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Mathematical Modeling of Lipase and Protease Production by *Penicillium restrictum* in a Batch Fermenter

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Abstract

This work presents a mathematical model that describes time course variations of extracellular lipase and protease activities for the batch fermentation of the fungus *Penicillium restrictum*, a new and promising strain isolated from soil and wastes of a Brazilian babassu coconut oil industry. The fermentation process was modeled by an unstructured model, which considered the following dependent variables: cells, fat acid, dissolved oxygen concentrations, lipase and protease activities, and cell lysate concentration. The last variable represents the amount of cells that has been lysed by the shear stress and natural cell death. Proteases released to the medium, as consequence of this process, enhance lipase inactivation. The model is able to predict the effects of some operation variables such as air flow rate and agitation speed. The mathematical model was validated against batch-fermentation data obtained under several operating conditions. Because substrate concentration has antagonistic effects on lipase activity, a typical optimization scheme should be developed in order to minimize these deleterious effects while maximizing lipase activity.

Index Entries: Lipase; protease; unstructured model; kinetics; *Penicillium restrictum*.

Introduction

Mathematical modeling is an important tool for optimization and control of biotechnological processes. Despite the major interest for lipase production, few works deal with the modeling of this enzyme production

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(1–3). Enzyme production by *Penicillium* presents some challenges for mathematical modeling because of the biomass morphological changes that occur during cultivation and the complex rheological characteristics of the fermentation broth *(4,5)*. Additionally, proteases are produced by these fungi, affecting lipase stability *(6)*. To take into account biomass growth, substrate consumption, lipase and protease production, and the interaction between these enzymes, an unstructured model was chosen because it is able to describe with a desirable degree of simplicity the most fundamental aspects of the fermentation process.

In previous works *(5,6)*, the potential of *Penicillium restrictum* for lipase production was characterized using different nutritional and operational conditions, leading to a large number of experimental data. Thus, the aim of this work was to model the complex behavior of the fermentation process, determining its main kinetic parameters.

Materials and Methods

Fermentation Process

Lipase and protease were produced during the fermentation of the fungus *Penicillium restrictum* upon variable conditions of stirring speed and aeration. Olive oil, used as the main carbon source, is hydrolyzed in the first hours of fermentation by the lipases contained in the inoculum, as observed by Serra et al. *(7)*. Thus, lipase is produced in large amounts associated with the consumption of fatty acids and biomass accumulates in the fermenter. When cell lysis is intensified, protease is increasingly detected in the medium, causing lipase activity to decrease.

Experimental System

Experiments were carried out in a 5-L New Brunswick Fermenter (Bigflo II) and the following operation conditions were used: stirring speed of 100, 200, and 300 rpm, specific air flow rate of 0.5, 0.75, and 1.0 wm, temperature of 30°C, initial pH of 5.5 (not controlled). Media composition, operation conditions, biomass and lipids concentrations, and lipase and protease activity determinations were described in previous works *(5,6)*.

Model Development

Qualitative Considerations

The proposed model considers that high lipase levels are associated with the consumption of free fatty acids, which occurs after oil hydrolysis, catalyzed by inoculum lipase, and complete glycerol utilization *(7)*.

In a previous work *(5)*, several fermentation tests were conducted at different aeration rates and agitation speeds. It was observed that cellular decay rose with the impeller speed, as a consequence of shear stress increase in the cultivation medium. Extracellular lipase production was associated

with growth rate and protease was late detected in the cultivation medium (after 96 h) associated with cell lysis. Protease levels seems to be responsible for lipase activity decay *(5,6).*

Mathematical Model

Equations and assumptions are presented below.

