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Nutrient uptake kinetics of filamentous microorganisms: Comparison of cubic, exponential, and Monod models

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Abstract - In practical situations where direct observation of the morphology and biomass distribution of filamentous microorganisms is not feasible, there is at present some uncertainty concerning the appropriate mathematical model to use to describe the substrate uptake and growth kinetics of these organisms. Some authors have argued that a "cubic" model was superior to available alternatives. In the present article, the experimental evidence upon which this claim was based is reanalyzed in detail. We identify a conceptual flaw in the rationale that led originally to that conclusion; we show that equally good fits to experimental data are obtained with the classical exponential model as with the cubic model. Finally, we demonstrate that a Monod-based nutrient uptake model outperforms both the cubic and the exponential models, and allows the full data set to be fitted, including a transition phase during which the rate of oxygen uptake decreases. Consequences of these observations, and the simplifications they afford, for the modeling of environmental or engineered systems are briefly addressed.

Key words: filamentous microorganisms, *Nocardia* sp., cubic growth, Monod equation.

INTRODUCTION

Microbiologists have known for many years that the growth curve of filamentous organisms is characterized by the same succession of phases (from "lag" to "decay") than that typical of bacteria (Rigelato, 1975; Griffin, 1981). It is generally agreed that the first three growth phases can be approximated reasonably well, as a whole, by logistic-type equations (*e.g.*, Koch, 1975; Scow *et al*., 1990). Consensus is lacking, however, about the best way to model mathematically, as an individual process, the growth that takes place once organisms

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inoculated in a new medium have overcome an initial lag period. In some cases (*e.g.*, Aiba and Kobayashi, 1971), the morphology and biomass distribution of filamentous organisms may be observed directly and may provide guidance as to the most suitable model. However, for many situations encountered in engineering practice (*e.g.*, polluted subsurface environments, large-scale engineered systems), such direct observation is not possible. A more "black-box", less mechanistic approach to the description of filamentous growth, based solely on observations of substrate disappearance and encompassing a class of models labeled as "unstructured", is the only feasible option.

Several authors (*e.g.*, Barclay *et al*., 1993; Garcia *et al*., 1997) consider that, after an initial lag period, filamentous microorganisms (fungi or actinomycetes) grow exponentially, following classical kinetics identical to that of unicellular organisms. Other researchers (*e.g.*, Prosser, 1982), however, argue that the growth of filamentous microorganisms follows a markedly different, "cubic" kinetics. A key reference in support of the latter viewpoint is a study by Marshall and Alexander (1960), based on measurements of oxygen uptake by 6 fungal strains. These authors interpreted the experimental results as evidence that the cubic growth model was better suited than the exponential one to describe fungal growth. Their conclusion is based on the premise that if filamentous fungi grow exponentially, the plot of the logarithm of oxygen uptake versus time should yield a straight line. Failure to observe such linearity was viewed by Marshall and Alexander (1960) as indication that fungi did not exhibit an exponential growth pattern.

In the present article, we assess the validity of this premise. We re-analyze the original data of Marshall and Alexander (1960) on a sound theoretical basis. We also compare a number of available models (the cubic and exponential models, commonly used to simulate the growth of filamentous organisms, and the Monod model, widely used to simulate the kinetics of microbial growth) to determine which one best describes the experimental results.

MATERIALS AND METHODS

When microorganisms grow exponentially, the substrate remaining in solution at any given time after the onset of growth is given by the following equation (*e.g.,* Simkins and Alexander, 1984; Alexander and Scow, 1989):

$$S = S_0 + X_0\left[1 - \exp(\mu_{\max} t)\right] \tag{1}$$

where S_0 is initial substrate concentration, S is substrate concentration, X_0 is the amount of substrate required to produce the initial population, $\mu_{\max}$ is the maximum specific growth rate, and t is the time after growth started.

