# A Kinetic Model for the Fungal Pellet Lifecycle

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*Many industrially significant microbial products are produced during secondary metabolism by fungal pellets. Most models of mycelial pellet growth, however, consider only the primary growth phase. During secondary metabolism, the white-rot fungus Phanerochaete chrysosporium produces extra-cellular lignin peroxidases and manganese-dependent peroxidases which have been implicated in the degradation of lignin and various xenobiotics. This unstructured kinetic model for mycelial pellet cultures of P. chrysosporium describes the culture dry weight, respiration, glucose consumption, nutrient nitrogen uptake, pellet size and oxygen effectiveness factor during the lag, primary growth, secondary metabolic, and death phases. During secondary metabolism, the intraparticle diffusion of oxygen limits metabolism. Parameters for respiration during secondary metabolism are validated using three separate measurements: 1. by measuring oxygen concentration profiles in mycelial pellets using oxygen microelectrodes; 2. by measuring the depletion of oxygen from the culture fluid using a respirometer; and 3. by measuring the daily evolution of carbon dioxide. The physiological state of the mycelial pellets is predicted for various culture conditions and is related to the production of LIP and MNP during secondary metabolism.*

## Introduction

Filamentous fungi commonly grow in the form of spherical mycelial pellets when cultivated in agitated submerged cultures (Metz and Kossen, 1977; Whitaker and Long, 1973). Industrial-scale processes utilizing mycelial pellets include the production of citric acid using *Aspergillus* species (Sodeck et al., 1981) and the production of antibiotics using *Penicillium* species (Whitaker and Long, 1973). The extensively studied, lignin degrading, white-rot fungus *Phanerochaete chrysosporium* also grows in the form of mycelial pellets (Liebeskind et al., 1990; Michel et al., 1990). This organism mineralizes a wide range of xenobiotic compounds including dioxins (Eaton, 1984), pentachlorophenol (Lin and Wang, 1990), and PCBs (Bumpus et al., 1985). *P. chrysosporium* produces extracellular lignin peroxidases (LIPs) and manganese-dependent peroxidases (MNPs) during secondary metabolism, and their production is significantly enhanced by hyperbaric oxygen (Barlev and Kirk, 1981; Dosoretz et al., 1990; Dosoretz and Grethlein, 1991). These enzymes have been implicated in the initial steps of lignin depolymerization and biodegradation (Kirk and Farrel, 1987) and in the decolorization of paper mill bleach plant effluents (Michel et al., 1991).

Previous studies have shown that oxygen mass-transfer limitations can influence the growth kinetics of mycelial pellets. Pirt (1966) described "cube-root" growth kinetics by mycelial pellets of *Penicillium chrysogenum* where growth occurs only in an outer zone of the pellet. Phillips (1966) calculated the critical diameter for growing mycelial pellets of *Penicillium* by assuming zero-order kinetics for oxygen. Kobayashi et al. (1973) solved the differential equations describing oxygen transfer and reaction in pellets of *Aspergillus niger* for Michaelis-Menton kinetics. Van-Suijdam et al. (1982) developed an unstructured growth model for mycelial pellets of *P. chrysogenum* in a bubble column. Their model considered biomass growth, a substrate balance, an oxygen balance, and pellet density. It incorporated functions for mass-transfer processes in both the pellet and in the bulk liquid and assumed zero-order kinetics

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Table 1. Summary of Studies of Pellet Growth and Respiration

| Reference | Organism | Oxygen Kinetics | Growth Phase |
|---|---|---|---|
| Yano et al. (1961) | *A. niger* | zero-order | primary |
| Phillips (1966) | *P. chrysogenum* | zero-order | primary |
| Pirt (1966) | *P. chrysogenum* | zero-order | primary |
| Moo-Young et al. (1972) | (enzymes) | Michaelis-Menten | |
| Kobayashi et al. (1973) | *A. niger* | *M.M.*; adaptive | primary |
| van Suijdam et al. (1982) | *P. chrysogenum* | zero-order | primary and death |
| Wittler et al. (1986) | *P. chrysogenum* | zero-order | |

for respiration. The above studies of pellet growth considered only the primary or exponential growth phase and largely ignored the secondary metabolic phase. In practice, many important products are secondary metabolites expressed after a limiting nutrient has been exhausted and primary growth has ended. A summary of previous studies of mycelial pellet respiration and mycelial pellet growth is presented in Table 1.

For reactor design and scale-up, the relationships among the rates of substrate utilization, respiration, biomass production and product production are important design considerations. Our objectives were to formulate a simple unstructured kinetic model, which considers oxygen mass-transfer limitations to describe the growth, respiration, and substrate utilization by mycelial pellets cultures of *P. chrysosporium* during the lag, primary growth, secondary metabolic and death phases. We measured the oxygen concentration profile in mycelial pellets using an oxygen microelectrode and determined the intrinsic kinetic parameters for respiration. These parameters were confirmed using overall culture respiration data and integrated into the unstructured model. The intrinsic kinetic parameters for respiration were also used to generate a three-dimensional plot relating the mycelial pellet radius and liquid-phase oxygen concentration to the effectiveness factor in the pellet, defined as the observed rate of respiration divided by the rate observed without mass-transfer limitations. The model shows that during secondary metabolism, the rate of substrate utilization is limited by the rate of oxygen transfer into the pellets.

The model was tested using a range of initial glucose, nutrient nitrogen and oxygen concentrations. A qualitative relationship between the physiological state of the fungal pellets and the production of the secondary metabolites LIP and MNP was found.

## Theoretical Studies

### *Kinetic model development*

In this section, the development of a kinetic model for batch, periodically oxygenated, mycelial pellet cultures of *P. chrysosporium* is described. The model will describe the unsteady-state glucose ($G$), nutrient nitrogen ($N$), oxygen ($C_l$), polysaccharide ($pG$), and carbon dioxide concentrations as well as the culture dry weight ($X$), the characteristic mycelial pellet radius ($R$), and the oxygen effectiveness factor ($E$), given the initial glucose, nutrient nitrogen and oxygen concentrations and the initial inoculum dry weight.

Typically, nitrogen-limited cultures of *P. chrysosporium* proceed through four stages. Following a short lag phase, a primary growth phase begins, during which the cultures grow exponentially. When nutrient nitrogen is exhausted from the medium and glucose is present in excess, the cultures enter a secondary metabolic phase during which the MNPs and then the LIPs are produced. Cell autolysis occurs during the death phase. We have presented a set of rate equations for each of the above phases. Culture respiration, which was found to be the rate-limiting process under most conditions, is described using a separate set of equations which are valid during both primary growth and secondary metabolic phases.

The critical model assumptions are that pellets are spherical, monodisperse and of constant density during the primary growth and secondary metabolic phases, that the transfer of oxygen into pellets occurs solely by means of molecular diffusion, and that cell respiration follows Michaelis-Menten kinetics.

*Lag Phase.* Relatively little change in the biomass, glucose, nitrogen or oxygen concentration is seen immediately after inoculation in agitated submerged cultures of *P. chrysosporium*. During this lag phase, tiny mycelial pellets form. The process of pellet formation, involving the aggregation of hyphal fragments, is complete within 8 hours after culture inoculation, and the total number of these spherical pellets does not increase after this period (Michel et al., 1990). Furthermore, these pellets exhibit a fairly uniform size which is dependent on the rate of agitation. The total specific pellet volume and hence pellet density are constant for pellet diameters up to 0.005 m (Liebeskind et al., 1990; Michel et al., 1990). This is in contrast to previous studies of *P. chrysogenum* where pellet density varies with pellet size (Kobayashi et al., 1973; van Suijdam et al., 1982) or position (Wittler et al., 1986). Thus, at a specific biomass concentration the characteristic pellet radius ($R$) and pellet volume ($V_{pel}$) can be calculated.

$$R = \left( \frac{X}{\frac{4}{3}\pi\rho\eta} \right)^{1/3} \tag{1}$$

$$V_{pel} = \frac{4}{3}\pi R^3 = \frac{X}{\pi\eta} \tag{2}$$

*Primary growth phase.* Growth proceeds exponentially after the lag phase if nutrients are nonlimiting. The growth rate ($R_x$) and the uptake rate of nutrient nitrogen ($R_n$) are given by the exponential growth law (van Suijdam et al., 1982):

$$R_x = \frac{dX}{dt} = \mu_e X \tag{3}$$

$$R_n = -\frac{dN}{dt} = Y_{n/x}\mu_e X \tag{4}$$

The oxygen concentration in the pellets may become growth-limiting. The effective specific growth rate ($\mu_e$) is related to the maximum growth rate ($\mu_{max}$) using the effectiveness factor for oxygen ($E$) (van Suijdam et al., 1982):

$$\mu_e = E\,\mu_{max} \tag{5}$$

If the pellets are small ($R = 0.0003$ m) and the oxygen concentration is high ($C_l = 32.9$ g/m$^3$), the effectiveness factor is near unity, and the biomass concentration can be calculated as a function of time by integrating Eq. 3.

$$\ln\left(\frac{X}{Xo}\right) = \mu_{max}(t - t_{lag}) = \mu_{max}\,t - \mu_{max}\,t_{lag} \tag{6}$$

A plot of $\ln(X/X_o)$ vs. time during the primary growth phase will reveal $\mu_{max}$ and $t_{lag}$ as its slope and $x$-intercept, respectively.