Cell Concentration Variation

$$\frac{dX}{dt} = \mu X - \alpha X + \beta X_e \tag{1}$$

$$\frac{dX_e}{dt} = \alpha X Y_{Xe/X} - \beta X_e \tag{2}$$

where,

$$\mu = \frac{\mu_{max} S}{BX + S}\left(1 - \frac{X}{X_{max}}\right) f(O) \tag{3}$$

$$\beta = k_\beta \, f(O) \tag{4}$$

and

$$f(O) = \frac{\log(1+O)}{\log(1+O^{sat})} \tag{5}$$

with the following initial conditions: $X_{t=ti} = X_0$ and $X_{e\,t=ti} = 0$,

In Eq. 1, X is the cell concentration and X_e is the concentration of lysate cells, μX is the growth rate, βX_e represents the lysate extract assimilation rate by cells and αX is the cell lysis rate. The term $Y_{Xe/x}$ (lysate cell conversion factor), shown in Eq. 2, is evidently equal to 1, as each lysate cell comes from an active one. Equation 3 represents specific growth rate that follows a modified Contois *(9)*. The empirical Eq. 5 was utilized to include the effect of oxygen availability on biomass rate Eqs. 3 and 4.

Experimental data *(5)* show that the lysis rate is related to the agitation speed (N) and that the biomass concentration tends to a final stable value, suggesting equilibrium between lysis and lysate extract absorption by the viable fungal cells. Furthermore, it was observed that cell lysis and consequent protease activity only occur after some hours of fermentation. To take into account these experimental evidences, the following equations were proposed:

$$\alpha = k_0 \, e^{(-k_1 \mu)} \tag{6}$$

$$k_0 = 0.01 + 10^{-4} N \tag{7}$$

α depends on k_0 and μ (Eq. 6), k_0 being a linear function of the agitation speed (Eq. 7). When μ tends to zero (substrate exhaustion), α is maximum (k_0).

Substrate Concentration Variation

The model considers that secondary substrate consumption (only fatty acids are taken as substrate) only occurs after 6 h of fermentation, as previously commented.

$$\frac{dS}{dt} = -q_s X \tag{8}$$

with the following initial condition: $S_{t = ti} = S_0$
where,

$$q_s = \frac{\mu}{Y_{X/S}} + m \tag{9}$$

$$m = \frac{m' S}{K_m + S}\left(\frac{X}{X_{max}}\right) f(O) \tag{10}$$

Substrate is utilized for cell growth and maintenance as represented by the above equations. Maintenance was represented by the empirical Eq. 10.

Dissolved Oxygen Variation

$$\frac{dO}{dt} = \frac{-q_s X Y_{X/S}}{Y_{X/O}} - \frac{\beta X_e}{Y_{Xe/X} Y_{X/O}} + K_{la}(O^{sat} - O) \tag{11}$$

where $O_{t = ti} = O_0$.

The terms shown in the above equation correspond to oxygen consumption for cell growth and cell maintenance, lysate assimilation, and oxygen supply to the medium by aeration and agitation. The volumetric oxygen transfer coefficient (K_{la}) values, at different air flow rates and agitation speeds, were obtained from Bandino et al. *(8)* that worked with a similar fermenter (Bigflo C 32) and the fungus *Penicillium chrysogenum.*

Lipase Activity

Extracellular lipase activity detected in the culture medium was represented by the following equation:

$$\frac{dU_L}{dt} = Y_{U_L/X} \mu X - k_p U_p \tag{12}$$

where $U_L\ (t = t_i) = U_{L0}$.

Lipase activity accumulation is a result of two contributions, i.e., lipase production associated with cell growth and enzyme activity decay caused by protease activity. $Y_{UL/X}$ is the lipase yield coefficient, k_p is a parameter that takes into account the effect of protease on lipase inactivation and U_L and U_P are, respectively the lipase and protease activities in the culture medium.

Table 1
Constants and Parameters Used in the Model

$Y_{x/s}$ (gg^{-1})	2.0
B (gg^{-1})	0.75
$Y_{Xe/X}$ (gg^{-1})	1.0
$Y_{X/O}$ (gmg^{-1})	0.00135
μ_{max} (h^{-1})	0.2
$Y_{U_L/X}$ (U_1g^{-1})	1.1
Y_{U_P/X_e} (U_pg^{-1})	0.63
Kβ (h^{-1})	0.06
Kp ($U_LU_P^{-1}h^{-1}$)	0.03
k1 (h)	200.0
K_m (gL^{-1})	0.75
m′ ($gg^{-1}h^{-1}$)	0.01
O^{sat} (mgL^{-1})	7.5
X_{max} (gL^{-1})	14.0

Protease Activity

Equation 13 represents the variation of protease activity in the medium.

$$\frac{dU_p}{dt} = Y_{U_P/X_e}\alpha X_e \tag{13}$$

where Up (t = ti) = 0.

