Effect of Evaporation Losses on Experimental Continuous Culture Results

By W. R. KING, C. G. SINCLAIR AND H. H. TOPIWALA

Department of Chemical Engineering, University of Manchester Institute of Science and Technology, Manchester

(Accepted for publication 8 January 1972)

SUMMARY

Evaporation can lead to errors in the measurement of parameters such as yield and maintenance coefficient, using continuous cultures at dilution rates below 0.05 h^{-1} .

INTRODUCTION

This paper shows how simple practical details, if ignored, can lead to false conclusions regarding the kinetic model used for a continuous culture system. The need to humidify the air, when operating a continuous culture system at low dilution rates, can be assessed by measuring the function $\phi = \bar{x}_e/\bar{x}$, where $\bar{x}_e$ and $\bar{x}$ are the cell concentrations, which would be observed for evaporating and non-evaporating conditions, respectively. As shown later, this function becomes significant at dilution rates below 0.05 h^{-1} , values which were used in work reported for example by Marr, Nilson & Clark (1963) to calculate a maintenance energy constant (a), by Schulze & Lipe (1964) and by Tempest, Herbert & Phipps (1967). This error can be avoided by fixing a condenser on the gas outlet of the fermenter.

The rate of evaporation, D_e , will be a function of several parameters, including the stirrer speed, the temperature and humidity of the inlet air, the temperature of the liquid medium, the flow rate of air and the design of the vessel. The effect of evaporation has been calculated for three different theoretical models (Fig. 2 and 3).

THEORY

In Fig. 1, F_0 is the inlet liquid flow rate, F_1 the exit liquid flow rate and F_2 the evaporation rate of water into the air stream, all in units of litres of liquid per hour. The basic assumptions of a completely stirred tank reactor (C.S.T.R.) are assumed to hold for the models developed (i.e. the organism and substrate concentrations in the vessel are completely homogeneous and equal to their exit concentrations).

Monod's model. This is based on Monod's model (Monod, 1942, 1950) for the growth rate, μ .

Organism balance:

$$V \frac{dx_e}{dt} = \frac{V\mu_m x_e S_e}{K_s + S_e} - F_1 x_e. \quad (1)$$

Accumulation = Input - Output.

Substrate balance:

$$V \frac{dS_e}{dt} = F_0 S_R - F_1 S_e - \frac{V\mu_m x_e S_e}{Y(K_s + S_e)}. \quad (2)$$

Overall balance:

$$F_0 = F_1 + F_2. \quad (3)$$

At steady state, $dx_e/dt = dS_e/dt = 0$.

From equation (1):

$$0 = \frac{\mu_m \bar{x}_e \bar{S}_e}{K_s + \bar{S}_e} - D_1 \bar{x}_e. \quad (4)$$

From equation (2):

$$0 = D_0 S_R - D_1 \bar{S}_e - \frac{\mu_m \bar{x}_e \bar{S}_e}{Y(K_s + \bar{S}_e)}. \quad (5)$$

From equation (3):

$$D_1 = D_0 - D_2, \quad (6)$$

where $D_1 = F_1/V$, therefore $\bar{S}_e = K_s D_1 / (\mu_m - D_1)$ and $\bar{x}_e = Y/D_1 (D_0 S_R - D_1 \bar{S}_e)$.

If $D_0 = D_1$, $\bar{x} = Y(S_R - \bar{S})$, giving the steady-state cell concentration of the normal Monod model without evaporation.

Therefore, the expression for the parameter, ϕ , becomes

$$\phi = \frac{\bar{x}_e}{\bar{x}} = \frac{D_0 S_R - D_1 \bar{S}_e}{D_1 (S_R - \bar{S})}.$$

At low values of D , $\bar{S}$ and $\bar{S}_e \ll S_R$, therefore $\phi \approx D_0/D_1 = D_0/(D_0 - D_2)$.

Endogenous model. The second model is based on Herbert's formulation of endogenous metabolism (Herbert, 1958), in which the constant, k , accounts for the oxidation of cell substance to CO_2 . This constant endogenous metabolism added to the anabolic metabolism of the culture gives the modified equation set for evaporation.

