A Computer Model Based on Reaction–Diffusion Equations for the Growth of Filamentous Fungi on Solid Substrate

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A model of two reaction–diffusion equations for biomass production and glucose consumption was developed. Experiments were carried out in order to estimate the different constants of the model and to compare the behavior of the system using two species of fungi, *Rhizopus oligosporus* and *Trichoderma viride T.S.* A mathematical study of the equations was performed. The model provides good predictive values, and the parameters have a precise biological significance. From the study of the phase plane biomass–glucose, in the case of *Rhizopus oligosporus* the existence of a secondary metabolism for the growth when glucose consumption is almost complete is proposed.

Introduction

Reaction–diffusion equations have a wide range of applications in biology. Examples are widely reported in epidemiology (Kermack and McKendrick, 1927; Macdonald and Bacon, 1982) and enzymology (Thomas, 1976; Kernevez et al., 1979). The typical reaction–diffusion equation is

$$\frac{\partial U}{\partial t} = f(U) + D\Delta U \qquad (1)$$

where U is a scalar or vector function of space time, f is a function describing the reaction term, Δ is the Laplace operator describing the space diffusion term of the equation, and D is the coefficient of diffusion. The reaction–diffusion equation is useful whenever the spatial distribution aspect of the phenomenon is relevant.

Growth and glucose consumption of filamentous fungi have been studied by numerous workers (e.g., Trinci, 1969). On solid medium, fungal growth is limited by the geometrical constraints of the substrate. As a consequence, it is natural to try to use reaction–diffusion equations to describe growth. Cultures are usually made on Petri dishes, which can be described by cylindrical coordinates. Georgiou and Shuler (1986) developed a model of growth and sporogenesis for *Aspergillus nidulans* that was based on the separation of the Petri dish into radial rings, where the phenomena were considered as constant in space and the extension of the colony from an inner ring to a more exterior ring was uniform with time. The glucose diffusion was calculated using the Fick equation. In the present report, it is shown that colony expansion can be incorporated into the basic equations of the model as a diffusional term. It is also possible to specify growth limitations such as glucose uptake relative to the colony surface on a solid substrate. The diffusional term for biomass has an interesting characteristic: the curve of radial expansion versus time has a linear relationship, as observed experimentally for a ponctually inoculated Petri dish (Gervais et al., 1988b).

Model Formulation

Hypotheses. The variables of the model are glucose concentration and biomass density (rather than dry weight of biomass). This allows the introduction of the concept notion of maximal surface for glucose uptake, which is relevant for solid substrate cultures. The model consists of a system of two equations involving glucose uptake, maintenance energy, and lysis of the biomass.

The basic hypotheses of the model are as follows: (1) The glucose uptake capacity of the fungus is constant in time and independent of mycelial age. (2) The colonization of a volume by the mycelium is directly related to the quantity of mycelium in the neighborhood of this volume. This implies that spatial growth is isotropic. (3) The mycelium can expand slightly into the substrate, suggesting that the area of contact between the fungus and the substrate could be greater than the upper surface area of the substrate. (4) The glucose taken up by the mycelium is used first for maintenance and afterward for growth. (5) The other components used by the fungus for development (O_2, N_2, etc.) are not limiting factors.

Equations. In order to work with biomass density and glucose concentration, it is convenient to examine these phenomena on an elementary surface, S.

(a) Let B be the biomass density on the surface S. The surface S_p of the glucose uptake is not S, but a fraction of S which is directly related (k_1) to the biomass up to a maximal proportion, k_0. Indeed, when a further increase in B occurs, only the part of B in contact with the substrate can take up glucose:

$$S_p = S \min(k_0, k_1 B) = SR_s \qquad (2)$$

(b) The mass of glucose, P_s, taken up by the biomass on the surface S is proportional to S_p, up to a maximal admissible flux of glucose:

$$\begin{aligned} P &= \min(k_2 G S_p, k_3 S_p) \\ &= S_p \min(k_2 G, k_3) \\ &= SR_s \min(k_2 G, k_3) = SP_s \end{aligned} \qquad (3)$$

where G is the glucose concentration.

(c) The quantity of glucose used by the biomass for its maintenance is proportional to B, up to the quantity P:

$$M = S \min(P_s, k_4 B) = SM_s \qquad (4)$$

(d) The quantity of glucose used for the growth is the quantity remaining after maintenance utilization:

$$C = S(P_s - M_s) = SC_s \qquad (5)$$

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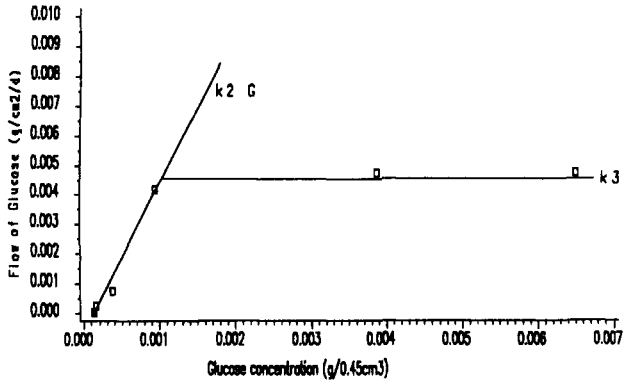

Figure 1. Experimental flow of glucose on tubes for *Rhizopus oligosporus*.

(e) The nonmaintained biomass is

$$B_{NM} = B - \frac{M_s}{k_4} \tag{6}$$

These mass balances lead to kinetic equations for glucose consumption and biomass evolution: Over a small time interval, dt, an amount of glucose C_s is converted into an amount of biomass, αC_s. A fraction β of the nonmaintained biomass B_{NM} disappears by lysis. The space expansion of the biomass is described by the diffusional term, $D_B \Delta B$. Glucose consumption in the same time interval is P_s, and the diffusional term for glucose is $D_G \Delta G$. This leads to the following equations:

$$\begin{cases} \dfrac{\partial B}{\partial t} = \alpha C_s - \beta B_{NM} + D_B \Delta B \\ \dfrac{\partial G}{\partial t} = -P_s + D_G \Delta G \end{cases} \tag{7}$$

where Δ is the Laplace operator.

This model can easily be expanded to cover more complicated mass-transfer systems, which include, for example, the use of more than one carbon source or a nitrogen source. However, initially it was used only for glucose consumption and then adapted in order to use more than one carbon source.

