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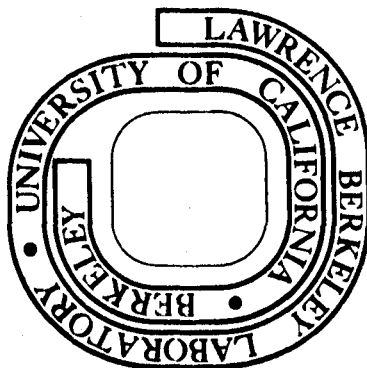
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EXPONENTIAL GROWTH KINETICS FOR POLYPORUS VERSICOLOR
AND PLEUROTUS OSTREATUS IN SUBMERGED CULTURE

Running head: Kinetic model tests for pellet-forming fungi

by

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Abstract

The simple mathematical models for batch culture of pellet-forming fungi in submerged culture are tested on growth data for Polyporus versicolor (ATCC 12679) and Pleurotus ostreatus (ATCC 9415). A kinetic model based on growth rate proportional to the two-thirds power of cell mass is shown to be satisfactory. A model based on growth rate directly proportional to cell mass fits 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 which are more sophisticated than the simple differential equation for exponential growth of unicellular organisms:

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

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

There are some important fermentations, however, 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 using pellet-forming fungi, it being usually assumed that pellet-forming fungi grow at a rate proportional to the 2/3rds 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 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

$$\frac{dx}{dt} = A \cdot x^{2/3} \quad (2)$$

where A is a constant.

The present study uses Polyporus versicolor and Pleurotus ostreatus, both fungi involved in lignin degradation and both of which form pellets in liquid culture. The fungi were grown in a five-liter fermentor on a glucose medium, and cell mass concentration was measured as a function of time. Results show that for small pellets, the assumption of growth being proportional to the 2/3rds power of cell mass is good, but not necessarily the best one to make.

MATERIALS AND METHODS

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

The inocula were prepared by culturing the organisms in the liquid media to be described for about 6 days, or until the mycelial mats taken from the solid culture produced several pellets in liquid culture.

Culture media. Polyporus versicolor was cultured on a medium containing (g/liter): glucose 10.0; yeast extract, 3.333;

KH_2PO_4 , 0.333; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.167; CaCl_2 0.01; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.001; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.001; and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.001 (4,8). Pleurotus ostreatus was cultured on a medium containing (g/liter): glucose, 10.0; KH_2PO_4 , 0.6; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5; K_2HPO_4 , 0.4; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.074; ferricitrate, 0.012; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.666; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.005; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.001; thiamine·HCl, 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 minutes. The glucose was sterilized separately, as was the $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

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

Sampling procedure

The fermentor was equipped with a sampling line positioned at the level of the impeller. Fermentation broth was withdrawn and 50 ml were centrifuged, and the solids washed three times in tared tubes. Tubes were dried in a 105°C oven overnight. Cell mass concentration is expressed as dry weight of

cells per volume of sample withdrawn from the fermentor.

RESULTS

For each of the eight batch fermentations listed in Table 1, the cell mass concentration was monitored over time. Complete data are available elsewhere (3). From the data, cell growth models represented in Equation (1) and Equation (2) were tested. For example, in Figure 1 is illustrated the increase in cell mass over time for P. versicolor grown at 35°C, plotted on rectangular coordinates. The model of Equation (1) is illustrated in a semilogarithmic plot shown in Figure 2. The best-fit least squares line has been calculated and is shown. The model of Equation (2), in which growth is proportional to the 2/3rds power of the cell mass, is illustrated in Figure 3. Again, the calculated best-fit line which minimizes the squares of the errors is shown.

For each fermentation, the parameters which define the best-fit line are listed in Table 1, according to the relevant models. A general form of the Equations is as follows:

$$\frac{dx}{dt} = C \cdot x^B \quad (3)$$

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

The coefficient of determination, R^2 , is given for each best-fit line in Table 1. The value indicates the proportion of the variance between calculated and measured cell mass concentrations that is explained by the independent variable

Table 1

Comparison of Models for Growth of Pellet-Forming Fungi

Organism	Temperature	$\frac{dx}{dt} = C \cdot X^B$		$\frac{dx}{dt} = \mu \cdot X$		$\frac{dx}{dt} = C \cdot X^{2/3}$	
		<u>B</u>	<u>R²</u>	<u>μ</u>	<u>R²</u>	<u>C</u>	<u>R²</u>
P. versicolor	20°C	0.97	0.985	0.056	0.985	0.045	0.962
p. versicolor	25°C	0.87	0.964	0.071	0.959	0.058	0.962
p. versicolor	28°C	0.83	0.946	0.081	0.942	0.064	0.942
p. versicolor	30°C	1.03	0.994	0.071	0.993	0.047	0.960
p. versicolor	33°C	0.85	0.996	0.053	0.994	0.056	0.993
p. versicolor	35°C	0.97	0.998	0.044	0.998	0.032	0.953
p. versicolor	37°C	0.80	0.988	0.012	0.980	0.010	0.984
Pl. ostreatus	28°C	1.02	0.997	0.017	0.997	0.016	0.963
		$\bar{B}=0.918$	$\bar{R}^2=0.984$			$\bar{R}^2=0.981$	$\bar{R}^2=0.964$
		$\sigma=0.090$	$\sigma=0.019$			$\sigma=0.020$	$\sigma=0.017$

