

A Review of Recent Developments in Modeling of Microbial Growth Kinetics and Intraparticle Phenomena in Solid State Fermentation

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ABSTRACT: Mathematical models are important tools for optimizing the design and operation of solid-state fermentation (SSF) bioreactors. Such models must describe the kinetics of microbial growth, how this is affected by the environmental conditions and how this growth affects the environmental conditions. This is done at two levels of sophistication. In many bioreactor models the kinetics are described by simple empirical equations. However, other models that address the interaction of growth with intraparticle diffusion of enzymes, hydrolysis products and O₂ with the use of mechanistic equations have also been proposed, and give insights into how these microscale processes can potentially limit the overall performance of a bioreactor. The current article reviews the advanced that have been made in both the empirical and mechanistic type kinetic models and discusses the insights that have been achieved through the modeling work and the improvements to models that will be necessary in the future.

KEYWORDS: Solid – state fermentation; Modeling; Growth and death kinetics

I. INTRODUCTION

Mathematical modeling is an essential tool for optimizing bioprocesses. Not only the models guide the design and operation of bioreactors but they can also provide insights into how the various phenomena within the fermentation system combine to control overall process performance. However, it is only over the last decade efforts have been made to develop mathematical models of solid-state fermentation (SSF) processes [3].

The various mathematical models that have been proposed to date in the area of SSF can be divided into two categories, macroscale models and microscale models. Macroscale models are those that concern themselves with the operation of bioreactors. They describe mass and heat transport processes across the substrate bed, but typically treat phenomena at the level of the individual particle in a highly simplified manner. As such, they could also be referred to as SSF bioreactor models. Microscale models are those that concern themselves with the phenomena that occur on and within individual particles and do not attempt to describe the performance of the bioreactor as a whole. Clearly, the performance of an SSF bioreactor can potentially be limited either by the phenomena that occur at the level of the whole bed or by the phenomena that occur within individual particles, so both types of models are important, despite their differing objectives.

The aim of SSF bioreactor models is to describe how the performance is affected by the various operating variables that can be manipulated in an attempt to control the process. For example, a mathematical model will enable predictions of how the flow rate, humidity and temperature of the inlet air will affect the temperature and water content of the substrate bed, and in turn, how these system variables will affect microbial growth and product formation. Therefore bioreactor models can be thought of as consisting of two sub-models. 1. The balance/transport sub-model and 2. the kinetic sub-model.

The balance/transport sub-model describes mass and heat transfer within and between the various phases of the bioreactor, while the kinetic sub-model describes how the growth rate of the microorganism depends on the key local environmental variables.

Mathematical modeling in SSF is reviewed in two parts. The first part reviews the balance/transport sub-models of bioreactors. The second part reviews both the kinetic sub-models of bioreactor models and microscale models of those processes associated with growth that occur at the level of individual particles. Both these reviews aim to give an insight into how the various phenomena that occur within the system can be described mathematically. In this paper we shall discuss the second part.

II. BASIC KINETIC EQUATIONS

Various kinetic profiles have been reported in SSF systems, including linear, exponential, logistic and fast-acceleration/slow-deceleration. These equations do not include the effect of nutrient concentration on growth. To do so would require modeling of intraparticle diffusion process. The empirical equations are simply fitted to experimental growth profiles by non-linear regression. The typical forms of these curves are shown in Fig.2.1

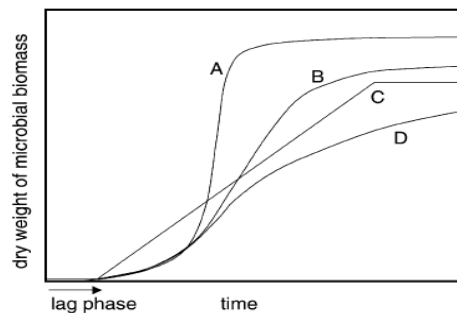


Fig. 2.1. The various empirical kinetic profiles that have been described in solid-state fermentation systems: (A) exponential; (B) logistic; (C) linear; (D) fast-acceleration/slow-deceleration. In applying non-linear regression to experimental data zero time will typically be taken as the end of the lag-phase.

