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Review

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 O_2 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 advances 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 kinetics; Diffusion-reaction; Filamentous fungi; Oxygen transfer

1. Introduction

Mathematical models have an important role to play in the optimization of solid-state fermentation (SSF) bioreactors [1]. Bioreactor models aim to describe the overall performance of the bioreactor and consist of two sub-models: a balance/transport sub-model that describes mass and heat transfer within and between the various phases of the bioreactor and a kinetic sub-model that describes how the growth rate of the microorganism depends on the key local environmental variables. One of two approaches may be taken to describing the growth kinetics. Simple empirical equations may be used or mechanistic models that attempt to describe intraparticle diffusion processes related to growth may be proposed. In fact, several mechanistic models have been proposed that do not concern themselves with overall bioreactor performance but instead focus on the question of how growth can be limited by events that occur at the level of individual particles. In this context, they have been referred to as microscale models [2].

The current work represents the second part in a two-part review of modeling in SSF. The first part reviewed approaches that have been used to modeling macroscale heat and mass phenomena in various bioreactor types [1]. The current part reviews the microscale phenomena, including both the simple empirical approaches to describing growth kinetics that have been used in most of the bioreactor models and microscale models that have been proposed independently of bioreactor models. The review aims to give an insight into how the various kinetic and intraparticle phenomena that occur within the system can be described mathematically. To this end key model equations are reproduced and discussed. However, the sets of equations that constitute the various models that are discussed are not reproduced in their entirety. Readers wishing to understand individual models in detail must refer to the original works. Also discussed are the insights that have been achieved through the modeling work, and improvements to models that will be necessary in the future.

2. State of the art of modeling growth with empirical growth kinetic models

The majority of current bioreactor models have simple empirical kinetic sub-models, since the heterogeneity within many bioreactors means that the balance/transport

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sub-model is already quite complex [1,2]. For example, significant gradients can be expected within a packed bed bioreactor with respect to temperature, moisture and the gas phase O_2 concentration [3,4]. The balance equations in dynamic models of such systems are partial differential equations, which require much more computational power to solve than the ordinary differential equations that describe the balances over well-mixed bioreactors. If the intraparticle diffusion of enzymes, hydrolysis products and O_2 were to be described in the model of such a bioreactor, the computational power required to solve the model would increase dramatically. Further, a greater experimental effort would be required to determine the model parameters associated with the microscale. This is typically avoided by using empirical kinetic sub-models [2]. One exception is the gas–solid fluidized bed bioreactor. This bioreactor provides good mixing and heat removal at the macroscale, greatly simplifying the balance equations, and allowing the assumption that all substrate particles are subjected to identical external conditions. As a result, the various models that have been proposed for this bioreactor use kinetic sub-models that consider intraparticle diffusion phenomena.

2.1. Basic kinetic equations

Various kinetic profiles have been reported in SSF systems, including linear, exponential, logistic and fast-acceleration/slow-deceleration [5]. The typical forms of these curves are shown in Fig. 1. Empirical equations that have been proposed to describe these curves are shown in Table 1 (Eqs. (1)–(8)). These equations do not include the effect of nutrient concentration on growth. To do so would require modeling of intraparticle diffusion processes. The empirical equations are simply fitted to experimental growth profiles by non-linear regression.

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 [6]. In this kinetic model, an exponential phase is followed by a deceleration phase. The quantity in square brackets in Eq. (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 Eq. (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.

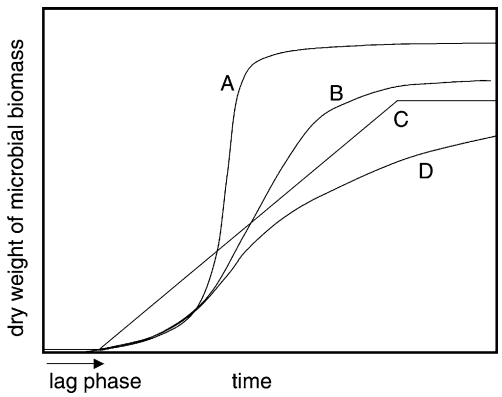

Fig. 1. The various empirical kinetic profiles that have been described in solid-state fermentation systems: (A) exponential; (B) logistic; (C) linear; (D) fast-acceleration/slow-deceleration. In applying non-linear regression to experimental data zero time will typically be taken as the end of the lag-phase.

The causes of the deceleration of growth are not specified in the simple empirical growth kinetic equations presented in Table 1. In developing their two-phase equation, Ikasari and Mitchell [6] suggested that the sudden deceleration at the end of the exponential phase might be associated with an event such as the meeting of hyphae from different expanding microcolonies, which could cause a sudden decrease in the number of actively extending hyphal tips. However, they did not propose a mechanism by which this effect might be mediated. Possible explanations are the accumulation of inhibitory metabolites in the medium, exhaustion of readily utilizable nutrients or the onset of oxygen limitation. The mechanisms by which these phenomena can cause deceleration of growth are discussed in more detail in Section 3 of this review, which addresses the modeling of intraparticle phenomena.

Mathematical models of SSF bioreactors most commonly use the logistic equation, including several of the most recently proposed models [7–17]. 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.

Table 1
Differentiated and integrated forms of the various empirical growth equations that have been applied to SSF systems[a]

	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)	$X = \frac{X_m}{1 + ((X_m/X_0) - 1)e^{-\mu t}}$	(7)
Two phase	$\frac{dX}{dt} = \mu X,\ t < t_a$	(4a)	$X = X_0 e^{\mu t},\ t < t_a$	(8a)
	$\frac{dX}{dt} = [\mu L e^{-k(t-t_a)}]X,\ t \geq t_a$	(4b)	$X = X_A \exp\left[\frac{\mu L}{k}(1 - e^{-k(t-t_a)})\right],\ t \geq t_a$	(8b)

[a] 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.

Many SSF processes involve filamentous fungi, which bind tightly to the substrate, usually making it impossible to determine the dry weight of fungal biomass directly since it cannot be weighed independently of the residual substrate. Therefore, many of the kinetic studies done to determine the kinetic model are either undertaken in artificial systems that allow microbial biomass measurement, such as membrane culture [18] or amberlite resin [19], or are undertaken in the real system but using indirect measurements of growth, such as O_2 consumption or glucosamine content. However, both approaches have problems. The growth kinetics in artificial systems may not reflect the growth kinetics in the real system, while indirect measurements may be hard to interpret. The use of O_2 consumption to monitor growth will be discussed later. The determination of the growth kinetics by measuring the content of a microbial biomass component within the fermenting solid mass can be complicated by two factors. Firstly, if the biomass component is also present in the substrate and is consumed during the fermentation, it is impossible to determine independently the contributions of growth and consumption to the observed changes in the level of this component within the fermenting mass. It is for this reason that glucosamine is often used as an indicator of growth, since it is present in the cell walls of most fungi, in the form of chitin, but is not found in the plant material commonly used as substrates in these fermentations. However, this introduces the second factor, which is that the level of components such as glucosamine within the fungal biomass may change during the fermentation. Nagel et al. [20], who used glucosamine as an indicator of growth, tried to overcome this problem by characterizing these changes in cell composition. They used a membrane culture system, in which fungal biomass could be measured, in order to establish an empirical equation for the glucosamine content of the biomass (G_x) as a function of time (t):

$$G_x = 44.61 + \frac{43.65}{1 + \exp(\{(t - \lambda) - 61.70\}/12.34)} \quad (9)$$

where λ is the lag time (h). Such a strategy allows glucosamine measurements made in the fermentation in the real system to be converted into a corresponding dry weight of fungal biomass. However, it is not necessarily the case that the temporal variations in the glucosamine content of the fungal biomass in the artificial and real systems are the same. The more closely the growth conditions provided by the artificial system mimic those experienced in the real system, the more likely they are to be similar, but it is never possible to be completely certain.