Organism balance:

$$V \frac{dx_e}{dt} = V \left[\frac{(\mu_m + k) S_e}{K_s + S_e} - k \right] x_e - F_1 x_e. \quad (7)$$

Substrate balance:

$$V \frac{dS_e}{dt} = F_0 S_R - F_1 S_e - \frac{V(\mu_m + k) x_e S_e}{Y_p (K_s + S_e)}. \quad (8)$$

As before, the steady-state equations are solved to give:

$$\begin{aligned} \bar{S}_e &= \frac{K_s (D_1 + k)}{\mu_m - D_1}, \\ \bar{x}_e &= \frac{Y_p (D_0 S_R - D_1 \bar{S}_e)}{D_1 + k}, \\ D_0 &= D_1 + D_2. \end{aligned}$$

For $D_0 = D_1$, we obtain the cell concentration under non-evaporating conditions.

$$\bar{x} = \frac{Y_p D_0 (S_R - \bar{S})}{D_0 + k}.$$

Therefore

$$\frac{\bar{x}_e}{\bar{x}} = \frac{D_0 S_R - D_1 \bar{S}_e}{(D_1 + k)(S_R - \bar{S})} \frac{D_0 + k}{D_0},$$

and at low dilution rates, $\phi \approx (D_0 + k)/(D_0 + k - D_2)$.

Maintenance model. This was suggested by Pirt (1965), who included a specific maintenance term (a) accounting for the consumption of the limiting substrate to provide energy to maintain integrity and viability, but no biomass.

This maintenance model, when modified for evaporation, gives the equation set.

Organism balance:

$$\frac{dx_e}{dt} = \frac{\mu_m S_e}{K_s + S_e} x_e - D_1 x_e. \quad (9)$$

Fig. 1. Evaporating model.

Table 1.

Type and reference	Air flow rate (l liquid/h)	Temp. (°C)	Agitator speed (rev./min)	D_2 (h ⁻¹)
Continuous culture (Pirt, 1957)	2.0	37	1030	0.003
Continuous culture (this work)	0.7	25	250	0.0013
Continuous culture (this work)	1.2	45	450	0.00275
250 ml shake flask with 100 ml of culture (this work)	—	30	125	0.00034

Substrate balance:

$$\frac{dS_e}{dt} = D_0 S_R - D_1 S_e - \frac{\mu_m x_e S_e}{Y_g(K_s + S_e)} - \frac{a x_e}{Y_g}. \quad (10)$$

As before, the steady-state equations are solved to give

$$\begin{aligned} \bar{S}_e &= \frac{K_s D_1}{\mu_m - D_1}, \\ \bar{x}_e &= \frac{Y_g (D_0 S_R - D_1 \bar{S}_e)}{D_1 + a}, \\ D_0 &= D_1 + D_2. \end{aligned}$$

For non-evaporating conditions,

$$\bar{x} = \frac{Y_g D_0}{D_0 + a} (S_R - \bar{S}),$$

and therefore

$$\phi = \frac{\bar{x}_e}{\bar{x}} = \frac{D_0 S_R - D_1 \bar{S}_e}{(D_1 + a)(S_R - \bar{S})} \frac{D_0 + a}{D_0};$$

and at low dilution rates, $\phi = (D_0 + a)/(D_0 + a - D_2)$, which is identical in form to the simplified expression for the endogenous metabolism models.

Fig. 2. Monod's model errors. Curve 1, $D_2 = 0.001$; curve 2, $D_2 = 0.002$; curve 3, $D_2 = 0.003$.

Fig. 3. Endogenous metabolism or maintenance model errors. —, k (or a) = 0.03; ---, k or (a) = 0.01; curve 1, $D_2 = 0.001$; curve 2, $D_2 = 0.002$; curve 3, $D_2 = 0.003$.

EXPERIMENTAL

Realistic values of the parameters in these expressions for ϕ may be obtained from the literature, except for the effective evaporation rate (D_2). Pirt (1957) has published a value for evaporation in vigorously agitated laboratory-scale continuous culture. This is included together with some experimental values for D_2 obtained by us (Table 1).

The parameter, ϕ , was calculated from the simplified expression derived in the theory section and plotted against D_0 for a practical range of D_2 , as shown in Fig. 2 and 3. The expression for ϕ is identical for the endogenous and maintenance models and therefore the effect of different values of ' k ' and ' a ', respectively, will be identical.

