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Exponential Growth Kinetics for *Polyporus versicolor* and *Pleurotus ostreatus* in Submerged Culture

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Simple mathematical models for a batch culture of pellet-forming fungi in submerged culture were tested on growth data for *Polyporus versicolor* (ATCC 12679) and *Pleurotus ostreatus* (ATCC 9415). A kinetic model based on a growth rate proportional to the two-thirds power of the cell mass was shown to be satisfactory. A model based on a growth rate directly proportional to the cell mass fitted the data equally well, however, and may be preferable because of mathematical simplicity.

The representation of batch culture data has been the subject of previous work by others (5, 9, 10). Generally, such studies have attempted to develop kinetic models that are more sophisticated than the simple differential equation for exponential growth of unicellular organisms

$$\frac{dX}{dt} = \mu \times X \quad (1)$$

where X represents the cell mass concentration, t represents time, and μ represents the maximum specific growth rate obtained in the batch culture during exponential growth. Models are often tested against data that are obtained by culture of unicellular organisms.

There are, however, some important fermentations which involve multicellular organisms, notably pellet-forming fungi. Such fungi produce small balls or pellets of mycelia in liquid culture. There has been considerably less kinetic modeling of batch culture with pellet-forming fungi, as it is usually assumed that pellet-forming fungi grow at a rate that is proportional to the two-thirds power of the cell mass (1, 2). This is based on the fact that such fungi should grow in proportion to their area, since nutrients must diffuse through a surface cell layer. Unfortunately, this widely assumed result seems to be based on an experiment that was conducted in shaken flasks and may not be applicable to all fermentations, particularly those with vigorous mechanical agitation (6).

This early study, using the fungus *Neurospora*, compared semilogarithmic, linear, and cube root plots and showed that a cube root best fitted the batch culture growth data (6). This corresponds to a growth rate model such that

$$\frac{dX}{dt} = A \times X^{2/3} \quad (2)$$

where A is a constant.

The present study uses *Polyporus versicolor* and *Pleurotus ostreatus*, two fungi that are involved in lignin degradation and both of which form pellets in liquid culture. The fungi were grown in a 5-liter fermentor on a glucose medium, and the cell mass concentration was measured as a function of time. Results show that for small pellets, the assumption that growth is proportional to the two-thirds power of cell mass is good but is not necessarily the best one to make.

MATERIALS AND METHODS

Test organisms. The fungi *P. versicolor* ATCC 12679 and *P. ostreatus* ATCC 9415 were used. Stock cultures were maintained on potato dextrose agar (Difco) with 0.5% yeast extract (Difco) and stored at refrigerated temperature.

The inocula were prepared by culturing the organisms in the liquid media (described below) for about 6 days or until the mycelial mats that had been taken from the solid culture produced several pellets in liquid culture.

Culture media. *P. versicolor* was cultured on a medium containing (in grams per liter): glucose, 10.0; yeast extract, 3.333; KH_2PO_4, 0.333; $MgSO_4 \cdot 7H_2O$, 0.167; $CaCl_2$, 0.01; $FeSO_4 \cdot 7H_2O$, 0.01; $MnSO_4 \cdot 4H_2O$, 0.001; $CuSO_4 \cdot 5H_2O$, 0.001; and $ZnSO_4 \cdot 7H_2O$, 0.001 (4, 8). *P. ostreatus* was cultured on a medium containing (in grams per liter): glucose, 10.0; KH_2PO_4, 0.6; $MgSO_4 \cdot 7H_2O$, 0.5; K_2HPO_4, 0.4; $CaCl_2 \cdot 2H_2O$, 0.074; ferricitrate, 0.012; $ZnSO_4 \cdot 7H_2O$, 0.666; $MnSO_4 \cdot 4H_2O$, 0.005; $CoCl_2 \cdot 6H_2O$, 0.001; and thiamine-hydrochloride, 0.0001 (7). Each medium was adjusted to pH 5.0 before inoculation. All pH adjustments were made with 4 N H_2SO_4 or NaOH. The media were autoclaved at 121°C for 20 min. The glucose was steri-

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lized separately, as were $CaCl_2 \cdot 2H_2O$ and $MgSO_4 \cdot 7H_2O$.

Fermentation conditions. All fermentations were conducted in a standard 5-liter fermentor which was equipped with a six-bladed turbine impeller. Agitation was constant at 500 rpm to insure turbulent mixing conditions. The temperature was controlled by passing water through hollow baffles. Sterile air was fed at a rate of 3 liters/min into a 3-liter volume of broth. The pH of the *P. versicolor* fermentation was not controlled, but that of the *P. ostreatus* fermentation was controlled at pH 5.0 by the addition of 4 N H_2SO_4 and 4 N NaOH.

