Morphologically Structured Model for Antitumoral Retamycin Production During Batch and Fed-Batch Cultivations of *Streptomyces olindensis*

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Abstract: A morphologically structured model is proposed to describe trends in biomass growth, substrate consumption, and antitumoral retamycin production during batch and fed-batch cultivations of *Streptomyces olindensis*. Filamentous biomass is structured into three morphological compartments (apical, subapical, and hyphal), and the production of retamycin, a secondary metabolite, is assumed to take place in the subapical cell compartment. Model accounts for the effect of glucose as well as complex nitrogen source on both the biomass growth and retamycin production. Laboratory data from bench-scale batch and fed-batch fermentations were used to estimate some model parameters by nonlinear regression. The predictive capability of the model was then tested for additional fed-batch and continuous experiments not used in the previous fitting procedure. The model predictions show fair agreement to the experimental data. The proposed model can be useful for further studies on process optimization and control.

Keywords: *Streptomyces*; morphologically structured models; antibiotic; retamycin; fed-batch cultivation; mathematical modeling

INTRODUCTION

A broad variety of secondary metabolites of commercial importance, including antibiotics, is produced by filamentous microorganisms, such as fungi and *Actinomycetes* in submerged cultures. *Streptomyces* spp. is one of the most important microorganisms used in antibiotic production. Retamycin is an anthracycline antibiotic complex with potent antitumor activity (Bieber et al., 1989), similar to daunorubicin and doxorubicin (adriamycin), showing positive results in the treatment of human leukemias (Asfora et al., 1972). Retamycin complex is a red powder with low solubility in water and high solubility in organic solvents (methanol, chloroform), and is produced as a secondary metabolite by submerged cultivations of *Streptomyces olindensis* (Lima et al., 1972). The isolation of new anthracyclins has been pursued in attempts to overcome its undesirable cardiotoxicity (Scotti and Hutchinson, 1995). Only a few studies have been published about this antibiotic, but retamycin showed promising results in the intercalation into DNA, which makes this antibiotic suitable to be used as a chemotherapy agent (Latorre, 2001).

The present article focuses on the development of a mathematical model able to describe the production of retamycin, the consumption of glucose, and the biomass growth during batch and fed-batch fermentations of *S. olindensis*.

Growth and production of secondary metabolites from filamentous microorganisms involves complex, and yet poorly understood processes, even for the most studied and commercially employed cases, such as penicillin production. Filamentous microorganisms present a multicompartment structure of morphologically heterogeneous hyphae. Cellular differentiation changes during growth and plays an important role on the production of secondary metabolites.

Models with different complexity have been presented in the literature for cultivation of filamentous microorganisms, although none has specifically focused on *S. olindensis*. The most studied processes of secondary metabolite production in filamentous microorganism cultivation are the production of penicillin and of cephalosporin C.

Modeling the growth and product formation characteristics of several microorganisms is a very challenging task. There are many different approaches to modeling the microbial kinetics (for an excellent review, see Nielsen and Villadsen, 1992). The model complexity can be varied from simple unstructured models to morphologically and intracellularly structured models (Nielsen and Villadsen, 1994).

Unstructured models are the simpler ones, in which the biomass is viewed as homogeneous and its properties are considered constant over time, being characterized only by the

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cell mass. These models in general also incorporate Monod kinetic terms or other empirical expressions. This type of model is less suited for the complex phenomena observed in processes involving filamentous microorganisms.

Simple structured models take into account variations in the growth and production properties of the cells, according to genetic, age-related, or morphological differences. More complex segregated models may account for a continuous variation of a cellular property, which may be related to individual cells, e.g., age, size, or intrinsic concentration of a certain cellular component.

Several studies about the modeling of the growth and morphology of filamentous microorganisms have been published (Nielsen, 1992). Examples of unstructured models for penicillin fermentation are the works of Bajpai and Reuβ 1980 and Menezes et al. (1994). Examples of structured models of different degrees of complexity applied to a number of filamentous microorganisms cultivations are found in the works by Megee et al. (1970), Matsumura et al. (1981), Nielsen (1993), Tiller et al. (1994), Meyerhoff and Bellgardt (1995), Paul and Thomas (1996), King (1997), Zangirolami et al. (1997), Paul et al. (1998), Bellgardt (1998), Zangirolami et al. (2002), and Birol et al. (2002). Several studies on filamentous microorganism cultivation have indicated that secondary metabolite production is associated with changes in morphological forms of the cells, thus favoring the morphologically structured model approach.

The aim of the present work was to develop a morphologically structured mathematical model for the growth, substrate consumption, and retamycin production during cultivation of *S. olindensis*. Model development was based on data from a series of batch and fed-batch runs carried out in a lab-scale fermentor.

MODEL DEVELOPMENT

The model proposed here is mostly based on the morphologically structured model presented by Nielsen (1993), and also incorporates some ideas from other related works (e.g., Zangirolami et al., 1997; Paul and Thomas, 1996; Paul et al., 1998). This model was chosen because it is simpler than other morphologically structured models proposed in the literature and was able to reasonably describe the growth of different types of filamentous microorganisms.

The morphological structure of the model is described in detail elsewhere (Nielsen, 1993; Nielsen and Villadsen, 1994) and is only summarized here. The hyphae is considered divided into three cell compartments or regions: apical (Z_a), subapical (Z_s), and hyphal (Z_h). The apical compartment is defined as the part of the hyphal element between the tip and the first septum. The subapical compartment is the following part of the hyphal element, just behind the apical compartment. Both apical and subapical compartments are active in substrate utilization and growth and are capable of producing the secondary metabolite (retamycin, in the case under consideration). The hyphal compartment is farthest from the tip and is inactive in terms of substrate utilization and secondary metabolite production.

Following Nielsen (1993), three metamorphosis processes are considered in the model: branching, tip extension, and differentiation. Branching represents the appearance of new apical compartments at certain points in the subapical compartment. Tip extension results in the formation of new subapical cells from apical cells. As the cell age increases, the cell becomes more and more vacuolated and changes its metabolism. In this process, called differentiation, inactive vacuolated hyphal cells are formed from the subapical cells.