2.2. Describing the effect of environmental conditions on growth

The aim of a bioreactor model is to describe the environmental conditions within the bioreactor as a function of time, and the response of the microorganism to these conditions. In bioreactor models, the two most important environmental variables are the temperature and the water activity of the bed, since these can be affected by the way in which the bioreactor is operated. To date the approach to modeling these effects has been through what can be referred to as the "constant condition" approach. In this approach, a range of cultures are incubated at different values of the environmental variable, with each culture being subjected to the same conditions throughout the whole fermentation. The kinetic profile for each culture is then analyzed in order to extract the values of the kinetic model parameters for those particular conditions. The growth parameters are then plotted against the environmental variable and an empirical equation is fitted.

For example, in applying this approach, Saucedo-Castaneda et al. [7] arrived at the following equations for two parameters of the logistic equation as functions of temperature:

$$\mu = \frac{2.694 \times 10^{11}\, e^{(-70225/8.314T)}}{1 + 1.300 \times 10^{47}\, e^{(-283356/8.314T)}} \quad (10)$$

and

$$X_m = -127.08 + 7.95(T - 273) - 0.016(T - 273)^2 - 4.03\times10^{-3}(T - 273)^3 + 4.73 \times 10^{-5}(T - 273)^4 \quad (11)$$

where T is the absolute temperature (K).

To date most models of SSF bioreactors have used this approach to describing the effect of environmental conditions on the parameters of the growth equation [7,9,11–17,21,22] although the form of the empirical equation that is used varies. Factorial design of experiments could potentially be used to evaluate the combined effect of more than one environmental condition, such as temperature and water activity, although this has not yet been done, with current equations being developed on the basis of one-by-one variations of environmental variables. In any case, use of these equations within the kinetic sub-model of a bioreactor model has the implicit assumption that the growth of the microorganism depends only on the current values of the environmental variables. However, in SSF bioreactors it is notoriously difficult to control the environmental variables at the optimum values for growth and the response to these variations might not be that described by the equations derived from the constant condition approach [23]. For example, significant variations in the temperature can be expected. Ikasari et al. [24] mimicked such changes by incubating a culture of *Rhizopus oligosporus* at its optimum temperature of 37 °C for 20 h, followed by incubation at 50 °C for another 10 h before returning the culture to 37 °C. After the return to 37 °C, the culture took 20 h to establish a growth rate similar to that of an otherwise identical culture incubated at 37 °C throughout. This result suggests that modeling approaches are needed that describe the growth rate at any instant not only as a function of the present values of the environmental variables, but also incorporating the deleterious effects of unfavorable growth conditions experienced in the past. Within the context of SSF no effort has been made either to propose such models or to undertake the experimental studies on which such models could be based. However, insights into possible mathematical strategies can be gained from studies undertaken in the context of food microbiology. Bovill et al. [25] modified the logistic equation by inclusion of a parameter (Q) that represented the physiological state of the cell:

$$\frac{dX}{dt} = \mu X\left(1 - \frac{X}{X_m}\right)\left(\frac{Q}{1+Q}\right) \quad (12)$$

A differential equation can then be developed that describes how the physiological state changes with time and how these changes are influenced by environmental conditions:

$$\frac{dQ}{dt} = f(T, a_w, Q) \quad (13)$$

Such an approach enables the past environmental conditions to affect the current growth rate, since the past conditions affect the current value of Q. The growth rate also depends on the current environmental conditions through the parameter μ. In effect, μ is the maximum possible value of the specific growth rate constant under a given set of environmental conditions, with the physiological state determining how closely the maximum value is approached. In this work the variable Q was never defined in more detail, being simply referred to as "a dimensionless quantity related to the physiological state of the cells", although it is possible to speculate that Q might represent the levels of intracellular enzymes that play key roles in the growth process.

2.3. Modeling of death kinetics

In SSF bioreactors the environmental conditions, especially the temperature, can attain values that are sufficiently adverse to cause death. The modeling of death kinetics in SSF systems has received relatively little attention due to the fact that the majority of SSF processes involve filamentous fungi. The mycelial mode of growth of fungi makes the definition of death more problematic than is the case for unicellular organisms, for which death can be both defined and measured experimentally through total and viable counts. If death is simply defined as a permanent loss of the ability to grow, then autolysis is not a necessary consequence of death and therefore death will not necessarily lead to a reduction in the amount of fungal biomass, so it is not a simple matter to quantify death experimentally. As a result, current models of death used in SSF bioreactor models are very simple. For example, Sangsurasak and Mitchell [12] assumed first-order death kinetics and segregated the microbial biomass into living and dead sub-populations:

$$\frac{dX_V}{dt} = \mu_G X_V\left(1 - \frac{X_V + X_D}{X_M}\right) - k_D X_V \quad (14a)$$

$$\frac{dX_D}{dt} = k_D X_V \quad (14b)$$

where X_V represents viable cells, X_D represents the dead cells and μ_G is the true specific growth rate, not the observed specific growth rate. It is not a simple matter to determine the true specific growth rate and the first-order death constant (k_D) for use in these equations. Szewczyk and Myszka [26] did this by fitting an equation containing two Arrhenius-type terms to a plot of observed specific growth rate versus temperature. One term described μ_G as a function of temperature and the other described k_D as a function of temperature:

$$\mu_{obs} = \mu_G - k_D = \mu_{G_0}\exp\left(-\frac{E_{aG}}{RT}\right) - k_{D_0}\exp\left(-\frac{E_{aD}}{RT}\right) \quad (15)$$

where μ_{G_0} and k_{D_0} are frequency factors and E_{aG} and E_{aD} are activation energies for the growth and death reactions, respectively. However, independent data of death kinetics was not available to confirm the validity of this approach, which tends to predicts small but significant death rates at the optimum temperature for growth.

