STUDIES ON CONTINUOUS FERMENTATION PART 1. THE CONCEPT OF THE MEAN CUMULATIVE-AGE OF MICROBES

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Most industrial fermentations are conducted batchwise. Recently, however, more attention has been paid to continuous fermentation, due primarily to the development of fermentation technology. The facts that vast numbers of research works pertaining to continuous fermentation have been published during the past few decades and that many International Symposia on this topic have been held in the past several years indicate the current interests in this field.

The performance of a batch fermentation is depicted usually by the concentrations of cells, products, carbohydrates, pH values, and so forth, plotted against the fermentation time. The microbes incessantly undergo changes of physiological phases in the batch culture. Theoretically, the physiological phases of microbes, when they are cultured continuously, are kept unchanged, irrespective of the fermentation time. In that sense, one cannot use the fermentation time as a scale of time with which the performance of the batch, and the continuous runs of the same fermentation, are assessed and compared.

It is the purpose of this paper to introduce a time-scale which can be shared by both the batch and the continuous runs in terms of evaluating the performance of both types of fermentation. The time-scale introduced here is based on an assumption that the age of cells is zero when they are newly formed, while the age of the mother cells becomes older with the duration of fermentation. The mean cumulative-age of a culture system at a specific time of fermentation is thus the cumulative age of the mother and daughter cells divided by the total cells present. It is evident that the age as defined in this paper is entirely different from the physiological age. Particularly with respect to bacteria, the concept of the cumulative-age is incompatible with the age concept which has been accepted by many people. Therefore, the calculation and discussion which follow in this account will be confined tacitly to multi-cellular microbes or yeasts in order to avoid undue repercussions from the physiological side.

Analytical studies are necessary to assess a fermentation in such a way that the design and operational basis of the continuous run can/may be revealed. The argument whether or not the concept of the mean cumulative-age of microbes as defined in this paper is valid remains to be investigated

and the experimental data which have been or will be published by many workers referred to. Nevertheless, this discussion will be useful for analyzing the process of fermentation, continuous or batch, from an angle which seems to have not yet been adopted.

SINGLE VESSELS

a) Batch

A growth curve of microbes is represented schematically in Fig. 1, in which aggregate mass (or number), x of microbes is plotted against time, T of fermentation on a semi-logarithmic scale. The schematic course of

Fig. 1. Schematic representation of growth of microbes.

Table 1. Growth of microbes (see Fig. 1).

Phase	Specific growth rate	Cell mass at the start of each phase	Period of time of fermentation	Mean cumulative-age of microbes at the start of each phase
I	$\mu_I > 0$	x_{0I}	$0 \sim T_I$	0
II	$\mu_{II} > 0$	x_{0II}	$T_I \sim T_{II}$	$\bar{A}_I^* (= \bar{A}_{II}^0)$
III	$\mu_{III} = 0$	x_{0III}	$T_{II} \sim T_{III}$	$\bar{A}_{II}^* (= \bar{A}_{III}^0)$
IV	$\mu_{IV} < 0$	x_{0IV}	$T_{III} \sim$	$\bar{A}_{III}^* (= \bar{A}_{IV}^0)$

fermentation in the figure is described as shown in Table 1. The term $\bar{A}$ (mean cumulative-age) in the table will be described below. The growth curve is subdivided into four phases in Fig. 1 only for convenience. This sort of subdivision will be made depending on a specific growth curve in question when necessary, and the subdivided phases do not necessarily have any particular relations with the physiological one—lag, logarithmic, stationary, and so on—. However, it will become clear from the following equations that the conditions assumed in Fig. 1 or in Table 1 can be applied to a practical problem as will be shown in the examples of calculation.

The definition of $\bar{A}$ and equations to calculate the value of $\bar{A}$ in each phase of Table 1 (or Fig. 1) are as follows:

Phase I ($\mu_1 > 0$)

At Δt starting from $T=0$, the mean cumulative-age, $\bar{A}_{\Delta t, I}$ is defined as below by neglecting the second order, $\sim \Delta t^2$ of time intervals.

$$\bar{A}_{T=0 \rightarrow T=\Delta t} = \bar{A}_{\Delta t, I} = \frac{x_{0I}\Delta t + (\mu_1 x_{0I}\Delta t)\Delta t'}{x_{0I} + \mu_1 x_{0I}\Delta t} = \frac{\Delta t}{1 + \mu_1 \Delta t}$$

where

$$0 \leq \Delta t' \leq \Delta t$$

Likewise at $2\Delta t$ starting from $T=0$,

$$\begin{aligned} \bar{A}_{T=0 \rightarrow T=2\Delta t} &= \bar{A}_{2\Delta t, I} = \frac{(x_{0I} + \mu_1 x_{0I}\Delta t)(\bar{A}_{\Delta t, I} + \Delta t) + \{\mu_1(x_{0I} + \mu_1 x_{0I}\Delta t)\Delta t\}\Delta t'}{(x_{0I} + \mu_1 x_{0I}\Delta t) + \mu_1(x_{0I} + \mu_1 x_{0I}\Delta t)\Delta t} \\ &= \frac{\bar{A}_{\Delta t, I} + \Delta t}{1 + \mu_1 \Delta t} = \Delta t \left\{ \frac{1}{1 + \mu_1 \Delta t} + \frac{1}{(1 + \mu_1 \Delta t)^2} \right\} \end{aligned}$$