The kinetic expressions for the morphological transformations are given as:

$$\text{Branching} \qquad Z_s \rightarrow Z_a \qquad u_1 = k_{u1} Z_s \tag{1}$$

$$\text{Tip extension} \qquad Z_a \rightarrow Z_s \qquad u_2 = k_{u2} Z_a \tag{2}$$

$$\text{Differentiation} \qquad Z_s \rightarrow Z_h \qquad u_3 = \frac{k_{u3} Z_s}{S K_{u3} + 1} \tag{3}$$

where Z_a, Z_s, and Z_h are the mass fraction of each morphological portion.

In the cultivations of *S. olindensis* used in the present work, yeast extract and Tris(hydroxymethyl)aminomethane (THAM) were the main nitrogen sources present in the fermentation medium. Yeast extract contains a large number of nutrients, thus making it difficult to specify each of the components contributing to the growth in this complex medium. In the present work the nitrogen sources from both the yeast extract and THAM were pooled together as a unique component, N (in terms of NH_3-equivalent):

$$N = \beta_1 C_{ye} + \beta_2 C_{THAM} \tag{4}$$

where β_1 and β_2 are the conversion factors of yeast extract and of THAM into NH_3-equivalent nitrogen substrate. The elemental composition for yeast extract is assumed to be $CH_{1.8}O_{0.5}N_{0.2}$ (Nielsen and Villadsen, 1994), which results in β_1 = 0.138 g NH_3/g DW yeast extract. The elemental composition of THAM is $C_4H_{12}O_3N$, which gives β_2 = 0.139 g NH_3/g THAM.

Growth of apical and subapical cells is described by a Monod type kinetics, including the effect of both glucose and nitrogen source in a multiplicative form (Merchuk and Asenjo, 1991):

$$\mu_a = \mu_s = k \left(\frac{S}{S + K_s} \right) \left(\frac{N}{N + K_N} \right) \tag{5}$$

where N is the concentration of nitrogen source. The same growth kinetics was considered for the apical and subapical cells ($\mu_a = \mu_s$). Zangirolami et al. (1997) considered the possibility that a fraction f_h of the hyphal cells be active for

growth and metabolite production. Here we adopted the simpler approach of the original work of Nielsen (1993) in which such a possibility is not considered ($f_h = 0$):

$$\mu = \mu_a Z_a + \mu_s Z_s \tag{6}$$

Zangirolami et al. (1997) considered only the effect of glucose in the growth kinetics. Birol et al. (2002) introduced the effect of oxygen, based on the earlier modeling work on penicillin production by Bajpai and Reuβ (1980). In (almost all) the experiments reported in the present work, the dissolved oxygen concentration was above 40% of saturation. Under these conditions the assumption of oxygen limitation-free is reasonable (Bajpai and Reuβ, 1980), and therefore the effect of oxygen on the kinetics was not considered. On the other hand, the experiments with different amounts of glucose and of other nutrients (yeast extract, nitrogen source) suggested that the effect of nitrogen source on the kinetics of both growth and production should be included in the model. This was done, as shown in the last factor in Eq. (5) for the growth kinetics, in a simple saturation-type form with only one additional parameter (K_N). Comparing previous works on modeling filamentous microorganism cultivation, different approaches are found: Menezes et al. (1994) considered the carbon-amino substrates present in complex fermentation media as combined with glucose in a single substrate; Zangirolami et al. (1997) considered the pool of nutrients from corn steep liquor as a separated (but similar to the glucose) substrate; and Bellgardt (1998) considered the ''pharma'' medium as a truly second nutrient.

The formation of retamycin from glucose and nitrogen is assumed to occur only at the subapical compartment, following a simple Monod type kinetics for the effect of glucose. The inhibitory effect of nitrogen on retamycin production is included in a multiplicative form (Merchuk and Asenjo, 1991):

$$r_p = k_2 \left(\frac{S}{S + K_2}\right)\left(\frac{K_N}{N + K_N}\right) Z_s \tag{7}$$

The uptake rate of glucose includes the consumption of glucose for biomass growth (through a stoichiometric coefficient α_1 g glucose/g biomass dry basis), for retamycin production (through a stoichiometric coefficient α_2 g glucose/g retamycin) and for biomass maintenance (m_s):

$$r_s = \alpha_1 \mu + \alpha_2 r_p + m_s f_s(S) \tag{8}$$

Consumption of glucose for biomass maintenance was modeled as substrate concentration-dependent, following ideas similar to those presented by Guardiola et al. (1995) and Paul et al. (1998). While glucose is available, a certain amount can be used to maintain the existing cells. The function $f_s(S)$ in Eq. (8) accounts for the decrease of the specific rate of glucose consumption for maintenance as glucose concentration vanishes. This dependence avoids nonfeasible situations (negative substrate concentrations). A simple Monod type function is used to represent such behavior:

$$f_s = \frac{S}{S + K_{ms}} \tag{9}$$

Furthermore, biomass can also be degraded for maintenance purposes at low substrate concentrations. Biomass degradation was assumed to occur only for the hyphal compartments. The specific rate of biomass degradation for maintenance increases in order to compensate for the lack of glucose in the culture. Following the work of Guardiola et al. (1995), this behavior can be accounted for by the following expression:

$$r_d = k_d[1 - f_s(S)]Z_h \tag{10}$$

The uptake rate of the nitrogen source substrate includes the consumption of nitrogen for biomass growth (through a stoichiometric coefficient α_3 g NH_3/g biomass dry basis) and for retamycin production (through a stoichiometric coefficient α_4 g NH_3/g retamycin):

$$r_N = \alpha_3(\mu - r_d) + \alpha_4 r_p \tag{11}$$

From the average elemental composition of retamycin complex $C_{40}H_{53}O_{13.5}N$ (Bieber et al., 1989), we estimated the coefficient $\alpha_4 = 0.022$ g NH_3/g retamycin. As cell lysis contributes to the reformation of nitrogen constituents, the rate of biomass degradation is discounted in the first term of Eq. (11).