Protease is an enzyme, which accumulates in the medium as a consequence of cell lysis. The above empirical equation represents the retarded presence of protease activity in the fermentation broth. The parameter $Y_{UP/Xe}$ should be estimated from the experimental data and may be considered a yield factor (protease activity from lysed cells).

Simulation Results

To calibrate the proposed model or, in other terms, to find its parameters, a set of experiments representing the different experimental conditions (specific air flow rates and agitation speeds) was chosen. Parameters were obtained by visual inspection, based on previous determinations *(5,6)*, except the yield coefficient biomass-oxygen ($Y_{x/o}$), which was taken from the literature *(9)*. The model differential equations were solved by using the STIFF3 algorithm *(10).* The parameters that calibrate the model and were used in the simulation procedure are shown in Table 1.

Simulation and comparison with experimental data, performed for experiments carried out at several conditions, are shown in Figs. 1–5. Simulated time course fermentation variables were: biomass and substrate concentration, lipase and protease activity.

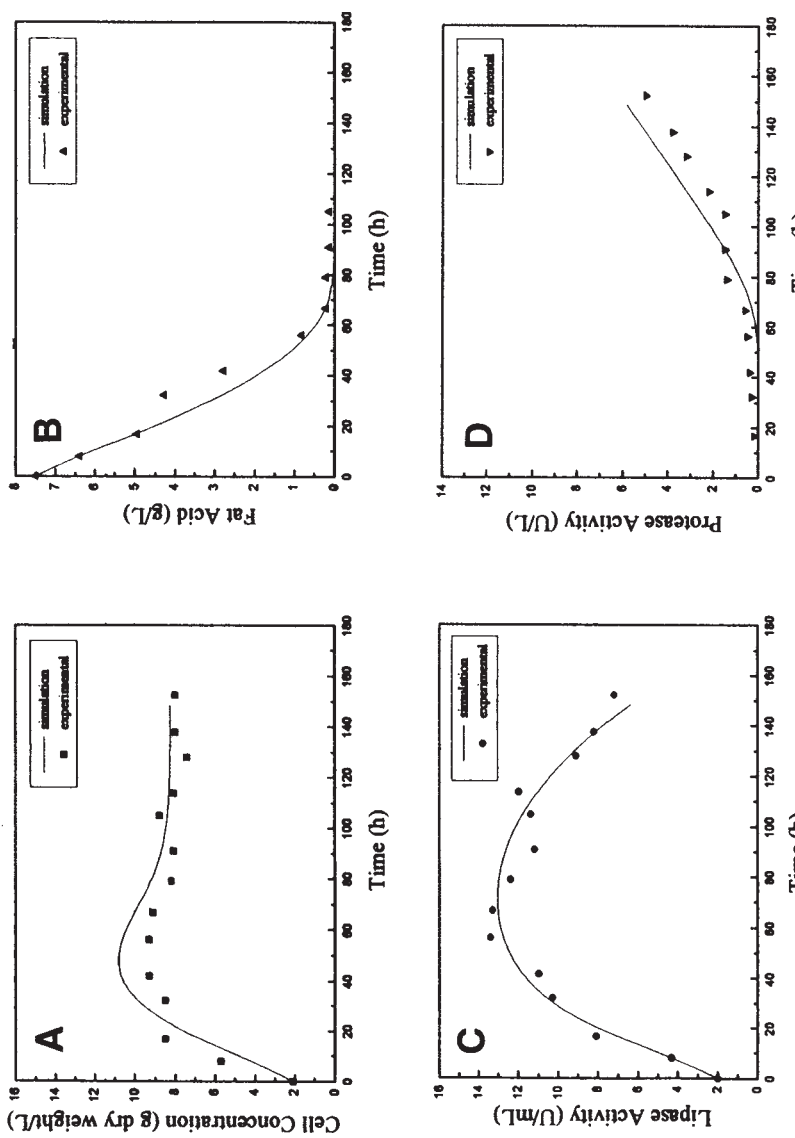

Fig. 1. Experimental time-course variation and simulation results. Fermentation carried out at 200 rpm, 0.5 vvm. Initial conditions: $X_0 = 2.1$ g/L, $S_0 = 7.5$ g/L, $O_0 = 6.0$ mg/L, $U_{L0} = 2.0$ U/L. Adopted $K_{la} = 60$ (h^{-1}). **(A)** biomass (■); **(B)** substrate (▲); **(C)** lipase activity (●); **(D)** protease activity (▼). Simulated results (—).

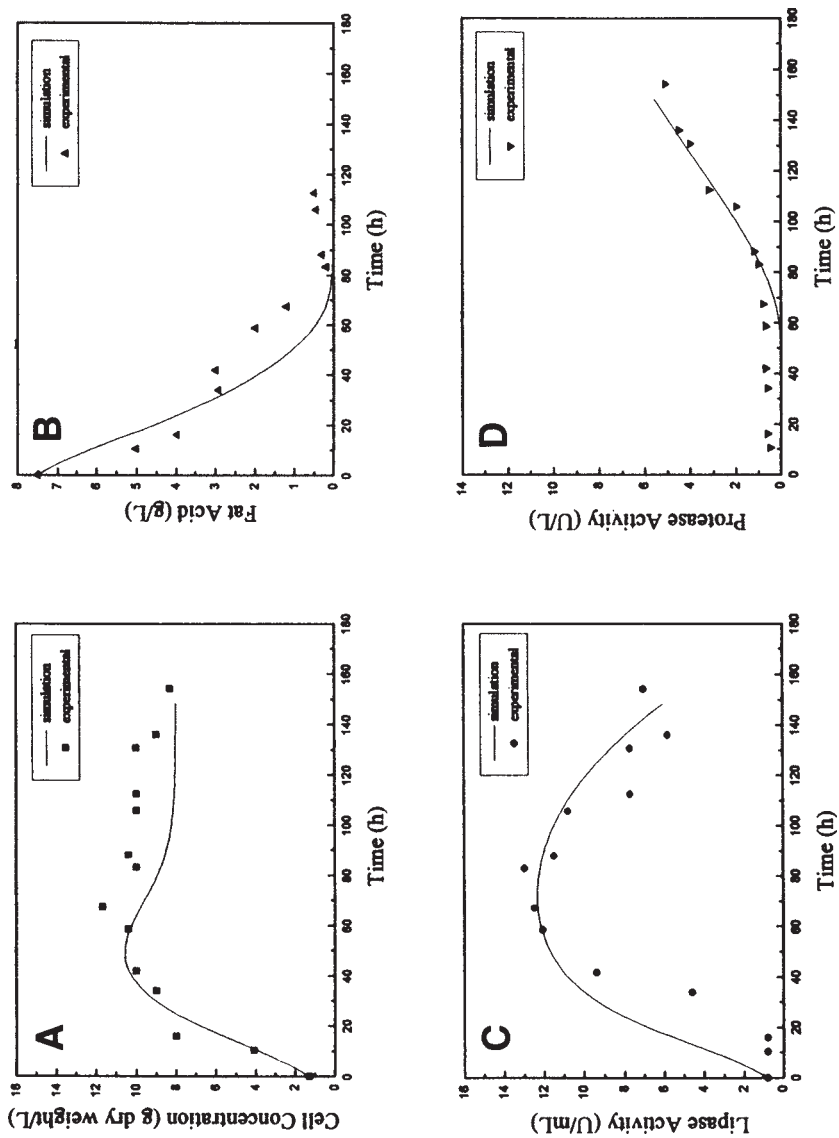

Fig. 2. Experimental time-course variation and simulation results. Fermentation carried out at 200 rpm, 0.75 vvm. Initial conditions: $X_0 = 1.3$ g/L, $S_0 = 7.5$ g/L, $O_0 = 6.0$ mg/L, $U_{L0} = 0.8$ U/L. Adopted $K_{la} = 72$ (h^{-1}). **(A)** biomass (■); **(B)** substrate (▲); **(C)** lipase activity (●); **(D)** protease activity (▼). Simulated results (—).