Sampling procedure. The fermentor was equipped with a sampling line which was positioned at the level of the impeller. Fermentation broth was withdrawn, a 50-ml portion was centrifuged, and the solids were washed three times in tared tubes. Tubes were dried overnight in a 105°C oven. Cell mass concentration is expressed as the dry weight of cells per volume of sample withdrawn from the fermentor. Reported values are the average of duplicate samples. The sampling proved to be highly reproducible.

RESULTS

The cell mass concentration was monitored over time for each of the eight batch fermentations listed in Table 1. Complete data are available elsewhere (3). From the data, cell growth models represented in equations 1 and 2 were tested. The model of equation 1 is illustrated in a semilogarithmic plot shown in Fig. 1. The best-fit, least-squares line has been calculated and is shown in Fig. 1. The model of equation 2, in which growth is proportional to the two-thirds power of the cell mass, is illustrated in Fig. 2. Again, the calculated best-fit line which minimizes the squares of the errors is shown.

For each fermentation, the parameters that define the best-fit line are listed in Table 1 according to the relevant models. A general form of the equations is

$$\frac{dX}{dt} = C \times X^B \qquad (3)$$

where C and B are constants. In addition to the two models which are described, Table 1 also lists the calculated values of B that best fit the data for each fermentation.

The coefficient of determination, R^2, is given for each best-fit line in Table 1. This value indicates the proportion of variance between the calculated and measured cell mass concentrations which is explained by the independent variable time. It has a maximum value of 1.0. Table 1 also lists the average exponent B which best fits the data and the averages of the coefficients of determination, as well as all standard deviations (σ).

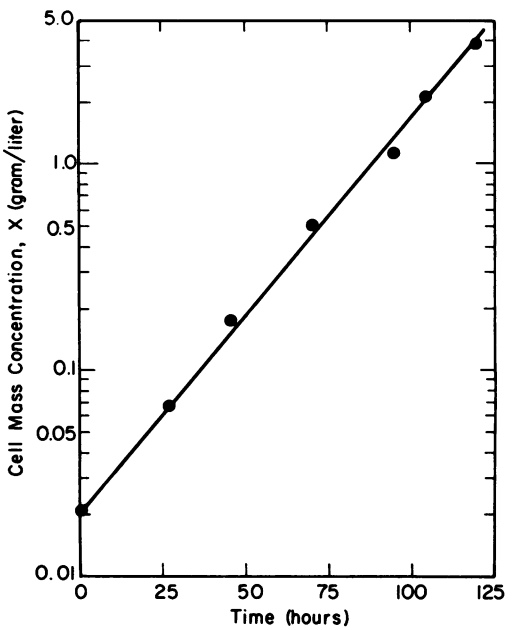

FIG. 1. *Cell mass concentration of P. versicolor grown in a fermentor at 28°C shown as a function of time in exponential growth. Coordinates are semilogarithmic, illustrating equation 1.*

TABLE 1. *Comparison of models for growth of pellet-forming fungi*

Organism	Temp (°C)	$\frac{dX}{dt} = C \times X^B$		$\frac{dX}{dt} = \mu \times X$		$\frac{dX}{dt} = C \times X^{2/3}$	
		B	R^2	μ (h^{-1})	R^2	C	R^2
P. versicolor	20	0.97	0.985	0.056	0.985	0.045	0.962
P. versicolor	25	0.87	0.964	0.071	0.959	0.058	0.962
P. versicolor	28	0.83	0.946	0.081	0.942	0.064	0.942
P. versicolor	30	1.03	0.994	0.071	0.993	0.047	0.960
P. versicolor	33	0.85	0.996	0.053	0.994	0.056	0.993
P. versicolor	35	0.97	0.998	0.044	0.998	0.032	0.953
P. versicolor	37	0.80	0.988	0.012	0.980	0.010	0.984
P. ostreatus	28	1.02	0.997	0.017	0.997	0.016	0.963
		$\bar{B} = 0.918$	$\bar{R}^2 = 0.984$		$\bar{R}^2 = 0.981$		$\bar{R}^2 = 0.964$
		$\sigma = 0.090$	$\sigma = 0.019$		$\sigma = 0.020$		$\sigma = 0.017$

