THEORETICAL MODEL FOR THE GROWTH BEFORE SPORULATION OF A FUNGAL POPULATION ON SOLID SUBSTRATE

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ABSTRACT
This paper deals with a non-linear system consisting of two ordinary differential equations describing differentiated fungal phases, a diffusion equation of the oxygen density. The equations are coupled due to the influence of the oxygen density in the biomass growth and the oxygen consumption by the biomass. Its solution is investigated using a perturbation technique. An asymptotic formula is given for phase-change times of biomass at any depth inside the reactor. Is determined the maximal thickness of substrate in order that tall present biomass be in the competent phase in the moment previous to the sporulation at the open surface.

Key words: population dynamics, linear diffusion, regular perturbations.

RESUMEN
Este trabajo se refiere a un sistema no lineal consistente en dos ecuaciones diferenciales ordinarias que describen fases diferenciadas de un hongo, y una ecuación de difusión para la densidad de oxigeno. Las ecuaciones están acopladas debido a la influencia de la densidad de oxigeno en el crecimiento de la biomasa y, al consumo del oxigeno por biomasa, la solución es investigada usando una técnica de perturbación. Se presenta una formula asintótica para los tiempos de cambios de fase en biomasa a cualquier profundidad dentro del reactor. Se determina el grosor máximo del sustrato para que toda la biomasa presente se encuentre en fase competente en el momento previo a la esporulación en la superficie superior abierta.

MSC: 92D25, 35K55

1. INTRODUCTION

Currently, appear in the literature a lot of mathematical simulations for the growth of a microbial colony. [Doelle **et al**. (1992)]. The majority of the existing mathematical models deal with the increase of biomass, but there is a modern tendency to consider also environmental effects on biomass differentiation [Axelrod (1972), Georgiou (1986)], and the influence of diffusion of metabolites in the media for homogeneous liquid cultures and solid state fermentation (SSF). Advantages of SSF are very well known. [Hesseltine (1987)]

Biomass is a fundamental parameter in the characterization of microbial growth. Direct determination of biomass in SSF is very difficult (see [Doelle **et al**. (1992)], Ch. 4]). There are not mathematical models in the literature representing the visual evidence of stratified values of retards in the (differentiated) growth and also in the change-of-phase time for a filamentous fungal (for instance *Trichoderma citrinoviride*) culture in SSF, on which we are interested as a way to obtain proteinized animal feed. Here we propose a model for the study of the influence on the (differentiated in the sense of Mitchell **et al.** (1990)) biomass growth of the oxygen diffusion through the solid substrate fixing evenly a truncated cylinder (without lid) with generatrix parallel to the vertical axis –called bio-reactor–.

A question follows , how can we determine the thickness of the reactor's substrate column for which all the biomass present inside the substrate stay in a ´competent´ phase when sporulation starts over the substrate's external surface- ? We shall call it the **critical thickness** (CT) of the reactor's substrate column.

Our model, presented in Section 3, is based on a system of differential equations derived from standard considerations as Monod law, diffusion and consumption of nutrients, a qualitative investigation and consumption of nutrients. A qualitative investigation of the solution of this system is given in Section 4 using a perturbation technique. There, we will estimate analytically the fungal mass at any depth and at any time inside the medium before sporulation. Further, we will show in Section 5 how we can do an estimate of the CT. In section 6 we give the conclusions. A description of the coefficients appearing in the model can be seen in the appendix.

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2. BASIC CONSIDERATIONS

Mathematically, the model is given by a system of three equation for three variables representing two differentiated phases of the biomass (DPB), called vegetative and competent respectively, and oxygen density.

The solid medium (or substrate) is composed homogeneously by sugar cane straw and zeolite, which bring the requirements of ammonium and glucose for the fungal growth. We recall that our concern is the evolution of the biomass before the appearance of the conidial phase at the external surface over the medium. Here we use a terminology like in Axelrod (1972) and Georgiou **et al**. (1986), but we consider that the differentiation in components of the biomass is a consequence of the changes in its physical characteristics during the growth, not connected necessarily with a secondary metabolite [Mitchell **et al.** (1990)].

