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INTERPRETING GAS KINETICS OF BATCH CULTURES

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SUMMARY

In the batch culture of bacteria with growth-linked gaseous products the kinetics of growth and gas accumulation are only simply related after several generations of growth. The relation between growth and gas accumulation is made quantitative and the correct method of interpreting the gas data for earlier generations is given.

INTRODUCTION

Various efforts have been made to use the data of gas production to interpret the growth kinetics of a batch culture, assuming a constant product yield (for example in methanogenesis, Balch and Wolfe, 1976; Zehnder and Brock, 1979; Schönheit et al., 1980). Such attempts are particularly useful when more direct methods are inapplicable for reasons such as poor plating recovery, inhomogeneous cultures etc. In these investigations a useful correspondence (sometimes implicitly assumed) is that an exponentially increasing biomass gives rise to an exponentially accumulating biogas. This assumption is examined and the correspondence shown to hold only after several generations of growth. A method is given which allows the determination of the growth kinetics from the gas data for earlier times. In the following it is assumed that all statements are made with reference to 1L of culture.

THEORY

During exponential growth the biomass x (g) increases with time according to

$$x = x_0 e^{\mu(t-t_0)} \qquad (1)$$

where x_0 is the biomass at time t_0 and μ is the specific growth rate, taken as constant. For a growth-linked product (Pirt, 1975) the product yield $Y_{p/x}$ (moles/g biomass) is constant and the quantity of product p (moles) accumulates as

$$p = p_0 e^{\mu(t-t_0)} \qquad (2)$$

where p_o is the quantity of product accumulated at time t_o. In (2) (see Pirt eqn. 16.15) it is assumed that $p_o = Y_{p/x}x_o$, ie only that quantity p_o is present which has resulted from the synthesis of x_o g of biomass.

For a completely soluble product measurements of the concentration p are sufficient to follow the biomass concentration x. In particular an exponentially increasing product concentration is indicative of an exponentially increasing biomass concentration.

However a complication arises when the product is gaseous and is lost from the culture. Assuming the gas is completely insoluble and gas collection starts from time t_o when the cell concentration is x_o (as in the case when the growth medium is inoculated with x_o g of cells and the gas tower 'zeroed', at time t_o), then the accumulation of gas, since time t_o, is given by $p' = p - p_o$. Then, from (2)

$$p' = p_o e^{\mu(t-t_o)} - p_o \qquad (3)$$

the unknown contribution $p_o = Y_{p/x}x_o$ going unaccounted. Unlike p, however, the quantity p' is not an exponentially increasing function of time during the exponential phase of growth (plots of log p' vs t are not linear). Nor can the quantity p (which is exponentially increasing) be calculated since p_o is unknown.

It can been seen from (2) and (3) that after a sufficiently long time interval $(t-t_o)$, p is nevertheless well appoximated by p'. In fact the relative error introduced in approximating p by p' is

$$(p-p')/p = e^{-\mu(t-t_o)} = 2^{-n} \qquad (4)$$

for an interval $(t-t_o)$ of n doubling times $t_d = (\log_e 2)/\mu$. For example, after 4 generations, the relative error has diminished to less than 7% (Fig. 1). Note from (4) the error involved is independent of the value $p_o = Y_{p/x}x_o$ and hence independent of the inoculum x_o.

In cultures where exponential growth is maintained over many generations it is therefore quite valid to approximate p by p' (ignoring earlier data) and apply the usual interpretation to the gas data (linear plots of log p' vs t indicate exponential growth).

However in cases where exponential growth is not maintained over many generations, but one is nevertheless interested in the growth kinetics for early times $(t-t_o) < 4\ t_d$, then the approximation of p by p' is poor and the usual interpretation of the gas data fails (plots of log p' vs t showing a characteristic downturn for such times, Fig. 1). This difficulty can be avoided if, rather than the accumulation, the rate of accumulation is followed.

The rates of accumulation r and r' (moles/h) corresponding to equations (2) and (3) respectively are given by