Constants. The following nine constants are sufficient for the complete definition of the model: k_0 (cm^2/cm^2), maximal ratio of the surface occupation by the mycelium; k_1 ($cm^2/g_B/cm^2$), ratio of the surface occupation by the biomass (g_B is grams of biomass); k_2 ($g_G/day/g_B = day^{-1}$), specific glucose uptake (g_G is grams of glucose); k_3 ($g_G/cm^2/day$), maximum flux of glucose; k_4 ($g_G/g_B/day = day^{-1}$), specific maintenance rate; α (g_B/g_G), conversion coefficient for glucose into biomass; β ($g_B/day/g_B = day^{-1}$), death rate for the non maintained biomass; D_B and D_G (cm^2/day), diffusion coefficients for biomass and glucose.

Materials and Methods

Experiments. ***Fungi.*** Two fungi were employed in this study, *Rhizopus oligosporus* C.B.S. 338.62, which is used to produce tempeh, and *Trichoderma viride T.S.*, recommended for protein production from vegetable byproducts.

Cultivation. Petri dishes (90 mm) and tubes (18 mm) were filled with 25 mL of PDA (potato dextrose agar). This medium was composed of glucose (20 g/L), a potato extract (4 g/L) as an organic nitrogen source, and agar (15 g/L). The dishes were inoculated at the center with 0.01 mL of a spore suspension (containing 10^5 spores). Tubes were inoculated by spreading the same amount of spores over the upper surface. (These spores were obtained from a 6-day cultivation on PDA medium, and 5 mL of physiological water was added to the Petri dish in order to collect the spores.) Both tubes and plates were stored in boxes containing 200 mL of water in order to maintain a constant humidity. Tube cultivation was carried out in order to obtain a rapid and complete occupation of the substrate upper surface and to estimate the amount of glucose needed for maintenance, when the biomass reached a constant value. This was used for the estimation of the model parameters.

Macroscopical Measurements. The radial extension rate and biomass were measured, whereby the diameters of the colonies were measured along two arbitrary perpendicular axes twice each day, and analyzed by linear regression to give the mean radial extension rate. The amount of biomass was obtained according to Gervais et al. (1988a): The agar medium was solubilized by adding 25 mL of distilled water and heating in a microwave oven until liquefaction of the medium occurred (approximately 70 °C) and then filtered, and the filter was rinsed twice with 25 mL of distilled water at 70 °C. The filter containing the biomass was dried for 12 h and finally weighed.

Growth Simulation Using the Model. Glucose was considered to be essentially distributed on the upper surface of the medium, since the Petri dish had a surface area of $\pi \times 4.5 \times 4.5 = 63.61$ cm^2 and was filled by 25 mL of medium, thus giving a thickness of 3.9 mm which is low in relation to glucose diffusion and uptake by the biomass. The complete system with boundary conditions corresponding to a Petri dish of radius l can be expressed in terms of polar coordinates by the following equations:

$$\begin{cases} \dfrac{\partial B}{\partial t} = \alpha C_s - \beta B_{NM} + D_B \dfrac{1}{r}\dfrac{\partial}{\partial r}\left(r \dfrac{\partial B}{\partial r}\right) \\ \dfrac{\partial G}{\partial t} = -P_s + D_G \dfrac{1}{r}\dfrac{\partial}{\partial r}\left(r \dfrac{\partial G}{\partial r}\right) \\ \left.\dfrac{\partial B}{\partial r}\right|_{r=0} = \left.\dfrac{\partial B}{\partial r}\right|_{r=l} = 0 \\ \left.\dfrac{\partial G}{\partial r}\right|_{r=0} = \left.\dfrac{\partial G}{\partial r}\right|_{r=l} = 0 \\ B(t=0,r) = B_0(r) \\ G(t=0,r) = G_0(r) \end{cases} \tag{8}$$

This system can be solved numerically for various B_0 values and can then be used for several types of culture seed, such as central seed or uniformly distributed seed. The isotropy in the angular variable allows this variable to be suppressed.

Approximation of the min Functions. The functions used in the model are not smooth. Georgiou and Shuler (1986) approximated these functions using Monod-type rate expressions, because Monod laws work more effectively with a biological approach. However, other types of approximation can be used to smooth the min functions, when fixing the slope at the origin (k) and the asymptote value (k_{max}).

In fact, for the surface colonization and glucose uptake, there are several reasons for choosing a specific way of smoothing the functions used in the model. (1) For surface colonization: If in a surface, S, the colonization is randomly distributed, this surface can be divided into n compartments. Suppose that the appearance of a new biomass element occurs randomly in each space. Then, if p biomass elements fall on n spaces, the probability for a space to

contain k elements follows the binomial law,

$$P(X=k) = C_p^k\left(\frac{1}{n}\right)^k\left(1-\frac{1}{n}\right)^{p-k}$$

and the expected number of spaces containing k elements:

$$N(X=k) = nP(X=k)$$

The surface covered is directly related to the number of spaces occupied by at least one element, namely,

$$N(X\geq 1) = n\left(1-\left(1-\frac{1}{n}\right)^p\right) \simeq n(1-e^{-p/n})$$

which takes the form

$$k_0(1-e^{k_1B/k_0}) \tag{9}$$

This gives an exponential approximation for the surface occupation.

(2) For glucose absorption: It is well-known (Lehninger, 1970) that glucose absorption occurs by facilitated diffusion. This phenomenon follows a Monod law; thus, the following approximation can be used:

$$\min(k_2G,k_3) \simeq \frac{k_2k_3G}{k_3+k_2G} \tag{10}$$

(3) A smooth approximation of the function $\min(P_s,k_4B)$ is left undefined. The precise role of this function will be studied in the following section.

According to these approximations, the present model can be related to the Herbert growth model in the following way. The growth term αC_s can be detailed as

$$\alpha C_s = \alpha\left(k_0(1-e^{-k_1B/k_0})\frac{k_2k_3G}{k_3+k_2G} - k_4B\right)$$

When only this term is relevant, the differential equation can be expressed as

$$\mu_{obsd} = \frac{1}{B}\frac{\partial B}{\partial t} = \alpha\frac{C_s}{B}$$

where μ_{obsd} is the observed growth coefficient as defined by Herbert (1958). When the problem of biomass contact with the substrate is negligible (liquid medium), the exponential term becomes k_1B and the equation becomes

$$\mu_{obsd} = \alpha\left(k_1\frac{k_3G}{k_3/k_2+G} - k_4\right)$$

This equation can be expressed in the following terms:

$$\mu_{obsd} = \mu_{true}^{max}\frac{G}{K_G+G} - \mu_e$$

where $\mu_{true}^{max} = \alpha k_1k_3$, $\mu_e = \alpha k_4$, and $K_G = k_3/k_2$. This is the Herbert equation for growth (see also Beeftink et al., 1990).

