COMPARISON OF ALTERNATIVE KINETIC MODELS FOR ESTIMATING THE SPECIFIC GROWTH RATE OF Gibberella fujikuroi BY IMAGE ANALYSIS TECHNIQUES.

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SUMMARY

A new equation is proposed in order to obtain a consistent and accurate correlation between morphometric data of growing mycelia of Gibberella fujikuroi in solid media and its observed specific growth rates ($\mu_{obs}$) in stirred fermenters. A critical analysis is made of previous data and models.

INTRODUCTION

There is growing interest to use the analysis of microscopic images of mycelial cultures for estimating the kinetic parameters of industrial fermentations (Packer and Thomas, 1990). The specific growth rate ($\mu$) has been found to be proportional to the specific rate of increase of the number of tips ($\phi$) resulting from the binary branching process (Trinci, 1974; Fiddy and Trinci, 1976). In other words, if $X$ is the amount of biomass at time, $t$, and $X=X_0$ when $t=0$, then the growth process can be followed up by the exponential equation (1)

$$X/X_0 = e^{\mu t} = 2^{\phi t} \quad \ldots(1)$$

The practical problem is then how to estimate $\phi$, using morphometric data obtained from mycelial cultures on agar plates or sparse liquid suspensions. Trinci (1974) and Steele and Trinci (1975) suggested the following phenomenological equation,

$$\mu_1 = u_r / L_{hyu} \quad \ldots(2)$$

where, $u_r$ is the maximal rate of extension of leading hyphae and, according to Steele and Trinci (1975), $L_{hyu} = L_t/N_t$, is the length of the growth unit, being $L_t$, the total length and $N_t$, the number of tips. This model assumes that most of the hyphae grow at steady state velocity $u_r$. Hence, the steady state branching frequency $\phi_s$ is given by

$$\phi_s = u_r / L_{hyu} \quad \ldots(3)$$
Viniegra-González et al., (1993) have suggested that \( L_{\text{hgu}} = (2N_t - 1)L_c / N_t \), being \( L_c \) a critical length after which all leading hyphae branch out, thus, for \( 2N_t >> 1 \), \( L_{\text{hgu}} = 2L_c \). The value of \( L_c \) is not always available for measurement in fully grown (dense) colonies, instead, \( L_{\text{av}} \) can be obtained as the average length of leading hyphae in the periphery of such colonies (Fig. 1). Here it is assumed that \( L_{\text{hgu}} \) is proportional to \( L_{\text{av}} \) (Eq.4) and this hypothesis is tested by the comparison of observed values of the specific growth rate of \( G. fujikuroi (\mu_{\text{obs}}) \) measured in fermenters to the calculated values using Eq.2 or other alternative models as the model proposed by González-Blanco et al., (1993) (Eq. 5) and the model proposed in this paper (Eq. 6).

\[
L_{\text{hgu}} = a_1 L_{\text{av}} \quad (4)
\]

\[
\mu_2 = \frac{u_r \ln(2)}{a_2 L_{\text{av}}} \quad (5)
\]

Previous models proposed that \( a_1 \) and \( a_2 = 1 \), but, recent work (Alvarez and Rodríguez, 1993) showed that in fact \( a_2 = 10 \). In order to account for this discrepancy, we propose a third model (Eq.6) to be compared with previous ones.

\[
\mu_3 = \frac{(u_r / L_c) \ln(2)}{\ln(L_c / L_0)} \quad (6)
\]

Where, \( L_0 \) is the initial length of a new branch with length \( L < L_c \) which is growing exponentially with kinetic constant \( \alpha = (u_r / L_c) \), according to Trinci (1974) and Prosser (1991). This model gives more weight to the branching of small segments in a mycelium having the branching rate \( \phi_e \) (Eq. 7) and neglects the contribution of \( \phi_s \) (Eq. 3) to the overall branching mycelial process.

\[
\phi_e = \frac{(u_r / L_c)}{\ln(L_c / L_0)} \quad (7)
\]

In order to use Eq. 6, together with morphometric data of mycelia, it is necessary to make two assumptions (Eqs. 8 and 9)

\[
L_c = a_3 L_{\text{av}} \quad (8)
\]

Where, \( a_3 \) is a positive empirical constant. Also, according to Prosser (1991), \( L_0 \), is of the size of a germinating spore which is close to twice the hyphal diameter \( D_h \).

\[
L_0 = 2D_h \quad (9)
\]