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Mathematical modeling of batch and semibatch reactors for the enzymic synthesis of amoxicillin

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Abstract

The performance of batch and semibatch reactors, under optimal operational conditions of amoxicillin (AN) enzymic synthesis at 25 °C and pH 6.5, were compared. Simulations for a batch reactor indicate that there are two sets of optimal operational conditions, if we consider productivity and global yield ($Y_{AN,AB}$) with respect to the ester substrate: (i) low initial concentration of ester and high initial concentration of 6-aminopenicillanic acid (6-APA); (ii) high initial concentration of ester and high initial concentration of 6-APA. Simulations for a semibatch reactor, using the hybrid-neural network model, indicated a consumption of the formed antibiotic. Such pattern was not observed experimentally and was caused by the lack of information during neural network training procedure. This problem was overcome by including new sets of experimental data in the learning phase, performed in a batch reactor in the presence of different initial concentrations of substrates and products. Results for the semibatch reactor indicated there are different sets of optimal operational conditions, if we consider local selectivity, performance index (I_{NH}), and global yield. Unfortunately, the point of highest local selectivity and performance index is not the one of highest global yield. Therefore, the choice of initial operational conditions for the batch or semibatch production of amoxicillin is connected to an economical evaluation. The semi-continuous operation of the integrated reactor (reaction + crystallization) provided better results than the batch mode.

Keywords: Pharmaceuticals; β-Lactamic antibiotic; Penicillin G acylase; Simulation; Artificial neural network; Mathematical modeling; Immobilized enzyme

1. Introduction

Amoxicillin (AN) is one of the major β-lactam antibiotics, with sales of US$ 2200 million as a bulk formulated drug in 1994 [1], due to its high spectrum of activity, high solubility, high rate of absorption, and its stability under acid conditions. Although industrial enzymatic synthesis is starting [2], today semisynthetic β-lactam antibiotics are usually prepared in industry by chemical methods. These reactions typically involve costly steps such as sub zero degree Celsius conditions (−30 °C) and toxic organic solvents like methylene chloride and silylation reagents [3]. Therefore, enzymic synthesis is becoming increasingly interesting as an industrial process because it reduces the number of reaction steps and decreases the amount and toxicity of waste products per kilogram of antibiotic. The use of enzymes such as penicillin G acylase (PGA) could be of great interest due to its high selectivity, specificity, and activity in mild reaction conditions (aqueous media, neutral pH, and moderate temperatures). This work focuses on the production of amoxicillin, by the reaction of *p*-hydroxyphenylglycyne methyl ester (POH-PGME) and 6-APA catalyzed by penicillin G acylase immobilized on glyoxyl-agarose at 25 °C and pH 6.5, see Fig. 1.

Previous works on the kinetics and mechanism of reaction [4–9] showed that the use of neural networks to represent the kinetics seems to be an alternative to simulate experimental data of amoxicillin synthesis, since the resulting set of mathematical equations of a deterministic model is very complex. Hybrid models were used in this work to simulate the performance of batch and semibatch reactors for the production of amoxicillin.

Studies on the reactor optimization must be done, since high product yield is required for the enzymatic synthesis to become competitive with the chemical synthesis. Therefore, in this paper, the performance of a batch and a semibatch

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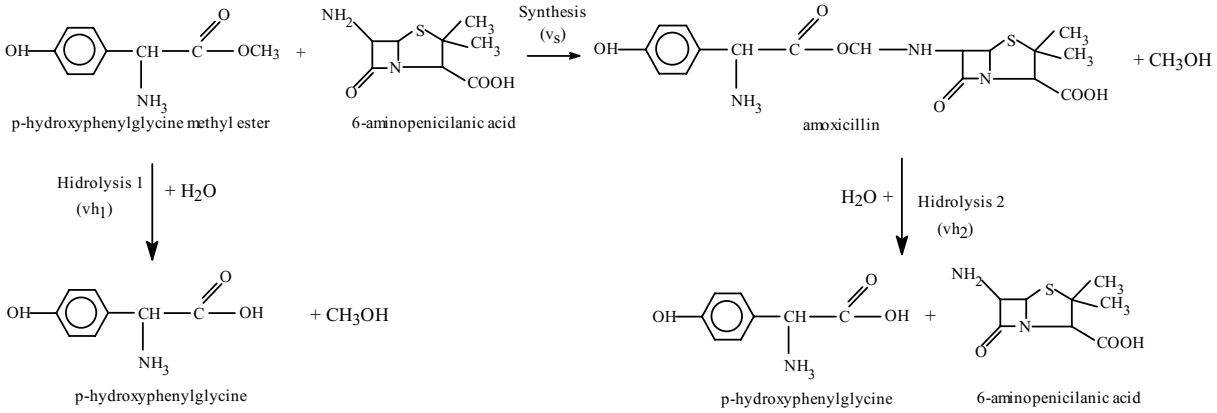

Fig. 1. Synthesis of amoxicillin using penicillin G acylase as catalyst.

reactor were compared. For this purpose, techniques of process modeling and simulation were used and the obtained results were compared to experimental data of synthesis, in order to validate the mathematical model.