Taking into account the kinetic and stoichiometric relations previously presented, the differential balance equations for the fractions of different morphological compartments, for the reactor volume, for the total biomass, for the product (retamycin), for glucose, and for the nitrogen source substrate, in a well-mixed fed-batch fermentor, are given as follows:

$$\frac{dZ_a}{dt} = u_1 - u_2 + \mu_a Z_a - (\mu - r_d)Z_a \tag{12}$$

$$\frac{dZ_s}{dt} = u_2 - u_1 - u_3 + \mu_s Z_s - (\mu - r_d)Z_s \tag{13}$$

$$\frac{dZ_h}{dt} = u_3 - (\mu - r_d)Z_h - r_d \tag{14}$$

$$\frac{dV}{dt} = F - F_{sampling} \tag{15}$$

$$\frac{dX}{dt} = (\mu - r_d)X - \frac{F}{V}X \tag{16}$$

$$\frac{dP}{dt} = r_p X - \frac{F}{V} P \tag{17}$$

$$\frac{dS}{dt} = (S_F - S)\frac{F}{V} - r_s X \tag{18}$$

$$\frac{dN}{dt} = (N_F - N)\frac{F}{V} - r_N X \tag{19}$$

Product hydrolysis was not considered in the model, as this effect was not clearly observed in the experimental data. Measurements of the excreted (extracellular) retamicyn, P_{ext}, along with the total (extracellular and intracellular) retamycin concentration, P, were done in the experiments and a simple linear relationship between both concentrations was found (see Fig. 1):

$$P_{ext} = K_p P \tag{20}$$

From the whole set of measurements of total and extracellular retamycin, the estimated value of $K_p = 0.839 \pm 0.009$ was obtained, as shown in Figure 1.

MODEL IDENTIFICATION

Materials and Methods

Microorganism

A mutant strain of *Streptomyces olindensis* ICB20 was supplied by Laboratório de Genética de Microrganismos/Instituto de Ciência Biomédicas/Universidade de São Paulo. The cells were stored in cryotubes containing glycerol 20%, at −20°C (Guimarães, 2000).

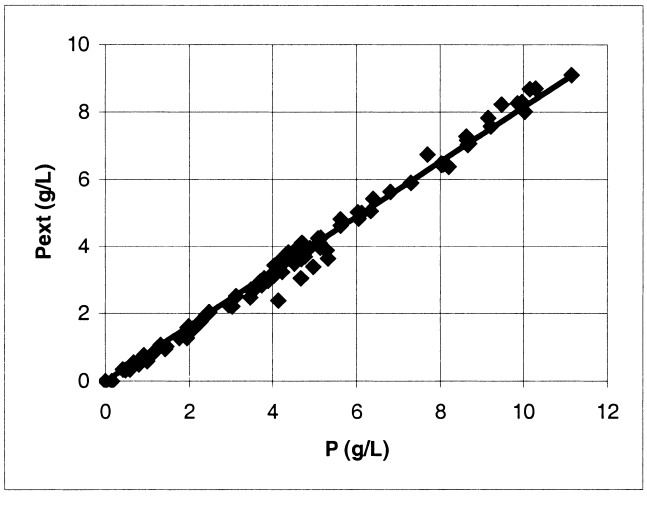

Figure 1. Extracellular retamycin as function of total (intracellular + extracellular) retamycin. Available points of all runs are included in the plot.

Inoculum Preparation

The inoculum for the fermenter runs was prepared in a New Brunswick rotary shaker at 200 rev/min and 30°C in two steps. The first step was carried out for 16 h and the second step was carried out for 24 h. A 10% inoculum was used in the bioreactor inoculation.

Culture Medium

The medium for the inoculum preparation and bioreactor cultivations contained the following nutrients (Guimarães, 2000): glucose (10.0 g/L), yeast extract (5.0 g/L), Tris(hydroxymethyl)-aminometan (3.09 g/L), casein hydrolysate (0.10 g/L), K_2SO_4 (0.25 g/L), and $MgCl_2.6H_2O$ (10.12 g/L). The pH was adjusted to 7.0. After medium sterilization, the following sterile solutions were added (for 250 mL of culture medium): KH_2PO_4 0.5% w/v (2.5 mL), $CaCl_2$ 5M (1.0 mL), and 0.5 mL of a trace elements solution (40 mg $ZnCl_2$, 200 mg $FeCl_3.6H_2O$, 10 mg $(NH_4)_6Mo_7O_{24}.4H_2O$, 10 mg $CuCl_2.2H_2O$, 10 mg $MnCl_2.4H_2O$, 10 mg $Na_2B_4O_7.10H_2O$ in 1,000 mL of distilled water).

Culture Conditions

Batch runs were performed in a 5-L New Brunswick Bioflo fermenter under the following conditions: initial volume = 4.0 L (for batch runs); agitation rate = 500 rev/min; air flow rate = 4 L/min; pH = 7.0; and temperature = 30°C. The fermenter was provided with two flat blade turbine disk impellers with four blades each.

Fed-batch runs were performed under the same conditions, except initial volume = 3.5 L and feed volume = 1.0 L. The feeding policy was done as described in Pamboukian and Facciotti (2002): after an initial batch period cultivation (varying from 13–21 h) the feed flow rate was started and varied according to an exponential function, then followed by a second batch period.

Experimental Methodology

Samples collected periodically from the fermenter were evaluated for biomass, glucose concentration, and retamycin concentration. Biomass (X) was evaluated after vacuum filtration and drying and glucose concentration (S) was determined by a glucose-oxidase method (Guimarães, 2000). The antibiotic retamycin (P) is a pigmented compound (red) and its concentration was measured colorimetrically as the absorbance at wavelength 547 nm after sample vacuum filtration and adjusting the pH to 6.3 (Guimarães, 2000). The values of retamycin concentration reported here were obtained from a calibration curve determined from a partially purified antibiotic mixture; thus, they should be regarded as such a mixture rather than as pure antibiotic.

Table I. Parameter set.