Future developments in the modeling of death kinetics of fungi in SSF could potentially be based on the symmetric branching model that was developed by Viniegra-Gonzalez et al. [27,28] and later refined by Ikasari et al. [24] for use in characterizing the effect of temperature. Viniegra-Gonzalez et al. [27,28] showed how the microscopic events of hyphal elongation and branching could be related to logistic growth kinetics if the length of hyphal extension prior to branching was always the same and each of the daughter hyphae were identical with each other and with the hypha that gave rise to them. Ikasari and Mitchell [6] extended this model to allow for the death of hyphal tips, giving rise to the deceleration phase of the two phase model in Table 1, the parameter k in Eq. (4b) representing a first-order tip-death coefficient. This model could be applied to growth profiles obtained at different temperatures, obtaining k as an empirical function of temperature, this relationship being incorporated into the kinetic sub-model of a bioreactor model. Ikasari et al. [24] determined k at 37 and 50 °C, but two data points are insufficient to determine the form of the relationship. Although the model is empirical, it is based on considerations of microscale growth processes that potentially could be investigated through microscope-based studies in which tip death was measured directly, possibly through the use of fluorescent vital stains.

A slightly different approach was taken by Smits et al. [21]. They did not model death explicitly, but rather proposed an equation that described how the specific respiration activity of the microbial biomass decreased over time, something which would be expected if a part of the biomass were dying. The specific rate of O_2 uptake (q_{O_2}) was modeled as depending on growth and maintenance:

$$q_{O_2} = \frac{1}{Y_{XO_2}}\mu(t) + m_{O_2} - D \tag{16}$$

where Y_{XO_2} is the yield of microbial biomass from O_2, $\mu(t)$ is the instantaneous specific growth rate and m_{O_2} is the maintenance coefficient for O_2. The term D describes a decrease in maintenance activity. Various expressions were suggested for D. One which gave realistic predictions was the temporary-decline model. In this model, there is no inactivation initially, that is, all the microbial biomass contributes to maintenance metabolism:

$$\text{for } 0 \le t < t_d, \quad D = 0 \tag{17a}$$

After time t_d is reached inactivation starts and increases with time:

$$\text{for } t_d \le t < t_r, \quad D = m_d(t - t_d) \tag{17b}$$

After time t_r, D takes on a constant value:

$$D = m_d(t_r - t_d) \tag{17c}$$

The form of the term D and the parameters of the model are determined by comparing experimental profiles for either the microbial biomass or a component of the biomass and the experimental results for O_2 uptake. Therefore, it is not necessary to undertake measurements distinguishing living and dead biomass. Since this model does not try explicitly to describe death kinetics, it may be the preferred approach until better experimental methods are developed to study the death of fungi.

2.4. 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 microbial biomass produced is less than 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:

$$\frac{dR}{dt} = -\frac{1}{Y_{XR}}\frac{dX}{dt} - m_R X \tag{18a}$$

$$\frac{dP}{dt} = Y_{PX}\frac{dX}{dt} + m_P X \tag{18b}$$

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 Eq. (18b) is written in terms of metabolic heat. It has often been assumed that heat production is directly proportional to growth [11,12,14,16,17], although this is probably not true. In any case, the inclusion of maintenance heat production is a simple matter [9].

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 [9,29]. Other models have ignored the decrease in total solids weight despite the fact that such decreases occur in reality [12,14,17]. This could be a shortcoming since bed shrinkage can potentially have important effects on bioreactor performance, such as promoting channeling in packed-beds [30].

At times Eqs. (18a) and (18b) are used in order to estimate the growth kinetics parameters from indirect measurements of either O_2 consumption or carbon dioxide evolution. For example, assuming logistic growth kinetics and that O_2 is consumed for growth and maintenance according to

$$\frac{dO_2}{dt} = Y_{OX}\frac{dX}{dt} + m_O X \tag{19}$$

then the cumulative O_2 consumption between time zero and time t is given by [31]

$$\mathrm{COU} = X_m\left[\frac{Y_{OX}}{((X_m/X_0)-1)e^{-\mu t}+1} - \frac{Y_{OX}}{(X_m/X_0)} + \frac{m_0}{\mu}\ln\left(\frac{((X_m/X_0)-1)e^{-\mu t}+1}{((X_m/X_0)-1)e^{-\mu t}}\right)\right] \tag{20}$$

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 O_2-microbial biomass stoichiometric coefficient (Y_{OX}) and the maintenance coefficient (m_0) 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 O_2 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 are significant temperature variations would carry with it the implicit assumption that Y_{OX} and m_0 do not vary with temperature, although this may not be a good assumption [32].

3. State of the art in modeling of intraparticle phenomena in SSF

A number of models have been proposed that, unlike the equations above that give simple empirical descriptions of observed growth curves, do attempt to describe how the growth can be affected by intraparticle diffusion of O_2, enzymes, hydrolysis products and other nutrients, and the role in the fermentation of other phenomena such as particle shrinkage and spatial microbial biomass distribution (Fig. 2). In a few cases, these models have formed the kinetic sub-models of bioreactor models, but in most cases the models were proposed without concern for overall bioreactor performance.

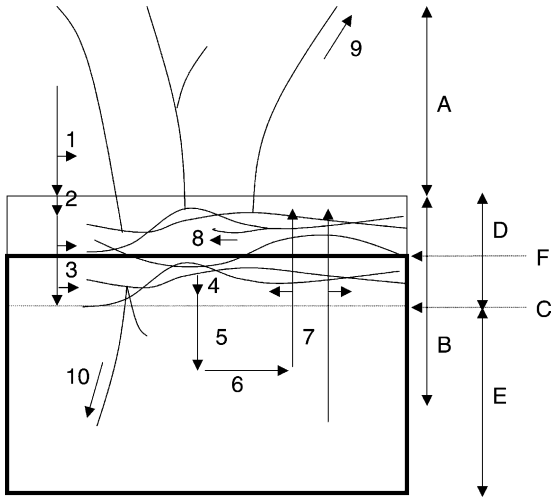

Fig. 2. Various of the microscale processes involved in growth in SSF systems and which have been investigated in modeling work. Key to processes: (1) diffusion of O_2 in a static air phase or convective flow, with uptake by aerial hyphae; (2) transfer of O_2 across a gas–liquid interface; (3) diffusion and reaction of O_2 within a liquid phase; (4) enzyme release; (5) enzyme diffusion; (6) hydrolysis of polymers by the enzyme, releasing soluble hydrolysis products; (7) diffusion of hydrolysis products, and other nutrients, with uptake by the microbial biomass; (8) increase in hyphal density at the surface; (9) growth of aerial hyphae; (10) growth of penetrative hyphae. Key to physical features: (A) aerial hyphae; (B) hyphae submerged within a liquid phase. Above the surface there are hyphae submerged within a thin liquid film that fills the spaces between the hyphae, below the surface the moisture is held within the solid matrix; (C) depth of penetration of O_2 into the substrate. The depth of penetration of O_2 varies during the fermentation depending on the respiratory activity of the microbial biomass and the thickness of the biomass layer, at times of peak O_2 consumption the O_2 may penetrate only partially into the liquid film above the surface; (D) aerobic zone; (E) anaerobic zone; (F) substrate surface. The surface may become indistinct or may even recede, especially if there is no inert polymer to give the particle structure while other macromolecules are being hydrolyzed.