The system of equations was solved numerically using a FORTRAN 77 program with the NAg D03PGF subroutine. Graphics were obtained from the SAS/GRAPH system. The fit of the parameters was obtained by minimization of the sum of squared, renormalized differences between simulated and experimental data, using an algorithm developed by Nelder and Mead (1965). This method is a refinement of the simplex method.

Solution for Two-Dimensional Diffusion. In the paragraph discussing growth simulation using the model, the equations were solved by assuming that glucose was essentially distributed in the upper surface of the medium. This is equivalent to the assumption that the diffusion coefficient of glucose in the direction of depth (z) is infinite. In order to give this coefficient its real value, a finite difference method was used. The equation for glucose became

$$\frac{\partial G}{\partial t} = -P_s + D_G\left[\frac{1}{r}\frac{\partial G}{\partial r} + \frac{\partial^2 G}{\partial r^2} + \frac{\partial^2 G}{\partial z^2}\right]$$

with the following boundary conditions:

$$\begin{cases} G(t=0,r,z) = G_0 \quad 0\leq r\leq l \quad 0\leq z\leq Z \\ \left.\frac{\partial G}{\partial r}\right|_{r=0} = \left.\frac{\partial G}{\partial r}\right|_{r=l} = 0 \\ \left.\frac{\partial G}{\partial z}\right|_{z=0} = 0 \\ -D_G\left.\frac{\partial G}{\partial z}\right|_{z=0} = P_s \end{cases} \tag{11}$$

The numerical solution was obtained by an explicit method. The equation of biomass had to be solved in the same manner, so that the set of simultaneous equations described previously becomes two equations for which biomass must first be solved. The values at each point of the discretization grid were integrated with respect to the elementary volume or surface in order to obtain the total glucose or biomass values. The observed curves were very close to one-dimensional curves, confirming that the vertical glucose diffusion in the medium is not limiting for the system.

Mathematical Study of the System without Diffusion

Experimentally, seeding of the entire surface of the Petri dish corresponds to the system of equations without diffusion. The system was first solved using the original equations in order to give a clear separation of the different phases of fungal development. The effects of smoothing surface occupation and glucose uptake in the equations were then examined.

The following changes of variables,

$$H = Gk_2/k_3$$
$$C = Bk_1/k_0$$
$$t_1 = k_0/k_2t$$

give the following dimensionless equations

$$\begin{cases} \frac{\partial H}{\partial t_1} = -f(C,H) \\ \frac{\partial C}{\partial t_1} = \zeta\left[f(C,H) - \min\left(f(C,H),\frac{C}{\epsilon}\right)\right] - \eta\left[C - \epsilon\min\left(f(C,H),\frac{C}{\epsilon}\right)\right] \end{cases} \tag{12}$$

where

$$\epsilon = \frac{k_1k_3}{k_4} \qquad \zeta = \alpha\frac{k_1k_3}{k_0k_2} \qquad \eta = \frac{\beta}{k_0k_2} \tag{13}$$

The function $f(C,H)$ is equal to $f(C,H) = \min(C,1) \times \min(H,1)$, when the functions describing surface occupation and glucose uptake are not smoothed. This function is equal to $f(C,H) = (1-e^{-c})H/(1+H)$ when these functions are smoothed as described above.

There are only three free parameters, and among them, ϵ plays an essential role in the behavior of the system. The extent of growth is controlled by the inequality $P_s > k_4B$, namely, $f(C,H) > C/\epsilon$. Since $f(C,H) \leq C$, when $\epsilon < 1$, $f(C,H) < C/\epsilon$ for all values of C and H. Consequently, there is no growth when $\epsilon < 1$.

Table I. Numbering the Domains of the Phase Plane According to the Comparison between C and 1, H and 1, and $f(C,H)$ and C/ϵ

	$f(C,H) > C/\epsilon$		$f(C,H) < C/\epsilon$	
	$C > 1$	$C < 1$	$C > 1$	$C < 1$
$H < 1$	1	2	5	6
$H > 1$	3	4	7	8

Equations and Solutions for the Nonsmoothed Model. The equations were solved for the nonsmoothed model, namely, when $f(C,H) = \min(C,1)\min(H,1)$. Table I separates the quadrant $H > 0$, $C > 0$ into eight various subdomains, using three comparisons between C and 1, H and 1, and $f(C,H)$ and C/ϵ, respectively. The position of ϵ with respect to 1 separates the subdomains into two exclusive classes as shown in Figure 2. When $\epsilon > 1$, subdomains 1–7 are reachable by the curve (H,C), and when $\epsilon < 1$, only subdomains 5–8 are represented by (H,C).

Case $\epsilon > 1$. Growth is only possible in this case, and it occurs in domains 1–4. Solution of the equation domain by domain is simple. For example, in domain 4 the exponential growth phase can be recognized. In this domain, the equations are

$$\begin{cases} \dot{C} = \zeta C(1 - 1/\epsilon) \\ \dot{H} = -C \end{cases} \tag{14}$$

for $H > 1$ and $C < 1$, which corresponds to a small amount of biomass and a large amount of nutrient, namely, the first phase after seeding.

In domains 2 and 6, the equations can be related to the epidemiological system described by Kermack and McKendrick (1927) (for a review, see Britton (1986)). For example, in domain 2, the equations are

$$\begin{cases} \dot{H} = -CH \\ \dot{C} = \zeta(CH - C/\epsilon) \end{cases}$$

(Here, glucose plays the role of the population susceptible to infection, and biomass represents the infective population.) An interesting fact is that glucose is never entirely consumed (see Figure 2a).

When $\epsilon > 1$, a curve exists describing the maxima of C. This curve is given by $\dot{C} = 0$ since $\dot{H} \neq 0$ for C and $H \neq 0$. $\dot{C} = 0$ is equivalent to $f(C,H) = C/\epsilon$, i.e. $\min(C,1)\min(H,1) = C/\epsilon$. This curve is the union of boundaries between domains 2 and 6, 1 and 5, and 3 and 7 (see Figure 2a).

Case $\epsilon < 1$. Growth is never possible, and only four domains (5–8) exist in this case (see Figure 2b).