Parameter	Value	Unit	Comments
k_{u1}	2.3	h^{-1}	Nielsen (1993)
k_{u2}	0.7	h^{-1}	Nielsen (1993)
k_{u3}	0.85	h^{-1}	Nielsen (1993)
K_{u3}	4.0	L/g glucose	Nielsen (1993)
β_1	0.138	g NH_3/g yeast extract	Elemental composition of yeast
β_2	0.139	g NH_3/g THAM	Elemental composition of THAM
f_h	0	g Z_h active/g Z_h total	Nielsen (1993)
k	0.33	h^{-1}	Attributed
K_S	0.03	g glucose/L	Nielsen (1993)
k_2	0.271 ± 0.007	h^{-1}	Estimated by NLR*
K_{S2}	0.1	g glucose/L	Attributed
K_N	0.713 ± 0.027	g NH_3/L	Estimated by NLR*
α_3	0.211 ± 0.003	g NH_3/g biomass DW	Estimated by NLR*
α_4	0.022	g NH_3/g P	Bieber et al. (1989)
α_1	1.117 ± 0.046	g glucose/g biomass DW	Estimated by NLR*
α_2	0.808 ± 0.151	g glucose/g P	Estimated by NLR*
m_s	0.019 ± 0.007	h^{-1}	Estimated by NLR*
K_{ms}	0.05	g glucose/L	Attributed
k_d	0.02	h^{-1}	Attributed
K_p	0.839 ± 0.009	g P extracellular/g P total	Estimated, see Figure 1*
Z_a at t = 0	0.70	g apical cells/g cells	Nielsen (1993)
Z_s at t = 0	0.20	g subapical cells/g cells	Nielsen (1993)
Z_h at t = 0	0.10	g hyphal cells/g cells	Nielsen (1993)

*Estimated parameters, ± standard deviation, NLR = nonlinear regression.

Parameter Estimation

From the 18 parameters originally present in the model, only six were estimated by the nonlinear fitting procedure. Using a similar approach used by other authors (Paul and Thomas, 1996; Zangirolami et al., 1997; Birol et al., 2002), some parameters were taken from the literature, while reasonable arbitrary values were attributed to other ones, and a smaller set of remaining parameters was estimated by fitting the model to the experimental data. As an additional difficulty, the present article is, to our best knowledge, the first one regarding the fermentation kinetics of *S. olindensis* ICB 20, so that in order to attribute values to some parameters, we rely on works that used other similar filamentous microorganisms.

Kinetic parameters related to the metamorphosis processes (k_{u1}, k_{u2}, k_{u3}, K_{Su3}) were assumed to be the same values provided by Nielsen (1993) for *Penicillium chrysogenum*. The same parameter values were assumed for the growth of apical and subapical compartments. The maximum specific growth rate (k) was modified from the value proposed by Nielsen (1993) in order to fit the data. Attributed values for the biomass degradation kinetics (k_d, K_{ms}) were in the range of the reported values for filamentous microorganisms (Guardiola et al., 1995; Matsumura et al., 1981; Menezes et al., 1994; Bellgardt, 1998). Values of yield and stoichiometric coefficients (α_4, β_1, β_2) were presented and discussed in the ''Model Development'' section.

The fractions of the morphological compartments used as initial condition in the simulation were taken as 70% apical, 20% subapical, 10% hyphal compartments, and were the same for all batch and fed-batch experiments. This corresponds to a rapid-growing culture, i.e., high Z_a *and* Z_s (Nielsen, 1993).

Six parameters (k_2, K_N, α_3, α_1, α_2, m_s) were estimated by fitting the model predictions to the experimental data of glucose (S), extracellular retamycin (P_{ext}) and biomass (X) concentrations, using the least-squares criterion:

$$min\ \phi = \sum_{j=1}^{Nruns} \sum_{i=1}^{Npoints} \Big[(S_{i,j,exp} - S_{i,j,calc})^2 + (X_{i,j,exp} - X_{i,j,calc})^2 + (Pe_{i,j,exp} - Pe_{i,j,calc})^2 \Big] \qquad (21)$$

The maximization of likelihood function provides a general formulation for the choice of an objective function in a parameter estimation problem. Depending on the error structure, different objective functions can be obtained,

Table II. Correlation matrix of the estimated parameters.

	k_2	K_N	α_3	α_1	α_2	m_s
k_2	1	0.38	0.21	0.24	−0.15	0.10
K_N	0.38	1	−0.26	0.60	−0.11	−0.04
α_3	0.21	−0.26	1	0.07	0.18	−0.08
α_1	0.24	0.60	0.07	1	0.21	−0.44
α_2	−0.15	−0.11	0.18	0.21	1	−0.96
m_s	0.10	−0.04	−0.08	−0.44	−0.96	1

as discussed by Biegler et al. (1986). Statistically more rigorous criteria would have been to use either the multiresponse estimation procedure of minimizing the determinant of the sum of squares and cross-products of the observations (Hunter, 1967), or a multifunctional error-in-variables method (Reilly and Patino-Leal, 1981). However, for the present case the simple least-squares criterion was adopted.

The calculated values are obtained by solving the system of ordinary differential Eqs. (12) to (19) by a standard variable-step fourth-order Runge-Kutta-Gill method with error control. The minimization of criterion [Eq. (21)] was done using Marquardt's (1963) method with numerical evaluation of the Jacobian matrix.