We suppose that, initially, there is a homogeneous distribution of spores in the substrate. In the process of degradation of the cane straw by the biomass, a small glucose quantity appears in medium, which are consumed during the growth. Nitrogen appears due to the liberation of ammonium ions of the zeolite matrix, by an exchange with the hydronium ions in medium. For any depth inside the reactor, we follow a surface model differentiated growth, in which the ammonium, glucose and oxygen are considered as nutrients. We assume constant values of glucose ad ammonium concentrations, only the oxygen density and the biomass are considered depending on the time and on the relative depth inside the substrate column.

The liberation of glucose to the medium due to the hydrolysis of the cellulose (sugar cane straw) is enzyme regulated by a control feedback, permitting the presence of a sufficient level of glucose for the fungal growth. So, the glucose is here neither a limiting substance or an inhibitor for the growth. Furthermore, the ammonium in the interior of the zeolite is liberated slowly as the culture needs and for this reason, its density in the media is sufficiently low and there is no inhibition due to a high salt concentration.. Also it is not here considered as a limiting substance.

In the vegetative biomass X_1 there is no cell differentiation. Competent biomass X_2 is characterized by the evidence of aerial hyphae an foot cells initiation, and immature conidiophorous which have not developed into fully-grown reproductive structures after sporulation this process follows with other DPB characterized by the appearance of mature conidiophorous and conidia, which we will not considered here.

We assume the diffusion process to be governed by a linear law [Vvedensky (1993), pag. 98]. This assumption is practically fulfilled at least in the laboratory stage.

Affinity and inhibition constants for the nutrient were determined experimentally following Monod kinetics, starting from the knowledge of the specific growth rates for different substrate concentrations. Specific growth rates ad specific decrement rates are determined from the knowledge of the variations of the weight of the fungal biomass in a given time interval, under exponential growth phase. Usually, it is determined from the amount of mycelial proteins.

3. EVOLUTION EQUATIONS

In the following we are going to consider only non-dimensional variables, according to appropriate scales: T*- time in which competent biomass turns on conidiopore at the top of the mass substrate. H - height of the substrate column in the reactor. α - expected (competent) biomass dry weight at the upper surface at time T*.

Let us consider the unknown distribution of DPB: $X_i(\zeta, \tau)$, (i = 1,2), depending on ζ, (relative depth) and τ (time), and let the total biomass distribution be given by $X = X_1 + X_2$. We denote the unknown oxygen density (relative to the atmospheric) by Ω (ζ, τ). Let us introduce, for brevity, the function $\Gamma(\zeta,\tau) = \varepsilon\Omega/(\varepsilon\Omega + 1)$ to be presented in (1) - (2) due to the consideration of the oxygen as a nutrient in a Monod-like growth law for the biomass, in which the corresponding non-dimensional affinity coefficient is equal to $1/\varepsilon$.

The system representing the DPB evolution at the ζ-level is given by two equations, each valid in disjoint time intervals divided by the time-value $t^*(\zeta)$ at which takes place, in the ζ-level, a change of the biomass phase. This time-value is a priori unknown . If $\tau < t^*(\zeta)$:

$$\frac{dX_1}{d\tau} = m\Gamma(\zeta, \tau)X_1 \qquad (1)$$

and, if $\tau > t^*(\zeta)$, a generalization of Monod law like in [Georgiou **et al**. (1986)] is considered:

$$\frac{dX_2}{d\tau} = [m \cdot \Gamma(\zeta, \tau) - n_1 - n_2]X_2 \tag{2}$$

In non-dimensional form, the equation for oxygen density taking into account diffusion and consumption is:

$$\frac{\partial \Omega}{\partial \tau} = d \cdot \frac{\partial^2 \Omega}{\partial \zeta^2} - e\Omega \cdot [X_1 + X_2] \tag{3}$$

Here d is a non-dimensional time-dependent function because the volume of biomass is growing and so. The pores volume is decreasing in time. However, it is assumed practically equal to the non -dimensional constant denoted J^2; and, e is a non-dimensional constant representing the oxygen consumption by the biomass.

The initial (known) condition for the system is

$$X_1[\zeta, 0] = X_1^0 \tag{4}$$

$$X_2[\zeta, t^*(\zeta)] = X_1[\zeta, t^*(\zeta)]$$

$$\Omega(\zeta, 0) = C_0(\zeta) \tag{5}$$

where $C_0(\zeta)$ is an initial oxygen density distribution inside the medium.