3.1. Modeling intraparticle diffusion

Early models describing the overculture of microorganisms on solid substrates were those of Georgiou and Shuler [33], which described growth at the surface of a flat slab of substrate with glucose as the substrate, and Mitchell et al. [34], which described a similar system but with the use of starch as a substrate, in which case it was necessary to describe the release of glucoamylase by the microorganism at the surface, the diffusion of the glucoamylase into the substrate, the hydrolysis of starch by the glucoamylase, the diffusion of the released glucose to the surface and the uptake of glucose at the surface by the microbial biomass.

These models had several limitations. Firstly, they described growth on an infinitely-wide, flat slab of substrate. This is not a severe limitation since it is a relatively simple matter to change the equations to take into account a spherical geometry, which is a much better approximation of particle shape in SSF systems. Secondly, they did not recognize any structure in the microbial biomass. Glucose

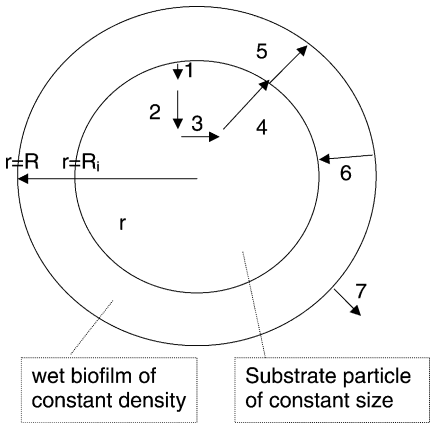

Fig. 3. The system modeled by Rajagopalan and Modak [35]. The processes modeled were: (1) enzyme release, which occurs only at the substrate/biomass interface; (2) diffusion of glucoamylase within the substrate particle; (3) hydrolysis of starch by the glucoamylase; (4) liberation of glucose and diffusion within the substrate particle; (5) diffusion of glucose within the biofilm and uptake by the microbial biomass; (6) diffusion of O_2 through the biofilm and uptake by the microbial biomass; (7) expansion of biofilm due to growth.

reaching the surface of the substrate was immediately taken up and converted into new microbial biomass. In effect the microbial biomass was treated mathematically as though it were an infinitely thin plane of infinite density at the substrate surface. Thirdly, they did not consider the possibility that O_2 availability and not glucose availability might limit the growth.

Rajagopalan and Modak [35] developed a model that took O_2 diffusion into account and gave a structure to the microbial biomass, treating it as a wet biofilm of constant density. The model described the various steps that are shown in Fig. 3. Diffusion of glucoamylase within the substrate particle was described as

$$\frac{\partial C_{\mathrm{E}}^{\mathrm{s}}}{\partial t} = \frac{D_{\mathrm{E}}^{\mathrm{s}}}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial C_{\mathrm{E}}^{\mathrm{s}}}{\partial r}\right) \tag{21}$$

where r is the radial position in the particle, $C_{\mathrm{E}}^{\mathrm{s}}$ is the concentration of enzyme within the substrate particle, $D_{\mathrm{E}}^{\mathrm{s}}$ is the effective diffusivity of the enzyme within the substrate and t is time. It was assumed that the enzyme was liberated into the substrate at the biofilm/particle boundary and could not cross this boundary into the biofilm, although there is no reason to suppose this would be the case in reality.

Hydrolysis of starch within the substrate particle was assumed to follow Michaelis–Menten kinetics:

$$\frac{\partial C_{\mathrm{S}}^{\mathrm{s}}}{\partial t} = -K_{\mathrm{cat}}C_{\mathrm{E}}^{\mathrm{s}}\left(\frac{C_{\mathrm{S}}^{\mathrm{s}}}{K_{\mathrm{S}}+C_{\mathrm{S}}^{\mathrm{s}}}\right) \tag{22}$$

where $C_{\mathrm{S}}^{\mathrm{s}}$ is the starch concentration within the substrate, K_{cat} the catalytic constant of the enzyme and K_{S} the Michaelis–Menten constant for starch.

Release of glucose by the action of glucoamylase and the diffusion of this glucose within the substrate particle were described by

$$\frac{\partial C_{\mathrm{G}}^{\mathrm{s}}}{\partial t} = \frac{D_{\mathrm{G}}^{\mathrm{s}}}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial C_{\mathrm{G}}^{\mathrm{s}}}{\partial r}\right) + K_{\mathrm{cat}}C_{\mathrm{E}}^{\mathrm{s}}\left(\frac{C_{\mathrm{S}}^{\mathrm{s}}}{K_{\mathrm{S}}+C_{\mathrm{S}}^{\mathrm{s}}}\right) \tag{23}$$

where $C_{\mathrm{G}}^{\mathrm{s}}$ is the glucose concentration within the substrate and $D_{\mathrm{G}}^{\mathrm{s}}$ is the effective diffusivity of glucose. The first term on the right hand side (RHS) describes diffusion and the second term the glucose generation.

The glucose that diffuses into the biofilm is consumed as it diffuses through the biofilm:

$$\frac{\partial C_{\mathrm{G}}^{\mathrm{f}}}{\partial t} = \frac{D_{\mathrm{G}}^{\mathrm{f}}}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial C_{\mathrm{G}}^{\mathrm{f}}}{\partial r}\right) - \mu_{\mathrm{m}}\rho_x Y_{\mathrm{G}/X}\left(\frac{C_{\mathrm{O_2}}^{\mathrm{f}}}{K_{\mathrm{O_2}}+C_{\mathrm{O_2}}^{\mathrm{f}}}\right)\left(\frac{C_{\mathrm{G}}^{\mathrm{f}}}{K_{\mathrm{G}}+C_{\mathrm{G}}^{\mathrm{f}}}\right) \tag{24}$$

where $C_{\mathrm{G}}^{\mathrm{f}}$ is the glucose concentration within the biofilm and $D_{\mathrm{G}}^{\mathrm{f}}$ is the effective diffusivity.The equation for diffusion and reaction of O_2 within the biofilm was

$$\frac{\partial C_{\mathrm{O_2}}^{\mathrm{f}}}{\partial t} = \frac{D_{\mathrm{O_2}}^{\mathrm{f}}}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial C_{\mathrm{O_2}}^{\mathrm{f}}}{\partial r}\right) - \mu_{\mathrm{m}}\rho_x Y_{\mathrm{O_2}/X}\left(\frac{C_{\mathrm{O_2}}^{\mathrm{f}}}{K_{\mathrm{O_2}}+C_{\mathrm{O_2}}^{\mathrm{f}}}\right)\left(\frac{C_{\mathrm{G}}^{\mathrm{f}}}{K_{\mathrm{G}}+C_{\mathrm{G}}^{\mathrm{f}}}\right) \tag{25}$$

where $C_{\mathrm{O_2}}^{\mathrm{f}}$ is the concentration of glucose in the microbial biomass film and $D_{\mathrm{O_2}}^{\mathrm{f}}$ is the effective diffusivity of O_2 within the biofilm. Oxygen was assumed to not be able to cross the biofilm/particle boundary, although there is no reason why this should be so.