Glucose serves as both the carbon and energy source for *P. chrysosporium* during primary growth. The utilization rate for glucose ($R_g$) includes factors for biomass production and respiration:

$$Rg = -\frac{dG}{dt} = R_{g\ \text{respiration}} + R_{g\ \text{growth}} \tag{7}$$

The amount of glucose used for growth during primary growth ($R_{g\ \text{growth}}$) can be calculated using a yield coefficient:

$$R_{g\ \text{growth}} = Y_{g/x}\frac{dX}{dt} \tag{8}$$

The rate of glucose uptake for respiration under aerobic conditions ($R_{g\ \text{respiration}}$) is proportional to the rate of oxygen utilization:

$$R_{g\ \text{respiration}} = Y_{g/O_2} R_{O_2} \tag{9}$$

*Secondary Metabolism.* In nitrogen-limited cultures, the onset of secondary metabolism coincides with the depletion of nutrient nitrogen from the medium (Jefferies et al., 1981). The production of extracellular MNPs and then LIPs occurs during this phase. The production of these enzymes as well as the total ligninolytic activity ($^{14}$C-lignin to $^{14}CO_2$) is significantly enhanced by high oxygen partial pressures (Barlev and Kirk, 1981; Dosoretz et al., 1990; Dosoretz and Grethlein, 1991; Reid and Seifert, 1982; Kirk and Farrell, 1987). In the classic case, no cell growth occurs during this phase. In cultures of *P. chrysosporium*, however, the culture dry weight slowly increases after nitrogen is depleted. Polymerization of glucose in the media to cell-bound and soluble 1-4 glucan has been shown (Dosoretz et al., 1990; Dosoretz and Grethlein, 1991; Leisola et al., 1982) and is affected by the oxygen partial pressure (Dosoretz et al., 1990). The primary metabolic activity of the cell during this period, however, is respiration. The total rate of glucose uptake during secondary metabolism ($R_g$) is a sum of the uptakes rates for respiration ($R_{g\ \text{respiration}}$), growth ($R_{g\ \text{growth}}$) and soluble polysaccharide production ($R_{g\ \text{polyG}}$).

$$R_g = -\frac{dG}{dt} = R_{g\ \text{respiration}} + R_{g\ \text{growth}} + R_{g\ \text{polyG}} \tag{10}$$

The fractions of the total glucose used for biomass, polysaccharide synthesis and respiration during secondary metabolism will be represented by the glucose metabolism factors; $f_x$, $f_{pg}$, and $f_r$ (Eqs. 11a, 11b and 11c):

$$R_{g\ \text{biomass}} = -f_x\frac{dG}{dt} \tag{11a}$$

$$R_{g\ \text{polyG}} = -f_{pg}\frac{dG}{dt} \tag{11b}$$

$$R_{g\ \text{respiration}} = -f_r\frac{dG}{dt} = Y_{g/O_2} R_{O_2} \tag{11c}$$

The total rate of glucose consumption ($R_g$) is obtained by substituting Eq. 11 into Eq. 10:

$$R_g = -\frac{dG}{dt} = -f_x\frac{dG}{dt} - f_{pg}\frac{dG}{dt} + Y_{g/O_2} R_{O_2} \tag{12}$$

Simplifying Eq. 12 gives the total rate of glucose consumption as a function of the rate of culture respiration.

$$R_g = -\frac{dG}{dt} = \left(\frac{Y_{g/O_2}}{1 - f_x - f_{pg}}\right) R_{O_2} \tag{13}$$

*Death Phase.* In aerobic batch cultures of many fungi, a decline in cell mass occurs due to cell autolysis when the carbon energy source is exhausted. In nitrogen-limited cultures of *P. chrysosporium*, proteases (shown to degrade LIPs and MNPs) are released during the beginning of the death phase (Dosoretz and Grethlein, 1991). Although glucose has been depleted, carbon dioxide evolution continues during the death phase. We assumed that polysaccharides produced during secondary metabolism are used as an energy source by nonautolyzed cells where $R_{pg}$ is the rate of consumption of polysaccharides:

$$R_{pg} = -\frac{dpG}{dt} = k_{pg} X \tag{14}$$

The rate of cell dry weight decrease ($R_x$) is expressed using a first-order rate constant ($k_{aut}$). The amount of biomass remaining after all biological activity has ceased is $X_f$ and is assumed to be a fraction of the maximum biomass attained:

$$R_x = -\frac{dX}{dt} = k_{aut}(X - X_f) \tag{15}$$

During the death phase, oxygen is no longer a limiting nutrient, and the effectiveness factor is not needed. To provide maintenance energy, polysaccharides formed during secondary metabolism are respired. The rate of polysaccharide respiration is $R_{O_2}$.

$$R_{O_2} = -\left(\frac{1}{Y_{g/O_2}}\right) - \frac{dpG}{dt} \tag{16}$$

### *Culture respiration and oxygen mass transfer*

A schematic representation of oxygen transfer in mycelial

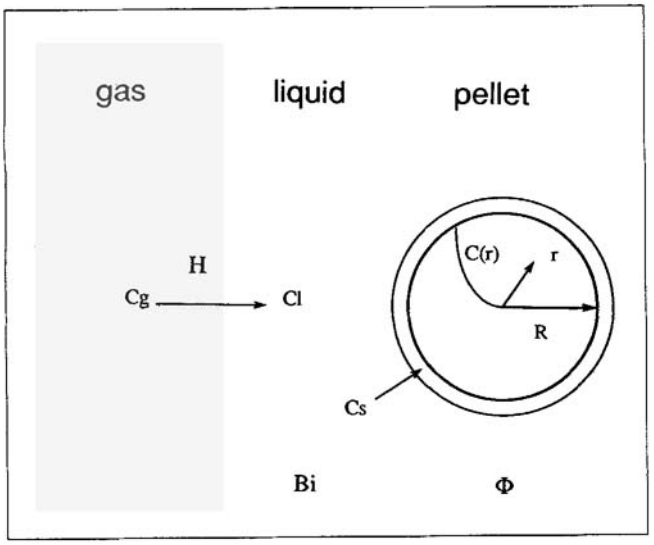


**Figure 1. Oxygen transfer in mycelial pellet cultures.**

pellet cultures is presented in Figure 1. Due to oxygen's low solubility in liquid media (32.9 g/m$^3$ in water at 40°C), it must continuously move from the gas phase to the interior portion of the mycelial pellets in order for respiration to occur.

### *Estimation of external and intraparticle mass-transfer resistances*

The contributions of the gas-liquid, liquid-pellet, and intraparticle mass-transfer limitations can be estimated by direct measurement of gas- and liquid-phase oxygen concentrations and by calculating the dimensionless Biot number ($Bi$) and the observable modulus ($\Phi$) (Bailey and Ollis, 1986).

If the rate of oxygen transport from the headspace to the liquid phase is much greater than the bulk rate of oxygen consumption, then the concentration of oxygen in the liquid phase and head space can be related using Henry's law:

$$C_l = H C_{hs} \tag{17}$$

The value of the Henry's law constant ($H$) can be estimated by dividing the solubility of oxygen in cell-free media by the ideal gas concentration in the headspace:

$$H = \frac{C_{sat} TRT}{MWP} \tag{18}$$

The external and intraparticle mass-transfer resistances can be compared based on the Biot number ($Bi$):

$$Bi = \frac{k_\ell R}{3 D_e} \tag{19}$$

For Biot numbers greater than 100, the effects of external mass-transfer resistance are not significant (Bailey and Ollis, 1986).