The boundary conditions are:

$$\Omega(0, \tau) = 1$$

$$\frac{\partial \Omega}{\partial \zeta}(1, \tau) = 0 \tag{6}$$

according to the fact that it is considered a constant oxygen density at the atmosphere over the medium and, that there is no flow through the (impenetrable) reactor's bottom.

4. INVESTIGATION OF THE SOLUTION

Equations (1) and (2) could be formally integrated assuming acknowledge of the initial condition s and Ω. We obtain, for $\tau < t^*(\zeta)$:

$$X_1(\zeta,\tau) = X_1^0(\zeta) \cdot \exp\left(\int_0^\tau m\Gamma(\zeta,t)dt\right) \tag{6a}$$

and for $\tau > t^*(\zeta)$:

$$X_2(\zeta,\tau) = X_1(\zeta, t^*(\zeta) \cdot \exp\left(\int_{t^*(\zeta)}^\tau [m\Gamma(\zeta,t) - n_1 - n_2]dt\right) \tag{7}$$

It is known that the (non-dimensional) oxygen affinity coefficient is high. Hence, we will consider the ratio: (atmospheric oxygen density)/(oxygen affinity coefficient) as the small parameter ε. Let us do a regular perturbation technique [Georgescu (1995)] for a qualitative investigation of the solution to the system (1) - (3). It should be noted that the parameter ε is non-zero. Additionally, it is not difficult to see that

$$d = J^2(1 + O(\varepsilon)) \tag{8}$$

where J^2 is a non-dimensional number involving essential characteristics of the system.

Hence, following standard techniques we consider the formal expansions:

$$X_j = X_j^0 + X_j^1 \varepsilon + X_j^2 \varepsilon^2 + \ldots \tag{9}$$

and,

$$\Omega = \Omega_0 + \Omega_1 \varepsilon + \Omega_1 \varepsilon^2 + \ldots \tag{10}$$

The equation for Ω_0 results:

$$\frac{\partial \Omega_0}{\partial \tau} = J^2 \cdot \frac{\partial^2 \Omega_0}{\partial \zeta^2} - eX^0 \Omega_0 \tag{11}$$

Noting that, for $\tau < t^*(\zeta)$:

$$X_1 = X_1^0(\zeta) \left[1 + \varepsilon\, m \int_0^{\tau} \Omega_0 \, dt + \ldots \right] \tag{12}$$

and, for $\tau > t^*(\zeta)$:

$$X_2 = X_2^0(\zeta, t^*(\zeta)) \left[1 + \varepsilon\, m \int_{t^*(\zeta)}^{\tau} \Omega_0 dt + \ldots \right] \exp(-(n_1 + n_2)(\tau - t^*(\zeta)) \tag{13}$$

we get that the initial values X_1^0 are the zero order terms in the corresponding expansions, and the first order ones depends only on Ω_0.

The solution to (11) - (5) - (6) can be obtained numerically of analytically. An analytical description of Ω_0 can be done using Fourier series:

$$\Omega_0(\zeta,\tau) = U(\zeta) + X_1^0 \sum_{n=0}^{\infty} a_n(\tau) \sin((n + 1/2)\pi\zeta) \tag{14}$$

$$U(\zeta) = \frac{\cosh((\zeta - 1) A)}{\cosh(A)}$$

$$A = \frac{eX^0}{J^2}$$

$$a_n = b_n \exp(- B_n \tau)$$

$$B_n = (J\pi(n + 1/2))^2 + eX^0$$

and the numbers b_n are determined by the Fourier development:

$$C_0(\zeta) - U(\zeta) = \sum_{n=0}^{\infty} b_n \sin((n+1/2)\pi\zeta) \, .$$

It is evident that $\lim_{\tau \to +\infty} \Omega_0(\zeta, \tau) = U(\zeta)$. Furthermore, we conclude that for large times the oxygen density at the reactor's bottom is strictly less than the atmospheric provided $(eX^0) \neq 0$.