Therefore, the mean cumulative-age, $\bar{A}_I (= \bar{A}_{T=0 \rightarrow T=\varepsilon \Delta t})$ will be defined by:

$$\begin{aligned} \bar{A}_{T=0 \rightarrow T=\varepsilon \Delta t} &= \bar{A}_I = \left\{ \frac{1}{1 + \mu_1 \Delta t} + \frac{1}{(1 + \mu_1 \Delta t)^2} + \cdots + \frac{1}{(1 + \mu_1 \Delta t)^\varepsilon} \right\} \Delta t \\ &= \left\{ \frac{1 - \left(\frac{1}{1 + \mu_1 \Delta t} \right)^{\varepsilon+1}}{1 - \left(\frac{1}{1 + \mu_1 \Delta t} \right)} - 1 \right\} \Delta t = \frac{1}{\mu_1} \left\{ 1 - \left(\frac{1}{1 + \mu_1 \Delta t} \right)^\varepsilon \right\} \\ &= \frac{1}{\mu_1} (1 - e^{-\mu_1 T}) \end{aligned} \quad (1)$$

provided:

$$\Delta t \rightarrow 0$$

Phase II ($\mu_{II} > 0$)

At Δt starting from $T=T_I$, the mean cumulative-age, $\bar{A}_{\Delta t, II}$ is defined as follows:

$$\bar{A}_{T=T_I \rightarrow T=T_I + \Delta t} = \bar{A}_{\Delta t, II} = \frac{x_{0II}(\bar{A}_I^* + \Delta t) + \mu_{II}x_{0II}\Delta t \Delta t'}{x_{0II} + \mu_{II}x_{0II}\Delta t} = \frac{\bar{A}_I^* + \Delta t}{1 + \mu_{II}\Delta t}$$

where

$$\bar{A}_I = \bar{A}_I^* \text{ at } T=T_I$$

At $2\Delta t$ starting from $T=T_I$,

$$\begin{aligned}\bar{A}_{T=T_I \rightarrow T=T_I+2\Delta t} &= \bar{A}_{2\Delta t, II} = \frac{(x_{0II} + \mu_{II}x_{0II}\Delta t)(\bar{A}_{\Delta t, II} + \Delta t) + \{\mu_{II}(x_{0II} + \mu_{II}x_{0II}\Delta t)\Delta t\}\Delta t'}{(x_{0II} + \mu_{II}x_{0II}\Delta t) + \mu_{II}(x_{0II} + \mu_{II}x_{0II}\Delta t)\Delta t} \\ &= \frac{\bar{A}_{\Delta t, II} + \Delta}{1 + \mu_{II}\Delta t} = \Delta t \left\{ \frac{1}{1 + \mu_{II}\Delta t} + \frac{1}{(1 + \mu_{II}\Delta t)^2} \right\} + \frac{\bar{A}_I^*}{(1 + \mu_{II}\Delta t)^2}\end{aligned}$$

Then,

$$\begin{aligned}\bar{A}_{T=T_I \rightarrow T=T_I+\varepsilon\Delta t} &= \bar{A}_{II} = \left\{ \frac{1}{1 + \mu_{II}\Delta t} + \frac{1}{(1 + \mu_{II}\Delta t)^2} + \dots + \frac{1}{(1 + \mu_{II}\Delta t)^\varepsilon} \right\} \Delta t + \frac{\bar{A}_I^*}{(1 + \mu_{II}\Delta t)^\varepsilon} \\ &= \frac{1}{\mu_{II}} \left\{ 1 - \left(\frac{1}{1 + \mu_{II}\Delta t} \right)^\varepsilon \right\} + \frac{\bar{A}_I^*}{(1 + \mu_{II}\Delta t)^\varepsilon} \\ &= \frac{1}{\mu_{II}} \{ 1 - e^{-\mu_{II}(T-T_I)} \} + \bar{A}_I^* e^{-\mu_{II}(T-T_I)}\end{aligned} \quad (2)$$

provided :

$$\begin{aligned}\Delta t &\rightarrow 0 \\ T_I &\leq T \leq T_{II}\end{aligned}$$

Phase III ($\mu_{III}=0$)

At Δt starting from $T=T_{II}$,

$$\begin{aligned}\bar{A}_{T=T_{II} \rightarrow T=T_{II}+\Delta t} &= \bar{A}_{\Delta t, III} = \frac{x_{0III}(\bar{A}_{II}^* + \Delta t) + \mu_{III}x_{0III}\Delta t}{x_{0III} + \mu_{III}x_{0III}\Delta t} \\ &= \bar{A}_{II}^* + \Delta t\end{aligned}$$

where

$$\bar{A}_{II} = \bar{A}_{II}^* \text{ at } T = T_{II}$$

Accordingly at $\varepsilon\Delta t$,

$$\bar{A}_{T=T_{II} \rightarrow T=T_{II}+\varepsilon\Delta t} = \bar{A}_{III} = \bar{A}_{II}^* + \varepsilon\Delta t = \bar{A}_{II}^* + (T - T_{II}) \quad (3)$$

where

$$T_{II} \leq T \leq T_{III}$$

Phase IV ($\mu_{IV}<0$)

At Δt starting from $T=T_{III}$,

$$\bar{A}_{T=T_{III} \rightarrow T=T_{III}+\Delta t} = \bar{A}_{\Delta t, IV} = \frac{(x_{0IV} + \mu_{IV}x_{0IV}\Delta t)(\bar{A}_{III}^* + \Delta t)}{x_{0IV} + \mu_{IV}x_{0IV}\Delta t} = \bar{A}_{III}^* + \Delta t$$

where

$$\bar{A}_{III} = \bar{A}_{III}^* \text{ at } T = T_{III}$$

Consequently,

$$\bar{A}_{T=T_{III} \rightarrow T=T_{III}+\varepsilon\Delta t} = \bar{A}_{IV} = \bar{A}_{III}^* + \varepsilon\Delta t = \bar{A}_{III}^* + (T - T_{III}) \quad (4)$$

where

$$T_{III} \leq T$$

b) *Continuous*

A mass balance of microbial cells is shown by the following equation.