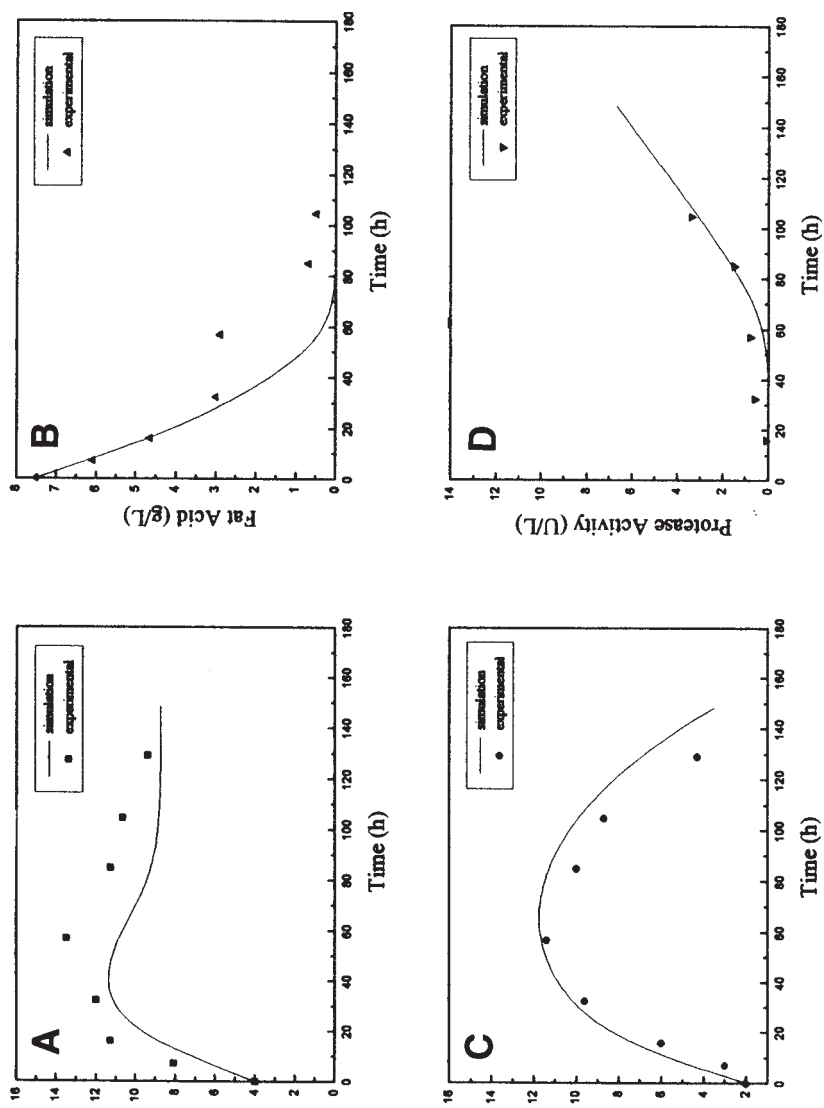

Fig. 3. Experimental time-course variation and simulation results. Fermentation carried out at 200 rpm, 1.0 vvm Initial conditions: $X_0 = 4.0$ g/L, $S_0 = 7.5$ g/L, $O_0 = 6.0$ mg/L, $U_{L0} = 2.0$ U/L. Adopted $K_{la} = 100$ (h^{-1}). **(A)** biomass (■); **(B)** substrate (▲); **(C)** lipase activity (●); **(D)** protease activity (▼). Simulated results (—).