DISCUSSION

The figures indicate that, for negligible endogenous metabolism, the accepted formulae connecting D , $\bar{x}_e$ and $\bar{S}_e$ need modification at low dilution rates and Herbert's model should read,

$$\begin{aligned}\bar{x}_e &= Y_p \frac{D_0 S_R - D_1 \bar{S}_e}{D_1 + k} \\ &= Y_p \frac{D_1}{D_1 + k} \left(\frac{D_0}{D_1} S_R - \bar{S}_e \right).\end{aligned}$$

The culture behaves exactly as if the dilution rate were D_1 , as indeed it is as far as the organism is concerned, with the ingoing substrate concentration of $D_0 S_R / D_1$. Even where the endogenous metabolism or maintenance energy constant is relatively large, the errors are not negligible. Steady state can be obtained at dilution rates down to 0.004 h^{-1} (Postgate & Hunter, 1962; Tempest *et al.* 1967) in bacterial cultures and that fungal cultures are operated at dilution rates as low as 0.0025 h^{-1} (Holme & Zacharias, 1965) with an endogenous metabolism constant k of nearly zero.

The low value for evaporation in shake flasks (Table 1; $D_2 = 0.0034 \text{ h}^{-1}$) must be considered in the context of the period of time the shake flask is on the rotary incubator. At this rate of evaporation the error in the measurement of organism concentration rises linearly with time and would be approximately 4% after five days.

We conclude therefore that errors in organism concentrations at low dilution rates can be significant especially for systems with low endogenous metabolism rates, when no special precautions are taken to reduce evaporation losses. When evaluating the endogenous metabolism constant (k) by plotting $\frac{1}{x}$ versus $\frac{1}{D_0}$, the slope of the line is equal to $(k - D_2) / Y_p S_R$ and not $k / Y_p S_R$. Thus, if evaporation were not taken into account the value of ' k ' (or ' a ') would be underestimated by an amount $D_2 \text{ h}^{-1}$.

Symbols

a	Substrate utilization constant due to maintenance requirements	h^{-1}
D	Dilution rate	h^{-1}
F_0	Inlet liquid flow rate	l/h
F_1	Exit liquid flow rate	l/h
F_2	Evaporation rate of water	l/h
k	Substrate utilization constant due to endogenous metabolism	h^{-1}
K_s	Monod's substrate constant, numerically equal to S at which the rate is $\frac{1}{2}\mu_m$	g/l
S	Substrate concentration under non-evaporating conditions	g/l

S_e	Substrate concentration under evaporating conditions	g/l
S_R	Inlet substrate concentration	g/l
t	Time	h
x	Organism concentration under non-evaporating conditions	g/l
x_e	Organism concentration under evaporating conditions	g/l
Y	Potential yield constant for the Monod model	dimensionless
Y_p	Potential yield constant for the endogenous metabolism model	dimensionless
Y_g	Potential yield constant for the maintenance model	dimensionless
$\mu(S)$	Specific growth rate	h^{-1}
μ_m	Maximum growth rate	h^{-1}
ϕ	$= \bar{x}_e/\bar{x}$	
(-)	Indicates steady-state values	

REFERENCES

- HERBERT, D. (1958). Some principles of continuous culture. In *Recent Progress in Microbiology. Symposium VII of the International Congress of Microbiology*, p. 381. Stockholm: Almquist & Wiksell.
- HOLME, F. & ZACHARIAS, B. (1965). Giberellic acid formation in continuous culture. *Biotechnology & Bioengineering* **7**, 405-415.
- MARR, A. G., NILSON, E. H. & CLARK, D. J. (1963). The maintenance requirement of *Escherichia coli*. *Annals of the New York Academy of Science* **102**, 536-548.
- MONOD, J. (1942). *Recherches sur la croissance des cultures bactériennes*. Paris: Hermann & Cie.
- MONOD, J. (1950). La technique de culture continue; theorie et applications. *Annales de l'Institut Pasteur* **79**, 390-410.
- PIRT, S. J. (1957). The oxygen requirement of growing cultures of an *Aerobacter* species determined by means of the continuous culture technique. *Journal of General Microbiology* **16**, 59-75.
- PIRT, S. J. (1965). The maintenance energy of bacteria in growing cultures. *Proceedings of Royal Society B* **165**, 224-231.
- POSTGATE, J. R. & HUNTER, J. R. (1962). The survival of starved bacteria. *Journal of General Microbiology* **29**, 233-263.
- SCHULZE, K. & LIPE, R. S. (1964). Relationship between substrate concentration, growth rate and respiration rate of *Escherichia coli* in continuous culture. *Archiv für Mikrobiologie* **48**, 1-20.
- TEMPEST, D. W., HERBERT, D. & PHIPPS, P. J. (1967). Studies on the growth of *Aerobacter aerogenes* at low dilution rates in a chemostat. In *Microbial Physiology and Continuous Culture*. London: Her Majesty's Stationery Office.