$$\frac{dx_1}{dt} = -F \frac{x_1}{V} + \mu_1 x_1 \quad (5)$$

In steady state, $\frac{dx_1}{dt}=0$

Then,
$$\mu_1 = \frac{F}{V} = \frac{1}{\theta} \quad (6)$$

where

F : feed rate, m³/hr V : liquid volume, m³ θ : holding time, hr

If a complete mixing of microbes within the vessel of continuous fermentation is assumed, each one of the cells whose population increases from x_1 to $(x_1 + \mu_1 x_1 \Delta t)$ during Δt has a probability, $\left(1 - \frac{\mu_1 x_1 \Delta t}{x_1 + \mu_1 x_1 \Delta t}\right)$ of remaining within the vessel to keep the value of x_1 constant. Assuming further that $\bar{A}_1 = \bar{A}_1^0$ at $T = T_0$,

$$\begin{aligned} \bar{A}_{T=T_0 \rightarrow T=T_0+\Delta t} = \bar{A}_{\Delta t} &= \frac{x_1 \left(1 - \frac{\mu_1 x_1 \Delta t}{x_1 + \mu_1 x_1 \Delta t}\right) (\bar{A}_1^0 + \Delta t) + (\mu_1 x_1 \Delta t) \left(1 - \frac{\mu_1 x_1 \Delta t}{x_1 + \mu_1 x_1 \Delta t}\right) \Delta t}{x_1} \\ &= \frac{1}{1 + \mu_1 \Delta t} (\bar{A}_1^0 + \Delta t) \\ \bar{A}_{T=T_0 \rightarrow T=T_0+2\Delta t} = \bar{A}_{2\Delta t} &= \frac{x_1 \left(1 - \frac{\mu_1 x_1 \Delta t}{x_1 + \mu_1 x_1 \Delta t}\right) (\bar{A}_{\Delta t} + \Delta t) + (\mu_1 x_1 \Delta t) \left(1 - \frac{\mu_1 x_1 \Delta t}{x_1 + \mu_1 x_1 \Delta t}\right) \Delta t}{x_1} \\ &= \Delta t \left\{ \frac{1}{1 + \mu_1 \Delta t} + \frac{1}{(1 + \mu_1 \Delta t)^2} \right\} + \bar{A}_1^0 \left(\frac{1}{1 + \mu_1 \Delta t} \right)^2 \\ \bar{A}_{T=T_0 \rightarrow T=T_0+\varepsilon \Delta t} = \bar{A}_1 &= \left\{ \frac{1}{1 + \mu_1 \Delta t} + \frac{1}{(1 + \mu_1 \Delta t)^2} + \dots + \frac{1}{(1 + \mu_1 \Delta t)^\varepsilon} \right\} \Delta t + \bar{A}_1^0 \left(\frac{1}{1 + \mu_1 \Delta t} \right)^\varepsilon \\ &= \frac{1}{\mu_1} \left\{ 1 - \left(\frac{1}{1 + \mu_1 \Delta t} \right)^\varepsilon \right\} + \bar{A}_1^0 \left(\frac{1}{1 + \mu_1 \Delta t} \right)^\varepsilon \\ &= \frac{1}{\mu_1} \{ 1 - e^{-\mu_1 (T - T_0)} \} + \bar{A}_1^0 e^{-\mu_1 (T - T_0)} \end{aligned} \quad (7)$$

provided: $\Delta t \rightarrow 0$

It is clear that Equations (1) (or (2)) and (7) have similar forms; this means that no substantial differences between the batch and the continuous operations are found so far as the values of specific growth rate constant in these equations and other initial values are the same regardless of the batch and continuous runs. It is also seen from Eq. (7) that the value of $\bar{A}_1$ approaches $1/\mu_1$ (holding time) when T becomes large, *i.e.*, as the continuous cultivation proceeds for a long period of time; $\bar{A}_1 = \bar{A}_1^\infty = 1/\mu_1$.

c) Example of calculation

An example of propagation of yeast cells is cited in Fig. 2. The data points are marked with cross symbols in the figure (1). Curves I and II are drawn tentatively through the data points as shown in the figure re-

Fig. 2. Propagation of yeast cells in molasses (I) (Batch run).

presenting Phases I and II respectively.

The values of the specific growth rate in each phase of this example

Fig. 3. T vs. $\bar{\lambda}$ (calculation) (see Fig. 2).

are as follows:

$$\mu_I = 0.268 \text{ hr}^{-1}, \quad \mu_{II} = 0.112 \text{ hr}^{-1}, \quad T_I = 15 \text{ hrs}$$

Substituting the above-mentioned values into Equations (1) and (2), the relation between T and $\bar{A}$ is calculated and plotted in Fig. 3.

The reason why a point of discontinuity is seen at $T=15$ hrs in Fig. 3 stems from the fact that Curves I and II are arbitrarily defined as shown in Fig. 2. If a smooth curve is drawn through the points in Fig. 2 and if the curve is expressed by an appropriate equation, the calculation of $\bar{A}$ based on the equation will result in a smooth curve without any point of discontinuity such as indicated in Fig. 3. The latter kind of example has been illustrated elsewhere (2). To recapitulate, the existence of the discontinuity of curves in Fig. 3 is of no serious significance, depending only on how to approximate a curve through the data points in Fig. 2.