The dimensionless observable modulus ($\Phi$) can be used to estimate the relative importance of the intraparticle mass-transfer resistances of substrates without prior knowledge of intrinsic kinetic parameters:

$$\Phi = \frac{v}{D_e C_l}\left(\frac{R^2}{9}\right) \tag{20}$$

For values of $\Phi$ less than 0.3, the effectiveness factor is approximately one and the diffusion and reaction of substrate in the pellet is reaction-limited. For $\Phi$ greater than 0.3, intraparticle diffusion resistance limits the reaction rate.

### *Intraparticle diffusion and reaction of oxygen in mycelial pellets*

The diffusion and reaction of oxygen in a spherical mycelial pellet were modeled using the following equation and boundary conditions:

$$\frac{dC}{dt} + D_e\left(\frac{d^2C}{dr^2} + \frac{2}{r}\frac{dC}{dr}\right) = v \tag{21}$$

$$@r = R,\ C = C_s$$

$$@r = 0,\ \frac{dC}{dr} = 0$$

$D_e$ is the diffusivity of oxygen in the mycelial pellet, and $v$ is the oxygen respiration rate. Various methods have been used to solve the pseudo-steady state form ($dC/dt = 0$) of this differential equation (Kobayashi et al., 1973; Moo-Young and Kobayashi, 1972; van Suijdam et al., 1982; Yano et al., 1961). The solutions are commonly represented in terms of an effectiveness factor, defined as the observed reaction rate divided by the rate without mass-transfer limitations. In sealed shake flask cultures the pseudo-steady-state assumption is not valid over long time periods (>1/2 h).

### *Prediction of oxygen concentration profile in mycelial pellets*

During the time period when a microelectrode is being used to measure the oxygen profile in a mycelial pellet, the pseudo-steady state form of Eq. 21 can be used. We used a finite difference approach to approximate this form of Eq. 21 (Carnahan et al., 1969). By choosing an appropriate rate equation for respiration ($v$), the concentration profile within the pellet can be calculated using a shooting algorithm. The oxygen concentration at the pellet center (where $dC/dr = 0$) in the range zero to $C_s$ is used to start the algorithm, and the surface concentration is calculated. The center concentration is changed until the calculated surface concentration converges to the known value ($C_n = C_s$). We used a small step size ($n = 100$) to obtain an accurate solution. This profile can be measured using an oxygen microelectrode (Revsbech and Ward, 1983). Intrinsic kinetic parameters are determined by curve fitting as described in the Results section.

### *Calculation of the effectiveness factor for Michaelis-Menten kinetics*

The generalized effectiveness factor ($E$), valid for any rate equation and spherical geometry, can be calculated at time $t$ by integrating the actual reaction rate over the pellet and comparing it to the rate with no diffusion resistance. For most microbial cultures, the Michaelis-Menten equation adequately

describes the respiration kinetics:

$$v = \frac{V_{\max} C_l}{[K_m + C_l]} \tag{22}$$

Since calculation of the effectiveness factor is time-consuming, the approximation derived by Moo-Young and Kobayashi (1972) was used to calculate the effectiveness factor for Michaelis-Menten kinetics ($E_{M.M}$). This approximation is based on the more easily calculated zero-order ($E_o$) and first-order ($E_1$) effectiveness factors.

*Michaelis-Menten Order*:

$$E_{M.M.} = \frac{E_o + \left(\frac{K_m}{C_s}\right) E_1}{1 + \left(\frac{K_m}{C_s}\right)} \tag{23}$$

If the intrinsic Michaelis-Menten kinetics are known, the zero-order ($E_o$) and first-order ($E_1$) effectiveness factors can be easily calculated using Eqs. 24 and 25 (Bailey and Ollis, 1986).

*Zero order*:

$$\begin{aligned} E_0 &= 1 && \text{for } R\left(\frac{\nu_{\max}}{6 D_e C_s}\right)^{1/2} \le 1 \\ E_0 &= 1 - \left(1 - \frac{6 D_e C_s}{\nu_{\max} R^2}\right)^{3/2} && \text{for } R\left(\frac{\nu_{\max}}{6 D_e C_s}\right)^{1/2} > 1 \end{aligned} \tag{24}$$

*First order*:

$$E_1 = \frac{1}{\phi}\left(\frac{1}{\tanh\phi} - \frac{1}{3\phi}\right) \quad \text{where } \phi = \frac{R}{3}\left(\frac{\nu_{\max}}{D_e K_m}\right)^{1/2} \tag{25}$$

Using the effectiveness factor, the rate of oxygen uptake by pellets of *P. chrysosporium* ($r_{O_2}$) can be calculated based on the liquid-phase oxygen concentration ($C_l$) (Eq. 29). A Monod expression is added to account for the decrease in the respiration rate at low glucose concentrations. This term is significantly different from one only at low glucose concentrations:

$$r_{O_2} = E_{M.M.}\left(\frac{\nu_{\max} C_l}{K_m + C_l}\right)\left(\frac{G}{K_s + G}\right) \tag{26}$$

The bulk rate of respiration ($R_{O_2}$) can be calculated using the characteristic pellet volume ($V_{pel}$), the liquid-phase culture volume ($V_l$) and the pellet number ($\eta$).

$$R_{O_2} = r_{O_2}\left(\frac{V_{pel}}{V_l}\right)\eta \tag{27}$$

During respiration, glucose is metabolized to carbon dioxide. The rate of $CO_2$ produced can be calculated using a yield coefficient ($= 44/32 = 1.38$). The carbon dioxide balance assumes that all $CO_2$ accumulates in the gas phase.

$$R_{CO_2} = Y_{CO_2/O_2} R_{O_2} \tag{28}$$

### *Unsteady-state oxygen balance*

In sealed shake flask cultures the gas phase is flushed daily with oxygen. As the oxygen is respired, the total amount in the flask decreases. A differential expression for the change in the liquid-phase oxygen concentration is obtained using Henry's law, a mass balance on total oxygen in the flask and Eq. 27 for the bulk rate of respiration:

$$\frac{dC_l}{dt} = \frac{R_{O_2} V_l}{V_l + H V_{hs}} \tag{29}$$

The unsteady-state concentration of oxygen in the liquid phase can be approximated by numerically integrating Eq. 31 and recalculating the respiration rate, the effectiveness factor, and the liquid-phase oxygen concentration at each successive time point.

## Experimental Studies

### *Culture conditions*

*P. chrysosporium* BKM-F-1767 (ATCC #24725) was cultured in defined media containing diammonium tartrate (2 to 117 g/m$^3$) as the nitrogen source and glucose (5,000 to 15,000 g/m$^3$) as the carbon source. The media also contained 2,000 g/m$^3$ of $KH_2PO_4$, 1,450 g/m$^3$ of $MgSO_4 \cdot 7H_2O$, 132 g/m$^3$ of $CaCl_2 \cdot 2H_2O$, 1.0 g/m$^3$ thiamine hydrochloride, 500 g/m$^3$ Tween 80 (not added in stationary starter cultures), 20 mM acetate (pH 4.5), and 0.4 mM veratryl alcohol. The following trace elements were also added: 142 g/m$^3$ of nitrolotriacetate trisodium salt, 70 g/m$^3$ of NaCl, 36.9 g/m$^3$ of $MnSO_4 \cdot H_2O$, 12.6 g/m$^3$ of $CoCl_2 \cdot 6H_2O$, 7.0 g/m$^3$ of $FeSO_4 \cdot 7H_2O$, 7.0 g/m$^3$ of $ZnSO_4 \cdot 7H_2O$, 1.1 g/m$^3$ of $CuSo_4 \cdot 5H_2O$, 0.7 g/m$^3$ of $AlK(SO_4)^2 \cdot 12H_2O$, 0.7 g/m$^3$ of $H_3BO_3$, and 0.7 g/m$^3$ of $Na_2MoO_4 \cdot 2H_2O$.

Media (85 mL) were dispensed into sterile 250-mL rubber stoppered Erlenmeyer flasks and inoculated with a 10% (v/v) homogenized mycelial inoculum which was grown in static cultures using the media described above with 10,000 g/m$^3$ glucose and 39 g/m$^3$ diammonium tartrate but without Tween 80. The cultures were agitated at 173 RPM and the headspace (165 mL) was flushed daily with oxygen (Michel et al., 1991).