RESULTS AND DISCUSSION

Table I presents the parameter set. The values of the six estimated parameters are present along with their respective standard deviation. Although some parameters are better estimated than others, all estimated parameters are significant at the 95% confidence level. It is interesting to note that the same parameter set fits all the experiments reasonably well, regardless of the natural batch-to-batch

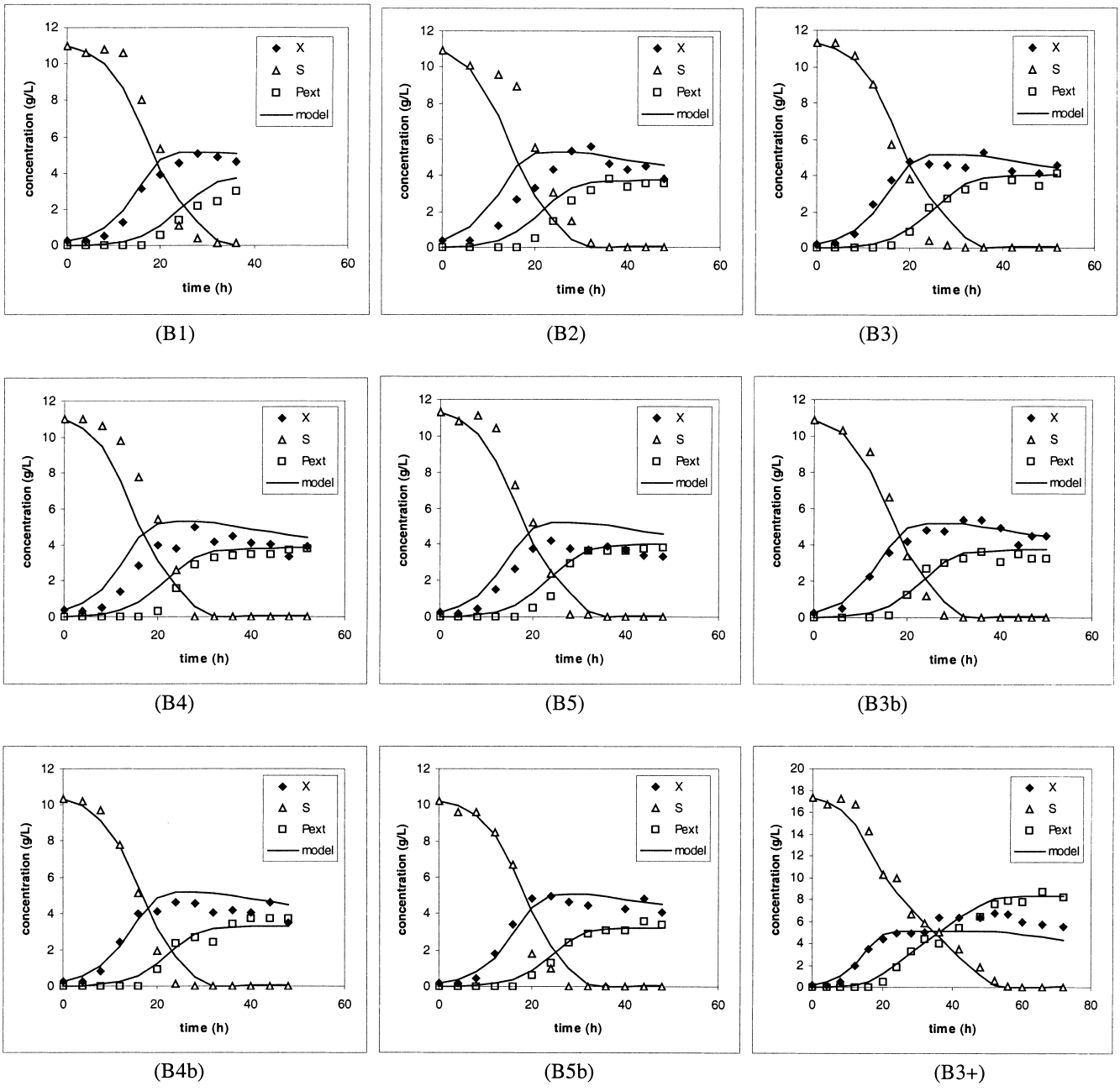

Figure 2. Comparison of simulated curves and experimental points for batch cultivations.

variation commonly found in cultivations of filamentous microorganisms. Table II shows the elements of correlation matrix of the estimated parameters. All correlations between the parameters are reasonably low, except that for the pair α_2 and m_s, which are strongly correlated.

Figures 2 and 3 shows experimental data and simulation results for several batch and fed-batch cultivations used for parameter estimation. The model predictions fit the data for biomass, glucose, and retamycin concentration remarkably well almost throughout the whole cultivation. The decrease in glucose concentration coincides with the increase in biomass concentration. Production of retamycin starts when the biomass growth rate begins to decrease. This point of changing the metabolism from growing to secondary metabolite production is related to the amount of available nitrogen nutrients in the fermentation medium. Retamycin production becomes effective when nitrogen substrate concentration is under a certain limit. This behavior was correctly predicted by the model and was indeed confirmed by experimental measurements of the remaining ammonium