Note that growth and therefore growth-related activities such as nutrient and O_2 uptake are assumed to be simultaneously limited by both O_2 and glucose. In the second term on the RHS of both of the previous two equations μ_{m} is the maximum specific growth rate, ρ_x the density of the microbial biomass and $Y_{\mathrm{O_2}/X}$ and $Y_{\mathrm{G}/X}$ the stoichiometric coefficients for O_2 and glucose, respectively. In both these equations, the first term on the RHS describes diffusion within the biofilm.

Growth of the microbial biomass causes the biofilm to expand. First the overall amount of microbial biomass was followed through integration of the growth equation across the biofilm:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \int_{R_i}^{R}\mu_{\mathrm{m}}\rho_x\left(\frac{C_{\mathrm{O_2}}^{\mathrm{f}}}{K_{\mathrm{O_2}}+C_{\mathrm{O_2}}^{\mathrm{f}}}\right)\left(\frac{C_{\mathrm{G}}^{\mathrm{f}}}{K_{\mathrm{G}}+C_{\mathrm{G}}^{\mathrm{f}}}\right)4\pi r^2\,\mathrm{d}r \tag{26}$$

and then the volume occupied by this microbial biomass in a spherical shell starting at the particle/biomass boundary at R_i was used to calculate the overall radius of the particle:

$$R = \sqrt[3]{\frac{3X}{4\pi\rho_x}+R_i^3} \tag{27}$$

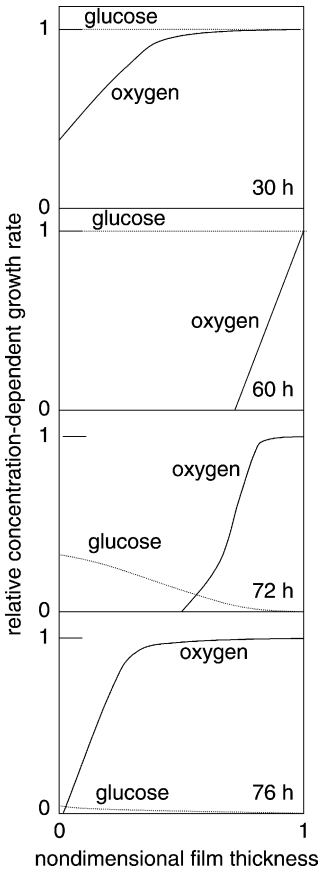

Fig. 4. Typical predictions of the model of Rajagopalan and Modak [35] regarding relative O_2 and glucose limitation in the biofilm. The *X*-axis represents the position within the biofilm with 0 representing the biomass/particle interface and 1 representing the air/biomass interface. The *Y*-axis represents the values of the Monod expressions $C^f_{O_2}/(C^f_{O_2} + K_{O_2})$ and $C^f_G/(C^f_G + K_G)$.

The particle was assumed to maintain its structure, as though an inert macromolecule were maintaining the physical structure.

The density of the biofilm was assumed to be equal to that of water and the diffusivities of glucose and O_2 in the biofilm were assumed to be equal to their diffusivities in water. Therefore, this system describes either a biofilm of unicellular organisms, or hyphae of a fungus embedded within a film of water at the surface. This model was used to show that for such a system O_2 limitation was a more serious problem for the microorganism than glucose limitation (Fig. 4). During the rapid growth period 70–80% of the biofilm can be in anaerobic conditions. Glucose limitation becomes most important at the outer surface of the biofilm and, even so, is only important late in the fermentation when the rate of glucose supply from the substrate becomes limiting. These assumptions and insights would not necessarily be true for aerial fungal hyphae, that is, hyphae that are not submerged within a liquid film. A model that describes aerial hyphal growth is described later in Section 3.3.

Only relatively few bioreactor models have kinetic sub-models that describe the intraparticle phenomena rather than simply using empirical growth kinetics. The model of Rottenbacher et al. [36] described a somewhat unconventional SSF system that involved pressed pellets of *Saccharomyces cerevisiae* within a fluidized bed bioreactor designed for the production of ethanol. Glucose was sprayed onto the surface of the fluidized bed. Due to the assumption of good mixing within the bed and good heat removal, the balance/transport model did not involve partial differential equations and the model focused on the intraparticle diffusion and reaction of glucose and the resultant growth and ethanol production. A similar model for this system was proposed by Bahr and Menner [37], although in this case it was assumed that the bed was fluidized in air, such that O_2 diffusion and reaction was also taken into account. The model predicted high O_2 and glucose concentrations in an outer shell of 100 μm depth, such that catabolite repression would be expected, and that both O_2 and glucose limitation would occur within the inner regions of the particle. The only model that involves the use of partial differential equations to describe both transport across the bed and intraparticle diffusion is the tray bioreactor model of Rajagopalan and Modak [22]. They used a version of the model presented in this section that described only O_2 diffusion within the particle and not enzyme production, diffusion and action and neither glucose diffusion. Their equations are presented and discussed in Mitchell et al. [1].

It is only recently that the intraparticle gradients predicted by such models have been characterized experimentally. Nagel et al. [38] used nuclear magnetic resonance to investigate water and glucose concentration gradients within substrate particles. Intraparticle gradients of O_2 have also been measured, using an O_2 microprobe [39]. There is general agreement between the model predictions and the experimental results.

The uptake of O_2 into the microbial biomass in SSF systems has received special attention lately. Thibault et al. [40] used an O_2 diffusion and reaction model to compare the rate of transfer of O_2 across the air/liquid interface at the surface of a biofilm with the rate of O_2 diffusion through the biofilm. They concluded that rather than characterize O_2 transfer across the gas–liquid interface with $K_L a$, which is done in liquid culture because this is the limiting step, it is preferable to use a "conductive biofilm coefficient", $K_F a$, where K_F is the ratio of the diffusivity of O_2 within the biofilm to the thickness of the aerobic portion of the biofilm. This is necessary because, differently from submerged fermentation, in SSF there is not a uniformly-mixed bulk liquid phase on the other side of the gas–liquid interface and therefore it is diffusion through the biofilm that is the limiting step in O_2 transfer and not the gas–liquid transfer step. Similarly, models of O_2 reaction and diffusion in biofilms have been used in combination with experimental data for overall O_2 consumption rates to show that in some cases aerial

hyphae do not contribute significantly to overall O_2 uptake [39] whereas in others they do [41].