TWO EQUI-VOLUME VESSELS IN SERIES

Suppose that two vessels in series, inoculated and cultured under the same conditions, be put into continuous operation at $T=I$. For simplicity the total mass and the mean cumulative-age of microbes flowing from the 1st into the 2nd vessel are assumed to be constant (x_1 and $\bar{A}_1^\infty$).

a) Estimation of the specific growth rate, μ_2 in the 2nd vessel

The rate of change, $\frac{dx_2}{dt}$ of the mass (or number) of microbes in the 2nd vessel is:

$$\frac{dx_2}{dt} = \frac{F}{V} x_1 - \frac{F}{V} x_2 + \mu_2 x_2 = \mu_1 x_1 - x_2 (\mu_1 - \mu_2) \quad (8)$$

Integrating Eq. (8) with the initial condition of $x_2 = x_1$ at $t=0$ ($T=I$),

$$\frac{x_2}{x_1} = \left\{ 1 - \frac{1}{1 - \left(\frac{\mu_2}{\mu_1} \right)} \right\} e^{-\mu_1 \left\{ 1 - \left(\frac{\mu_2}{\mu_1} \right) \right\} t} + \frac{1}{1 - \left(\frac{\mu_2}{\mu_1} \right)} \quad (9)$$

In steady state,

$$\frac{x_2}{x_1} = \frac{\mu_1}{\mu_1 - \mu_2} \quad (10)$$

The value of μ_2 can be estimated from Eq. (10), provided that the values of x_2/x_1 and μ_1 are given. It is also easy to compute from Eq. (9) the time, t required, for a steady state is realized in the 2nd vessel after the initiation of the continuous operation at $T=I$ ($t=0$).

b) Calculation of the mean cumulative-age in the 2nd vessel

Mass (or number) of cells flowing into the 2nd vessel during Δt is:

$$\frac{F}{V} x_1 \Delta t = \mu_1 x_1 \Delta t$$

Mass (or number) of cells generated in the 2nd vessel during Δt is: $\mu_2 x_2 \Delta t$. The following equation implies that each cell has a probability, p of remaining within the 2nd vessel, provided that the cells are mixed completely and the value of x_2 is kept unchanged.

$$\begin{aligned} p &= 1 - \frac{(\mu_1 x_1 + \mu_2 x_2) \Delta t}{x_2 + (\mu_1 x_1 + \mu_2 x_2) \Delta t} = \frac{x_2}{x_2 + (\mu_1 x_1 + \mu_2 x_2) \Delta t} \\ &= \frac{1}{1 + \left\{ \mu_1 \left(\frac{x_1}{x_2} \right) + \mu_2 \right\} \Delta t} = \frac{1}{1 + K_2 \Delta t} \end{aligned} \quad (11)$$

where
$$K_2 = \mu_1 \left(\frac{x_1}{x_2} \right) + \mu_2 \quad (11)'$$

Consequently, as was conducted in the preceding section,

$$\begin{aligned} \bar{A}_{T=\Gamma \rightarrow T=\Gamma+\Delta t} &= \bar{A}_{\Delta t} = \frac{\mu_1 x_1 \Delta t p \bar{A}_1^\infty + x_2 p (\bar{A}_2^0 + \Delta t) + \mu_2 x_2 \Delta t p \Delta t'}{x_2} \\ &= \mu_1 \left(\frac{x_1}{x_2} \right) \Delta t p \bar{A}_1^\infty + p \bar{A}_2^0 + p \Delta t \\ \bar{A}_{T=\Gamma \rightarrow T=\Gamma+2\Delta t} &= \bar{A}_{2\Delta t} = \frac{\mu_1 x_1 \Delta t p \bar{A}_1^\infty + x_2 p (\bar{A}_{\Delta t} + \Delta t) + \mu_2 x_2 \Delta t p \Delta t'}{x_2} \\ &= \mu_1 \left(\frac{x_1}{x_2} \right) \Delta t p \bar{A}_1^\infty + p \left\{ \mu_1 \left(\frac{x_1}{x_2} \right) \Delta t p \bar{A}_1^\infty + p \bar{A}_2^0 + p \Delta t + \Delta t' \right\} \\ &= \mu_1 \left(\frac{x_1}{x_2} \right) \bar{A}_1^\infty \Delta t (p + p^2) + p^2 \bar{A}_2^0 + \Delta t (p + p^2) \end{aligned}$$

where $\bar{A}_2^0$ = mean cumulative-age of cells in the 2nd vessel at $T=\Gamma$
Then,

$$\begin{aligned} \bar{A}_{T=\Gamma \rightarrow T=\Gamma+\varepsilon \Delta t} &= \bar{A}_2 = \mu_1 \left(\frac{x_1}{x_2} \right) \bar{A}_1^\infty \Delta t (p + p^2 + \dots + p^\varepsilon) + p^\varepsilon \bar{A}_2^0 + \Delta t (p + p^2 + \dots + p^\varepsilon) \\ &= \Delta t (p + p^2 + \dots + p^\varepsilon) \left\{ 1 + \mu_1 \left(\frac{x_1}{x_2} \right) \bar{A}_1^\infty \right\} + p^\varepsilon \bar{A}_2^0 \\ &= \frac{1}{K_2} \{ 1 - e^{-K_2(T-\Gamma)} \} \left\{ 1 + \mu_1 \left(\frac{x_1}{x_2} \right) \bar{A}_1^\infty \right\} + e^{-K_2(T-\Gamma)} \bar{A}_2^0 \end{aligned} \quad (12)$$

provided: $\Delta t \rightarrow 0$

If T becomes large, Eq. (12) is simplified as follows:

$$\bar{A}_2^\infty = \frac{1}{K_2} \left\{ 1 + \left(\frac{x_1}{x_2} \right) \right\} \quad (12)'$$

where $\mu_1 = \frac{1}{\bar{A}_1^\infty}$, $\bar{A}_2 = \bar{A}_2^\infty$

Substituting Equations (10) and (11)' into Eq. (12)',

$$\bar{A}_2^\infty = \frac{1}{\left\{ \mu_1 \left(\frac{\mu_1 - \mu_2}{\mu_1} \right) + \mu_2 \right\}} \left(1 + \frac{\mu_1 - \mu_2}{\mu_1} \right) = \frac{1}{\mu_1} \left(2 - \frac{\mu_2}{\mu_1} \right) \quad (13)$$