Effect of Smoothing. Equation 12 is now used with

$$f(C,H) = \frac{H(1 - e^{-C})}{1 + H} \tag{15}$$

Parameter ϵ is also essential for the behavior of the system. If $\epsilon < 1$ and $f(C,H) < C/\epsilon$, then the equations are

$$\begin{cases} \dot{C} = -\eta\epsilon(C/\epsilon - f(C,H)) \\ \dot{H} = -f(C,H) \end{cases} \tag{16}$$

and there is no growth, since $\dot{C}$ is always negative. If $\epsilon > 1$, the curve $\dot{C} = 0$ is described by the equation

$$\frac{H(1 - e^{-C})}{1 + H} = \frac{C}{\epsilon} \tag{17}$$

This curve comes close to that of the nonsmoothed model when ϵ increases (see the details of calculation in the Appendix).

Traveling Wave Fronts. An interesting aspect of reaction–diffusion equations is that some of them admit traveling wave fronts. A traveling wave front (TWF) is a solution taking the form $U(x + vt)$, where x is the space variable, t is the time variable, and v is the speed of the wave. These solutions are generally considered as asymptotic forms of solutions of the initial boundary value problem. TWF describe, for example, the propagation phenomena of epidemics (see Britton (1986) for more details). The existence of TWF for the present equations may be discussed in two cases: unlimited and limited nutrient.

Unlimited Nutrient. In this case, the function $f(C,H)$ is equal to $1 - e^{-C}$. Since it is not known how to measure β precisely, it will be assumed that $\beta = \alpha k_4$. This assumption does not change the behavior of the solutions and simplifies the equations. The following changes of variables,

$$C = k_0/k_1 B \qquad t_1 = \beta t \qquad x = \sqrt{\beta/D_B}$$

give the dimensionless equation

$$\dot{C} = \epsilon(1 - e^{-C}) - C + C_{xx}$$

where

$$C_{xx} = \frac{\partial^2 C}{\partial x^2}$$

is the one-dimensional form of the Laplace operator.

If we set $C = C(x + vt)$, the TWF equation becomes

$$\ddot{C} - v\dot{C} + \epsilon(1 - e^{-C}) - C = 0$$

Let C_ϵ be the solution of $\epsilon(1 - e^{-C}) - C = 0$; set $D = C/C_\epsilon$ and $\omega = 1 - C_\epsilon/\epsilon$ to finally obtain the equation

$$\ddot{D} - v\dot{D} + \frac{1 - \omega^D}{1 - \omega} - D = \ddot{D} - v\dot{D} + f(D) = 0 \tag{18}$$

with $0 < \omega < 1$. This equation is a generalized Fisher equation (see the Appendix for the definition of this equation). The existence of TWF for this equation was proved by Britton (1986) for $v \geq 2(k)^{1/2}$ where $k = \sup_{u \in (0,1)} f(u)/u$.

In the present case, $f(D) = (1 - \omega^D)/(1 - \omega) - D$, and it is easy to see that f satisfies the conditions required for a generalized Fisher equation with $k = \epsilon - 1$. The TWF corresponds to uniform filling of the surface with a maximal density of biomass being equal to C_ϵ. This solution distinguishes the difference between the solid and liquid substrates. For the latter, the model would give infinite biomass when the quantity of nutrient is unlimited.

Limited Nutrient. The previous changes of variables give the system

$$\begin{cases} \dot{C} = \epsilon f(C,H) - C + \Delta C \\ \dot{H} = -\mu f(C,H) \end{cases} \tag{19}$$

with $\mu = k_0/\alpha k_2 k_4$ and where the diffusion of H has been neglected for simplicity. The system is too complicated to find a TWF analytically. Nevertheless, using the constants estimated in the section on approximation of the min functions, it can be solved numerically on a very large domain. The curves obtained show that in the case of central seeding, after a short growth phase, the biomass evolution has approximately the behavior of a TWF (see Figures 3 and 4). Thus, fungal development takes the form of an expanding ring of biomass. This phenomenon can be related to the work of Gervais and Sarette (1990), who also observed an expanding ring for aroma production.