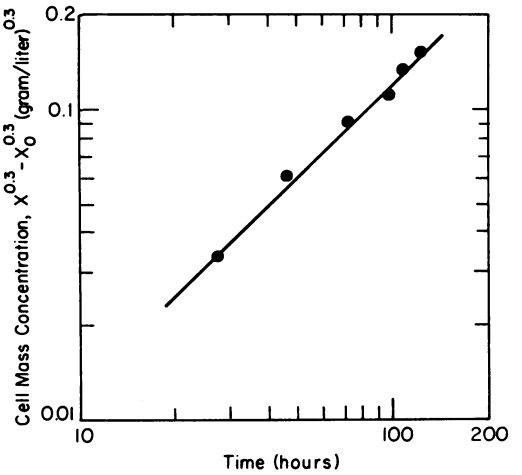

FIG. 2. *Cell mass concentration of P. versicolor grown in a fermentor at 28°C shown as a function of time during exponential growth. Coordinates are logarithmic. The ordinate is the cell mass plotted as* $X^{0.3} - X_0^{0.3}$, *where* X_0 *is the cell mass concentration at time zero, as required to illustrate equation 2.*

DISCUSSION

It has been previously assumed that pellet-forming fungi grow at a rate that is proportional to the two-thirds power of the cell mass. The present results show that this is a reasonably good model but is not necessarily the best or the simplest one. The average R^2 for this model is 0.964, which means that 96.4% of the variation in cell mass can be explained by the best-fit line calculated from the model.

Although such an R^2 is quite acceptable, it is seen in Table 1 that the average R^2 for the simpler model of an exponential B set that is equal to 1 is even higher, a value of 0.981. Thus, there is no improvement to selecting a two-thirds power for cell growth dependence on cell mass in preference to a power of 1.

By varying B to determine the best linear correlation of the data, one obtains an average best exponent B of 0.918. This is close to the B value of 1.0 that is used in equation 1. The difference in an R^2 value between 0.984 and 0.981 is only significant at the 60% confidence level, based on a standard comparison of means using Student's t-test. This would not merit the use of the exponent 0.92 in the model in preference to a value of 1.0. Equation 1 has the additional benefits of more facile mathematical manipulation and more intuitive association with biological phenomena.

The difference between the R^2 values in equations 1 and 2 is significant at the 95% confidence level. The main conclusion of this work, however, is that it does not make a great deal of difference which model one chooses. All models result in very acceptable coefficients of determination. Furthermore, if one were interested in determining the temperature which yields the highest exponential-phase growth rate, the choice of either the equation 1 or 2 model would result in 28°C being selected. For both models, the maximum growth rate occurs at the same temperature (28°C), although the values of the maxima differ.

The effect of agitation on the fungus pellets is probably important. Fungus pellets grown in shaken flasks are often relatively large, with a diameter of 0.5 to 1.0 cm. In the fermentor, however, the average diameter is rarely greater than 1 or 2 mm. It seems that the agitation tends to break apart the pellets and keep them small. This breaking apart of pellets would tend to liberate small mycelial pieces of component cells which can then multiply rapidly without a mass transfer limitation to the supplies of nutrients and oxygen.

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LITERATURE CITED

1. **Aiba, S., A. E. Humphrey, and N. Millis.** 1965. Biochemical engineering, 1st ed. Academic Press Inc., New York.
2. **Aiba, S., A. E. Humphrey, and N. Millis.** 1973. Biochemical engineering, 2nd ed. Academic Press Inc., New York.
3. **Carroad, P. A., and C. R. Wilke.** 1976. Studies of lignin-degrading fungi and enzymatic delignification of cellulosic materials. Lawrence Berkeley Laboratory Report LBL-4490. University of California, Berkeley.
4. **Dion, W. M.** 1952. Production and properties of a polyphenol oxidase from the fungus *Polyporus versicolor*. Can. J. Bot. 30:9-21.
5. **Edwards, V. H., and C. R. Wilke.** Mathematical representation of batch culture data. Biotechnol. Bioeng. 10:205-232.
6. **Emerson, S.** 1950. The growth phase in *Neurospora* corresponding to the logarithmic phase in unicellular organisms. J. Bacteriol. 60:221-223.
7. **Eriksson, K.-E., and K. Larsson.** 1975. Fermentation of waste mechanical fibers from a newsprint mill by the rot fungus *Sporotrichum pulverulentum*. Biotechnol. Bioeng. 17:327-348.
8. **Fahraeus, G., V. Tullander, and H. Ljuggren.** 1958. Production of high laccase yields on culture of fungi. Physiol. Plant. 11:631-643.
9. **Kono, T.** 1968. Kinetics of microbial cell growth. Biotechnol. Bioeng. 10:105-131.
10. **Tanner, R. D.** 1970. An enzyme kinetic model for describing fermentation processes. Biotechnol. Bioeng. LL:831-843.