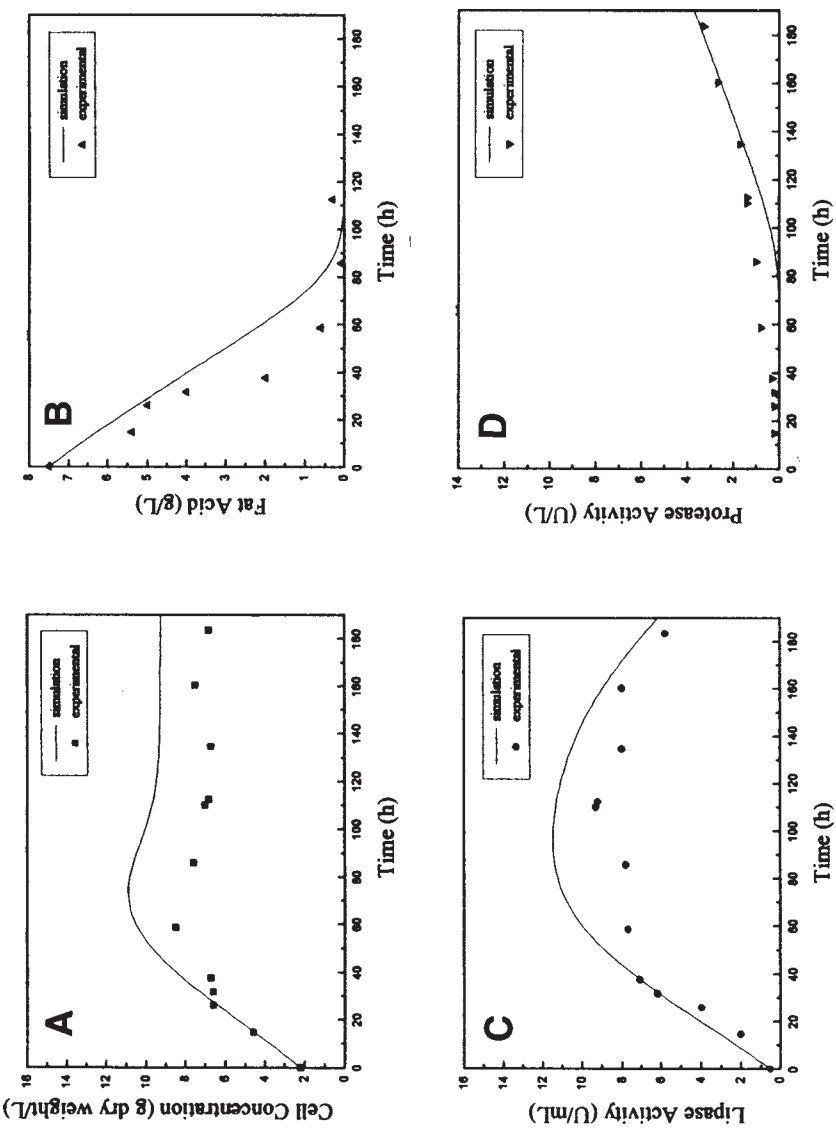

Fig. 4. Experimental time-course variation and simulation results. Fermentation carried out at 100 rpm, 0.5 vvm Initial conditions: X_0 = 2.2 g/L, S_0 = 7.5 g/L, O_0 = 6.0 mg/L, U_{L0} = 0.5 U/L. Adopted K_{la} = 20 (h^{-1}). **(A)** biomass (■); **(B)** substrate (▲); **(C)** lipase activity (●); **(D)** protease activity (▼). Simulated results (—).