### *Analytical assays*

Glucose was measured as reducing sugar by the dinitrosalicylic acid method using D-glucose as the standard. Ammonium concentration was determined using an ammonium electrode (Orion model #95-12). For determining mycelial dry weight cultures were vacuum-filtered through tared GF/C grade filter paper, rinsed with 100-mL deionized water and dried at 105°C to a constant weight. LIP activity was determined by measuring veratryl alcohol oxidation to veratryl aldehyde (Tien and Kirk, 1984). MNP activity was determined using phenol red assay as described previously (Michel et al., 1991).

### *Oxygen concentration*

The oxygen profile within the mycelial pellets was measured using an oxygen microelectrode with a tip diameter of approximately 3 $\mu$m (Revsbech and Ward, 1983). The electrode

**Table 2. Physical Characteristics of a Submerged Mycelial Pellet Culture of *P. Chrysosporium***

| Characteristic | Value |
|---|---|
| Temperature | 39°C |
| Liquid Phase Volume ($V_l$) | $8.5\ e^{-5}\ m^3$ |
| Head Space Volume ($V_{hs}$) | $1.65\ e^{-4}\ m^3$ |
| Pellet number* ($\eta$) | $7.3 \pm 1.1\ e^{5}\ m^{-3}$ |
| Pellet density* ($\rho$) | $6.5 \pm 1.1\ e^{4}\ g/m^3$ |
| Diffusivity** ($D_e$) | $2.9\ e^{-9}\ m^2/s$ |
| Henry's law constant ($H$) | 37.8 |
| Solubility of oxygen at 39°C | $32.9\ g/m^3$ |

* Values were experimentally determined and reported as ± 1 standard deviation.
** Literature value for the diffusivity of oxygen in water at 39°C (Perry et al., 1984).

was calibrated with oxygen and nitrogen sparged into distilled water. All measurements were made at 39°C. Pellets were removed and placed onto a plate of agar solidified culture fluid (15 mL). Liquid extracellular culture fluid (15 mL) was added to the surface of the agar and the system was equilibrated to air. Oxygen measurements were made by inserting the microelectrode into a pellet using a micromanipulator (0.01-mm gradations). The procedure was monitored using a stereo dissecting microscope. The micromanipulator was also used to measure the diameter of the pellet being probed.

The oxygen concentrations in the culture fluid and head space were measured using a polarographic oxygen electrode (Ingold, model #531). Respiration rate measurements were made by pouring the culture into a hydraulically full stirred vessel (respirometer) and measuring the decrease in oxygen concentration with time.

The gas in the headspace was sampled with a pressure-lok syringe, and carbon dioxide was measured using gas chromatography (column, temp, flow rate) (GOW-MAC).

### *Pellet measurement*

A known volume of culture fluid with pellets was poured into a Petri dish, photographed, and enlarged. The total number of pellets was counted and the diameters of a group of 20 pellets were measured, scaled, and averaged using the Sauter equation (Michel et al., 1990).

## Results

Cultures of *P. chrysosporium* were grown at 39°C in agitated 250-mL Erlenmeyer flasks. The physical characteristics of the cultures used to determine the kinetic parameter values are presented in Table 2. Kinetic coefficients for growth and respiration were determined in cultures initially containing 39 g/m$^3$ ammonium and 11,000 g/m$^3$ glucose. The models were simultaneously solved using finite difference techniques. The determined growth model parameter values are summarized in Table 3.

### *Kinetic coefficients for primary growth*

To determine $\mu_{max}$ and the length of the lag time, the natural log of the biomass divided by the average initial biomass concentration was plotted for the first 24 hours after inoculation (Figure 2). The slope and the $x$-intercept were determined using a linear regression technique. As given by Eq. 6, the length of the lag time ($x$-intercept) was 7.9 h, and the maximum growth rate constant $\mu_{max}$ (slope), was $3.9\ e^{-5}\ s^{-1}$.

The yield coefficient for nutrient nitrogen (Eq. 4) was calculated by dividing the total nutrient nitrogen consumed by the cell dry weight at the end of the primary growth phase. The yield coefficient for glucose (Eq. 8) was estimated based on the following assumptions. In fungi, inorganic nutrients account for 1% to 10% of the dry biomass (Aiba et al., 1973); in nitrogen-limited cultures, nutrient nitrogen accounts for only 1% to 3% of the biomass. Therefore, the yield coefficient for glucose ($Y_{g/x}$) during balanced growth was set at 0.92 g/g.

### *Kinetic coefficients for secondary metabolism*

The glucose metabolism factors (Eq. 11) are used to calculate the proportion of glucose used for polysaccharide production, biomass and respiration during secondary metabolism. Dosoretz et al. (1990) showed that in submerged oxygen flushed

**Table 3. Kinetic Parameter Values**

| Parameter | Definition | Value | Equations |
|---|---|---|---|
| *Respiration* | | | |
| $\nu_{max}$ | Michaelis-Menten coefficient | $0.76 \pm 0.10$ g/m$^3$ pellet/s | 21, 24, 25, 26 |
| $K_m$ | Michaelis-Menten coefficient | $0.5 \pm 0.3$ g/m$^3$ | 21, 23, 25, 26 |
| $Y_{g/O_2}$ | Theoretical yield coefficient | 0.92 g/g | 9, 13, 16 |
| $Y_{CO_2/O_2}$ | Theoretical yield coefficient | 1.38 g/g | 28 |
| *Primary Growth* | | | |
| $t_{lag}$ | Length of the lag phase | $2.8 \pm 0.2\ e^{4}$ s | 6 |
| $\mu_{max}$ | Growth rate constant | $3.9 \pm 0.3\ e^{-5}\ s^{-1}$ | 5, 6 |
| $Y_{g/x}$ | Growth yield for glucose | $0.92 \pm 0.1$ g/g | 8 |
| $Y_{n/x}$ | Growth yield for ammonium | $0.04 \pm 0.1$ g/g | 4 |
| *Secondary Metabolism* | | | |
| $f_{pg}$ | Polysaccharide from glucose | $0.04 \pm 0.03$ g/g | 11a, 12, 13 |
| $f_x$ | Biomass from glucose | $0.12 \pm 0.01$ g/g | 11b, 12, 13 |
| $f_r$ | Glucose for respiration | $0.84 \pm 0.03$ g/g | 11c |
| *Death Phase* | | | |
| $k_{aut}$ | Autolysis rate constant | $2.5 \pm 0.5\ e^{-6}\ s^{-1}$ | 15 |
| $k_{pg}$ | Polysaccharide formation rate | $0.4 \pm 0.1\ e^{-6}\ s^{-1}$ | 14 |

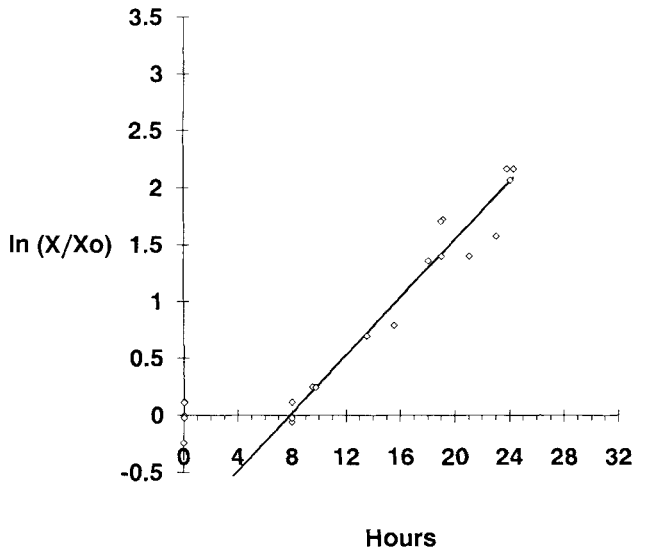


**Figure 2. Plot of $\ln(X/X_o$, avg) vs. culture age during the lag phase and primary growth phases of agitated, submerged cultures of *P. chrysosporium* for the determination of $\mu_{max}$ and $t_{lag}$.**

◇, experimental data; —, a linear regression fit of the data points ($\mu_{max} = 3.9\ e^{-5}\ s^{-1}$, $t_{lag} = 7.9$ h).