Differentiated and integrated forms of the various empirical growth equations that have been applied to SSF systems

Differentiated form

Integrated form

Linear $\frac{dX}{dt} = K$ -----(1)

$X = Kt + X_0$ -----(5)

Exponential $\frac{dX}{dt} = \mu X$ -----(2)

$X = X_0 e^{\mu t}$ -----(6)

Logistic $\frac{dX}{dt} = \mu X \left(1 - \frac{X}{X_m} \right)$ -----(3)

$X = \frac{X_m}{1 + \left(\left(\frac{X_m}{X_0} \right) - 1 \right) e^{-\mu t}}$ -----(7)

$$\text{Two phase} \quad \frac{dX}{dt} = \mu X, t < t_a \quad \text{-----(4a)} \quad X = X_0 e^{\mu t}, t < t_a \quad \text{-----(8a)}$$

$$\frac{dX}{dt} = \left[\mu L e^{-k(t-t_a)} \right] X, t \geq t_a \quad \text{-----(4b)} \quad X = X_A \exp \left[\frac{\mu L}{k} (1 - e^{-k(t-t_a)}) \right], t \geq t_a \quad \text{---(8b)}$$

X is microbial biomass, t is time, K is the linear growth rate, μ is the specific growth rate constant, X_0 is the initial biomass and X_m is the maximum possible microbial biomass. The symbols t_a , L and k in the two – phase model are explained in the text.

The linear, exponential and logistic equations have been used for some time. The two phase equation was developed more recently in order to describe the fast - acceleration/slow - deceleration profile [4]. In this kinetic model, an exponential phase is followed by a deceleration phase. The quantity in square brackets in equation (4b) represents the specific growth rate during the deceleration phase, which decreases due to two factors. Firstly a sudden deceleration is assumed at the instant that the switch, from exponential to deceleration phase occurs at time t_a , with the parameter L representing the ratio of the specific growth rate at the start of the deceleration phase to the specific growth rate during the previous exponential phase. Secondly, further deceleration is described by an exponential decay in specific growth rate throughout the deceleration phase, described by the exponential term, in equation (4b), with a first-order rate constant of k. One difficulty with applying this model is that the exponential period is typically short and has quite low microbial biomass concentrations, meaning that there are few experimental points and a relatively large error. Therefore, it might be difficult to determine the parameters for the exponential phase accurately. This could present a problem because μ from the exponential phase is used in the analysis of the deceleration phase, although it is also possible to combine μ and L and estimate this combination as a single parameter, μ_L , from the deceleration phase data.

Mathematical models of SSF bioreactors most commonly use the logistic equation, including several of the most recently proposed models [5] - [12]. This is done on the basis of mathematical simplicity, because often the logistic equation can, in a single equation, give an adequate approximation of the whole growth curve, including the lag phase and the cessation of growth in the latter stages of the fermentation. With the other kinetic models the growth curve must be separated into various phases, with a different equation for each phase. For example, for the linear model, lag and stationary phases might be recognized on either side of the linear growth region, and would be described by setting the growth rate equal to zero.

It may be difficult to undertake experimental growth studies to determine the kinetic equations and their parameters.

1. Modeling of death kinetics

The simplest assumption is that death is a first order process giving the equation[2]:

2. Modeling of death kinetics

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$$\frac{dC_{XAD}}{dt} = r_d = K_d C_{XAV} \quad \text{----- (3.1)}$$

Where C_{XAV} and C_{XAD} are the absolute concentrations of viable and dead biomass, respectively, and K_d is the specific death rate coefficient (h^{-1}).

This term might simply be subtracted from the equation for the production of viable biomass. In the case in which growth follows logistic kinetics then the equation for total biomass production might be:

$$\frac{dC_{XAT}}{dt} = \mu C_{XAV} \left(1 - \frac{C_{XAT}}{C_{XAM}} \right) \quad \text{-----(3.2)}$$

Where C_{XAT} is the absolute concentration of total biomass (ie, both viable and dead).

