Lambda(\zeta, N)\, d\zeta\right] \qquad (53)$$

where $g(0)$ is determined from Equation (14a)

$$g(0) = 1 \Big/ \int_0^\infty \exp\left[-\frac{\tau}{\theta} - \int_0^\tau \Lambda(\zeta, N)\, d\zeta\right] d\tau \qquad (54)$$

Equation (30a) determines the steady state population density corresponding to a given value of θ. Substitution of (51) into (30a) gives the required result

$$\frac{1}{2} = \int_0^\infty \Gamma(\tau, N) \exp\left[-\frac{\tau}{\theta} - \int_0^\tau [\Gamma(\zeta, N) + \Theta(\zeta, N)]\, d\zeta\right] d\tau \qquad (55)$$

In general, the relation between N and θ given by this equation must be determined by trial-and-error numerical integration. An example will be given below.

The fraction of cells which are viable, v, can be determined from either (31a) or (32a); the result is

$$v = g(0) \Big/ \left[g(0) + f(0) \int_0^\infty \Theta(\tau, N) \exp\left[-\frac{\tau}{\theta} - \int_0^\tau [\Gamma(\zeta, N) + \Theta(\zeta, N)]\, d\zeta\right] d\tau\right] \qquad (56)$$

where $f(0)$ and $g(0)$ are determined by (52) and (54). Hence, the procedure to be followed is:

1. Determine N for the desired value of θ from Equation (55).
2. Calculate $f(0)$ and $g(0)$ from Equations (52) and (54).
3. Calculate v from Equation (56).
4. Calculate the age distribution probability densities from Equations (51) and (53).

The procedure is illustrated by an example below.

The steady state solution of the equations for the probability density $f(\tau)$ has a simple interpretation. Consider a group of viable cells which were formed by fission at time $t = t'$. These cells have age zero at that time, and the number of them per unit volume is proportional to $f(0)$. At time $t = t' + \tau$, some of the cells will have died, others will have been washed out, others will have formed daughter cells, and the remainder will still be in the propagator. The number of cells remaining is proportional to $f(\tau)$. Hence, by Equation (51), the ratio

$$\frac{f(\tau)}{f(0)} = e^{-\frac{\tau}{\theta}} e^{-\int_0^\tau \Gamma(\zeta, N) d\zeta} e^{-\int_0^\tau \Theta(\zeta, N) d\zeta} \qquad (57)$$

is the joint probability of the following events:

(c). A cell formed at time t' has not been washed out by time $t' + \tau$.

(d). A cell formed at time t' has not undergone fission by time $t' + \tau$.

(e). A cell formed at time t' has not died by time $t' + \tau$.

Equation (57) shows that the events (c), (d), and (e) are independent with individual probabilities

$$P_{nw} = e^{-\tau/\theta} \qquad (58)$$

$$P_{ng} = e^{-\int_0^\tau \Gamma(\zeta, N) d\zeta} \qquad (59)$$

$$P_{nd} = e^{-\int_0^\tau \Theta(\zeta, N) d\zeta} \qquad (60)$$

* It is assumed that mutations do not occur with sufficient frequency to alter materially the nature of the culture, and that the steady state is stable; a discussion of the latter point is given below.

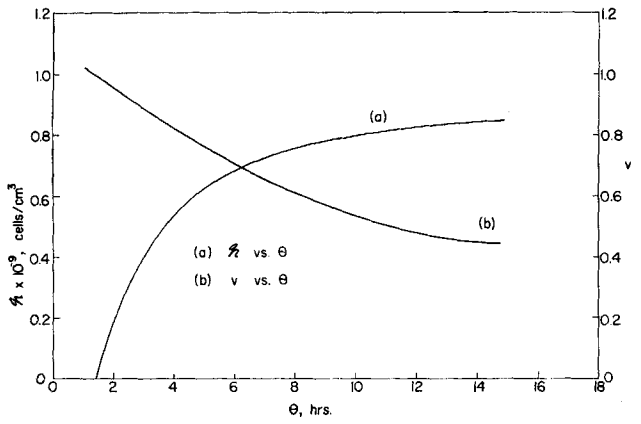

Fig. 1. Variation of population density (a) and fraction viable (b) with residence time in a continuous fermentor. Calculated as described in text.

The events

(c'). A cell formed at time t' has been washed out of the propagator by time $t' + \tau$.

(d'). A cell formed at time t' has undergone fission by time $t' + \tau$.