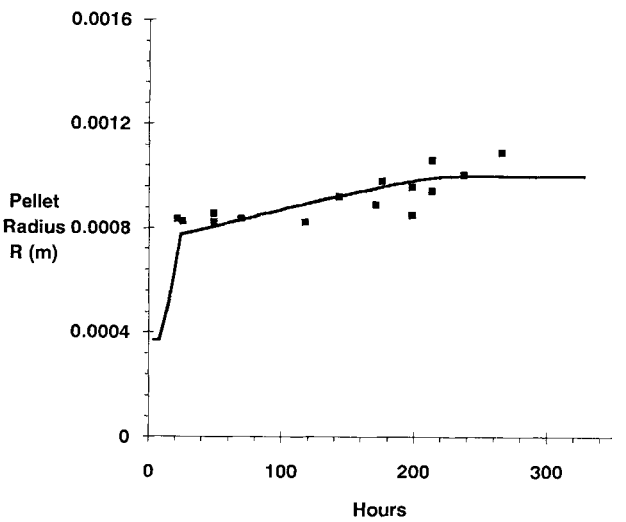


**Figure 3. Pellet radius in submerged cultures of *P. chrysosporium* grown using initial condition 2.**

—, kinetic model prediction; ■, experimental data points.

cultures initially containing 10,000 g/m$^3$ glucose, approximately 400 g/m$^3$ of soluble polysaccharide is formed by the time of glucose depletion. Hence, the time-averaged percentage of glucose consumed for soluble polysaccharide synthesis is 4 wt. % ($f_{pg} = 0.04$). A small amount of biomass is produced during secondary metabolism corresponding to approximately 12% of the glucose consumed ($f_x = 0.12$). The balance of the glucose consumed is used for respiration ($f_r = 0.84$). These factors are assumed to have fixed values during secondary metabolism for the purposes of the model; however, in reality these factors may change due to oxygen starvation or other environmental conditions (see discussion).

The yield coefficients for oxygen and carbon dioxide from glucose used for respiration (Eqs. 9 and 31) were calculated based on the overall stoichiometry of respiration ($Y_{CO_2/O_2} = 1.38$, $Y_{g/O_2} = 0.92$).

$$6\,C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$$

### *Kinetic coefficients for the death phase*

The rate constant for autolysis ($k_{aut}$) was estimated by curve fitting biomass data after glucose was depleted from the medium (Eq. 15). The rate constant for polysaccharide utilization ($k_{pg}$) was calculated by measuring the rate of $CO_2$ evolution after glucose was depleted from the medium using the yield coefficients for respiration (Eqs. 14 and 16). The final biomass concentration ($X_f$) was determined by measuring the biomass concentration 20 days after no respiration was detectable (Eq. 15).

### *Pellet size and density*

The pellet density ($\rho$) was calculated from Eq. 1 using experimental values for the characteristic pellet radius, pellet number, and culture dry weight. Other investigators (Yano et al., 1961; van Suijdam et al., 1982) have shown that during the growth phase, the pellet density may change as the pellet size increases. For pellets of *P. chrysosporium*, however, the pellet density was constant during the primary and secondary metabolism, up to a pellet radius of 2.5 mm (this study; Liebeskind et al., 1990; Michel et al., 1990). This could be explained by the relatively short growth phase and smaller pellets obtained in nutrient-limited cultures. The time course of the experimentally determined pellet radii and the model-predicted pellet radii using parameter values from Table 3 is presented in Figure 3. During the death phase, pellet density decreases due to autolysis.

### *Estimation of mass-transfer resistances*

In most studies of respiration by mycelial pellets the oxygen concentration at the pellet surface ($C_s$) is assumed to be equal to the bulk liquid concentration ($C_l$) (Metz and Kossen, 1977). This assumption was validated in theory by calculating the Biot number (Eq. 19) and directly by means of an oxygen microelectrode. The Biot number for oxygen, assuming a hypothetical worst case of very small pellets ($R = 0.5$ mm) and a low value for the mass-transfer coefficient ($k_l = 0.0005$ m/s), is 250. For a Biot number greater than 100, the external mass-transfer resistance is negligible compared to the intraparticle mass-transfer resistance (Bailey and Ollis, 1986), and the pellet surface concentration is approximately equal to the bulk liquid concentration. Measurements using an oxygen microelectrode confirmed this finding. There was a negligible decrease in the oxygen concentration as the microelectrode was moved from the bulk liquid phase to the pellet surface.

To confirm Henry's law equilibrium (Eq. 18), the gas-phase and liquid-phase oxygen concentrations were measured using a polarographic oxygen electrode. The liquid-phase oxygen concentration was never significantly different than the corresponding gas-phase equilibrium concentration. This implies that the rate of oxygen transfer into the liquid phase by means of diffusion is much faster than the bulk rate of culture respiration and that Eq. 18 is valid. Van Suijdam et al. (1982)

Table 4. **Observable Moduli for Substrates of *P. Chrysosporium* Using Worst Case Assumptions**

| | Oxygen | Glucose | Ammonium |
|---|---|---|---|
| $\nu$ (g/m$^3$ pellet/s) | 0.18 | 0.29 | 0.01 |
| $D_e$ (m$^2$/s) | 2.9 e$^{-9}$ | 9 e$^{-10}$ | 2.1 e$^{-9}$ |
| $C_l$ (g/m$^3$) | 1.5 | 500 | 3 |
| $R$ (m) | 0.0015 | 0.0015 | 0.0008 |
| $\Phi$ | 10.5 | 0.16 | 0.13 |

reported that in mycelial pellet cultures of *P. chrysogenum*, the gas-liquid mass-transfer coefficient ($k_{la}$) dropped rapidly above pellet volume fractions of 40%. In this study, the pellet volume fraction never exceeded 10%.

The dominant mass-transfer resistance in shake flask cultures of *P. chrysosporium* is the intraparticle diffusion of oxygen. This can be shown by calculating the observed moduli ($\Phi$) for oxygen using Eq. 20. For $\Phi$ values less than 0.3 the resistance to intraparticle mass transfer is negligible. Typical observed oxygen uptake rates with worst case assumptions of large pellets ($R = 0.0015$ m), and liquid-phase oxygen concentration of 1.5 g/m$^3$, gives a $\Phi$ value of 10.5 (Table 4). By comparison, the observable modulus for glucose is 0.16 using typical observed glucose uptakes rates with worst case assumptions of large pellets ($R > 1.5$ mm) and a low bulk glucose concentration ($G < 500$ g/m$^3$). The observed modulus for ammonium during primary phase growth using worst case assumptions is 0.13 (Table 4).

### *Determination of kinetic coefficients for oxygen*

The intrinsic respiration kinetics (Eq. 22) were determined by three independent methods.

1. Oxygen profiles were measured within individual mycelial pellets using an oxygen microelectrode, and the data were modeled using a computer simulation. Using this approach, the range of values of $V_{max}$ was $0.76 \pm .10$ g/m$^3$/s and the range of values of $K_m$ was $0.5 \pm 0.3$ g/m$^3$. A typical oxygen concentration profile and a computer simulation curve are presented in Figure 4. In pellets with a diameter greater than 1 mm, the oxygen concentration decreased to undetectable levels well before the pellet center. The rate of autolysis, however, was probably negligible, since both culture dry weight and the rate of respiration increased during secondary metabolism under all conditions studied.

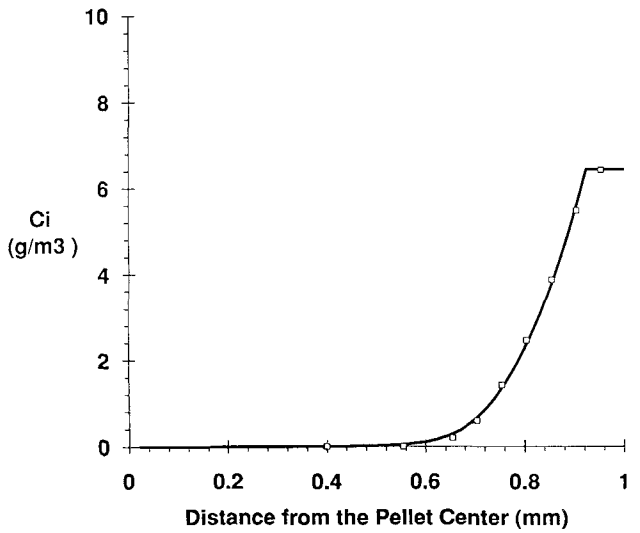


**Figure 4. Oxygen concentration profile in a mycelial pellet of *P. chrysosporium* (0.00185-m-dia.) as determined using an oxygen micro-electrode.**

—, predicted by the kinetic model ($K_m = 0.5$ g/m$^3$; $V_{max} = 0.76$ g/m$^3$/s; $D_e = 2.9$ e$^{-9}$ m$^2$/s.)