Generally, since $0 \leq \mu_2 \leq \mu_1$, $\bar{A}_1^\infty \leq \bar{A}_2^\infty \leq 2\bar{A}_1^\infty$.

If $\mu_1 = \mu_2$ in Eq. (13), $\bar{A}_1^\infty = \bar{A}_2^\infty$; if $x_2 = \infty$ in Eq. (12)', $\bar{A}_1^\infty = \bar{A}_2^\infty$, because the value of K_2 is equal to μ_1 from Equations, (10) and (11)' in a steady state in terms of x_2 (and x_1). It is evident from these facts that when $\mu_1 = \mu_2$, the continuous fermentation in two vessels in series is meaningless (see the former fact) or impossible (see the latter) from the viewpoint of the mean cumulative-age. When $\mu_2 = 0$, $\bar{A}_2^\infty = 2\bar{A}_1^\infty$.

If values of $\bar{A}$ greater than $2\bar{A}_1^\infty$ are required, a multi-stage series of vessels of more than two will be needed.

c) Example of calculation

The experimental data regarding alcohol fermentation of molasses in two vessels in series are cited from the literature (3) in Table 2. The continuous feeding started with a condition of $V/F = 12$ hrs in this example at 18th hr.

Using the four data points in a batch operation which preceded the continuous cultivation and assuming that three different values of the specific growth rate, μ represent the growth pattern in this range of $T = 0$

Table 2. Alcohol fermentation of molasses; two vessels in series (3).

Time T (hrs)	1st Vessel					2nd Vessel			
	Total sugar (g/100ml)	Yeast cells x_1 (1/ml)	pH	V_1/F (hrs)	V_1 (m^3)	Total sugar (g/100ml)	Yeast cells x_2 (1/ml)	V_2/F (hrs)	V_2 (m^3)
0	12.36	$\times 10^6$ 1	4.38	—	$\times 10^{-3}$ 8	—	$\times 10^6$ —	—	$\times 10^{-3}$ —
6	11.76	11	—	—	8	—	—	—	—
12	8.64	42	4.26	—	8	—	—	—	—
18	3.10	89	—	—	8	—	—	—	—
20	2.95	—	4.08	12	8	—	—	—	—
23	2.80	—	—	12	8	—	—	—	—
24	2.73	—	—	12	8	—	—	—	—
38	2.89	119	4.13	12	8	1.23	127	12	8
40	2.77	125	4.08	12	8	1.20	130	12	8
44	2.65	120	4.05	12	8	1.18	125	12	8
48	2.55	118	4.03	12	8	1.18	112	12	8
54	2.58	125	4.05	12	8	1.16	125	12	8
56	2.58	120	4.05	12	8	1.34	110	6	4
58	2.54	115	4.03	12	8	1.41	120	6	4
60	2.55	125	4.03	12	8	1.56	115	6	4
62	2.62	118	4.05	12	8	1.90	116	6	4
64	2.60	118	4.05	12	8	1.60	110	6	4

Fig. 4. T vs. $\bar{A}$ (see Table 2).

to $T=18$ hrs, the values of μ were determined from each segmental period of 6 hrs as follows:

$$\mu_I = 0.400 \text{ hr}^{-1}, \quad \mu_{II} = 0.223 \text{ hr}^{-1} \quad \text{and} \quad \mu_{III} = 0.125 \text{ hr}^{-1}.$$

The relation between T and $\bar{A}$ covering the period from $T=0$ to $T=18$ hrs was then calculated using Equations (1) and (2) (see Fig. 4).

The specific growth rate, μ_I in the 1st vessel is assumed to be $1/12 \text{ hr}^{-1}$ after the start of the continuous cultivation because the concentration of yeast cells in the 1st vessel was nearly constant under the operating condition of $V/F=12$ hrs (see Eq. (6)). Curve A in Fig. 4 regarding the 1st vessel was calculated by substituting

$$\mu_I = 1/12 = 0.0833 \text{ hr}^{-1} (= \mu_I \text{ in Eq. (7)}),$$

$$\bar{A}_1^0 = 5.92 \text{ hrs, and } T_0 = 18 \text{ hrs}$$

into Eq. (7).

Assuming $x_2/x_1 \doteq 1.0$ in Table 2, that is, assuming $\mu_2=0$ (see Eq. (10)), the relation between $\bar{A}_2$ and T was calculated using Eq. (12) for $V_1=V_2$. Since the value of $\bar{A}_1$ of the cells flowing from the 1st into the 2nd vessel changes with time as is apparent from Fig. 4, Curves B, C and D in the figure were prepared by substituting $\bar{A}_1^\infty=6, 9$ and 12 hrs respectively into Eq. (12) ($\bar{A}_2^0=5.92$ hrs, $T=18$ hrs). Although an exact course of $\bar{A}_2$ with time, T was not determined in this illustrative calculation, it is considered

that the mean cumulative-age, $\bar{A}_2$ of the cells in the 2nd vessel will change starting from S in Fig. 4, passing across Curve C and finally along Curve D till the time when the value of V_2 of the 2nd vessel changed by one-half at 56th hr as shown in Table 2.