(e'). A cell formed at time t' has died by time $t' + \tau$.

have probabilities

$$P_w = 1 - e^{-\tau/\theta} \tag{61}$$

$$P_g = 1 - e^{-\int_o^\tau \Gamma(\zeta, N)d\zeta} \tag{62}$$

$$P_d = 1 - e^{-\int_o^\tau \Theta(\zeta, N)d\zeta} \tag{63}$$

but the events are not independent, since their joint probability is

$$1 - e^{-\tau/\theta}\ e^{-\int_o^\tau \Gamma(\zeta, N)d\zeta}\ e^{-\int_o^\tau \Theta(\zeta, N)d\zeta} \tag{64}$$

Expressions (58) and (61) are implicit in the work of Denbigh (*7*); hence, they need not be discussed further, except to remark that they are a consequence of the assumption of perfect mixing in the propagator. Equation (62) for the probability of cell fission will have the following characteristics: At small values of τ, P_g will be near zero, since there is little probability that a young cell will divide. As τ approaches τ_g (τ_g = mean generation time) P_g must show a rapid increase. For large τ, P_g should approach the asymptotic value of unity. Experimental data which show this sort of variation have been obtained by Hershey and Bronfenbrenner (*12*). One expects that the probability P_d will show a similar sort of dependence on τ.

Numerical Example

Assume that for some organism

$$\Gamma(\tau, N) = \begin{cases} 0, & \tau < \tau_g \\ k_g, & \tau > \tau_g \end{cases} \tag{65}$$

$$\Theta(\tau, N) = k_d \tag{66}$$

$$\Lambda(\tau', N) = 0 \tag{67}$$

where k_g, k_d, and τ_g are functions of N

$$k_g/k_{go} = 1 - \phi \tag{68}$$

$$(\tau_g - \tau_{go})/(\tau_{g\infty} - \tau_{go}) = \phi \tag{69}$$

$$k_d/k_{d\infty} = \phi \tag{70}$$

and

$$\phi = \frac{1 - \exp\{-\beta(N/N_\infty)\}}{1 - \exp\{-\beta\}} \tag{71}$$

That is, the maximum possible population density is N_∞.

Age distributions and average metabolic rates may now be calculated from Equations (51), (53), and (35); the fraction of cells viable is given by Equation (56).

Figures 1 to 4 have been plotted for the particular values $\beta = 0.1$, $N_\infty = 1 \times 10^9$ cells/cc., $\tau_{g\infty} = 2$ hr., $\tau_{go} = 0.5$ hr., $k_{go} = 2$ hr.$^{-1}$, and $k_{d\infty} = 0.1$ hr.$^{-1}$. Figure 1 shows the relation between N and θ as computed by trial-and-error calculation. The minimum possible holding time is 1.334 hr. This figure gives the same type of curves as those obtained experimentally by Herbert et al. (*11*) for continuous propagation.

Figure 2 is a plot of productivity vs. θ. The productivity is N/θ, the number of cells produced per unit volume and time. The figure shows an optimum holding time (approximately 3.5 hr.) for maximizing production of cells.

Figure 4 gives the mean ages of viable and nonviable cells; these are defined by

$$\langle\tau\rangle = \int_o^\infty \tau\ f(\tau, N)d\tau;$$

$$\langle\tau'\rangle = \int_o^\infty \tau'\ g(\tau', N)d\tau' \tag{72}$$

If autolysis of dead cells occurred, the curve for $\langle\tau'\rangle$ would be similar to that for $\langle\tau\rangle$.

Finally for a process (production of X)with mean rate per cell

$$\langle R_X\rangle = \langle R_X\rangle_\infty\ (1 - e^{-\gamma\tau}) \tag{73}$$

one can calculate the average rate of production of X from Equation (35). Figure 2 shows how this average rate ($\bar{R}_X$) varies with holding time for $\langle R_X\rangle_\infty = 1 \times 10^{-11}$ moles of X per hour per cell, $\gamma = 0.346$ hr.$^{-1}$. An optimum holding time (approximately 10.5 hr.) is again observed; the optimum is different from that for maximization of cell production.