$$r = r' = \mu p_o e^{\mu(t-t_o)} \qquad (5)$$

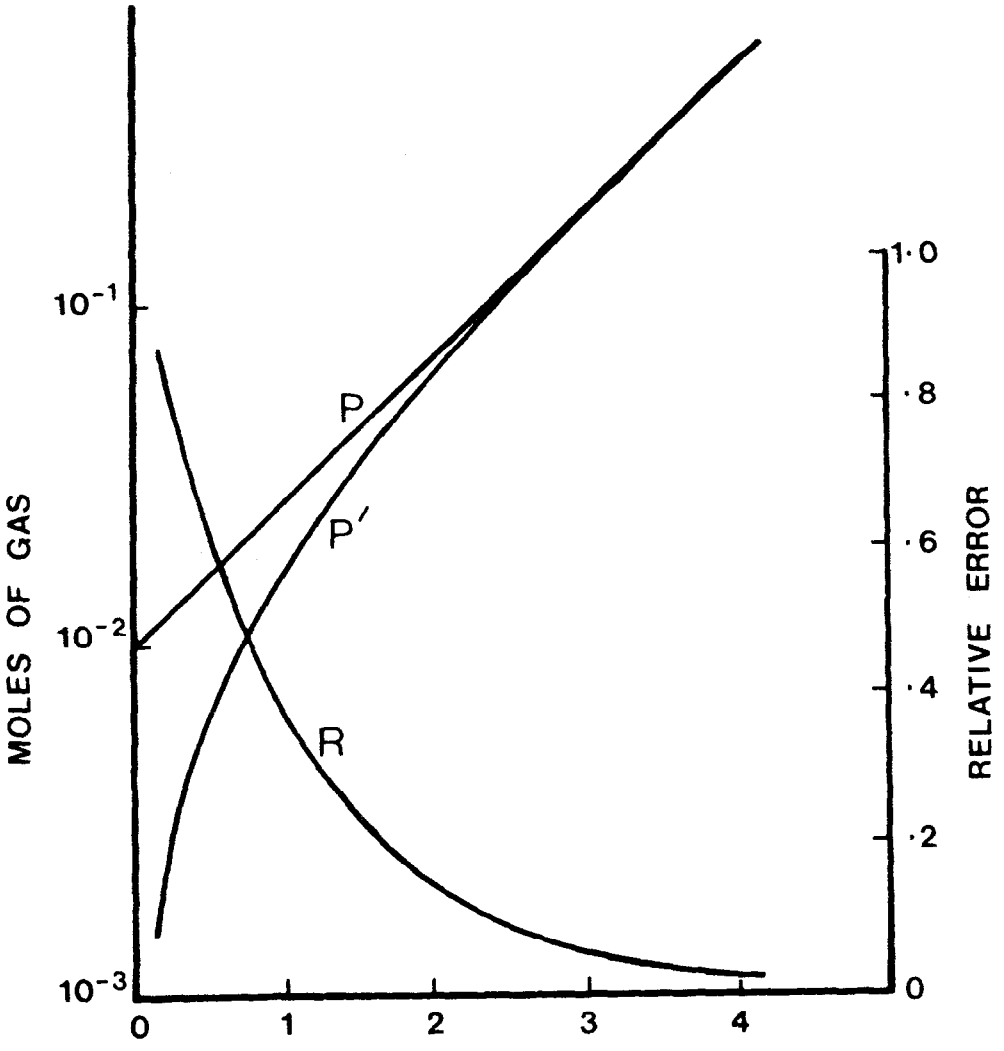

Figure 1 **NUMBER OF GENERATIONS (Since $t = t_o$)**

During exponential growth the accumulated gas p (moles) increases exponetially and plots of $\log_e$ p vs t are linear. However with batch culture often the quantity measured is $p' = p-p_o$, the accumulated gas since $t = t_o$, which is not exponential and plots of $\log_e$ p' vs t only approach linearity when the relative error $R = (p-p')/p$ becomes small.

so that taking logarithms

$$\log_e r' = \log_e (\mu p_o) + \mu(t-t_o) \qquad (6)$$

Plots of $\log_e$ r' vs t are then linear during the exponential growth phase. Standard methods of 'best-fit' can be applied to estimate the parameters μ and p_o allowing one to 'recover' the quantity $p = p' + p_o$ which is in direct proportion to x.

This method has been applied to the interpretation of the kinetics of methanogenesis from an acetate enrichment culture containing Methanosacina barkeri (Kirsop et al., 1983). Rates of

methane production were estimated with the use of time lapse photography. An early exponential phase lasting up to 4 generation times was found to be present, the existence of which is effectively masked by the use of the approximation p′ for p. If the correction is not made the early increase in p′ appears linear.

DISCUSSION

It now seems that with this interpretaion much of the published (and previously discounted gas data) can be made to yield a closer agreement with the direct estimates of growth. In cases where direct estimates of biomass are impractical the presence or extension of exponential growth may become evident where previously it was not. The ability to monitor the growth kinetics of a specific component by a simple analysis of its gas production can be of great value in the study of complex cultures (Winter, 1980), particularly if the interpretation can be made on a sound basis.

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