C_{XAT} appears in the numerator of the term within the parentheses since it is assumed that the biomass-associated limitation of growth is due to the total biomass concentration and not simply the viable biomass concentration. This could be true for the case in which growth is limited by the availability of nutrients.

Subtracting the rate of death equation (3.1) from the overall rate of biomass production equation (3.2) gives the rate of increase of viable biomass:

$$\frac{dC_{XAV}}{dt} = \mu C_{XAV} \left(1 - \frac{C_{XAT}}{C_{XAM}} \right) - k_d C_{XAV} \quad \text{-----(3.3)}$$

$$\frac{dC_{XAV}}{dt} = \mu C_{XAV} \left(1 - \frac{C_{XAV} + C_{XAD}}{C_{XAM}} \right) - k_d C_{XAV} \quad \text{-----(3.4)}$$

Arrhenius equation can be used to express the effect of environmental condition such as temperature on growth:

$$\mu = A_g \exp \left(\frac{-E_{ag}}{R(T + 273)} \right) \quad \text{-----(3.5)}$$

and on death:

$$K_d = A_d \exp \left(\frac{-E_{ad}}{R(T + 273)} \right) \quad \text{-----(3.6)}$$

$$\mu_{obs} = \mu - k_d$$

$$\mu_{obs} = A_g \exp \left(\frac{-E_{ag}}{R(T + 273)} \right) - A_d \exp \left(\frac{-E_{ad}}{R(T + 273)} \right) \quad \text{-----(3.7)}$$

Where T is the temperature ($^{\circ}C$), A_g and A_d are the frequency factors for growth and death (h^{-1}) and E_{ag} and E_{ad} are the activation energies for growth and death ($Jmol^{-1}$)

Typical profiles that could be expected for these two rate constants against temperature are shown in fig 3.1

Critical temperature:

The amount of viable biomass cannot increase at or above this temperature. The effect of the term $\left(1 - \frac{C_{XAT}}{C_{XAM}} \right)$ in equation (4.3) means that, as C_{XAT} increases, this critical temperature falls to lower and lower values.

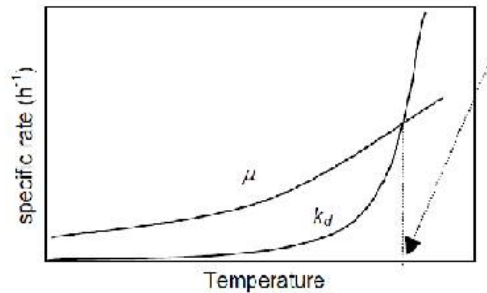


Fig. 3.1. Typical behavior that might be expected for the specific growth rate and specific death rate parameters as a function of temperature according to the Arrhenius equations (3.5) and (3.6)

III. MODELING THE EFFECT OF GROWTH ON THE ENVIRONMENT

It is usually of interest to model how the growth of the microorganism causes changes in its environment, because, in turn, the growth of the microorganism depends on the conditions in its local environment. Growth of the microorganism is associated with the consumption of O_2 and nutrients and the production of waste metabolic heat, water, carbon dioxide and various products. The growth process also leads to an overall decrease in the dry matter of the fermenting solids, because the mass of dry substrate consumed, at least for carbon sources such as carbohydrates for which the yield coefficient (Y_{XS}) is less than 1.

The typical manner to model these effects in bioreactor models is to assume growth associated and non-growth associated components. The general equations for a reactant R or a product P are therefore:

$$\frac{dR}{dt} = -\frac{1}{Y_{XR}} \frac{dX}{dt} - m_R X \quad \text{-----(4.1)}$$

$$\frac{dP}{dt} = Y_{PX} \frac{dX}{dt} + m_P X \quad \text{-----(4.2)}$$

Where Y_{XR} and Y_{PX} are stoichiometric coefficients and m_R and m_P are maintenance coefficients. In the case of some products, m_P is referred to as the non-growth associated production rate constant. All coefficients are written as positive numbers.

The identity of the components that are incorporated into the model depends on what the model proposes to describe. In some cases only a kinetic equation and an energy balance are written, in which case equation(4.2) is written in terms of metabolic heat. It has often been assumed that heat production is directly proportional to growth [7], [9], [11], [12], although this is probably not true. In any case, the inclusion of maintenance heat production is a simple matter.