STABILITY OF THE STEADY STATE

Finn and Wilson (*8*) found that the logarithm of the population density of a continuous culture of yeast oscillated about a mean. They attributed this phenomenon to a time lag in the adjustment of growth rate to changes in population density. They calculated the order of magnitude of the time lag by a method developed by Baron (*3*) and found it to be about ½ hr. Clearly, this is much too

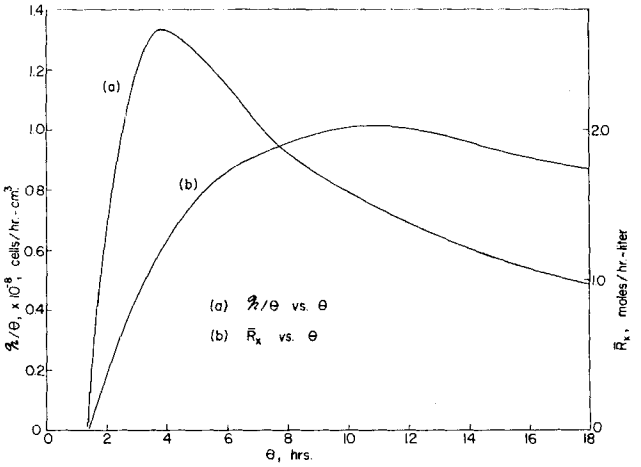

Fig. 2. Productivity of number cells (a) and of species X(b) with residence time in a continuous fermentor. Calculated as described in text.

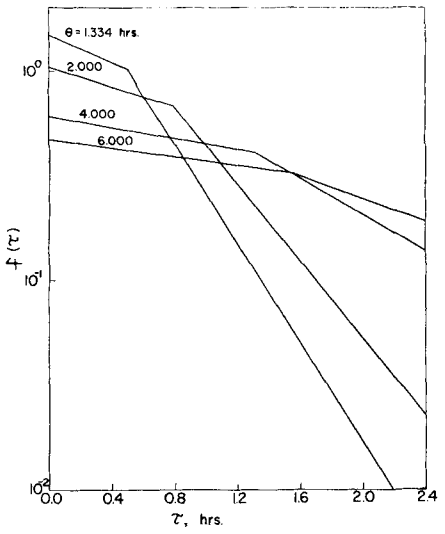

Fig. 3. Age distribution probability density for viable cells in a continuous fermentor.

large to be attributed to a diffusional process, and they therefore concluded that the effect was inherent in the growth of the organism.

The analysis of Finn and Wilson was based on Equations (1) and (2). In order to proceed, they had to introduce the ad hoc assumption that the value of C to be used is not that at the current time, t, but at the earlier time, $t - t'$. The quantity t' is then the time lag mentioned above.

Both of the postulates of Finn and Wilson are already contained in the more general Equations (23), (25), (30), and (31) derived above. Hence, it should be possible to decide from these equations, without introducing further assumptions, whether a given situation will be stable, unstable, or will exhibit oscillations of the type observed. The terms stable and unstable used here have the same meaning as in nonlinear mechanics: A system is stable if after a small perturbation from a steady state it returns to that state; a system is unstable if a small perturbation from a steady state leads to a new steady state.

An attempt was made to investigate the stability of a continuous microbial propagator by the methods in nonlinear mechanics as developed for chemical reactors by Bilous and Amundson (4) and Aris and Amundson (1). In this method, one first calculates the steady state behavior of the system and then supposes that a small (strictly speaking, an infinitesimal) perturbation from the steady state occurs. Since the perturbation is small, the unsteady state equations describing the system may be linearized by expanding them in Taylor series, and it may be possible to decide, from the character of solutions of the linearized equations, whether the perturbation will decay, grow, or oscillate.

If one supposes that the probability functions $\Gamma(t, \tau, N)$ and $\Theta(t, \tau, N)$ are independent of current time, t, even in the unsteady state, then it is a simple matter to obtain the linearized equations for a continuous microbial propagator. It appears that the resulting equations predict stable behavior, with no oscillations, although a rigorous proof of this was not obtained. This is in agreement with the results of Finn and Wilson; when K was assumed dependent on population density at the current time, no oscillations were predicted. Hence, one concludes tentatively that instability would be predicted only if the time dependence of Γ and Θ is retained; that is, one must assume that the probabilities of cell fission and death depend on the past histories of cells. If the analytical difficulties inherent in the required equations could be overcome, much information about microbial proliferation could be obtained from studies of the Finn and Wilson phenomenon.

DISCUSSION AND CONCLUSIONS

A few words are needed about the form of the generation function, Γ, for steady state cultures. The assumption made was [Equation (65)]

$$\Gamma(\tau, N) = k_g S(\tau - \tau_g)$$

and k_g and τ_g are functions of N given by Equations (68) and (69). That this form is reasonable can be seen if we revert to mechanistic arguments once more. A cell just formed by cell fission contains enzymes with which to form fission-inducing substances (called P earlier). The amount of such enzymes probably varies from cell to cell (that is, it is a random variable). Hence, the rate of formation of P is also a random variable. In any case, formation of the amount of P requisite for cell division takes a finite time; thus, there should be no cell division until some minimum age, τ_g, is attained. This is precisely what Equation (65) expresses. Moreover, the minimum time τ_g should increase with increasing population density, since the latter decreases the concentration of substrate A from which P is formed, and thereby decreases the rate of formation of P in a single cell. Equation (69) is an attempt to account for this.