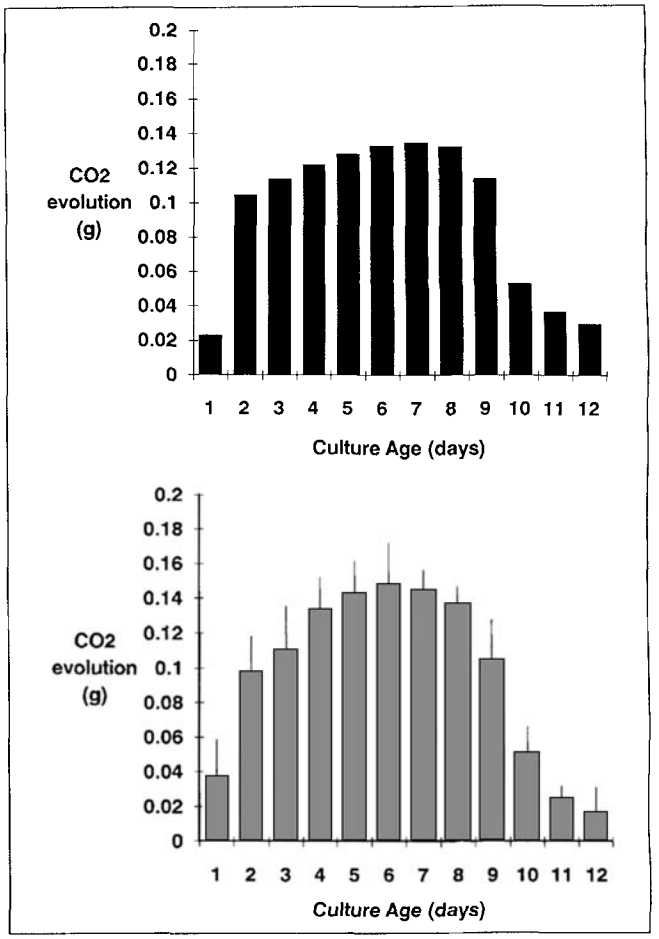


**Figure 5. Carbon dioxide evolution in mycelial pellet cultures of *P. chrysosporium* (condition 2).**

■, predicted by the kinetic model ($K_m = 0.5$ g/m$^3$; $V_{max} = 0.76$ g/m$^3$/s; $D_e = 2.9$ e$^{-9}$ m$^2$/s); ⊠, experimental data.

2. The carbon dioxide concentration in the culture headspace was measured daily and compared to that predicted by the integrated kinetic model. The model curve using the parameter values presented in Table 3 ($V_{max}$ value of 0.76 g/m$^3$/s, $K_m$ of 0.5 g/m$^3$ and pellet density value of 65,000 g/m$^3$ pellet) closely follows the $CO_2$ production data (Figure 5).

3. The rate of oxygen uptake by cultures was determined using a respirometer. One entire culture was transferred to a hydraulically full stirred vessel and the depletion of oxygen was measured with time. The slope of the curve was measured at various concentrations to obtain velocity data. The apparent $V_{max}$ (g/m$^3$ media/s) and the apparent $K_m$ (g/m$^3$) were calculated using the method of Wilkinson (1961) (Figure 6). During primary growth, the apparent $V_{max}$ was $0.04 \pm 0.02$ g/m$^3$ me-

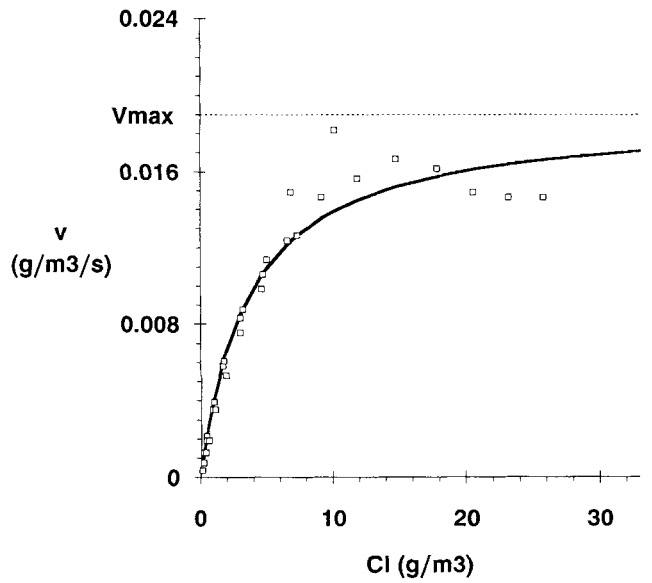


**Figure 6. Typical plot of the rate of oxygen depletion ($v$ g/m$^3$ media/s) vs. the liquid-phase oxygen concentration ($C_l$ g/m$^3$) by agitated cultures of *P. chrysosporium* as measured using a respirometer.**

—, curve fit using the method of Wilkinson (1961) for three consecutive measurements; □, experimental data.

dia/s. During secondary metabolism, the apparent $V_{max}$ ranged from 0.014 to 0.031 g/m$^3$ media/s, while the apparent $K_m$ ranged from 1.5 to 8 g/m$^3$. The apparent $V_{max}$ dropped to $0.007 \pm .002$ g/m$^3$ media/s one day after culture glucose was depleted and to $0.0001 \pm .0001$ g/m$^3$ media/s ten days later. By using appropriate pellet density and culture dry weight values, the apparent $V_{max}$ was converted to a specific apparent $V_{max}$ ($0.87 \pm 0.33$ g/m$^3$ pellet/s). Note that mass-transfer effects are not considered in the apparent $V_{max}$ and apparent $K_m$ values; however, if the effectiveness factor is near unity (the case at high $C_l$ concentrations), apparent $V_{max}$ values should be comparable to the intrinsic $V_{max}$ determined using oxygen profile modelling.

### *Model test cases and enzyme production*

Batch cultures were grown with five different initial conditions to test the model (Table 5). In one set of experiments, three different initial glucose concentrations were used (conditions 1, 2 and 3). The model closely predicted the glucose and biomass data for the three conditions (Figure 7). Nutrient nitrogen was completely depleted for each condition within 24 hours. The initial level of glucose had little effect on the specific rate of glucose or nitrogen uptake, the culture dry weight, or the pellet size. The initial glucose concentration primarily affected the length of secondary metabolism.

**Table 5. Initial Conditions for Model Test Cases**

| Condition | Ammonium (g/m$^3$) | Glucose (g/m$^3$) |
|---|---|---|
| 1 | 39 | 5,000 |
| 2 | 39 | 10,000 |
| 3 | 39 | 15,000 |
| 4 | 2 | 11,000 |
| 5 | 117 | 11,000 |

As previously shown by Jeffries et al. (1981), the production of MNP and LIP is a secondary metabolic event. In the cultures used in this study, secondary metabolism was triggered by the depletion of nutrient nitrogen. For conditions 1 (5 g/m$^3$ glucose), 2 (10 g/m$^3$ glucose), and 3 (15 g/m$^3$), MNP activity first appeared on day 2 and reached a maximum on day 3 of 2,500 U/L. The LIP activity in condition 1 cultures reached a maximum of 140 U/L on day 5. In conditions 2 and 3, LIP activity reached a maximum between days 5 and 6 and 260 U/L and 310 U/L, respectively. A rapid decrease in biomass concentration and the disappearance of MNP and LIP activity coincided with the time of glucose depletion. This occurred at hour 140, 220 and 300 for conditions 1, 2 and 3, respectively.

A second set of initial conditions was tested in a subsequent experiment (conditions 4 and 5) to determine the model's ability to predict changes in the nutrient nitrogen concentration. When cultures were grown with only 2 g/m$^3$ nutrient nitrogen (condition 4), little total biomass was produced and glucose was consumed slowly (Figure 7d). In cultures grown in 117 g/m$^3$ ammonium (condition 5), nutrient nitrogen was depleted within 30 hours, glucose was depleted within 190 hours and the biomass concentration reached 3 g/m$^3$ (Figure 7e). Control cultures grown in 39 g/m$^3$ ammonium (condition 2) produced the highest LIP activity (220 U/L) even though less biomass was produced than in the condition 5 cultures. In conditions 4 and 5, the maximum LIP activity was one quarter of that observed in condition 2 cultures. In all cases, the LIP activity decreased to undetectable levels one day after the glucose concentration dropped below 400 g/m$^3$.