3.2. Models recognizing the shrinking of particles

For many substrates the physical structure of the particle is degraded during the fermentation, and this may have important effects on the bed properties within a bioreactor, affecting bioreactor performance. Nandakumar et al. [42] modeled this by assuming that the overall particle size, that is, microbial biomass plus residual substrate, remained constant, with the new microbial biomass filling the space freed by the consumption of the particle. Consumption of the particle only occurred at a sharp biomass/substrate interface and was limited by the rate of diffusion of O_2 through the microbial biomass layer to this interface. They arrived at the equation:

$$t = \left[\frac{e_b L^2}{2bD_e C_A}\right]\left(1 + \frac{l_c^2}{L^2} - 2\frac{l_c}{L}\right) \tag{28}$$

where b is the stoichiometric coefficient, e_b is the molar density of the substrate, L is the overall particle size, which remains constant, D_e is the effective diffusivity of O_2 in the microbial biomass layer, C_A is the O_2 concentration at the outer surface of the microbial biomass layer and l_c is the length of the undegraded core of residual substrate. However, in applying their model to experimental data, they simply treated the term within the square brackets as an empirical constant, which represents the time required for complete degradation of the particle, and determined the value of this constant by non-linear regression of the equation against the experimental data.

A more sophisticated model was developed by Rajagopalan et al. [43], extending the model of Rajagopalan and Modak [35] that described the intraparticle diffusion of glucose and O_2. As with the model of Nandakumar et al. [42] there is a sharp biomass/particle interface, but the reaction is not limited to this interface because of the liberation of enzyme into the substrate and diffusion of the glucose into the biofilm. The following equation was derived to describe the transient particle radius $R_i(t)$:

$$R_i(t) = \sqrt[3]{R_{i_0}^3 - \frac{3\left[\int_0^1 D_G^s (\partial C_G^s/\partial r) 4\pi R_{i_0}^2 \, dt\right]}{4\pi\rho_G}} \tag{29}$$

where all the symbols have been previously defined above except for ρ_G, which is the original concentration of the starch expressed as the equivalent mass per unit volume of glucose and R_{i_0}, which is the initial radius of the substrate particle. The volume of the residual particle (V_S) is then given as

$$V_S = \tfrac{4}{3}\pi(R_{i_0}^3 - R_i(t)^3)\rho_G \tag{30}$$

However, there are many assumptions in such a model that might be reasonable for structurally simple substrates such as beads of starch, but that might not apply well to more complex substrates. Mechanistic modeling of substrate particle size reduction will remain a challenge.

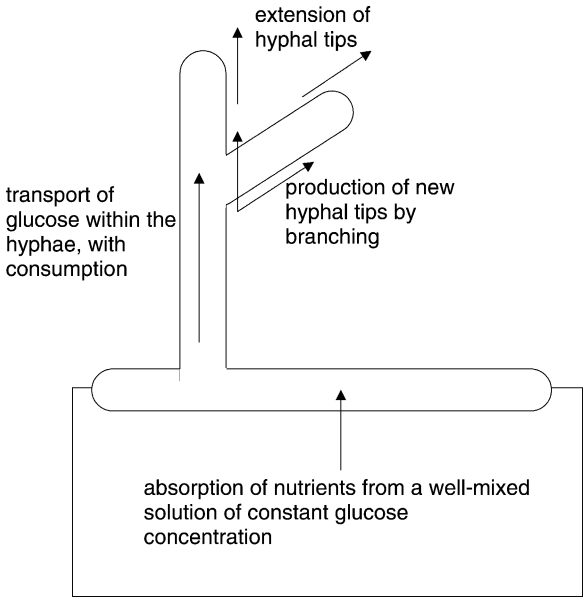

Fig. 5. Phenomena described in the model of Nopharatana et al. [44] describing the growth of aerial fungal biomass above the substrate surface.

3.3. Models recognizing the uneven distribution of microbial biomass above the substrate surface

Nopharatana et al. [44] presented a model to describe the growth of fungal hyphae above the surface, specifically attempting to describe how the hyphal biomass distribution with height above the surface might change over time, and how this would depend on the diffusion of glucose from the substrate surface to the tips extending into the airspace (Fig. 5). Since the space between hyphae was assumed to be filled with air, O_2 supply was assumed not to be limiting. The model considered only the vertical coordinate, and therefore all points in a horizontal plane parallel to the surface were assumed to be identical. Note that although fungal growth occurs as a result of the extension and branching of individual hyphal tips, the model did not attempt to describe this. The space above the substrate surface was not separated into distinct air and fungal biomass phases, but rather a single phase was assumed, with height above the surface being characterized by average spatial fungal biomass, glucose and tip concentrations.

An equation is necessary to describe the generation and movement of tips:

$$\left.\frac{\partial n}{\partial t}\right|_x = \alpha_t\left(1 - \frac{B}{B_m}\right)\left(\frac{G|_{x-\delta}}{k_t + G|_{x-\delta}}\right) + D_b\left(1 - \frac{B}{B_m}\right)\left(\frac{G|_{x-\delta}}{k_t + G|_{x-\delta}}\right)\frac{\partial n}{\partial x} \tag{31}$$

This equation postulates that the number of tips at a particular height (x) is the result of two processes. The first term on the RHS describes the production of new tips by branching, the rate of this process depending on the glucose concentration at a distance of δ behind the tip ($G|_{x-\delta}$), according to a Monod relationship, in which k_t is the saturation constant, and on the fungal biomass concentration (B), becoming inhibited as the fungal biomass approaches a maximum packing density (B_m). The maximum specific tip production rate is given by α_t. The second term on the RHS describes the movement of tips into and out of a particular height. This movement, due to extension of tips, is modeled as a diffusion-like process, assuming that the rate of extension of tips depends on the overall glucose concentration and the overall fungal biomass concentration.

The production of fungal biomass depends on the extension of tips:

$$\left.\frac{\partial B}{\partial t}\right|_x = n v_{max} a \rho \left(1 - \frac{B}{B_m}\right)\left(\frac{G|_{x-\delta}}{k_t + G|_{x-\delta}}\right) \quad (32)$$

where v_{max} is the maximum possible tip extension rate. The rate of extension of a single tip is v_{max} times the quantities in the parentheses and therefore is assumed to depend both on the fungal biomass concentration and the glucose concentration. Multiplying the tip extension rate by the cross-sectional area of a hypha (a) and the dry weight of fungal biomass per unit hyphal volume (ρ) gives the production of dry weight per unit time.

A balance is also written for diffusion and consumption of glucose within the hyphae:

$$\left.\frac{\partial G}{\partial t}\right|_x = D_{hyp}\frac{\partial}{\partial x}\left(B\frac{\partial(G/B)}{\partial x}\right) - \frac{1}{Y_{XG}}\left.\frac{\partial B}{\partial t}\right|_x - m_G B \quad (33)$$

where D_{hyp} is the effective diffusivity of glucose in the hyphae. The first term on the RHS describes the diffusion of glucose within the hyphae, which depends on the concentration gradient of glucose within the hyphae, and not the concentration gradient of glucose in space. Therefore, this term is written in terms of $\partial(G/B)/\partial x$ and not $\partial G/\partial x$. The second term describes the consumption of glucose for growth and the third term represents the consumption of glucose for maintenance. Y_{XG} is the yield of fungal biomass from glucose and m_G is the glucose maintenance coefficient.