After calculating the difference $(S_0 - S)$ and taking the logarithm of both sides of the resulting expression, one obtains an expression for the logarithm of substrate uptake:

$$\log\left(S_0 - S\right) = \log\left[X_0\left\{\exp\left(\mu_{\max} t\right) - 1\right\}\right] \tag{2}$$

The presence of the factor "-1" in the logarithm of the right side of this expression causes log $(S_0 - S)$ not to be, in general, a linear function of the time t, as wrongly assumed by Marshall and Alexander (1960).

To compare the model prediction with experimental results, a first step is to properly account for an initial period, clearly present in the experiments of Marshall and Alexander (1960) and traditionally referred to as "lag", during which no noticeable substrate uptake occurs. This may be done by introducing a lag time, t_0, explicitly in the equation for substrate disappearance, as follows:

$$S_0 - S = X_0\left[\exp\left\{\mu_{\max}(t - t_0)\right\} - 1\right] \quad (3)$$

If fungal growth follows a cubic kinetics, i.e., if fungal mass at time $(t\text{-}t_0)$ is proportional to $(t\text{-}t_0)^3$ (*e.g.*, Prosser and Touch, 1991), then substrate uptake is described as follows:

$$S_0 - S = \left\{a\left(t - t_0\right)\right\}^3 \quad (4)$$

where *a* is an arbitrary proportionality constant.

In addition we used a third modeling approach, where the growth rate of the exponential phase is allowed to depend on substrate concentration, *e.g.*, following Tessier's or Monod's equation. Under these equations, with the mathematically more tractable Monod equation, the differential form of substrate disappearance can be expressed as:

$$\frac{dS}{dt} = \mu_{\max}\frac{S\left(S_0 + X_0 - S\right)}{K_s + S} \quad (5)$$

where K_s is the Monod saturation constant. The integral form of this equation is:

$$K_s \ln\left(\frac{S}{S_0}\right) = \left(S_0 + X_0 + K_s\right)\ln\left(\frac{S_0 + X_0 - S}{X_0}\right) - \left(S_0 + X_0\right)\mu_{\max}t \quad (6)$$

After rearranging and introducing a lag time t_0, one gets

$$t = \frac{1}{\mu_{\max}}\left(\frac{K_s}{S_0 + K_0}\right)\ln\left(1 - \frac{S_0 - S}{S_0}\right) + \frac{1}{\mu_{\max}}\left(1 + \frac{K_s}{S_0 + X_0}\right)\ln\left(1 + \frac{S_0 - S}{X_0}\right) + t_0 \quad (7)$$

which unfortunately cannot be transformed into an expression for $(S_0 - S)$ as a function of t_0, and therefore has to be fitted with experimental data of *t* versus $(S_0 - S)$.

Each of the equations above (with t_0 as an additional adjustable parameter) was used to fit the original data from the experiments of Marshall and Alexander (1960), in which they monitored the uptake of oxygen by *Nocardia* sp. over time. Other data sets, such as that of Scow *et al*. (1990) were used to confirm that the conclusions reached were not uniquely associated with Marshall and Alexander's (1960) experiments. Since these additional data sets yielded identical conclusions, they will not be mentioned further in the following.

RESULTS AND DISCUSSION

Application of Equation (3) to the full data set of oxygen uptake by *Nocardia* sp. over time provides a reasonable fit (Fig. 1a), yet it is clear that the model predictions deviate from experimental data between hours 30 and 35, perhaps due to growth limitation (Marshall and Alexander, 1960). If the timeframe considered in the curve fitting only extends until 30 hours, the fit is better (Table 1).