Substituting Ω_0 in (12) follows the calculation of DPB. If another term in the asymptotic development is needed, the next step is the determination of Ω_1, being the solution to (11) with respective null boundary

conditions. This solution could be obtained numerically or analytically (by Fourier series) taking into account the obtained Ω_0. After this, one could perform calculations to obtain X_j^1. For our purpose, only the zero order approximation is needed.

5. ESTIMATE FOR t*(ζ)

A knowledge of the function t*(ζ) is necessary in the understanding the causes of the biomass growth delay inside the substrate.

Now we will derive formulas for times t*(ζ). To do so, we consider that t*(ζ) is the time needed at level ζ to obtain a quantity of vegetative biomass equal to the present at the substrate top in time t*(0). We recall that t*(0) is a datum in this problem.

We obtain the following implicit formula:

$$\int_0^{t^*(\zeta)} \Omega_0(\zeta,t)dt = \int_0^{t^*(0)} \Omega_0(0,t)dt \ . \tag{15}$$

It is evident, from the positiveness of Ω_0, that

$$t^*(\zeta) \geq t^*(0) \tag{16}$$

From (15) and the implicit function theorem it is possible to obtain the values t*(ζ) ($0 < \zeta \leq 1$) provided the existence of a $\tau' = \tau'(\zeta)$ such that $\Omega_0(\zeta, \tau') \neq 0$. In any case, this occurs “near” the upper boundary. We will present numerical results solving (15) in a future paper.

A direct consequence of (15) is the feasibility of the estimation of the CT, which is crucial if we are looking for the profitable culture. To do so, we should find the value J^2_{min} for which t*(1) = 1.

More precisely, as Ω_0 implicitly depends on J^2, one should solve the following equation derived from (15):

$$\int_0^1 \Omega_0(1,t;J^2)\, dt = t^*(0). \tag{17}$$

As usual, coefficients b_n are small and b_n are large, justifying the principal role of U(ζ) in (17), hence it can be rewritten approximately as

$$\frac{1}{\mathrm{ch}\left(\sqrt{\frac{eX^0}{J^2_{min}}}\right)} = t^*(0)$$

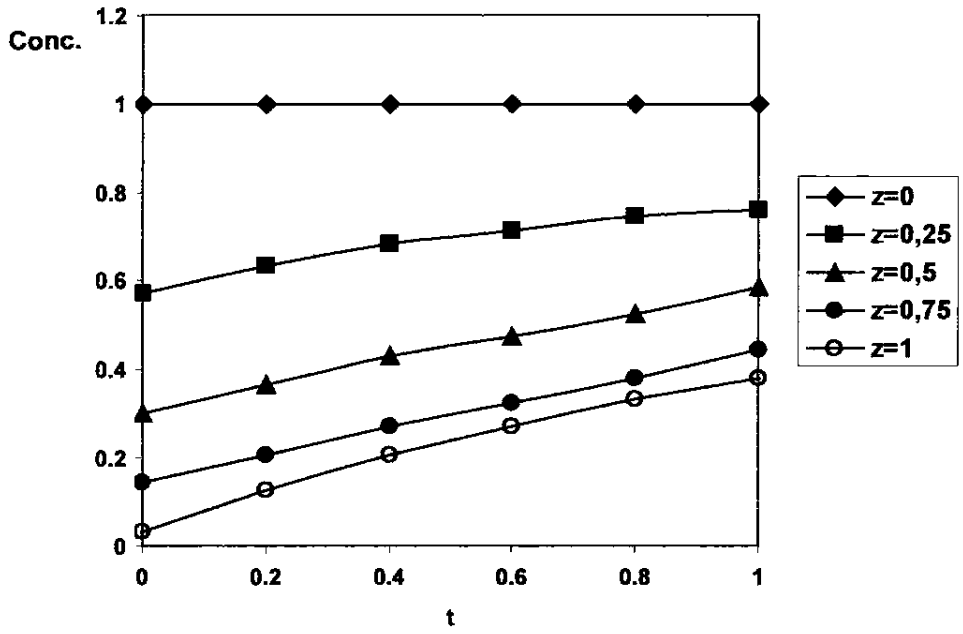
or more easily

$$\sqrt{\frac{eX^0}{J^2_{min}}} = \ln\left[\frac{1}{t^*(0)} + \sqrt{\left(\frac{1}{t^*(0)}\right) - 1}\right] \tag{18}$$

6. CONCLUSIONS

In this paper we have shown a method to estimate the oxygen density (see (10) and (14)) inside the substrate. The results are shown in Figure 1, and they agree very well with numerical calculations (see Alonso (1997)). Further, we determine the quantities of DPB (12) - (13) at different levels inside the substrate, at different times. The delay in growth of X_1 at different levels is shown in Figure. 2. Formula (14) explains the question about the delay of growth of the biomass inside the substrate, which is due to a gradient in the oxygen density.