Regarding $V_2=1/2 V_1$, the relation between T and $\bar{A}_2$ was calculated (see Curve E in Fig. 4), substituting the following values into Eq. (16) (see later):

$$m=2, \quad K_m=K_2=\mu_1, \quad I'=18 \text{ hrs}, \quad \zeta_m=\zeta_2=\frac{1}{2}, \quad \mu_m=\mu_2=0,$$

$$\bar{A}_{m-1}^\infty=\bar{A}_1^\infty=12 \text{ hrs}, \quad \bar{A}_m^0=\bar{A}_2^0=5.92 \text{ hrs}.$$

The exact course of $\bar{A}_2$ at the transient period of reducing the volume from $V_2=V_1$ to $V_2=1/2 V_1$ was left unclarified. However, the value of $\bar{A}_2$ will be represented eventually by Curve E.

MULTI-STAGE VESSELS IN SERIES

Suppose that multi-stage vessels, each volume of which is $V_1, \zeta_2 V_1, \dots, \zeta_{m-1} V_1$, and $\zeta_m V_1$, and m in total number of vessels be inoculated and cultured under the same condition individually. A continuous cultivation starts at $T=I'$ by feeding continuously a fresh cultural medium into the 1st vessel and by connecting appropriately the multi-stage vessels in series. Suppose, for simplicity, that the values of x_{m-1} and $\bar{A}_{m-1}$ of the microbes flowing from the $(m-1)$ th vessel into the m th vessel are constant at $T=I'$.

a) Estimation of the specific growth rate, μ_m in the m th vessel

The rate of change, $\frac{dx_m}{dt}$ of microbes in the m th vessel is:

$$\frac{dx_m}{dt} = \frac{F}{\zeta_{m-1} V_1} x_{m-1} - \frac{F}{\zeta_m V_1} x_m + \mu_m x_m = \frac{\mu_1}{\zeta_{m-1}} x_{m-1} - \frac{\mu_1}{\zeta_m} x_m + \mu_m x_m$$

In steady state, $\frac{dx_m}{dt}=0$.

Then,

$$\frac{x_m}{x_{m-1}} = \frac{-\frac{\mu_1}{\zeta_{m-1}}}{\mu_m - \frac{\mu_1}{\zeta_m}} = \left(\frac{\zeta_m}{\zeta_{m-1}} \right) \left(\frac{\mu_1}{\mu_1 - \zeta_m \mu_m} \right) \quad (14)$$

b) Calculation of the mean cumulative-age in the m th vessel

Referring to Eq. (11), a probability, p of cells remaining in the m th vessel will be:

$$p = 1 - \frac{\left(\frac{\mu_1}{\zeta_{m-1}} x_{m-1} + \mu_m x_m \right) \Delta t}{x_m + \left(\frac{\mu_1}{\zeta_{m-1}} x_{m-1} + \mu_m x_m \right) \Delta t} = \frac{\frac{x_m}{x_{m-1}}}{\frac{x_m}{x_{m-1}} + \left\{ \frac{\mu_1}{\zeta_{m-1}} + \mu_m \left(\frac{x_m}{x_{m-1}} \right) \right\} \Delta t}$$

$$= \frac{1}{1 + \left(\frac{\mu_1}{\zeta_{m-1}} \frac{x_{m-1}}{x_m} + \mu_m \right) \Delta t} = \frac{1}{1 + K_m \Delta t} \quad (15)$$

where

$$K_m = \frac{\mu_1}{\zeta_{m-1}} \frac{x_{m-1}}{x_m} + \mu_m \quad (15)'$$

Following the same procedure as was described in the preceding section, $\bar{A}_m$ will be described as follows:

$$\begin{aligned} \bar{A}_m &= \frac{1}{K_m} \{1 - e^{-K_m(T-\Gamma')}\} \left\{ 1 + \frac{\mu_1}{\zeta_{m-1}} \left(\frac{x_{m-1}}{x_m} \right) \bar{A}_{m-1}^\infty \right\} + e^{-K_m(T-\Gamma')} \bar{A}_m^0 \\ &= \frac{1}{K_m} \{1 - e^{-K_m(T-\Gamma')}\} \left\{ 1 + \left(\frac{\mu_1}{\zeta_m} - \mu_m \right) \bar{A}_{m-1}^\infty \right\} + e^{-K_m(T-\Gamma')} \bar{A}_m^0 \end{aligned} \quad (16)$$

provided:

$$\Delta t \rightarrow 0,$$

$\bar{A}_m^0$ = mean cumulative-age of cells in the m th vessel at $T = \Gamma'$

As the number of m increases, it seems to be difficult to realize the situation which corresponds to Eq. (16). However, it is of more significance to assess the values of $\bar{A}_m$ from Eq. (16) when T becomes large so that $\bar{A}_m = \bar{A}_m^\infty$. Namely,

$$\bar{A}_m = \bar{A}_m^\infty = \frac{1}{K_m} \left\{ 1 + \frac{\mu_1}{\zeta_{m-1}} \left(\frac{x_{m-1}}{x_m} \right) \bar{A}_{m-1}^\infty \right\} = \frac{1}{K_m} \left\{ 1 + \left(\frac{\mu_1}{\zeta_m} - \mu_m \right) \bar{A}_{m-1}^\infty \right\} \quad (16)'$$