When, for comparison sake, Equation (4) is used to fit the experimental data for *Nocardia* sp. within the whole time span, or within the first 30 hours only (Fig. 1b), the fits appear slightly better in both cases than with the exponential growth model. In particular, the curve obtained by fitting Equation (4) to the data points until 30 hours is able to approximate experimental data reasonably well up to about 34 hours, unlike Equation (3). Nevertheless, the differences in the respective RMSE and R^2 values (Table 1) are very small, especially between the fits up to 30 hours. These small differences may be explained partially by contrasting behaviors at early times. Indeed, Equation (4) leads to estimated lag durations of the order of only 3.02 to 5.89 hours (Table 1), whereas the

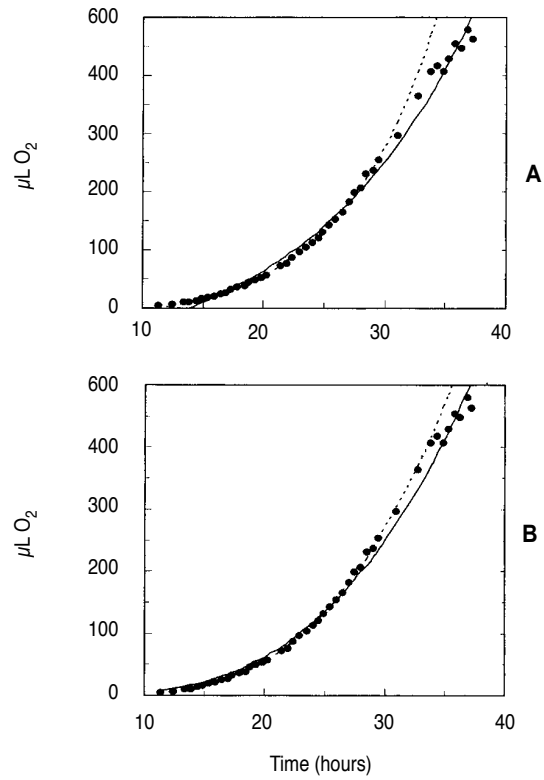

FIG. 1 – Fits of exponential (A) and cubic (B) equations to data of Marshall and Alexander (1960) relative to oxygen uptake during the growth of *Nocardia* sp.; •, Experimental data; ——, whole data; – – –, until 30 hours.

TABLE 1 – Fitted parameter values for Fig. 1 and 2, coefficients of determination (R^2) and root means square errors (RMSE)

Equation	Fitting	R^2 range	RMSE	t_0	μ_{max}	X_0	S_0	Ks
Exponential	Whole period	0.99128	14.37	13.780 ± 0.539	0.072882 ± 0.004739	111.63 ± 18.30		
Exponential	Until 30 h	0.99881	2.58	11.431 ± 0.317	0.13064 ± 0.00306	26.761 ± 2.597		
Cubic	Whole period	0.99208	13.70	3.0213 ± 0.5195				
Cubic	Until 30 h	0.99883	2.57	5.8927 ± 0.1626				
Monod	Whole period	0.99936	5.65	10.865 ± 0.271	0.20891 ± 0.03378	19.041 ± 2.814	509.11 ± 17.78	119.74 ± 85.92

exponential model leads to corresponding estimates that are appreciably higher, at 13.78 and 11.43 hours.

For all the used models it was necessary to assume a significant lag time in order to get a reasonable fit of the measured data. Lag duration is usually explained by the duration of dormant state of fungi, and, more likely in the present case, by the change of substrate and the subsequent adaptation of the organisms to the new substrate (Schlegel, 1985). The lag phase is usually determined from the biomass growth curve. As Marshall and Alexander (1960) measured substrate consumption only, the length of the lag phase was used as a fitting parameter. The actual lag time in the experiment might have been smaller than the fitted values, but the substrate consumption caused by the initial activity of the microorganisms might have been too small to be detected. The data support the assumption that the growth of filamentous organisms can be described by the same three phases usually used to describe the growth of unicellular organisms: a lag phase with a duration of approximately 10 hours, an exponential growth phase lasting approximately 30 hours, and a transition to a stagnation phase during which growth rate decreases because of substrate limitation.