Once the minimum age, τ_g, has been attained, cells begin to divide. At ages slightly greater than τ_g, cells with somewhat less P-forming enzymes begin to divide, and so on. The number of cells arriving at the critical concentration of P in a given time interval must decrease as the population density is increased. Again, this is due to the lowered substrate concentration in batch culture, and hence rate of P formation in single cells. Equation (68) was an attempt to give this idea a quantitative formulation.

The next step in the development of the ideas expressed herein might be to postulate a number of mechanisms for the maturation and fission processes and from them to derive expressions parallel to (65), (68), and (69). It is possible that such mechanisms will not have to be excessively complicated. One could then decide from experimental data which mechanism best reproduces the observations and so gain some idea of the interplay of medium composition and cellular proliferation.

This paper concentrated on the reproductive phenomenon of cells; little attention has been given to growth.

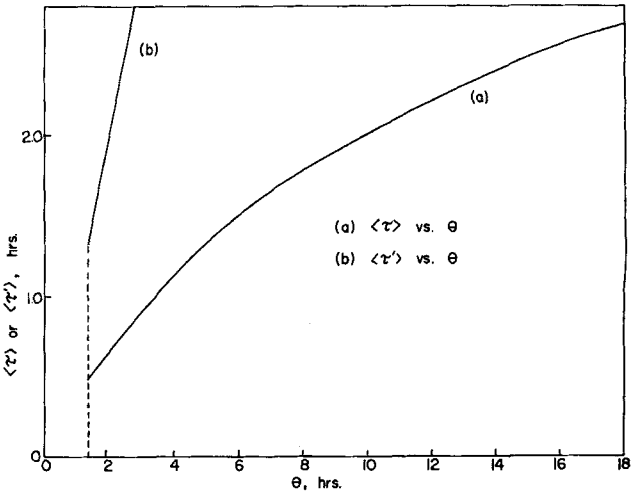

Fig. 4. Mean ages of viable (a) and nonviable (b) cells as functions of residence time.

Growth and reproduction are not synonymous, but neither are they independent of each other. Hence, a study of the interaction of growth and reproduction should be made.

Finally, there are a number of topics, such as the formation of mutant strains or the interaction of two or more populations in a single culture, which have not been touched on at all. To be sure, problems of this nature have been treated by many authors, but the lack of the age distribution concept in those theories means that they can be no more than approximations. This is because the susceptibility of organisms to attack by other organisms or mutation-causing influences varies with the ages of the individuals concerned, a fact which can be observed on a macroscopic level in the interaction of, for example, the fox and the rabbit population. The equations developed above provide a basis for proper consideration of such interactions.

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NOTATION

C = concentration of microbial mass
C_B, C_Q = concentration of chemical species B and Q
C_S = concentration of limiting substrate
D = probability density of size distribution for cells of age zero
F = function
f = probability density of age distribution for viable cells
G_G = probability density of growth rate distribution function
G_i = probability density of distribution function for i^{th} metabolic rate
g = probability density of age distribution function for nonviable cells
J = Jacobian determinant
K = multiplication rate constant
K_S = constant in Michaelis-Menten equation
k_g = defined by Equation (65)
k_d = defined by Equation (66)
k_{go}, $k_{g\infty}$ = limiting values of k_g at zero and limiting population densities, respectively
$k_{d\infty}$ = limiting value of k_d
N = population density
N_∞ = limiting population density
P_{nw}, P_{ng}, P_{nd} = probabilities defined by Equations (58) to (60)
P_w, P_g, P_d = probabilities defined by Equations (61) to (63)
Q = volumetric flow rate of nutrient
R, R_G = growth rate
R_B, R_Q = rate of production of chemical species B and Q
$-R_S$ = rate of substrate consumption
R_i = rate of i^{th} metabolic process
$\langle R_i \rangle$ = mean rate of i^{th} metabolic process in cells of age τ
$\overline{R_i}$ = total rate of i^{th} metabolic process
$\langle R_X \rangle$ = mean rate of production of X by cells of age τ
$\langle R_X \rangle_\infty$ = limiting value of $\langle R_X \rangle$ as τ becomes infinite
$\overline{R_X}$ = total rate of production of X
s = cell size
s_o = cell size for cells of age zero
$\langle s \rangle$ = mean cell size for cells of age τ
t = laboratory time
U = probability density of size distribution function for cells of age τ
V = volume of propagator
v = probability that a cell is viable
W = probability density of size distribution function
w = extent of growth