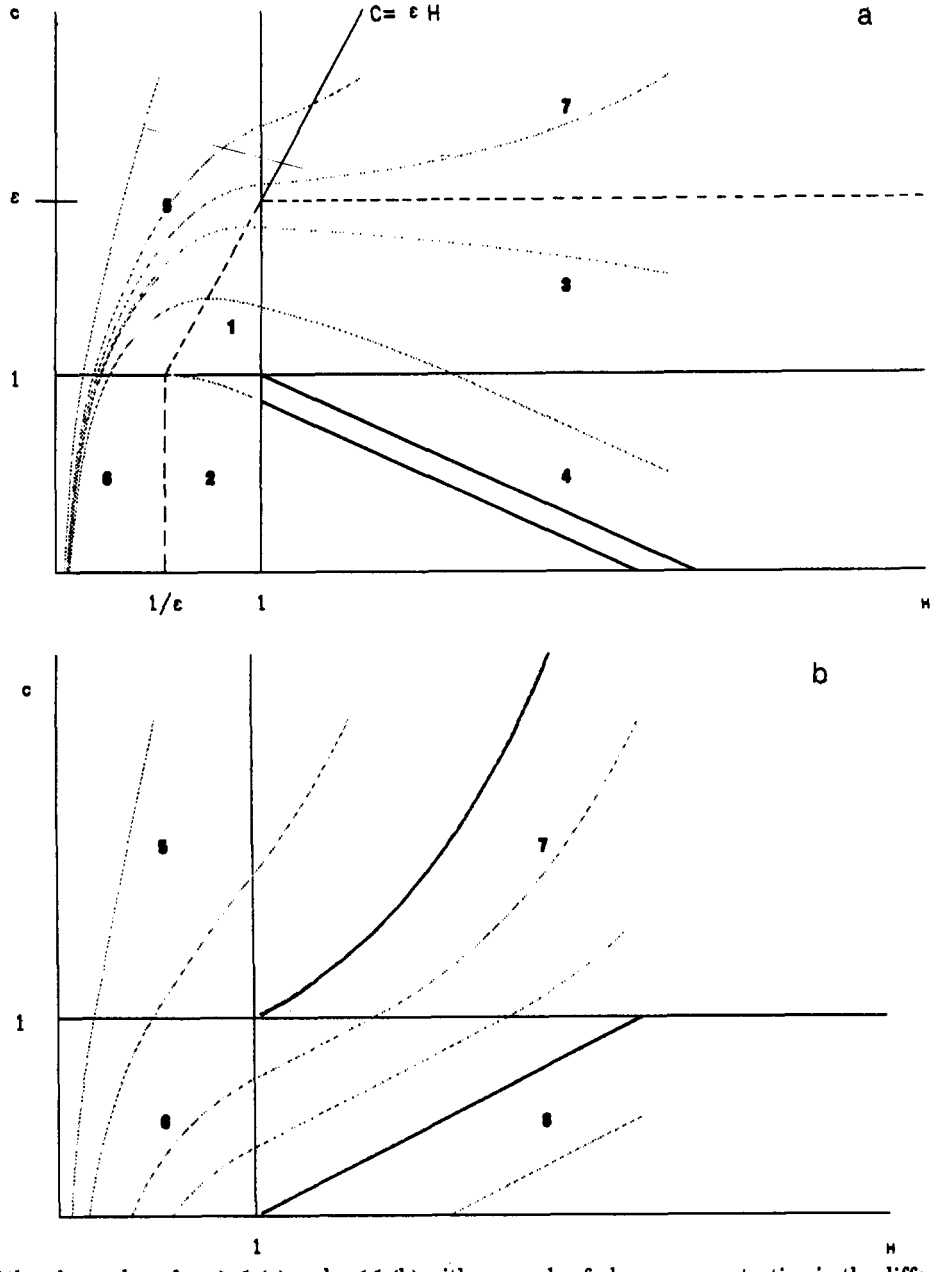

Figure 2. Domains of the phase plane for $\epsilon > 1$ (a) and $\epsilon < 1$ (b) with a sample of phase curves starting in the different domains (the dotted lines). The dashed line in a is the curve of maxima for C.

Results

Experimental Estimation of the Coefficients. The yield coefficient α is usually calculated as the ratio of the total biomass at the end of the cultivation divided by the total amount of glucose in the media. It should then be equal to

$$87.2 \times 10^{-3}\ g_B/0.5\ g_G = 0.183$$

(see Table III). This value will be corrected in the final discussion of the model by examining the experimental phase plane.

The maintenance coefficient k_4 is obtained when glucose uptake and the biomass in tube cultures reach a constant value: $k_4 = 0.334\ g_G/g_B/\text{day}$.

The coefficient k_0 is the result of the assumption that the flux of glucose, F_T, in the tubes is the maximum uptake capability of the fungus. It was observed that the fungal penetration into the medium, F_T/π, represents the real flux of glucose when only the active surface of mycelium is considered. Then, k_0 is the product of the real flux of glucose multiplied by the flux of glucose in Petri dishes (F_{PD}), divided by this flux in the tubes:

$$k_0 = \frac{F_T}{\pi}\frac{F_{PD}}{F_T} = \frac{F_{PD}}{\pi} = \frac{4.57}{\pi} = 1.46\ \text{cm}^2/\text{cm}^2$$

The glucose uptake parameters k_2 and k_3 are estimated from the experimental flux of glucose F, which is a function of the glucose concentration, G. This flux F was measured in tubes. The value obtained was considered to be maximal, as for the estimation of k_0. Parameter k_3 was then estimated by

$$k_3 = F_T = 17.86\ \text{g/cm}^2/\text{day}$$

By taking into account the relation

$$FG = \frac{4.97G}{1 + 1.09G}$$

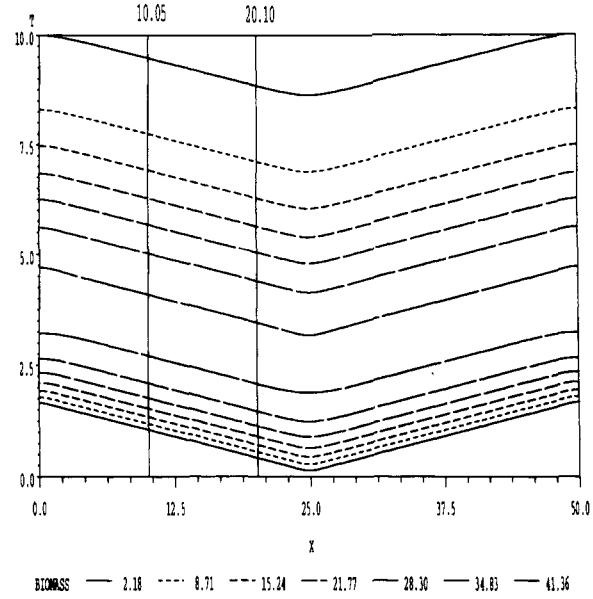

Figure 3. Contour plot of the traveling wave front of biomass.

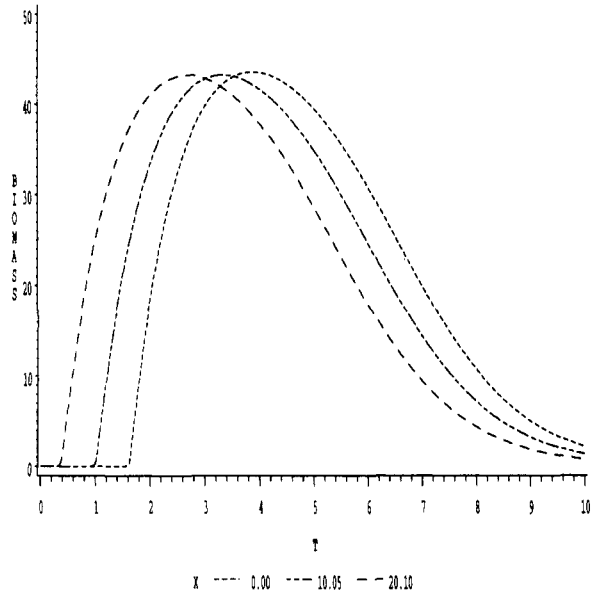

Figure 4. Time plots of the traveling wave front of biomass for some x repaired in Figure 3.

(cf. Figure 1), parameter k_2 was estimated as

$$k_2 = \frac{4.97}{F_{PD}/F_T} = \frac{4.97}{0.26} = 19.11 \text{ day}^{-1}$$

The amount $\nu\lambda$ (g/day) passes through a unit area equal to the hyphal growth section, where ν is the hyphal growth and λ is the conversion coefficient of biomass into mycelial length. The flux of biomass through 1 cm^3 is then $\nu\lambda/S$. Biomass density decreases from ρ to 0 at the peripheral growth zone (x cm in width). Thus,

$$D_B = \frac{\nu\lambda}{S}\frac{x}{\rho}$$

Mycelia were assumed to be largely composed of water (ρ = 1). The hyphal diameter was estimated to be close to 10 μm ($\lambda = 0.78 \times 10^{-6}$ g/cm and $S = 0.78 \times 10^{-6}$ cm^2). The value of x was set equal to 0.1 cm. This leads to D_B = 0.1682 cm^2/day.

k_1 could not be estimated, so its approximate value was obtained by the fitting program described in the section on approximation of the min functions.