Even though typically attempts are not made within bioreactor models to predict intraparticle concentration profiles of nutrients and the dependence of growth on local nutrient concentrations, due to the complexity that this would introduce, overall consumption of the solid substrate may be of interest in order to predict gross changes in the substrate bed such as bed shrinkage. Some models take this dry weight loss into account. Other models have ignored the decrease in total solids weight despite the fact that such decreases occur in reality [9], [12]. This could be a short coming since bed shrinkage can potentially have important effects on bioreactor performance, such as promoting channeling in packed-beds.

At times equations(4.1) and (4.2) are used in order to estimate the growth kinetics parameters from indirect measurements of either O₂ consumption or carbon dioxide evolution. For example, assuming logistic growth kinetics and that O₂ is consumed for growth and maintenance according to

$$\frac{dO_2}{dt} = Y_{OX} \frac{dX}{dt} + m_o X \quad \text{-----(4.3)}$$

then the cumulative O₂ consumption between time zero and time t is given by

$$COU = \left[\frac{1}{Y_{OX} \left[1 + \left(\left(\frac{X_m}{X_0} \right) - 1 \right) e^{-\mu t} \right]} \right] + \frac{m_o}{\mu} \log \left[\frac{1 + \left(\left(\frac{X_m}{X_0} \right) - 1 \right) e^{-\mu t}}{\left(\left(\frac{X_m}{X_0} \right) - 1 \right) e^{-\mu t}} \right] \quad \text{-----(4.4)}$$

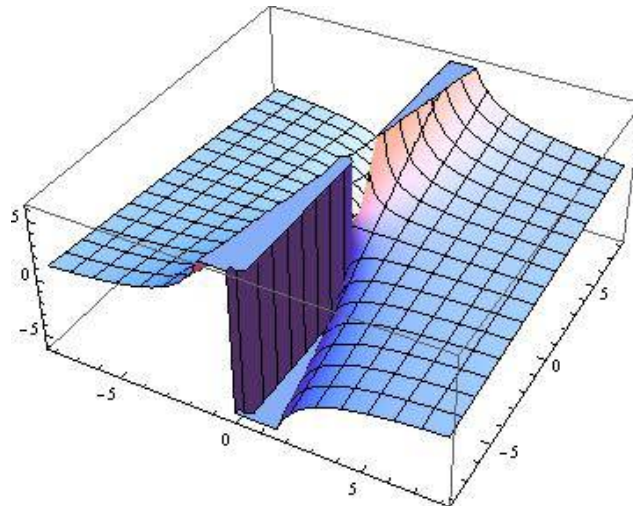


Figure 4.1 Oxygen Consumption and Carbon Dioxide Production

Although this equation appears quite complex, if the growth kinetics have been determined then the parameters X_m , X_0 and μ can be inserted into the equation, leaving the O₂ - microbial biomass stoichiometric coefficient (Y_{OX}) and the maintenance coefficient (m_o) as the only unknown parameters. Non-linear regression can be used to find the values of these two parameters that give the best agreement with the experimentally – determined cumulative O₂ uptake profile. Similar analyses could be made for kinetic types other than logistic kinetics, the only requirement being a growth rate equation that can be integrated analytically. Such an approach requires the measurement of microbial biomass in order to arrive at the growth kinetic equation and therefore, for the majority of SSF systems, in which direct microbial biomass measurement is not feasible, such experiments would have to be done in an artificial system that allows biomass measurement. Applying this method to a real fermentation in a bioreactor in which there is significant temperature variations would carry with it the implicit assumption that Y_{OX} and m_o do not vary with temperature, although this may not be a good assumption.

IV. CONCLUSION

Future improvements in computing power may make it feasible to use routinely bioreactor models that describe mechanistically both the mass and heat transfer phenomena at the macroscale and the particle - level phenomena.

The potential of these models to give insights into the complex manner in which macroscale and microscale phenomena interact to control growth in SSF systems.

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