From Equations (16) and (16)',

$$\bar{A}_m^\infty - \bar{A}_m = (\bar{A}_m^\infty - \bar{A}_m^0) e^{-K_m(T-\Gamma')} \quad (16)''$$

Substituting Equations (14) and (15)' into Eq. (16)',

$$\begin{aligned} \bar{A}_m^\infty &= \left(\frac{1}{\frac{\mu_1}{\zeta_{m-1}} \frac{x_{m-1}}{x_m} + \mu_m} \right) \left\{ 1 + \frac{\mu_1}{\zeta_{m-1}} \left(\frac{\zeta_{m-1}}{\zeta_m} \right) \left(\frac{\mu_1 - \zeta_m \mu_m}{\mu_1} \right) \bar{A}_{m-1}^\infty \right\} \\ &= \frac{1}{\frac{\mu_1}{\zeta_{m-1}} \frac{\zeta_{m-1}}{\zeta_m} \left(\frac{\mu_1 - \zeta_m \mu_m}{\mu_1} \right) + \mu_m} \left(1 + \frac{\mu_1}{\zeta_m} \bar{A}_{m-1}^\infty - \mu_m \bar{A}_{m-1}^\infty \right) \\ &= \frac{\zeta_m}{\mu_1} \left(1 + \frac{\mu_1}{\zeta_m} \bar{A}_{m-1}^\infty - \mu_m \bar{A}_{m-1}^\infty \right) = \zeta_m \bar{A}_1^\infty + \bar{A}_{m-1}^\infty - \zeta_m \bar{A}_1^\infty \mu_m \bar{A}_{m-1}^\infty \\ &= \bar{A}_{m-1}^\infty + \zeta_m \bar{A}_1^\infty (1 - \bar{A}_{m-1}^\infty \mu_m) \end{aligned} \quad (17)$$

It is expected from Eq. (17) that the values of $\bar{A}_m^\infty$ increase linearly with the number of vessels when the values of $\mu_i = \mu_{i+1} = \dots = \mu_m$ are zero; this situation corresponds to Eq. (3) or (4) in a batch operation. Another way of analyzing the mean cumulative-age of microbes in the multi-stage vessels starts from a "material balance" in terms of $\bar{A}_{m-1}$, and $\bar{A}_m$ regarding the m th vessel. This analysis, which is omitted here, may be consulted (4).

c) Example of calculation

The experimental data on a continuous alcohol fermentation using molas-

ses in three vessels in series are cited in Table 3 (3). In this example the continuous feeding started at $T=16$ th hr with a condition of $V/F=7$ hrs. The relation between T and $\bar{A}_1$ for the 1st vessel was calculated from Eq. (7), assuming that $\bar{A}_1^0=0$ and $\mu_1=1/7 \text{ hr}^{-1}$, because the values of x_1 were nearly constant after $T=24$ hrs as shown in Table 3. The result of the calculation is shown by Curve A in Fig. 5. Since the value of the specific growth rate of yeast is not clear in a batch run before $T=16$ th hr, Curve A below Point S in Fig. 5 was represented by a broken line.

It is evident from Table 3 that the values of the specific growth rate, μ_2 and μ_3 in the 2nd and 3rd vessels are not zero in this example. The values of μ_2 and μ_3 were estimated from Equations (10) and (14) respectively, assuming that the values of x in each vessel were $\bar{x}_2=71 \times 10^6$ and $\bar{x}_3=99 \times 10^6$ cells/ml in steady state.

From Eq. (10),

$$\frac{\mu_2}{\mu_1} = 1 - \frac{\bar{x}_1}{\bar{x}_2} = 1 - \frac{58 \times 10^6}{71 \times 10^6} = 0.18$$

$$\mu_2 = (0.18)(\mu_1) = (0.18)\left(\frac{1}{7}\right) = 0.026 \text{ hr}^{-1}.$$

From Eq. (14), taking $m=3$ and $\zeta_{m-1}=\zeta_m=1$,

$$\frac{\mu_3}{\mu_1} = 1 - \frac{\bar{x}_2}{\bar{x}_3} = 1 - \frac{71 \times 10^6}{99 \times 10^6} = 0.28$$

$$\mu_3 = (0.28)(\mu_1) = (0.28)\left(\frac{1}{7}\right) = 0.040 \text{ hr}^{-1}.$$

The procedure of calculating the relation between T and $\bar{A}_2$ for the 2nd vessel will be described first. It was confirmed from a preliminary calculation using Eq. (9) that a period of about 10 hrs will be required

Fig. 5. T vs. $\bar{A}$ (see Table 3).

Table 3. Alcohol fermentation of

Time T (hrs)	1st Vessel						
	Total sugar (g/100 ml)	Yeast cells x_1 (1/ml)	pH	V_1/F (hrs)	V_1 (m^3)	Total sugar (g/100 ml)	Yeast cells x_2 (1/ml)
0	12.49	$\times 10^6$ 1	4.51	—	$\times 10^{-3}$ 7	12.49	$\times 10^6$ 1
16	6.46	—	4.01	—	7	—	—
24	6.17	62	4.48	7	7	2.44	75
26	6.17	56	4.41	7	7	2.44	69
27	6.17	60	4.35	7	7	2.44	71
28	6.17	55	4.35	7	7	2.44	68
29	6.17	59	4.32	7	7	2.37	68
31	6.17	56	4.25	7	7	2.37	72

before a steady state of $\frac{\bar{x}_2}{\bar{x}_1} = \frac{71 \times 10^6}{58 \times 10^6} (=1.22)$ is attained after the continuous feeding started at Point S in Fig. 5. It is also clear from the figure that the value of $\bar{A}_1$ of cells from the 1st into the 2nd vessel varies from about 6.2 to 7.0 hrs as the continuous operation proceeds. Therefore the construction of Curve B in Fig. 5 was made by a stepwise calculation, substituting the following values into Eq. (12).