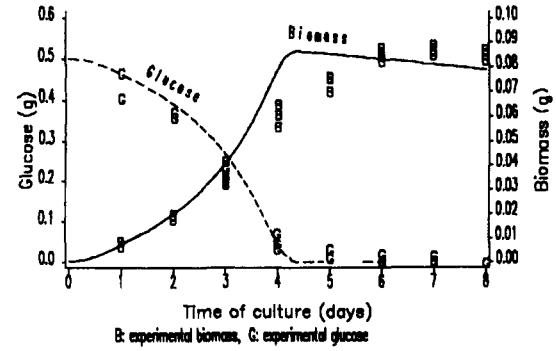

Figure 5. Kinetics of biomass and glucose for *Rhizopus oligosporus*.

Expansion of the Colony. Gervais et al. (1988b) reported that with central seeding radial growth is constant within the confines of the Petri dish. The radius of the colony was defined by the value r, where the biomass density reaches an arbitrary low value (10^{-6} in the simulation), and the calculated radial growth was verified in this way.

Biomass and Glucose Observations. The results for *Rhizopus oligosporus* and *Trichoderma viride T.S.* are shown in Figures 5 and 6, respectively, in relation to time with simulated biomass, total simulated quantity of glucose, observed biomass with three repetitions, and observed total quantity of glucose for a central seed with three repetitions. The different values for the estimated parameters for *Rhizopus oligosporus* and *Trichoderma viride T.S.* are presented in Table II. The experimental estimations for *Rhizopus oligosporus* using tube cultivations, when introduced in the computer program, were not substantially modified by the fitting algorithm, and the high value of k_0 confirms that this fungus expands in the substrate, in agreement with experimental observations.

By integration over the Petri dish surface, the global fungus biomass and glucose consumption can be estimated by the present model. Radial growth obtained by simulation (Figure 7) was close to the observed rate of 700 μm/h. This model can also be used to describe the biomass density and the glucose consumption in any part of the Petri dish. However, various parameters were estimated to fit the experimental data, but new experiments are currently underway to obtain real values for the parameters, in order to confirm the present estimations. Specifically, new techniques of bioluminescence would provide estimations of lysed and nonlysed biomass in order to refine the fit of the model for the period of decreasing biomass.

In the case of *Trichoderma viride T.S.*, the fit of the model to the experimental data is good. However, for the period of decreasing biomass, the fit is not perfect and it means that the mechanisms of lysis are not completely understood. The most plausible explanation is that the lysis of the biomass is never completely achieved and that lysis occurs very slowly for a part of the biomass. Considering *Rhizopus oligosporus*, it was observed from the experimental curves that the biomass growth is still high, although the glucose disappeared almost entirely. This problem will be addressed in the Discussion.

Discussion

Problem of Biomass Fitting for *Rhizopus oligosporus*. In order to study the bias observed on the biomass in Figure 5, the phase plane, namely, the diagram of biomass versus glucose, was examined. Figure 8 shows

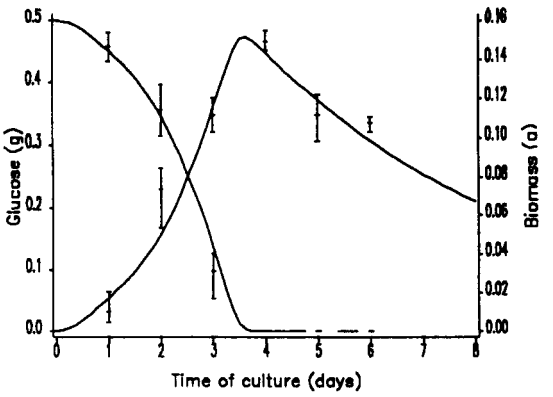

Figure 6. Kinetics of biomass and glucose for *Trichoderma viride T.S.*

Table II. Parameters Experimentally Estimated and Optimized: First Model

parameter	exptl estmn for *R. oligosporus*	adjusted for *R. oligosporus*	adjusted for *T. viride*
k_0 (cm^2/cm^2)	1.46	5.1	1.8
k_1 ($cm^2/g_B/cm^2$)		500	170
k_2 ($g_G/day/g_B$)	19.1	22.0	40.0
k_3 ($g_G/cm^2/day$)	0.018	0.047	0.128
k_4 ($g_G/g_B/day$)	0.334	0.334	0.300
α (g_B/g_G)	0.183	0.183	0.360
β ($g_B/day/g_B$)		0.01	0.2
D_{fungus} (cm^2/day)	0.168	0.160	0.11

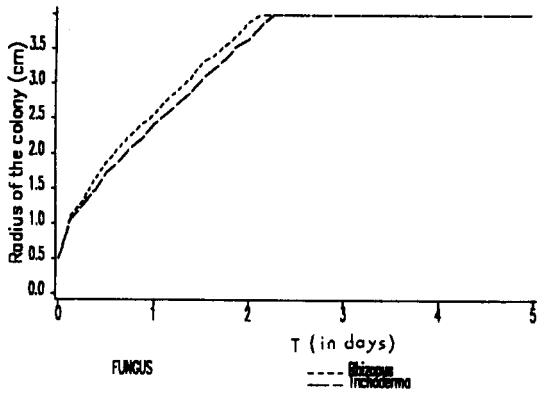

Figure 7. Evolution of the radius of the colony for *Rhizopus oligosporus* and *Trichoderma viride T.S.*

that before the fourth day the slope of the biomass versus glucose curve is constant and equal to the experimental conversion coefficient α. From the fourth day onward, α increases significantly, which explains why the previous fitting of biomass is not as good as that of glucose. This suggests that a new type of metabolism occurs when the amount of glucose becomes lower than 0.05 g. This may be explained by a change in oxygenation initiated by the colony at the edge of the Petri dish and could modify the conversion coefficient, although this seems doubtful since the colony reaches the edge of the Petri dish during the second day whereas α changes begin on the fourth day. Alternatively, changes in metabolism could be due to the accumulation of lipid reserves during early growth with the later consumption of such reserves. Since in the biomass determination lipids are measured as part of the biomass, such an explanation may also be discounted.