## Discussion

The oxygen partial pressure in submerged agitated cultures has been shown to affect the amount of the ligninolytic system produced (Barlev and Kirk, 1981), the rate of glucose depletion, polysaccharide production and final biomass level (Dosoretz et al., 1990), and rate of lignin degradation (Reid and Seifert, 1982). Our hypothesis was that the diffusion of oxygen is the limiting process during secondary metabolism of *P. chrysosporium* in agitated cultures and that this limitation may affect LIP and MNP production during secondary metabolism. To our knowledge, this is the first model to unite the four life-cycle stages of fungal pellets with a chemical engineering analysis of oxygen limitations. It is the first quantitative model describing the important lignin degrading fungus *Phanerochaete chrysosporium*, and it is the first study of the relationship between the physiology of fungal pellets and the production of secondary metabolites.

The intrinsic kinetics of respiration for pellets of *P. chrysosporium*, $V_{max}$ and $K_m$, determined by three separate methods are in agreement. The oxygen utilization by fungal pellets during the primary growth phase has been investigated previously. In the zero-order model for pellets of *P. chrysogenum* developed by Phillips (1966), a rate constant of 0.56 g/m$^3$ pellet/s was reported. Van Suijdam et al. (1982) reported a maximum oxygen uptake rate of approximately 0.8 g/m$^3$ pellet/s for pellets of *P. chrysogenum*. These values compare to a $V_{max}$ value for pellets of *P. chrysosporium* of 0.76 g/m$^3$ pellet/s. Both Yano et al. (1961) and Kobayashi et al. (1973) reported

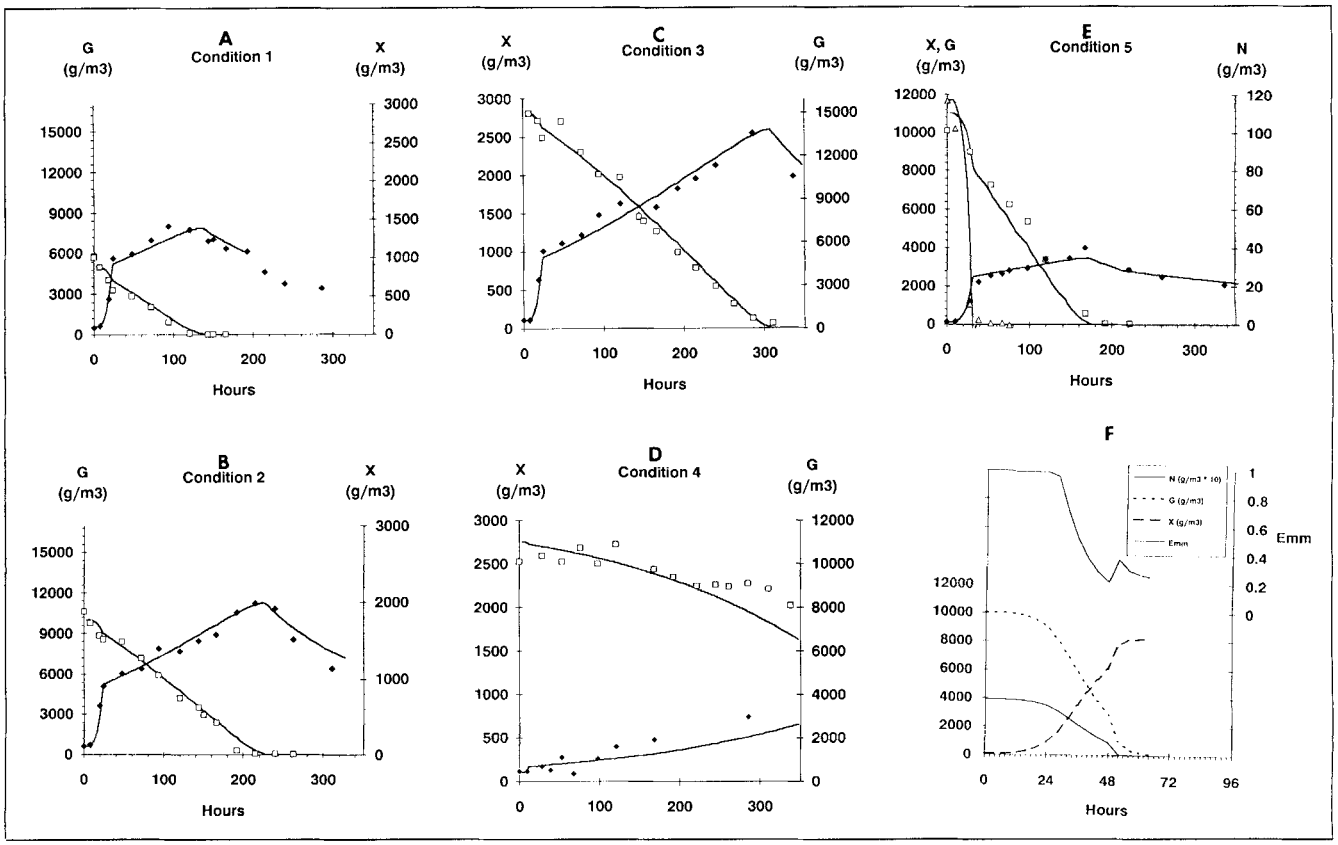


**Figure 7. Culture dry weight (*X*), glucose concentration (*G*), and nutrient nitrogen (ammonium) concentration (*N*) as a function of time in cultures of *P. chrysosporium* grown under various initial glucose and nutrient nitrogen conditions.**

—, predicted by the kinetic model; □, experimental data for glucose; ◆, experimental data for culture dry weight; △, experimental data for ammonium.

*A. Condition 1*: $G_o = 5{,}000\ g/m^3$; $N_o = 39\ g/m^3$.
*B. Condition 2*: $G_o = 10{,}000\ g/m^3$; $N_o = 39\ g/m^3$.
*C. Condition 3*: $G_o = 15{,}000\ g/m^3$; $N_o = 39\ g/m^3$.
*D. Condition 4*: $G_o = 11{,}000\ g/m^3$; $N_o = 2\ g/m^3$.
*E. Condition 5*: $G_o = 11{,}000\ g/m^3$; $N_o = 117\ g/m^3$.
*F. Model simulation for cultures grown in high nutrient nitrogen conditions*: $G_o = 10{,}000\ g/m^3$; $N_o = 390\ g/m^3$.

that the maximum specific oxygen uptake rate ($QO_{2max}$) for filamentous *A. niger* is $6.1 \times 10^{-5}$ g/g dry weight/s. By comparison, for pellets of *P. chrysosporium* $QO_{2max}$ was $1.2 \times 10^{-5}$ g/g dry weight/s. The respiration kinetics determined in this work were valid for both primary and secondary metabolism (see Figure 5). Wittler et al. (1986) showed that oxygen transfer in pellets of *P. chrysogenum* may occur by means of convection and eddy diffusion as well as by molecular diffusion. These methods of oxygen transfer were not considered in the model presented here for mildly agitated shake flask cultures. However, in larger-scale fermentations, reactor hydrodynamics may significantly influence the oxygen mass transfer within the pellets. The low $K_m$ value for oxygen of $0.5 \pm 0.3$ g/m$^3$ found for pellets of *P. chrysosporium* is also in line with other studies. The value of $K_m$ reported for pellets of *A. niger* by Kobayashi et al. (1973) was 0.1 g/m$^3$ and yeast cells typically have a $K_m$ of approximately 0.05 g/m$^3$.

Because of the very low value of $K_m$ for oxygen reported for many cultures, a zero-order model ($K_m = 0$) for respiration has been used by many investigators (Phillips, 1966; van Suijdam et al., 1982; Yano et al., 1961; Wittler et al., 1986). If a zero-order model is assumed for pellets of *P. chrysosporium*, then a critical pellet radius can be calculated where the center of the pellet first becomes anoxic. This radius is 0.86 mm in oxygen saturated medium and 0.40 mm in air saturated medium. For Michaelis-Menten kinetics, a three-dimensional surface provides a tool for quickly estimating the oxygen effectiveness factor for mycelial pellets of *P. chrysosporium* as a function of the pellet radius and the liquid-phase oxygen concentration (Figure 8).