The model suggests that during the early stages of growth two things occur (Fig. 6): the fungal biomass concentration at the surface increases and hyphae extend above the surface, forming a sigmoidal-shaped fungal biomass concentration profile with height. When the fungal biomass concentration at the surface reaches the maximum possible value then the shape of this biomass concentration profile does not change, but moves as a constant-shaped wavefront to greater heights. Unfortunately, there is no experimental data giving fungal biomass concentration profiles as a function of height above the substrate surface against which these predictions can be compared. Even without this experimental validation, the modeling work done and the assumptions made do raise interesting questions. One of the most pertinent questions is whether the movement of glucose within the hyphae, from the surface to the tips, actually occurs by a diffusion process, or whether there is some sort of translocation mechanism such as cytoplasmic streaming.

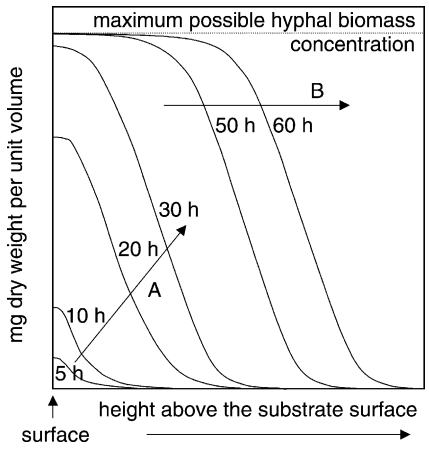

Fig. 6. Typical predictions of the model of Nopharatana et al. [44] with respect to the development of the fungal biomass concentration profile above the surface during the fermentation. Two phases are apparent: (A) initially the biomass concentration both increases at the surface and extends to greater heights; (B) once the maximum concentration is reached at the substrate surface a biomass concentration front of constant shape moves to higher and higher heights.

The model represents growth in a simplified system. The situation would be expected to be even more complex in a real SSF system in which hyphae extending from various surfaces surrounding a common void space would soon meet each other. Further, it was assumed that the hyphae at the substrate surface were in contact with a constant glucose concentration. To be more realistic it would be necessary to incorporate equations that describe how the various processes occurring within the substrate control the rate of supply of glucose to the surface, in the manner done by Mitchell et al. [34].

4. Conclusions

Simple models of growth kinetics have evolved little over the past decade, and systematic analyses of growth profiles in SSF systems have not been undertaken. This can be attributed to the experimental difficulties faced in growth kinetic studies. Further, to be useful in bioreactor models, such equations should describe how growth is affected by variations in key environmental parameters such as temperature and water activity but current models do this in a very simple fashion and are based on limited experimental data. Much more experimental work is required to elucidate how growth and death depend on these factors over wide ranges, and how temporal variations in these factors affect the physiological state of the microorganism.

Models that describe intraparticle diffusion and reaction suggest that oxygen limitation of at least part of the microbial biomass for part of the fermentation is an intrinsic characteristic of SSF processes. However, during the later stages of the fermentation glucose can be limiting at the outer surface of biofilm. Also it is quite possible that the diffusion of glucose within aerial fungal hyphae controls their extension above the substrate surface. These predictions agree reasonably with the little experimental work that has been done to confirm them to date. More experimental work is required to confirm the accuracy of these predictions for a range of different microbe–substrate systems.

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 model of Rajagopalan and Modak [22] shows 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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References