In fact, the most plausible explanation could involve the following: PDA culture medium is that which contains potato extract as a nitrogen source. It is known that the fungus can use the carbon of the nitrogen source as a carbon source (Sorenson and Hesseltine, 1966), and therefore the observed variation in yield may be explained by this

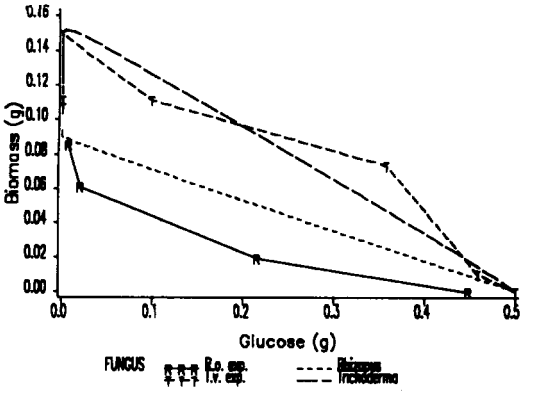

Figure 8. Phase plane of biomass versus glucose for *Rhizopus oligosporus* and *Trichoderma viride T.S.*

phenomenon. Experiments were also performed on Morton medium (Morton, 1960), which contained a synthetic source of nitrogen instead of the potato extract, in order to obtain pure glucose consumption and to verify the validity of the first model for *Rhizopus oligosporus*. These cultures were unsuccessful due to a lack of oligo elements in the Morton medium, which is an entirely synthetic medium. Currently, attempts are being made to obtain a suitable medium that would allow the growth of *Rhizopus oligosporus* on a single carbon source, and other sugars are also being studied in order to determine the order of carbon consumption.

The plane phase for *Trichoderma viride T.S.* (Figure 8), with greater experimental variations, appears to be unbiased. The model can be used without modification for *Trichoderma viride T.S.* However, in the case of *Rhizopus oligosporus*, this model is no longer valid in its present form, and a second source of carbon was introduced in the equations when glucose concentration is low.

Expansion for Two Nutrient Sources with a Hierarchy of Consumption for *Rhizopus oligosporus*. A second source, C_N, following the same kinetic equations as those defined for glucose, was introduced into the equations as follows. The C_N uptake is

$$P_s' = R_s \mathbf{H} \min(k_2' C_N, k_3')$$

where **H** is the hierarchization function. This function was chosen to be very simple since there is no available information about the change of metabolism:

$$\mathbf{H} = \sup(-G/\delta + 1, 0) \qquad (20)$$

The total quantity of nutrient used for maintenance becomes

$$M_s = \min(k_4 B, P_s + P_s')$$

and the quantity used for growth becomes

$$C_s = P_s + P_s' - M_s$$

The equations then become

$$\begin{cases} \dfrac{\partial B}{\partial t} = \dfrac{\alpha P_s + \alpha' P_s'}{P_s + P_s'} C_s - \beta B_{NM} + D_B \Delta B \\ \dfrac{\partial G}{\partial t} = -P_s + D_G \Delta G \\ \dfrac{\partial C_N}{\partial t} = -P_s' + D_N \Delta C_N \end{cases} \qquad (21)$$

These equations contain new coefficients: k_2' and k_3', which take on the roles of k_2 and k_3, respectively, for the second source of carbon; α' is the conversion coefficient for the second source of carbon and is equivalent to α; D_N

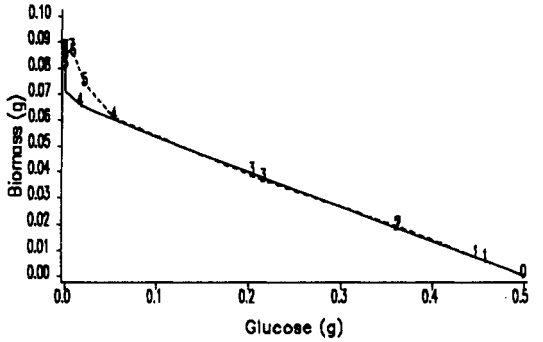

Figure 9. Phase plane of biomass versus glucose for *Rhizopus oligosporus* for the second model (the numbers on the curves indicate the time in days).

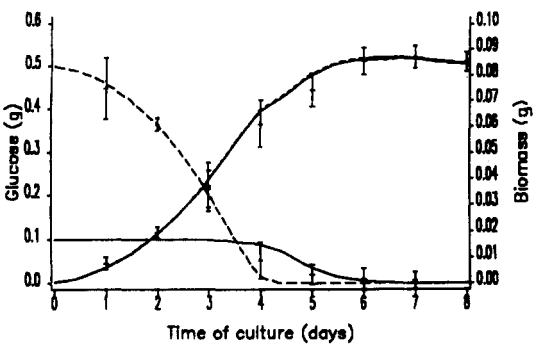

Figure 10. Kinetics of biomass and glucose for *Rhizopus oligosporus* for the second mode.

is the diffusion coefficient of the second carbon source in the medium; and δ is the limit of glucose concentration for the beginning of consumption of the second carbon source.

Figures 9 and 10 present growth kinetics, glucose and second source consumption, and the phase plane for the new model. The variations of the radius of the colony did not change since the surface growth occurs before the almost-complete glucose consumption. Model fitting is better for biomass, particularly for the phase plane which follows the change of the slope. Table III contains the estimated values for the parameters in *Rhizopus oligosporus*. Consideration of these parameters shows that the coefficient α is now 0.133 instead of 0.183 since this coefficient was calculated as $B_{max}/G_{initial}$ and the slope of the linear part of the phase plane is only 0.133.