Dosoretz et al. (1990) found that in air-flushed submerged cultures of *P. chrysosporium*, the rates of glucose depletion and carbon dioxide production decreased by 33% and 70%, respectively, compared to oxygen flushed cultures, while the biomass concentration reached 2,900 g/m$^3$ by day 8 and soluble polysaccharides doubled. A model simulation for air-flushed cultures using the kinetic parameters from Table 3 closely approximated the data for carbon dioxide production reported by Dosoretz et al. (1990), but underpredicted the glucose utilization rate and the biomass concentration. Since under air,

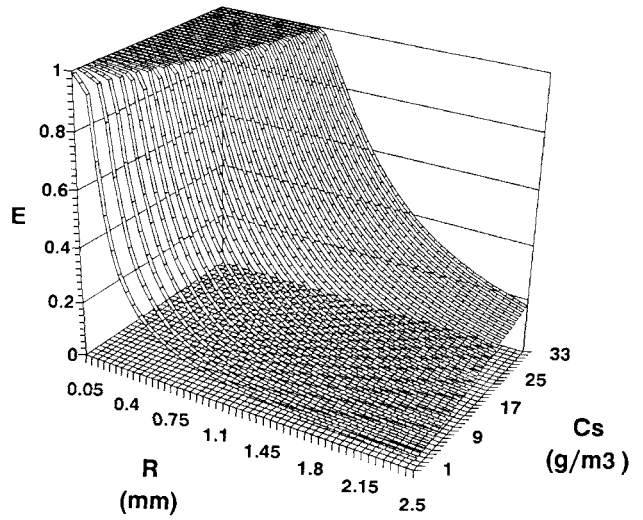


**Figure 8. Three-dimensional representation of the oxygen effectiveness factor (*E*) for mycelial pellets of *P. chrysosporium* as a function of the liquid phase oxygen concentration and the characteristic pellet radius.**

more cells would be oxygen limited and unable to respire glucose, the yield coefficients for glucose metabolism with air are different. By adjusting the glucose metabolism factors accordingly ($f_{pg}=0.25$, $f_x=0.3$), the model closely predicts the results reported for air-flushed cultures by Dosoretz et al. (1990). This finding suggests a relationship between the level of polysaccharides produced and the oxygen tension in the cultures.

The production of the secondary metabolites LIP and MNP has been shown to be a function of mineral concentration (Michel et al., 1991), genetic and cultural factors (Kirk and Farrell, 1987), the time elasped since primary growth has ended, media conditions, the presence of proteases in the extracellular medium (Dosoretz et al., 1990) the rate of culture agitation (Michel et al., 1990) and the level of intracellular cyclic AMP (Boominathan et al., 1992). Our intent was to provide a way of qualitatively evaluating the physiology of fungal pellets during secondary metabolism as a function of nutrient and oxygenation conditions and relate the physiological status of the fungal pellets to the production of the secondary metabolites: MNP and LIP. Certain culture conditions are known to significantly reduce the production of these enzymes. For example, Dosoretz et al. (1990) showed that in air-flushed cultures (where the oxygen effectiveness factor ranges from 0.1 to 0.3 based on this model), no LIPs and one third less MNPs were produced. Michel et al. (1990) found that oxygenated cultures, where large pellets (6-mm-dia.) form due to low agitation rates (effectiveness factor less than 0.1), also fail to produce LIPs. Similarly, Barlev and Kirk (1981) found that in stationary cultures of *P. chrysosporium* where a thick mycelial mat forms, the ligninolytic activity in air-flushed cultures was approximately 20% of that observed in oxygen-flushed cultures. Model simulations for these conditions show that these cultures are severely oxygen-limited. This oxygen limitation has a strong negative effect on LIP and MNP production.

Nitrogen sufficient cultures are also deficient in their ability to produce both LIPs and MNPs. A simulation for batch cultures grown in high nitrogen (390 g/m$^3$ ammonium), shows that culture glucose is exhausted and autolysis begins between days 2 and 3 (Figure 7f). Therefore, under the nitrogen sufficient condition the cells are no longer in a secondary metabolic state when LIPs and MNPs are normally produced in control cultures. The feeding of glucose to such cultures may not ameliorate this situation, since the pellets have already grown to a large size (>2 mm dia.), in which much of the center of the pellet is oxygen-limited.

In contrast to the aforementioned cases, fungal pellets of *P. chrysosporium*, which are not oxygen-limited (effectiveness factors near unity) and where secondary metabolism endures for at least a three-day period (for example, condition 2), produce high levels of both LIP and MNP.

In conclusion, the analysis of the mass-transfer limitations in mycelial pellets of *P. chrysosporium* indicates that intraparticle oxygen mass transfer is rate-limiting during secondary metabolism. The integrated life-cycle model presented here accurately predicts culture dry weight, substrate utilization, and potential oxygen limitations in mycelial pellet cultures, during both primary growth and secondary metabolism under a variety of initial conditions, and qualitatively explains the effects of these conditions on the production of the secondary metabolites: LIP and MNP.

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## Notation

$Bi$ = Biot number
$C$ = oxygen concentration, g/m$^3$
$C_i$ = oxygen concentration in pellet, g/m$^3$
$C_g$ = gas-phase oxygen concentration, g/m$^3$
$C_l$ = liquid-phase oxygen concentration, g/m$^3$
$C_s$ = oxygen concentration at pellet surface, g/m$^3$
$C_{sat}$ = oxygen equilibrium concentration in media, g/m$^3$
$D_e$ = effective diffusivity in pellets, m$^2$/s
$E_0$ = effectiveness factor for zero-order kinetics
$E_1$ = effectiveness factor for first-order kinetics
$E$ = effectiveness factor for oxygen
$E_{M.M.}$ = effectiveness factor for M.M.-order kinetics
$f_{pg}$ = glucose metabolism factor, g/g
$f_r$ = glucose metabolism factor, g/g
$f_x$ = glucose metabolism factor, g/g
$G$ = glucose concentration, g/m$^3$
$H$ = Henry's law constant
$i$ = index value for numerical method
$k_l$ = mass-transfer coefficient for oxygen, m/s
$K_m$ = kinetic coefficient for oxygen, g/m$^3$
$K_s$ = kinetic coefficient for glucose, g/m$^3$
$MW$ = molecular weight, g/mol
$n$ = number of steps in numerical method
$N$ = ammonium concentration, g/m$^3$
$P$ = pressure, atm
$pG$ = polysaccharide concentration, g/m$^3$
$QO_{2max}$ = maximum specific oxygen uptake rate, $gO_2/g_{pellet}/s$
$r$ = distance from pellet center, m
$R$ = ideal gas law constant, m$^3$·atm/mol/K
$R$ = characteristic pellet radius, m
$R_{CO_2}$ = bulk rate of carbon dioxide production, g/m$^3$/s

$r_{O_2}$ = rate of respiration by pellets, g/m$^3$ pellet/s
$Rg$ = bulk rate of glucose uptake, g/m$^3$/s
$R_{O_2}$ = bulk rate of oxygen consumption, g/m$^3$/s
$R_n$ = bulk rate of ammonium uptake, g/m$^3$/s
$R_{pg}$ = bulk rate of polysaccharide synthesis, g/m$^3$/s
$R_x$ = bulk rate of biomass production, g/m$^3$/s
$T$ = temperature, K
$t_{lag}$ = length of the lag phase, s
$V$ = reaction rate equation for oxygen, g/m$^3$ pellet/s
$V_{hs}$ = head space volume, m$^3$
$V_l$ = liquid-phase volume, m$^3$
$V_{max}$ = rate constant for oxygen, g/m$^3$ pellet/s
$V_{pel}$ = characteristic pellet volume, m$^3$
$X_f$ = final biomass concentration, g/m$^3$
$Y_{CO_2/O_2}$ = $CO_2$ produced per oxygen consumed, g/g
$Y_{g/O_2}$ = glucose used per oxygen consumed, g/g
$Y_{g/x}$ = growth yield coefficient for glucose, g/g
$Y_{n/x}$ = growth yield coefficient for nitrogen, g/g

### *Greek letters*

$\phi$ = first-order modulus
$\Phi$ = observed mass-transfer modulus
$\eta$ = pellet number, m$^3$
$\mu_e$ = effective growth constant, s$^{-1}$
$\mu_{max}$ = growth rate constant, s$^{-1}$
$\rho$ = pellet density (dry weight/pellet volume), g/m$^3$

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