However it is difficult to determine when each phase ends exactly. To a large extent, the decision to ignore data beyond 30 hours when fitting Equations (3) and (4) is arbitrary. Even though it is clear that growth becomes limited at some time around 30 hours, it is not clear exactly when this limitation becomes significant. By successive approximations, it may be possible to find a timeframe that produces maximal R^2 values or minimal RMSE values. However, a more reasonable approach would be to find a way to fit the entire data set using a single expression that accounts for all growth phases simultaneously.

This can be achieved with a number of structured models, for example involving two or more interacting compartments. A computationally simpler approach is to use unstructured models, among which the Monod model remains by far the most commonly used. The fit of Equation (7) to the whole experimental data set (Fig. 2) is excellent, and is sizably better than that of either the exponential or the cubic equations. The R^2 values (calculated on the basis of residuals at given times t, to enable a sound comparison with the previously calculated R^2 values), and, more clearly, the RMSE values reflect this better fit.

The results summarized in Table 1 indicate that the choice of a model affects severely the values obtained for common parameters. The lag phase, t_0, determined by the Monod equation is 10.87 hours, slightly shorter than the 11.4 hours needed in the experiment, before substrate consumption became measurable. The exponential model also produces lag times that are of the same order of magnitude as the lag time determined experimentally. By contrast, the values found with the cubic model are significantly shorter. For the maximum specific growth rate, μ_{max}, a comparison is only possible between the exponential model and the Monod model. Values determined using the exponential model were significantly smaller (0.07 h^{-1} and 0.13 h^{-1}, respectively) than that produced by the Monod model (0.21).

In summary, the above comparison of models shows that it is not necessary to invoke a cubic growth model to fit data of substrate uptake by filamentous microorganisms. The classical exponential growth model performs equally well

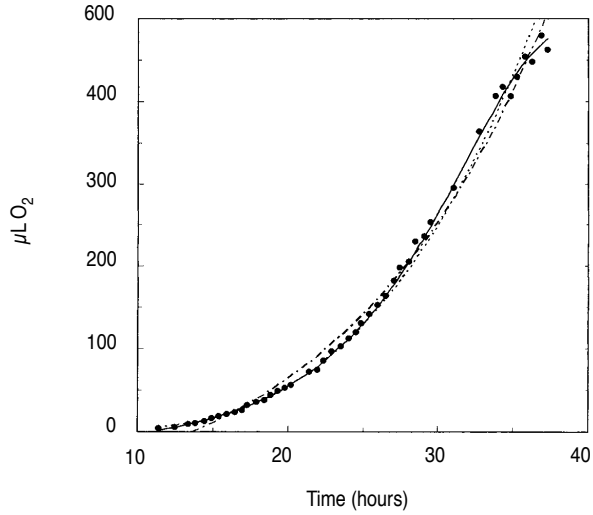

FIG. 2 – Oxygen uptake during growth of *Nocardia* sp. (Marshall and Alexander, 1960) fitted by three equations. •, Experimental data; ——, Monod kinetics (R^2 = 0.99936); ·····, cubic equation (R^2=0.99208); –·–·–·, exponential equation (R^2= 0.99128).

as the cubic growth model, and a more complete model, based on Monod's equation and traditionally used to describe substrate uptake by unicellular microorganisms, outperforms both the exponential and cubic models. This applicability of the classical Monod-based formulation should simplify greatly the practice of modeling the nutrient uptake and growth kinetics of filamentous organisms in complex systems, where direct observation of biomass morphology and distribution is not feasible; not only can theoretical considerations related originally to unicellular organisms (*e.g.*, Baveye *et al*., 1989) be extrapolated to filamentous fungi and actinomycetes, but, also, readily-available software can be used to describe the fate of biodegradable solutes in the increasingly numerous situations (*e.g.*, Laughlin and Stevens, 2002) where fungi and actinomycetes are now believed to be significantly implicated.

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