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time. It has a maximum value of 1.0. Table 1 also lists the average of the exponent B which best fits the data and the averages of the coefficients of determination, as well as all standard deviations.

DISUCSSION

It has been previously assumed that pellet-forming fungi grow at a rate proportional to the 2/3rds power of cell mass. The present results show that this is a reasonably good model, but 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 B set equal to 1 is even higher, at a value of 0.981. Thus, there is no improvement to selecting a 2/3rds 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 B of 0.918. This is close to the B value of 1.0 used in Equation (1). The difference in 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 a 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 in R^2 values between 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. Also Table 1 illustrates that both B values of 1 and of 2/3 yield maximum growth rates at the same temperature, 28°C.

The effect which agitation has on the fungus pellets is probably important. Fungus pellets grown in shaken flasks are often relatively large, of diameter 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 to 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. Given the industrial significance of pellet-forming fungi and their possible uses as sources of food, this study demonstrates the need for more thorough investigation of cell growth kinetics of pellet-forming fungi under conditions of turbulent mixing.

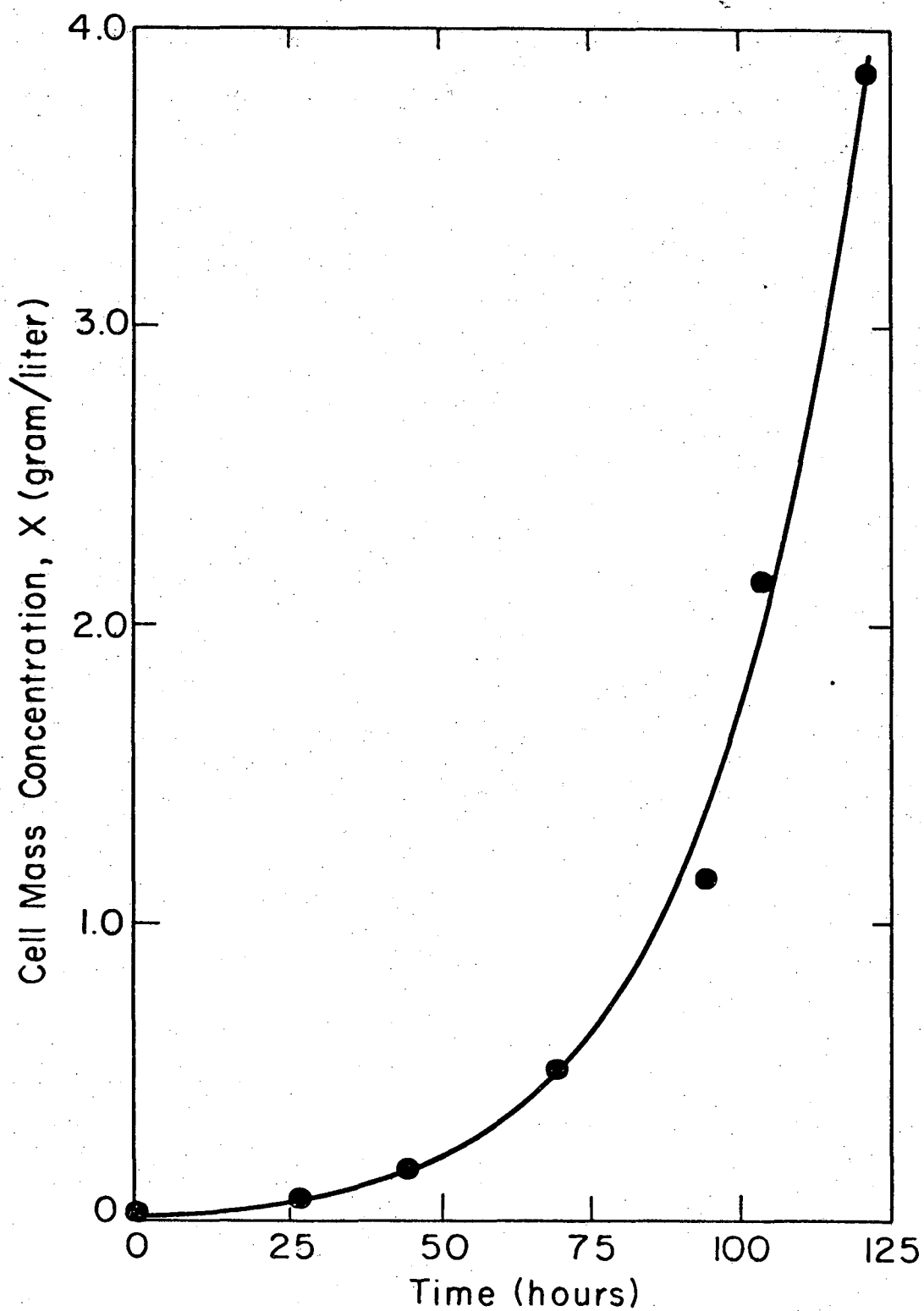
Acknowledgment

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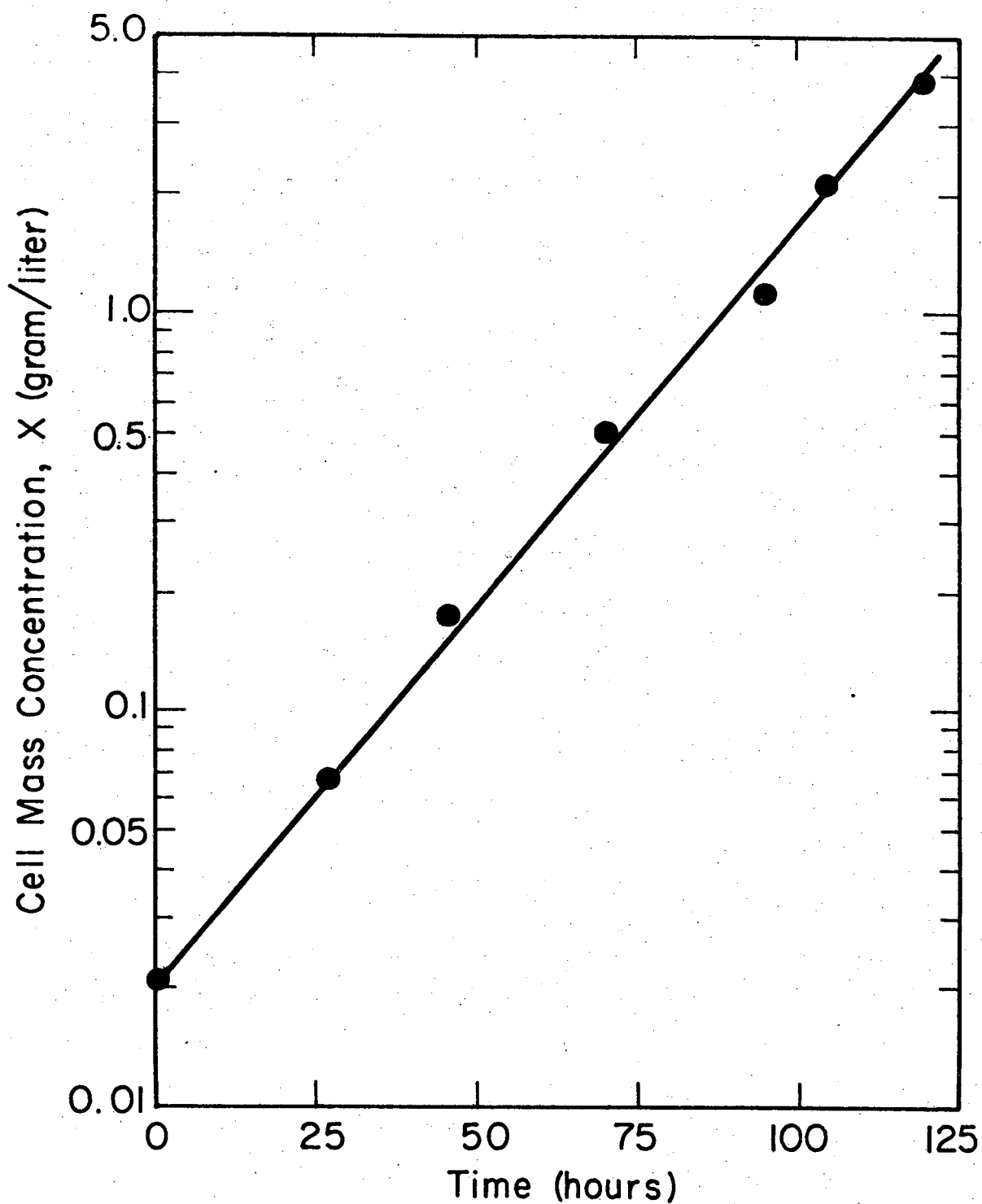
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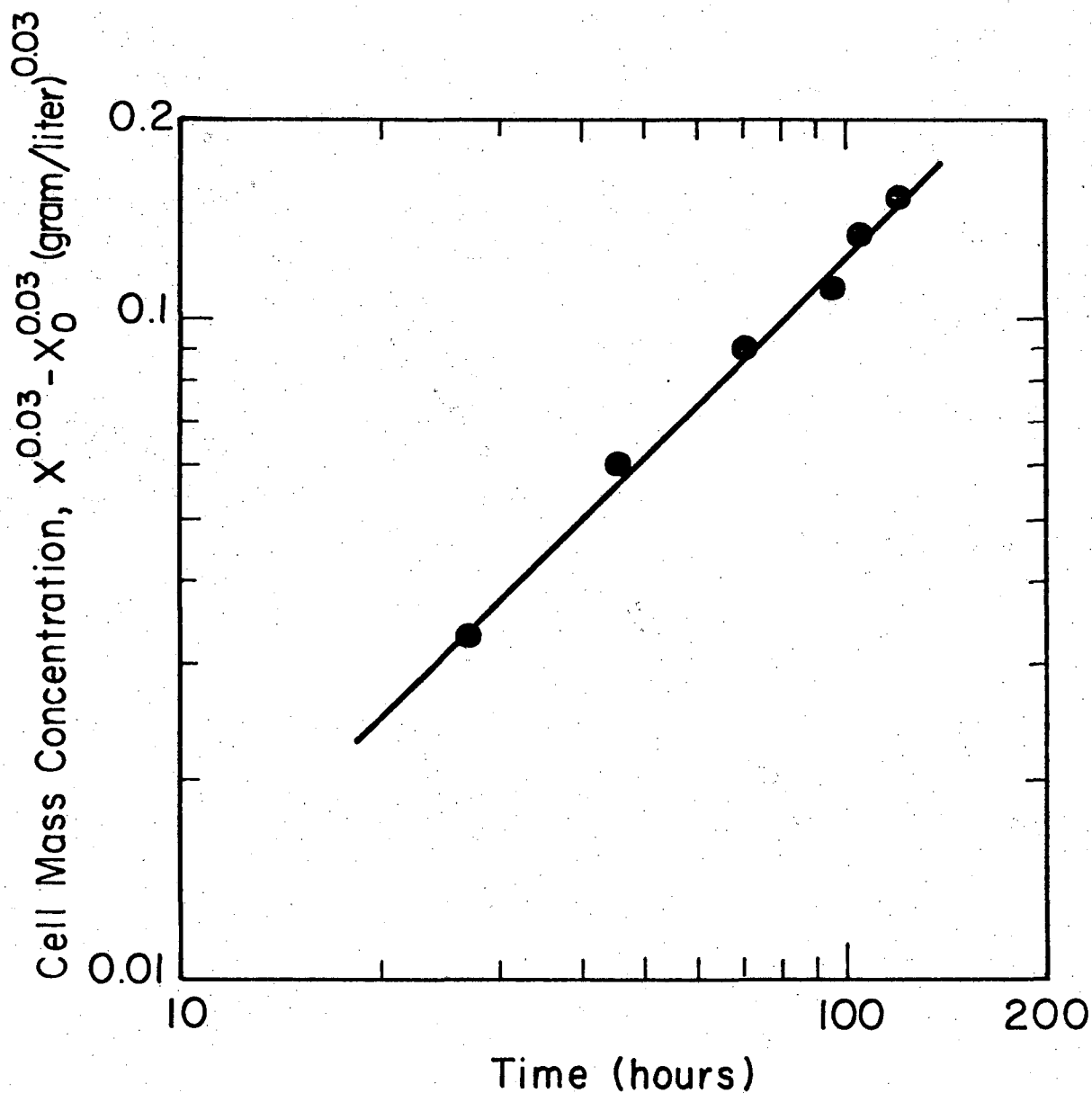
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Fig. 1. Cell mass concentration of *P. versicolor* grown in a fermentor at 28°C, shown as a function of time in exponential growth. Coordinates are rectangular.



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Fig. 2. Cell mass concentration of *P. versicolor* grown in a fermentor at 28°C, as a function of time in exponential growth. Coordinates are semilogarithmic, illustrating Equation 1.



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Fig. 3. Cell mass concentration of *P. versicolor* grown in a fermentor at 28°C, shown as a function of time during exponential growth. Coordinates are semilogarithmic and ordinate is cell mass plotted as $(X^{0.3} - X_0^{0.3})$ as required to illustrate Eq. 2, where X_0 is cell mass concentration at initial time.

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