Greek Letters

β = yield coefficient; constant in Equations (68) to (70)
Γ = fission probability function
γ = constant in Equation (73)
ζ = dummy variable of integration
Θ = death probability function
θ = mean residence time
Λ = autolysis probability function
μ_m = maximum specific growth rate
τ = biological age
τ' = age of a dead cell
$\langle \tau \rangle$ = mean age of viable cells in a culture
$\langle \tau' \rangle$ = mean age of dead cells in a culture
τ_g = defined by Equation (65)
τ_{go}, $\tau_{g\infty}$ = limiting values of τ_g at zero and limiting population densities, respectively

LITERATURE CITED

1. Aris, R., and N. R. Amundson, *Chem. Eng. Sci.*, **7**, 121 (1958).
2. Barner, H. D., and S. S. Cohen, *J. Bact.*, **72**, 115 (1956).
3. Baron, T., *Proc. Midwestern Conf. on Fluid Mech.*, **1**, 216 (1951).
4. Bilous, O., and N. R. Amundson, *A.I.Ch.E. Journal*, **1**, 513 (1955).
5. Campbell, A., *Bact. Rev.*, **21**, 263 (1957).
6. Danckwerts, P. V., *Chem. Eng. Sci.*, **2**, 1, (1953); pp. 93-102 in "Chemical Reaction Engineering," Pergamon Press, London (1957).
7. Denbigh, K. G., *Trans. Faraday Soc.*, **40**, 352 (1944).
8. Finn, R. K., and R. E. Wilson, *J. Agr. Food Chem.*, **2**, 66 (1954).
9. Halvorson, H. Orin, private communication to author.
10. Henrici, A. T., "Morphologic Variation and the Rate of Growth of Bacteria," Charles C. Thomas, Springfield, Illinois (1928).
11. Herbert, D., R. Elsworth, and R. C. Telling, *J. Gen. Microbiol.*, **14**, 601 (1956).
12. Hershey, A. D., and J. Bronfenbrenner, *J. Gen. Physiol.*, **21**, 721 (1938).
13. Hinshelwood, C. N., "The Chemical Kinetics of the Bacterial Cell," The Clarendon Press, Oxford (1946).
14. Hotchkiss, R. D., *Proc. Natl. Acad. Sci.*, **40**, 49 (1954).
15. Lapidus, L., "Digital Computation for Chemical Engineers," McGraw-Hill, New York (1962).
16. Lark, K. G., and O. Maaløe, *Biochim. Biophys. Acta*, **15**, 345 (1954).
17. Malmgren, B., and C. G. Hedén, *Acta Pathl. Microbiol. Scand.*, **24**, 448 (1947); **24**, 472 (1947).
18. Maxon, W. D., *Appl. Microbiol.*, **3**, 110 (1955) .
19. Monod, J., "Recherches sur la Croissance des Cultures Bactériennes," Herman and Cie, Paris (1942); *Ann. Inst. Pasteur*, **79**, 390 (1950); *Ann. Rev. Microbiol.*, **3**, 371 (1949).
20. Morimura, Y., *Physiol. Plantarum*, **13**, 20 (1960).
21. Nihei, T., T. Sasa, S. Miyachi, K. Suzuki, and H. Tamiya, *Arch. Mikrobiol.*, **21**, 156 (1954).
22. Novick, A., and L. Szilard, *Science*, **112**, 715 (1950); *Proc. Natl. Acad. Sci.*, **36**, 78 (1950). See also Novick, A., *Ann. Rev. Microbiol.*, **9**, 97 (1955).
23. Ping Shu, *J. Biochem. Microbiol Technol. Eng.*, **3**, 95, (1961).
24. Reusser, J., *Appl. Microbiol.*, **9**, 361 (1962); **9**, 366 (1962).
25. Rudd, D. F., in press (*Ind. Eng. Chem., Fundamentals*).
26. Sorokin, C., *Plant Physiol.*, **36**, 232 (1961).
27. Tamiya, H., T. Iwamura, K. Shibata, E. Hase, and T. Nihei, *Biochim. Biophys. Acta*, **12**, 23 (1953).

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