[1] D.A. Mitchell, O.F. von Meien, N. Krieger, Recent developments in modeling of solid-state fermentation: heat and mass transfer in bioreactors, Biochem. Eng. J. 13 (2002) 137–147.
[2] D.A. Mitchell, D.M. Stuart, R.D. Tanner, Solid-state fermentation—microbial growth kinetics, in: M.C. Flickinger, S.W. Drew (Ed.), The Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation, vol. 5, Wiley, New York, 1999, pp. 2407–2429.
[3] M.K. Gowthaman, N.P. Ghildyal, K.S.M.S. Raghava Rao, N.G. Karanth, Interaction of transport resistances with biochemical reaction in packed bed solid-state fermenters: the effect of gaseous concentration gradients, J. Chem. Technol. Biotechnol. 56 (1993) 233–239.
[4] N.P. Ghildyal, M.K. Gowthaman, K.S.M.S. Raghava Rao, N.G. Karanth, Interaction between transport resistances with biochemical reaction in packed bed solid-state fermenters: effect of temperature gradients, Enzyme Microb. Technol. 16 (1994) 253–257.
[5] P. Sangsurasak, M. Nopharatana, D.A. Mitchell, Mathematical modeling of the growth of filamentous fungi in solid-state fermentation, J. Sci. Ind. Res. 55 (1996) 333–342.
[6] L. Ikasari, D.A. Mitchell, Two-phase model of the kinetics of growth of *Rhizopus oligosporus* in membrane culture, Biotechnol. Bioeng. 68 (2000) 619–627.
[7] G. Saucedo-Castaneda, M. Gutierrez-Rojas, G. Bacquet, M. Raimbault, G. Viniegra-Gonzalez, Heat transfer simulation in solid substrate fermentation, Biotechnol. Bioeng. 35 (1990) 802–808.
[8] K.S.M.S. Ragheva Rao, M.K. Gowthaman, N.P. Ghildyal, N.G. Karanth, A mathematical model for solid-state fermentation in tray bioreactors, Bioprocess Eng. 8 (1993) 255–262.
[9] J. Sargantanis, M.N. Karim, V.G. Murphy, D. Ryoo, R.P. Tengerdy, Effect of operating conditions on solid substrate fermentation, Biotechnol. Bioeng. 42 (1993) 149–158.
[10] K.W. Szewczyk, The influence of heat and mass transfer on solid-state fermentation, Acta Biochim. Polon. 40 (1993) 90–92.
[11] S. Rajagopalan, J.M. Modak, Heat and mass transfer simulation studies for solid-state fermentation processes, Chem. Eng. Sci. 49 (1994) 2187–2193.
[12] P. Sangsurasak, D.A. Mitchell, Incorporation of death kinetics into a 2-D dynamic heat transfer model for solid-state fermentation, J. Chem. Technol. Biotechnol. 64 (1995) 253–260.
[13] S.D.M. Hasan, J.A.V. Costa, A.V.L. Sanzo, Heat transfer simulation of solid-state fermentation in a packed-bed bioreactor, Biotechnol. Tech. 12 (1998) 787–791.
[14] P. Sangsurasak, D.A. Mitchell, Validation of a model describing 2-dimensional heat transfer during solid-state fermentation in packed bed bioreactors, Biotechnol. Bioeng. 60 (1998) 739–749.
[15] V.M. Ashley, D.A. Mitchell, T. Howes, Evaluating strategies for overcoming overheating problems during solid-state fermentation in packed bed bioreactors, Biochem. Eng. J. 3 (1999) 141–150.
[16] D.A. Mitchell, A. Pandey, P. Sangsurasak, N. Krieger, Scale-up strategies for packed-bed bioreactors for solid-state fermentation, Process Biochem. 35 (1999) 167–178.
[17] D.A. Mitchell, O.F. von Meien, Mathematical modeling as a tool to investigate the design and operation of the Zymotis packed-bed bioreactor for solid-state fermentation, Biotechnol. Bioeng. 68 (2000) 127–135.
[18] D.A. Mitchell, H.W. Doelle, P.F. Greenfield, Suppression of penetrative hyphae of *Rhizopus oligosporus* by membrane filters in a model solid-state fermentation system, Biotechnol. Tech. 3 (1989) 45–50.
[19] R. Auria, S. Hernandez, M. Raimbault, S. Revah, Ion exchange resin: a model support for solid state growth fermentation of *Aspergillus niger*, Biotechnol. Tech. 4 (1990) 391–396.
[20] F.J.I. Nagel, J. Tramper, S.N. Marjolein, M.S.N. Bakker, A. Rinzema, Model for on-line moisture-content control during solid-state fermentation, Biotechnol. Bioeng. 72 (2001) 231–243.
[21] J.P. Smits, H.M. van Sonsbeek, J. Tramper, W. Knol, W. Geelhoed, M. Peeters, A. Rinzema, Modelling fungal solid-state fermentation: the role of inactivation kinetics, Bioprocess Eng. 20 (1999) 391–404.
[22] S. Rajagopalan, J.M. Modak, Modeling of heat and mass transfer for solid-state fermentation process in tray bioreactor, Bioprocess Eng. 13 (1995) 161–169.
[23] D.A. Mitchell, M. Berovic, N. Krieger, Biochemical engineering aspects of solid state bioprocessing, Adv. Biochem. Eng./Biotechnol. 68 (2000) 61–138.
[24] L. Ikasari, D.A. Mitchell, D.M. Stuart, Response of *Rhizopus oligosporus* to temporal temperature profiles in a model solid-state fermentation system, Biotechnol. Bioeng. 64 (1999) 722–728.
[25] R. Bovill, J. Bew, N. Cook, M. D'Agostino, N. Wilkinson, J. Baranyi, Predictions of growth for *Listeria monocytogenes* and *Salmonella* during fluctuating temperature, Int. J. Food Microbiol. 59 (2000) 157–165.
[26] K.W. Szewczyk, L. Myszka, The effect of temperature on the growth of *A. niger* in solid-state fermentation, Bioprocess Eng. 10 (1994) 123–126.
[27] G. Viniegra-Gonzalez, G. Saucedo-Castaneda, F. Lopez-Isunza, E. Favela-Torres, Symmetric branching model for the kinetics of mycelial growth, Biotechnol. Bioeng. 42 (1993) 1–10.
[28] G. Viniegra-Gonzalez, C.P. Larralde-Corona, F. Lopez-Isunza, A new approach for modelling the kinetics of mycelial cultures, in: E. Galindo, O.T. Ramirez (Eds.), Advances in Bioprocess Engineering, Kluwer Academic Publishers, Dordrecht, 1994, pp. 183–189.
[29] D.M. Stuart, Solid-state fermentation in rotating drum bioreactors, Ph.D. Thesis, The University of Queensland, Brisbane, Australia, 1996.

[30] F.J. Weber, J. Tramper, A. Rinzema, A simplified material and energy balance approach for process development and scale-up of *Coniothyrium minitans* conidia production by solid-state cultivation in a packed-bed reactor, Biotechnol. Bioeng. 65 (1999) 447–458.
[31] L.P. Ooijkaas, R.M. Buitelaar, J. Tramper, A. Rinzema, Growth and sporulation stoichiometry and kinetics of *Coniothyrium minitans* on agar media, Biotechnol. Bioeng. 69 (2000) 292–300.
[32] L. Ikasari, D.A. Mitchell, Oxygen uptake kinetics during solid-state fermentation with *Rhizopus oligosporus*, Biotechnol. Tech. 12 (2) (1998) 171–175.
[33] G. Georgiou, M.L. Shuler, A computer model for the growth and differentiation of a fungal colony on solid substrate, Biotechnol. Bioeng. 28 (1986) 405–416.
[34] D.A. Mitchell, D.D. Do, P.F. Greenfield, H.W. Doelle, A semi-mechanistic mathematical model for growth of *Rhizopus oligosporus* in a model solid-state fermentation system, Biotechnol. Bioeng. 38 (1991) 353–362.
[35] S. Rajagopalan, J.M. Modak, Evaluation of relative growth limitation due to depletion of glucose and oxygen during fungal growth on a spherical solid particle, Chem. Eng. Sci. 50 (1995) 803–811.
[36] L. Rottenbacher, M. Schossler, W. Bauer, Modelling a solid-state fluidized bed fermenter for ethanol production with *Saccharomyces cerevisiae*, Bioprocess Eng. 2 (1987) 25–31.
[37] D. Bahr, M. Menner, Solid-state-fermentation of starter cultures in fluidized bed. Part 2. Mathematical modeling, Bioforum 18 (1995) 366–372.
[38] F.J.I. Nagel, H. van As, J. Tramper, A. Rinzema, Water and glucose gradients in the substrate measured with NMR imaging during solid-state fermentation with *Aspergillus oryzae*, Biotechnol. Bioeng., 2002, submitted for publication.
[39] J. Oostra, E.P. le Comte, J.C. van den Heuvel, J. Tramper, A. Rinzema, Intra-particle oxygen diffusion limitation in solid-state fermentation, Biotechnol. Bioeng. 74 (2001) 13–24.
[40] J. Thibault, K. Pouliot, E. Agosin, R. Perez-Correa, Reassessment of the estimation of dissolved oxygen concentration profile and $K_L a$ in solid-state fermentation, Proc. Biochem. 36 (2000) 9–18.
[41] Y.S.P. Rahardjo, F.J. Weber, E.P. le Comte, J. Tramper, A. Rinzema, Contribution of aerial hyphae of *Aspergillus oryzae* to respiration in a model solid-state fermentation system, Biotechnol. Bioeng., 2002, in press.
[42] M.P. Nandakumar, M.S. Thakur, K.S.M.S. Raghavarao, N.P. Ghildyal, Mechanism of solid particle degradation by *Aspergillus niger* in solid-state fermentation, Proc. Biochem. 29 (1994) 545–551.
[43] S. Rajagopalan, D.A. Rockstraw, S.H. Munson-McGee, Modeling substrate particle degradation by *Bacillus coagulans* biofilm, Bioresour. Technol. 61 (1997) 175–183.
[44] M. Nopharatana, T. Howes, D.A. Mitchell, Modelling fungal growth on surfaces, Biotechnol. Tech. 12 (1998) 313–318.