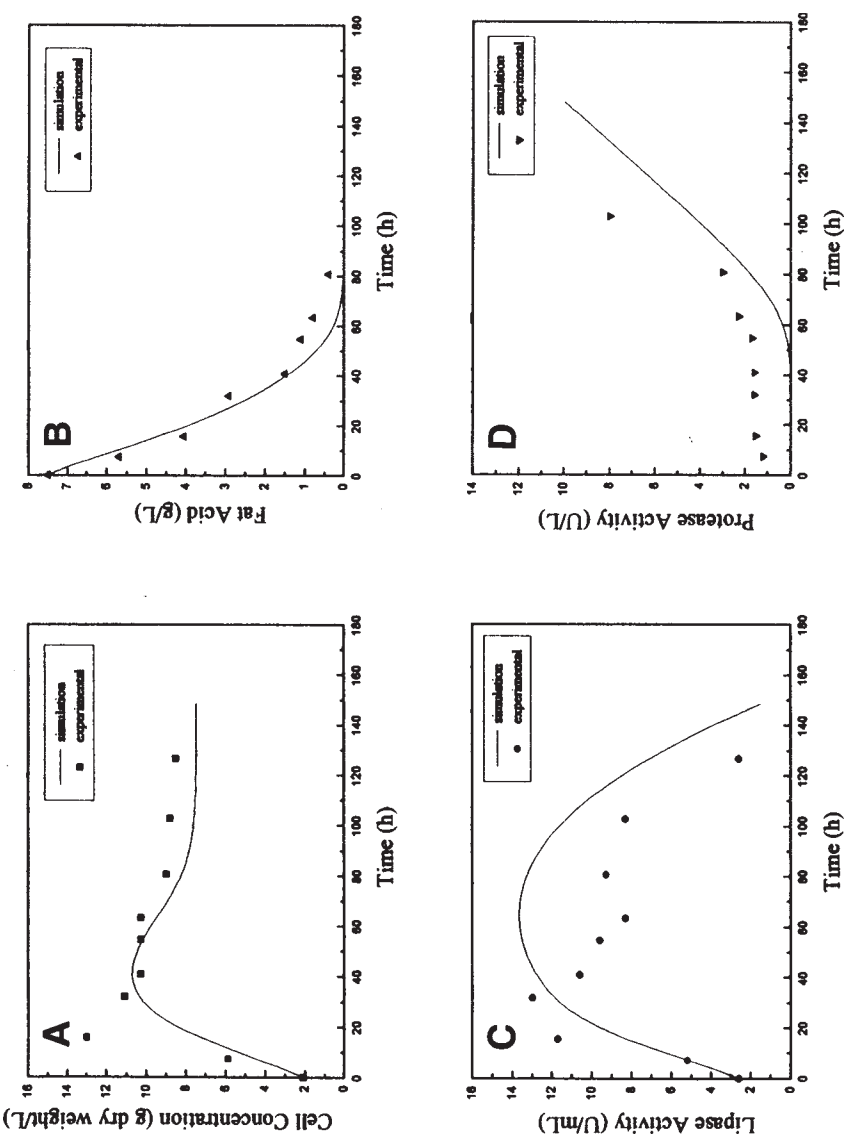

Fig. 5. Experimental time-course variation and simulation results. Fermentation carried out at 300 rpr4 0.5 vvm Initial conditions: X_0 = 2.1 g/L, S_0 = 7.5 g/L, O_0 = 6.0 mg/L, U_{L0} = 2.6 U/L. Adopted K_{la} = 160 (h^{-1}). **(A)** biomass (■); **(B)** substrate (▲); **(C)** lipase activity (●); **(D)** protease activity (▼). Simulated results (—).

Lipase and protease production by *Penicillium restrictum* was well-simulated by the model, for different fermentation conditions. The same was observed for biomass and substrate variation. For the experiments conducted at 200 rpm and 0.5 wm, which was the best condition for lipase production *(5)*, the simulation succeeded particularly well (Fig. 1).

Oxygen consumption during fermentation was only simulated because dissolved oxygen measurement was very difficult to perform in such complex and viscous medium. The simulation indicates that dissolved oxygen falls from 80% of the saturation value, at the beginning of the fermentation (6 h), reaches 20% of that value at the end of the fungal growth phase, and rises when the stationary growth phase is reached. That behavior is typical of filamentous fungal fermentation *(11).*

In conclusion, it can be stated that an unstructured model that describes the lipase and protease production by *Penicillium restrictum* has been developed, calibrated, and validated. The combination of process qualitative information obtained from experiments and the development of an adequate mathematical model is an important tool for process operation and optimization. As lipase, the target product of the fermentation process, is affected by substrate concentration and protease activity *(5)*, a typical scheme of process operation should be developed, based on model information, in order to minimize these effects and maximize lipase production.

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