Step No.	μ_1 (hr^{-1})	μ_2 (hr^{-1})	T (hrs)	$\bar{A}_1^\infty$ (hrs)	$\bar{x}_2/\bar{x}_1$	$\bar{A}_2^0$ (hrs)
1	0.143	0.026	16~21	6.4	1.06	6.2
2	0.143	0.026	21~26	6.7	1.17	9.2
3	0.143	0.026	26~	7.0	1.22	10.8

The values of $\bar{A}_1$ used in the above calculation are the arithmetical mean of those which could be estimated from Curve A in Fig. 5 for each step, while the values of $\bar{x}_2/\bar{x}_1$ adopted were the interpolation $\Delta(\bar{x}_2/\bar{x}_1)=1.22-1.00$ with respect to T . The values of $\bar{A}_2^0$ shown above were obtained by successive calculation, starting from $\bar{A}_2^0=\bar{A}_1^\infty=6.2$ hrs in Step No. 1. It was ascertained that the curve of $\bar{A}_2$ did not have any significant difference from Curve B in Fig. 5 even if $\bar{A}_1^\infty=6.6$ hrs ($=6.2+7.0/2$), $\bar{x}_2/\bar{x}_1=1.22$ and $\bar{A}_2^0=6.2$ hrs were substituted into Eq. (12) without resorting to the stepwise calculation.

Similarly, the construction of Curve C in Fig. 5 was made by the following stepwise calculation using Eq. (16) ($m=3$, $\zeta_{m-1}=1$).

Step No.	μ_1 (hr^{-1})	μ_3 (hr^{-1})	T (hrs)	$\bar{A}_2^\infty$ (hrs)	$\bar{x}_3/\bar{x}_2$	$\bar{A}_3^0$
1	0.143	0.040	16~21	7.7	1.07	6.2
2	0.143	0.040	21~26	10.0	1.20	9.4
3	0.143	0.040	26~31	11.3	1.32	11.8
4	0.143	0.040	31~	12.7	1.39	13.5

molasses; three vessels in series (3).

2nd Vessel			3rd Vessel				
pH	V_2/F (hrs)	V_2 (m^3)	Total sugar (g/100 ml)	Yeast cells x_3 (1/ml)	pH	V_3/F (hrs)	V_3 (m^3)
4.51	—	$\times 10^{-3}$ 7	12.49	$\times 10^6$ 1	4.51	—	$\times 10^{-3}$ 7
—	—	—	—	—	—	—	—
4.36	7	7	—	—	—	—	—
4.30	7	7	1.00	—	—	7	7
4.30	7	7	1.00	96	4.24	7	7
4.30	7	7	1.00	107	4.24	7	7
4.33	7	7	1.00	85	4.24	7	7
4.29	7	7	1.00	106	4.24	7	7

In the above calculation Eq. (9), with its terms x_1 , x_2 and μ_2 being replaced with x_2 , x_3 and μ_3 respectively, was used to estimate the period of time required before the value of $\bar{x}_3/\bar{x}_2$ became constant starting from Point *S* in Fig. 5. The period needed was about 15 hrs.

It is worthwhile to point out the fact that ultimate values of $\bar{A}_2$ and $\bar{A}_3$ i.e., $\bar{A}_2^\infty$ and $\bar{A}_3^\infty$ are not two and three times as large as that of $\bar{A}_1^\infty$ in Fig. 5 as are apparent from Equations (13) and (17).

SUMMARY

The mean cumulative-age of microbes was defined, and a procedure of calculating the age thus defined was presented; examples of the calculation were given. A distinction between batch and continuous operations in fermentations will be revealed by using the concept of the mean cumulative-age of microbes.

Although an ultimate objective—whether or not the concept can be applied in the design and operation of a continuous fermentation—remains to be discussed, the cumulative-age concept and the procedure of calculating the microbial age are deemed worthy of attention in analyzing the experimental data, particularly of mycelial fermentations.

NOMENCLATURE

F : feed rate (m^3/hr)

K_2 : $\mu_1 \left(\frac{x_1}{x_2} \right) + \mu_2$ (hr^{-1})

K_m : $\frac{\mu_1}{\zeta_{m-1}} \frac{x_{m-1}}{x_m} + \mu_m$ (hr^{-1})

x : total mass (or number) of microbes or microbial concentration

- t : time (hr)
 Δt : time interval (hr)
 $\Delta t'$: $0 \leq \Delta t' \leq \Delta t$
 T : fermentation time (hr)
 V : volume of medium in vessel (m^3)
 Γ, Γ' : time (hr)
 ε : number of time intervals
 ζ : volume ratio of vessels in series
 θ : holding time
 $\bar{A}$: cumulative-age of microbes (hr)
 $\bar{A}$: mean cumulative-age of microbes (hr)
 $\bar{A}_{m-1}^0$: mean cumulative-age of cells in the $(m-1)$ th vessel at the start of continuous operation
 μ : specific growth rate (hr^{-1})

subscripts

- 1, 2, 3,—: 1st, 2nd, 3rd — vessels in series
 I,II,III,—: growth phases in batch operation

superscripts

- : arithmetical average
 $^{\circ}$: initial condition
 ∞ : value of $\bar{A}$ when $T = \infty$
 $*$: value of $\bar{A}$ at a particular time, T

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