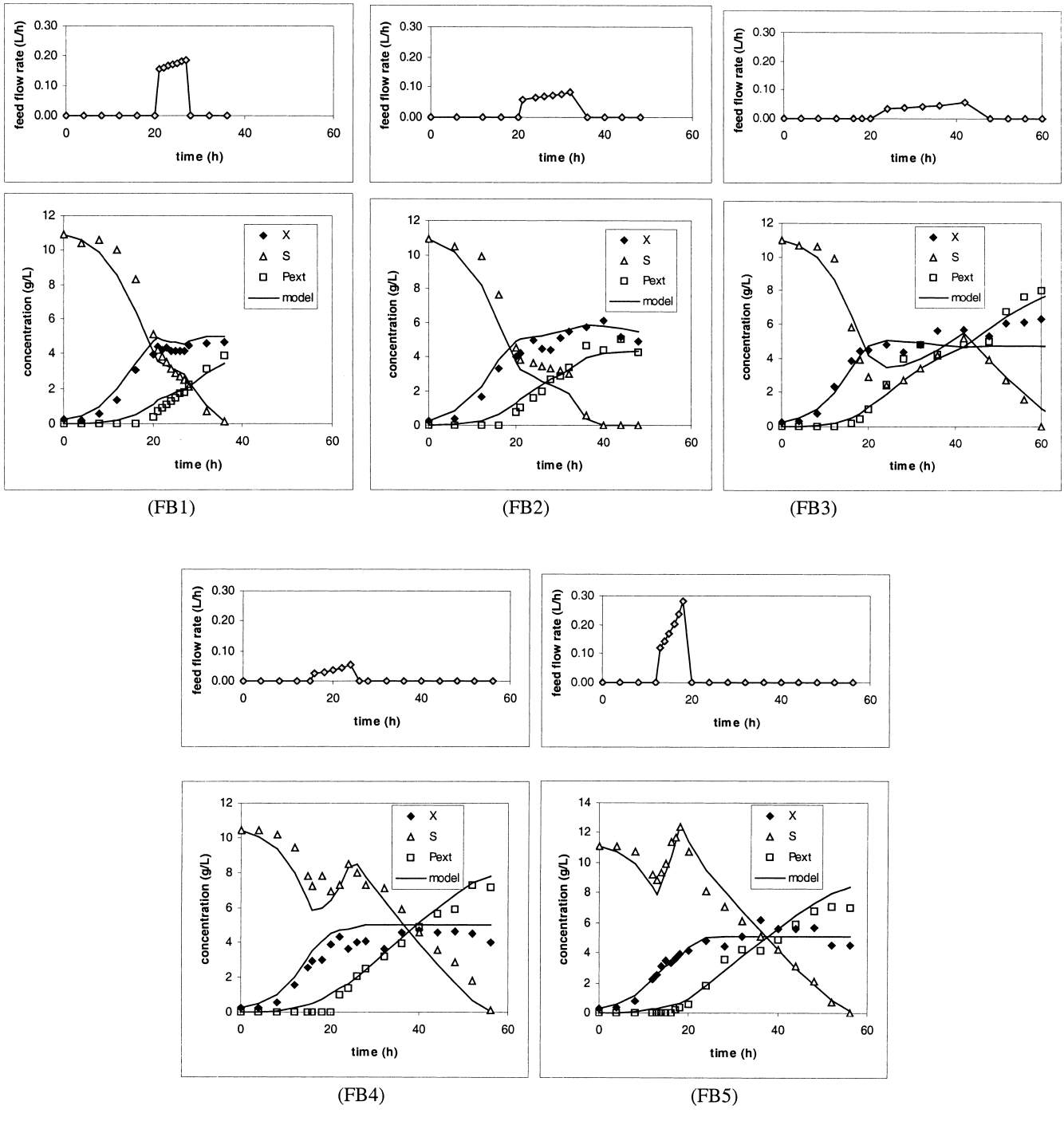

Figure 3. Comparison of simulated curves and experimental points for fed-batch cultivations.

in the medium during the cultivations (Pamboukian, 2003). It is important to mention, at this point, that all runs (batch and fed-batch) were fitted using the same set of parameters, and the same set of initial conditions assumed for the morphological compartments at the beginning of the cultivation. An adjustment of parameters specific for each run would result in even better representation. Nevertheless, we decided to use the same set of parameters for all runs in order to test the fitting capability of the proposed model.

Figure 4 shows a typical simulated time evolution of the morphological compartments. As glucose concentration decreased, differentiation processes are favored, resulting in an increase in Z_h and a consequent decrease in Z_a and Z_s. Unfortunately, it was not possible to experimentally quantify the fractions of cell compartments exactly as used in the mathematical model (apical, subapical, and hyphal) during the cultivations. Morphological characterization measurements, in terms of pellets, clumps, branched filaments, and unbranched filaments, were done by Pamboukian and Facciotti (2004) using image analysis and a sample of such images is presented in Figure 5. Pellets dominated at the beginning of the run, because of the inoculum preparation, and during the cultivation its amount decreases with a concomitant increase of clumps and, mainly, of free hyphae. At the end of the fermentation almost no pellets are present in the culture medium.

Additional model validation was done by comparison of simulated predictions with experiments not previously used at the parameter estimation step. Some results are presented in Figure 6 for fed-batch experiments and in Figure 7 for continuous experiments. In any case, the behavior predicted by the model follows the trends of the experimental data well. The only remarkable exception is the model overprediction for retamycin concentration at the fed-batch run FB5b. However, in this particular run a very fast growth took place, causing a high oxygen consumption, which resulted in a strong reduction of the dissolved oxygen (the measured dissolved oxygen concentration was very low, 10% of saturation in this run). This was the only run in which oxygen limitation was experimentally observed. Oxygen limitation during the growth phase restrains the synthesis of primary metabolites that are precursors for the secondary metabolite production, and consequently is prejudicial to the antibiotic production (Martins, 2001).

In general, the predictions for the continuous runs were quantitatively worse than those for the batch and fed-batch cultivations, but the qualitative trends were still correctly represented by the model. These validation tests with an independent dataset provide a fair check of the quality of the model predictions. It is noteworthy that the same set of parameters was used in the simulation of both the batch/fed-batch and the continuous runs. Certainly, the quantitative agreement between simulation and experimental results could be improved either if a subset of the parameters would be adjusted for the continuous runs or if some of the