Figure 1. Oxygen concentration.

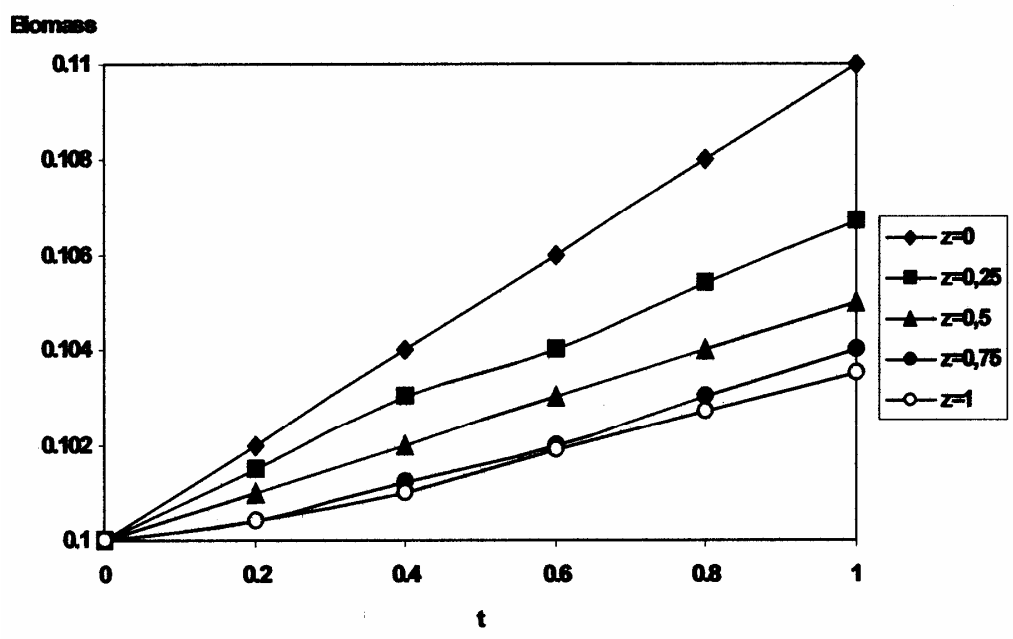


Initial distribution: $C_0(z) = \exp(-2z)$

$J^2 = 147.652$

$eX^0 = 518.587$

Figure 2. Total biomass by depth-levels.