Table III. Parameters for *Rhizopus oligosporus*: Second Model

parameter	*R. oligosporus*
k_0 (cm^2/cm^2)	5.0
k_1 ($cm^2/g_B/cm^2$)	850
k_2 ($g_G/day/g_B$)	16.1
k_3 ($g_G/cm^2/day$)	0.04
k_4 ($g_G/g_B/day$)	0.334
α (g_B/g_G)	0.138
β ($g_B/day/g_B$)	0.01
D_{fungus} (cm^2/day)	0.160
k_2' ($g_G/day/g_B$)	1.2
k_3' ($g_G/cm^2/day$)	0.025
α' (g_B/g_G)	0.25

Table IV. Experimental Values over 8 Days of Biomass and Glucose for *Trichoderma viride T.S.* and *Rhizopus oligosporus*

	R. oligosporus		*T. viride T.S.*	
day	biomass (g) (mean std)	glucose (g) (mean std)	biomass (g) (mean std)	glucose (g) (mean std)
0	0.0003 (0.000)	0.5000 (0.000)	0.0003 (0.000)	0.5000 (0.000)
1	0.0076 (0.001)	0.4479 (0.036)	0.0105 (0.009)	0.4582 (0.023)
2	0.0192 (0.001)	0.3635 (0.007)	0.0738 (0.018)	0.3577 (0.041)
3	0.0368 (0.005)	0.2153 (0.021)	0.1115 (0.009)	0.0980 (0.040)
4	0.0610 (0.004)	0.0529 (0.020)	0.1493 (0.005)	0.0000 (0.000)
5	0.0742 (0.003)	0.0208 (0.012)	0.1117 (0.012)	0.0000 (0.000)
6	0.0855 (0.003)	0.0090 (0.012)	0.1075 (0.004)	0.0000 (0.000)
7	0.0872 (0.002)	0.0072 (0.009)		
8	0.0852 (0.002)	0.0000 (0.000)		

Conclusions

This model is sufficiently flexible to describe growth in many species of filamentous fungi. It can be expanded to cover well-stirred reactors by the suppression of the diffusional terms in the equations. Another quality is that few parameters are used. The parameter $\epsilon = k_1k_3/k_4$ and its position with respect to 1 appear to be essential for growth, and ϵ is equal to μ_{true}^{max}/μ_e of the Herbert model. The values of ϵ for the two fungi are very close (72.53 for *Trichoderma viride* and 70.36 for *Rhizopus oligosporus*).

The next step will be the study of these parameters in relation to different physicochemical parameters, particularly the water activity of the medium. However, the present model can be improved by refining the various functions in eqs 9, 10, and 21. Another improvement for this model concerns the hypothesis of growth and glucose consumption homogeneity in time (first hypothesis in the Model Formulation section). The age of the mycelium can influence these variables, and for solid substrates, age can be related to the spatial position of the mycelia.

The problem of age is also important in the continuation of this model. It is known that sporogenesis (Ellison et al., 1981) and aroma production (Gervais and Sarette, 1990) occur at a particular mycelial age. In the present model, biomass density is only described by its value, and there is no method available at present to distinguish the age of any particular part of this biomass density. We are now working on the function of a pyramid-shaped diagram of ages based on $\partial B/\partial t$, in order to use it for the differentiation phenomena.

Notation

B biomass density (g cm^{-2})
B_{NM} nonmaintained biomass (g cm^{-2}).
C reduced biomass (Bk_1/k_0) (g)
C_s glucose concentration used for growth (g cm^{-2})
C_N concentration of the second source of carbon (g cm^{-2})
D coefficient of diffusion (cm^2 day^{-1})
D_N diffusion coefficient for the second source of carbon (cm^2 day^{-1})
D_B diffusion coefficient for biomass (cm^2 day^{-1})
D_G diffusion coefficient for glucose (cm^2 day^{-1})
D_N diffusion coefficient for the second source of carbon (cm^2 day^{-1})
F flux of glucose from the medium to the mycelium (g cm^{-2} day^{-1})
F_{PD} flux of glucose from the medium to the mycelium (case of Petri dish) (g cm^{-2} day^{-1})
F_T flux of glucose from the medium to the mycelium (case of tubes) (g cm^{-2} day^{-1})
G glucose concentration (g cm^{-2})
H reduced glucose (Gk_2/k_3) (NDP)
k_0 maximal ratio of the surface occupation by the mycelium (NDP)
k_1 ratio of the surface occupation by the biomass (day^{-1})
k_2 specific capability of glucose uptake (day^{-1})
k_3 maximum flow of glucose (g cm^{-2} day^{-1})

k_4	specific quantity of glucose necessary to maintain the biomass (day^{-1})
k_2'	same as k_2 for the second source of carbon (day^{-1})
k_3'	same as k_3 for the second source of carbon (g cm^{-2} day^{-1})
l	radius of the Petri dish (cm)
M_s	quantity of glucose used for maintenance (g cm^{-2})
NDP	nondimensional parameter
P_s	quantity of glucose taken up by the biomass (g cm^{-2})
P_s'	quantity of second carbon source taken up by the biomass (g cm^{-2})
r	radius of the colony (cm)
S	elementary surface (cm^2)
S_p	surface of the glucose uptake (cm^2)
t	time (day)
t_1	reduced time (k_0/k_2t) (day)

Greek Letters

α	conversion coefficient glucose → biomass (NDP)
α'	same as α for the second source of carbon (NDP)
β	death rate for the nonmaintained biomass (day^{-1})
δ	lower limit of glucose concentration for second carbon source consumption (g cm^{-2})
η	reduced parameter of the model (β/k_0k_2) (NDP)
λ	conversion coefficient of biomass into mycelium length (g cm^{-1})
ν	hyphal growth rate (cm day^{-1})
ζ	reduced parameter of the model [$\alpha(k_1k_3/k_0k_2)$] (cm^{-1})
ϵ	reduced parameter of the model (k_1k_3/k_4) (cm^{-2})

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Appendix

Definition of the Generalized Fisher Equation. The Fisher equation is defined by $\dot{u} = u(1 - u) + u_{xx}$. This corresponds to the logistic model with diffusion, and the generalized Fisher equation is $\dot{u} = f(u) + u_{xx}$, where $f(0) = f(1) = 0$, $f > 0$ on [0,1], $f'(0) > 0$, and $f'(1) < 0$.

Proof of the Convergence of the Curve $\dot{C} = 0$ of the Smoothed Model to the One of the Nonsmoothed Model when ϵ Increases. Equation 17 can be solved in H by

$$H = \frac{1}{\epsilon\left(\frac{1 - e^{-C}}{C}\right) - 1}$$

The value at $C = 0$ of the curve is $H = 1/(\epsilon - 1) > 1/\epsilon$ and approximates $1/\epsilon$ when ϵ is high.

The asymptote of this curve, when H tends toward infinity, is given by the equation

$$1 - e^{-C} = C/\epsilon$$

In order to compare this value of C with ϵ, we set $C = \epsilon - u$ with low values of u. The equation becomes

$$e^{-\epsilon+u} = u/\epsilon$$

By approximating e^{-u} with $1 - u$, the following approximation for u is obtained:

$$u \simeq \frac{\epsilon e^{-\epsilon}}{1 - \epsilon e^{-\epsilon}}$$

This value goes to 0 when ϵ goes to infinity, confirming that the asymptote of this curve closes to the value ϵ when ϵ increases.

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