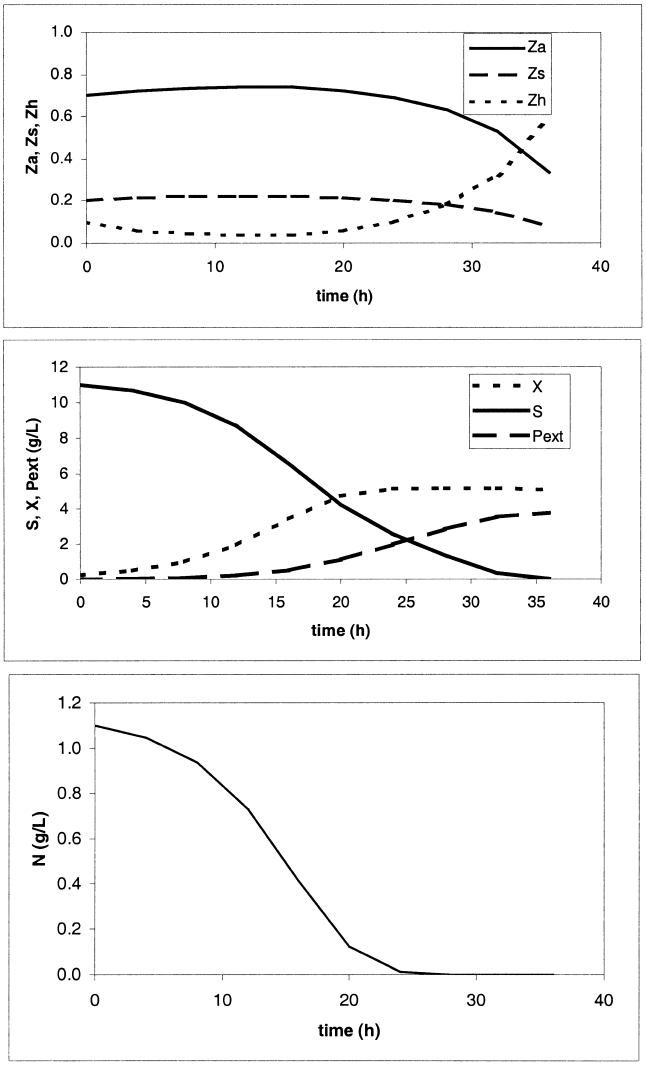

Figure 4. Typical simulation results for a batch cultivation, including the prediction of the morphological cell fractions.

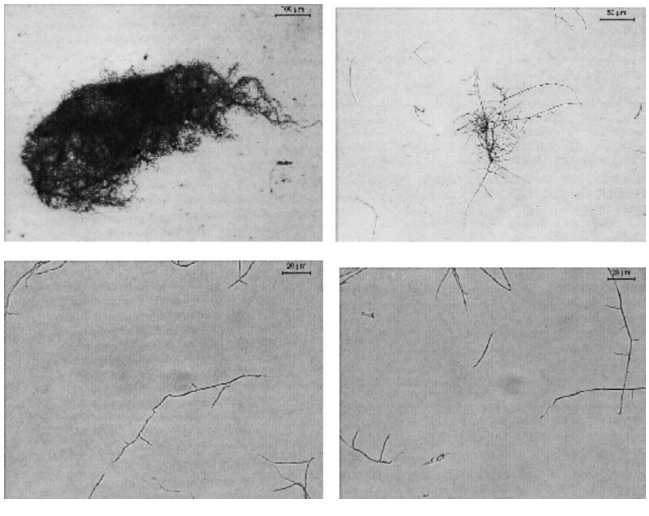

Figure 5. Different morphological forms of the microorganism: pellets, clumps, branched, and unbranched filaments.

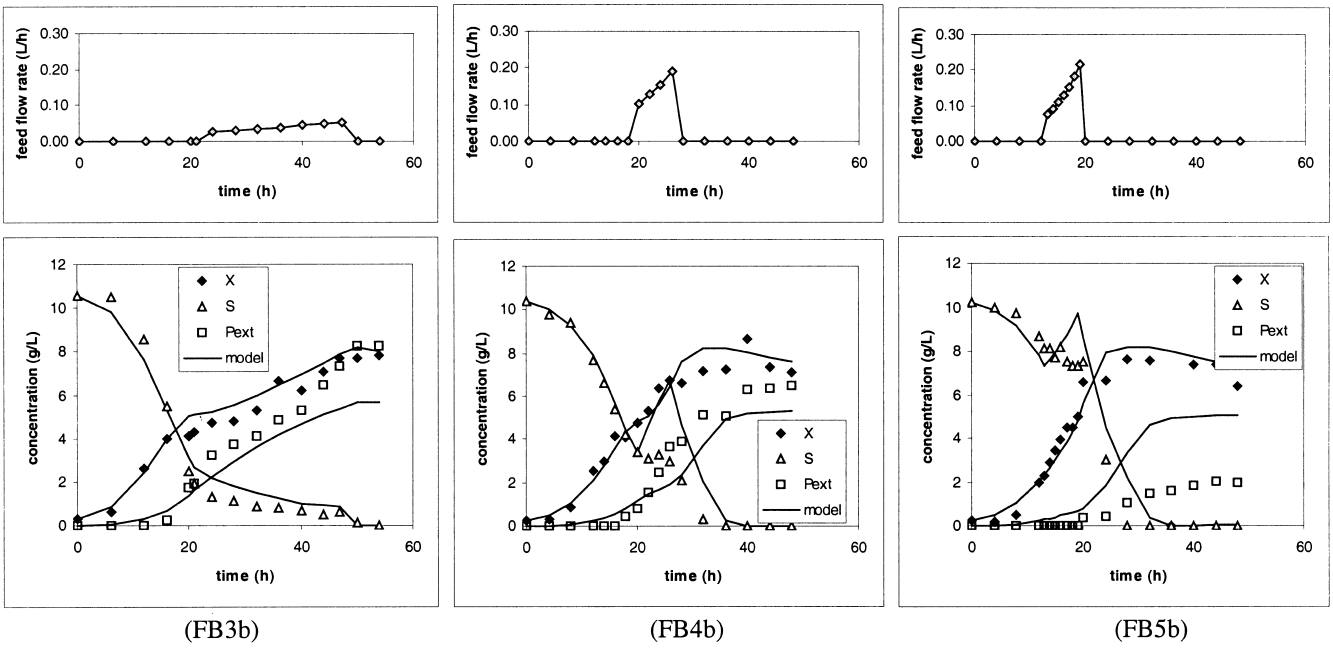

Figure 6. Comparison of simulated curves and experimental points for fed-btach cultivations not used during the parameter estimation step.

parameters taken from the literature or attributed would be included as additional fitting parameters.

The analysis of the experimental data shows that the values of growth rate are negatively correlated to the values of antibiotic production rate, i.e., the higher the growth rate, the lower the product formation rate, and vice versa. This behavior is typically found in fermentation processes for production of secondary metabolites. Several works emphasize the importance of keeping low values of specific growth rate in order to obtain high specific rates of secondary metabolite production (e.g., Ates et al., 1997; Melzoch et al., 1997; Seidel et al., 2002a,b).