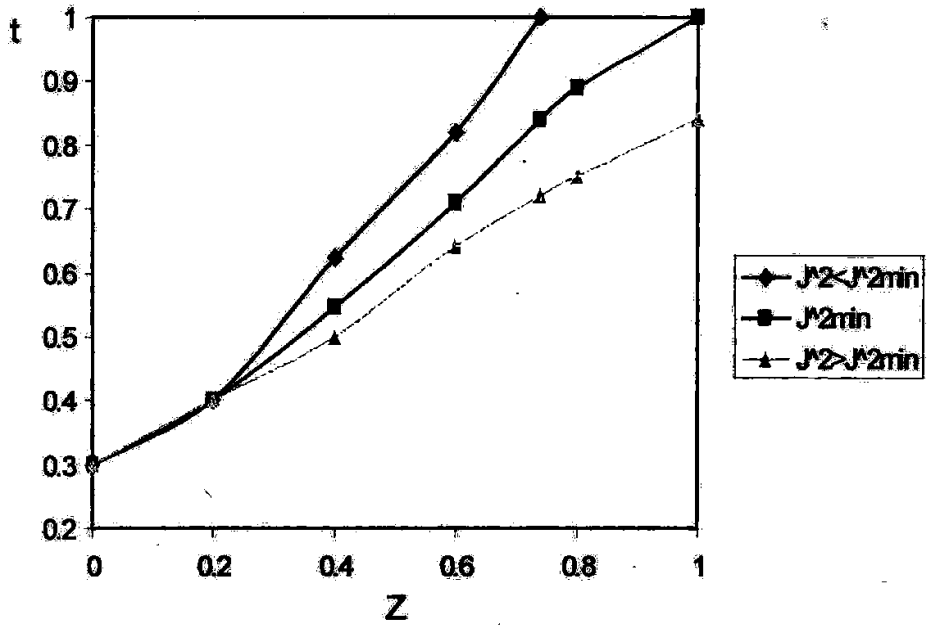
Initial distribution: $X_1^0 = 0.1$

$J^2 = 147.652$

$eX_1^0 = 518.587$

Further, we show in (15) a way to estimate the biomass change-of-phase times inside the substrate and consequently, in (17), a way to determine the CT substituting J^2_{min} in the equation defining J^2 (see appendix). From (Alonso (1997)) we know that a typical diagram of the phase-change times is given in Figure 3 as is expected.

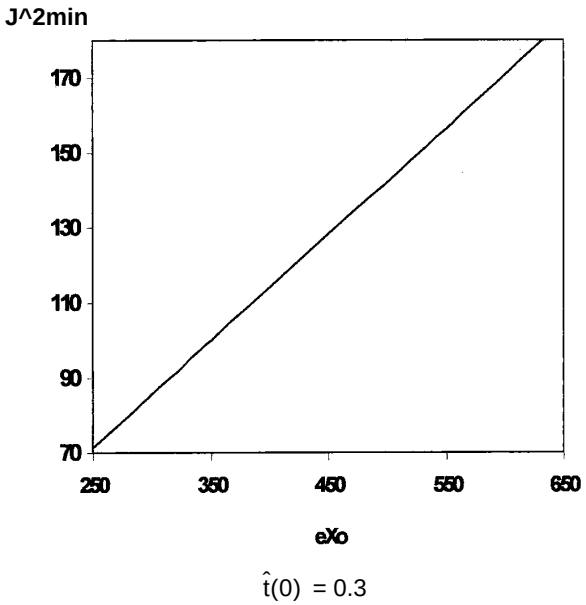
Figure 3.Phase-change times. From vegetative to competent.



Typical portrait for J^2 near J^2_{min}

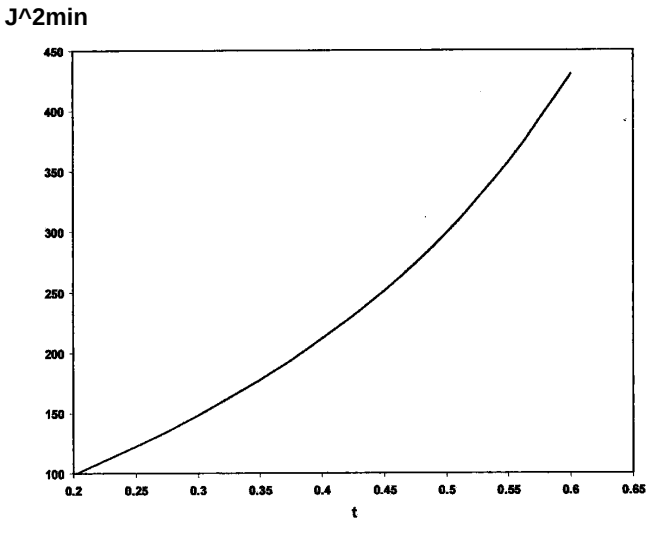
The parameter J^2, which involves several physical and biological characteristics, is crucial in the model. We show that condition $J^2 > J^2_{min}$ is enough to guarantee the presence of competent biomass at the bottom reactor before the appearance of conidia over the medium. Theoretical values of J^2_{min} with respect to eX^0 and t*(0) are given in Figure 4 and Figure 5.

Figure 4. Theorical J^2_{min}.



$\hat{t}(0) = 0.3$

Figure 5. Theorical J^2_{min}.



$eX_1^0 = 518.587$

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