CONCLUSION

A morphologically structured model was applied to represent the main variables during the production of retamycin by *Streptomyces olindensis* ICB20. The model

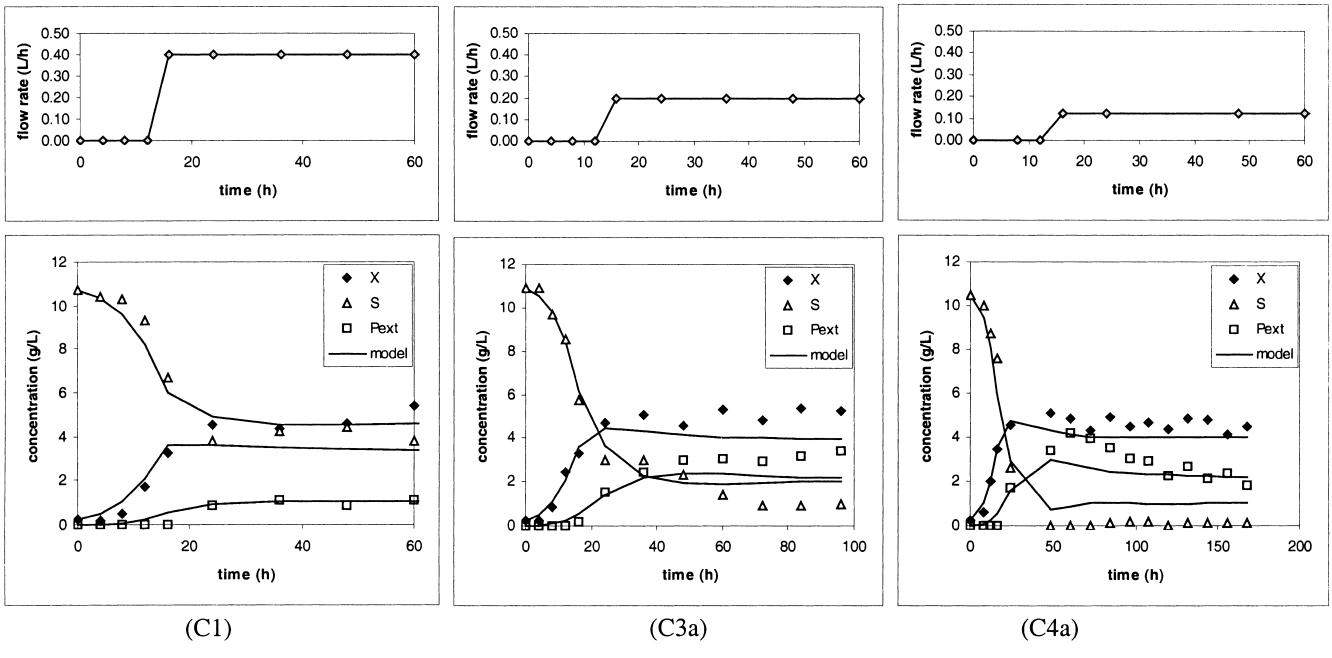

Figure 7. Comparison of simulated curves and experimental points for continuous cultivations not used during the parameter estimation step.

was based on previous works on modeling of cultivations of filamentous microorganisms, along with some improvements adapted to the present case. In order to increase the prediction capability of the model, the number of parameters was limited to a reduced set (six fitted parameters) by attributing to some of the model parameters values taken from the literature for similar filamentous microorganisms. The remaining parameters were estimated in order to fit the model to the experimental data of a number of batch and fed-batch runs. The prediction capability of the model was further validated with some additional fed-batch and a few continuous runs not used during the parameter estimation step. The model gives a fairly good quantitative description of the retamycin production, biomass, and residual glucose in both batch and fed-batch fermentations, and even for a limited number of continuous runs. The model can thus be useful for further studies on process optimization and control.

NOMENCLATURE

C_{THAM}	concentration of tris (hydroxymethyl) aminometan (g/L)
C_{ye}	concentration of yeast extract (g/L)
$f_s(S)$	function defined by equation (9)
F	volumetric feed flow rate (L/h)
$F_{sampling}$	volumetric flow rate of sampling (L/h)
k_2	rate constant for retamycin production (h^{-1})
k	rate constant for growth reaction of apical and subapical cells (h^{-1})
k_d	rate constant for biomass degradation (h^{-1})
K_s, K_2, K_{ms}, K_N	saturation constants (g/L)
K_p	partition coefficient between extracellular and total retamycin
k_{u1}	rate constant for branching reaction (h^{-1})
k_{u2}	rate constant for tip extension reaction (h^{-1})
k_{u3}	rate constant for differentiation reaction(h^{-1})
K_{u3}	saturation constant for differentiation reaction (L/g glucose)
m_s	maintenance coefficient (h^{-1})
N	nitrogen source substrate concentration (g NH_3/L)
N_F	nitrogen source substrate feed concentration (g NH_3/L)
P	concentration of total (extracellular + intracellular) retamycin (g/L)
P_{ext}	concentration of extracellular retamycin (g/L)
r_d	specific rate of biomass degradation (h^{-1})
r_p	specific rate of retamycin production (g/(g DW.h))
r_s	specific rate of glucose consumption (g/(g DW.h))
r_N	specific rate of nitrogen consumption (g NH_3/(g DW.h))
S	concentration of glucose (g/L)
S_F	concentration of glucose in the feed (g/L)
t	time (h)
u_1	rate of branching reaction (h^{-1})
u_2	rate of tip extension reaction (h^{-1})
u_3	rate of differentiation reaction (h^{-1})
V	volume (L)
X	biomass concentration (g DW/L)
Z_a, Z_h, Z_s	mass fraction of apical, hyphal, and subapical compartments (g/g DW)
α_1	stoichiometric coefficient for glucose (biomass formation) (g glucose/g DW)
α_2	stoichiometric coefficient for glucose (retamycin formation) (g glucose/g retamycin)
α_3	stoichiometric coefficient for nitrogen (biomass formation) (g NH_3/g DW)
α_4	stoichiometric coefficient for nitrogen (retamycin formation) (g NH_3/g retamycin)
β_1	conversation factor for yeast extract into NH_3 equivalent (g NH_3/g yeast extract)
β_2	conversation factor for THAM into NH_3 equivalent (g NH_3/g THAM)
μ	total specific growth rate (g/(g DW.h)
μ_a, μ_s	specific growth rate for apical and subapical cells, respectively (g/(g DW.h)

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