2. Materials and methods

2.1. Materials

D-*p*-Hydroxyphenylglycine methyl ester (POHPGME) was synthesized in Universidad de Salamanca, Spain. Amoxicillin was from a commercial brand: Glamoxyl, Spain. Penicillin G Acylase from *Escherichia coli* and *p*-hidroxyphenylglycine (POHPG) were donated by Antibioticos S.A., Spain. Gel of agarose 6%, cross-linked (6 BCL), $\phi_{av} = 175 \pm 50\,\mu m$ (S.D.), was donated by Hispanagar S.A., Spain. All other chemicals were of laboratory grade from different commercial suppliers.

2.2. Methods

2.2.1. Immobilization of PGA on agarose

PGA was immobilized on agarose gel 6%, cross-linked (Hispanagar, Spain) through multi-point links [10,11]. Glyceryl gels ($Ag-O-CH_2-CHOH-CH_2OH$) were produced by etherification of agarose with glycidol (2,3-epoxypropanol), and oxidized with sodium periodate, resulting in glyoxyl gels ($Ag-O-CH_2-CHO$). In a typical procedure, 150 ml of gel was suspended in 30 ml of distilled water. The 50 ml of a solution of NaOH 1.7N, with 28.5 mg/ml of sodium borohydride were added to this suspension. Glycidol was then slowly added up to a final concentration of 2 M. The suspension was gently agitated during 18 h at 25 °C. For oxidation, the glyceryl gel was suspended in distilled water (1:10) and sodium periodate was added up to 75 μmol per ml of gel, under mild agitation for 2 h. Further PGA (amine)–agarose (aldehyde) multiple-point attachment was obtained by reaction at pH 10 (bicarbonate buffer), in the presence of 100 mM of phenylacetic acid (PAA), during 3 h, at 20 °C. Final reduction of the Schiff bases (reaction of the terminal and lysine amine groups of the enzyme with aldehyde groups in the support) was obtained by adding sodium borohydride (1 mg/ml of solution), during 30 min at room temperature. After each step, the gel was filtered and washed with distilled water.

2.2.2. Gel enzymic load

Enzyme activity was assessed via colorimetric analysis using *p*-dimetilaminobenzaldehide (PDAB). 6-APA released by the hydrolysis of penicillin G (5%), in 50 mM phosphate buffer, pH 8.0 at 38 °C, reacts with PDAB to yield a colored product, measured at 415 nm. The enzymatic load of the gel was calculated by measuring the difference between enzymatic activities of the supernatant (free enzyme) before and after immobilization. Here, 1 IU corresponds to the amount of enzyme that hydrolyzes 1 μmol of penicillin G (5% mass/volume) per min at pH 8.0 and 38 °C.

2.2.3. Analysis

Concentrations of POHPGME, POHPG, 6-APA, and amoxicillin were measured by HPLC: C18 column (Waters Nova-Pak C18 60 Å 4 μm, 3.9 mm × 150 mm) and 1 ml/min mobile phase, containing 35% acetonitrile, 2% SDS (lauryl sodium sulphate), H_3PO_4 10 mM, $K_2H_2PO_4$ 5 mM, at 25 °C and $\lambda = 215$ nm.

2.2.4. Batch experiments of amoxicillin synthesis

Amoxicillin synthesis was performed in a 30 ml batch reactor with mechanical stirring by reacting *p*-hydroxyphenylglycyne methyl ester and 6-APA catalyzed by penicillin G acylase immobilized on glyoxyl-agarose at 25 °C and pH 6.5 in 25 mM phosphate buffer. In all experiments, a jacketed reactor was used and an automatic titrator controlled the pH. The same amount of biocatalyst was used in all assays: 1.5 g of gel of catalyst, containing 30 IU of immobilized PGA g^{-1}. This enzymic load (30 UI/g of gel) was selected in order to avoid intra-particle diffusional delays [5].

2.2.5. Semibatch experiments of amoxicillin synthesis

Semibatch assays of amoxicillin synthesis were performed at 25 °C and pH 6.5, in 30 ml of a 25 mM phosphate buffer solution. In this case, both substrates (*p*-hydroxyphenylglycyne methyl ester and 6-APA) were added to semibatch reactor in order to keep their concentration constant with time. Powder substrate was used instead of a liquid solution in order to keep higher concentrations inside the reactor. The feed of substrates was not performed at the same time: a known amount of solid (6-APA or POHPGME) was added to the vessel at different times. A jacketed stirred reactor was used and an automatic titrator controlled the pH. The same amount of biocatalyst was used in all assays: 1.0 g of catalyst containing 30 IU of immobilized PGA/g of gel.

2.3. Mathematical models

2.3.1. Semi-empirical kinetic model

The semi-empirical model that was used in this work is described in detail elsewhere [4–6,12]. Shortly, its main hypotheses are: antibiotic synthesis only occurs when 6-aminopenicillanic acid (6-APA) binds to the enzyme before the formation of the acyl-enzyme complex (EA) and the rate of formation of the acyl-enzyme complex is not influenced by the presence of 6-APA. Taking into account those hypotheses, the rate equations become as follows:

Rate of antibiotic hydrolysis (ν_{h2}):

$$\nu_{h2} = \frac{k_{cat2} C_E C_{AN}}{K_{m2}(1 + (C_{AB}/k_{AB}) + (C_{NH}/k_{NH}) + (C_{AOH}/k_{AOH})) + C_{AN}} \quad (1)$$

Rate of ester consumption (hydrolysis + synthesis, ν_{AB}):

$$\nu_{AB} = \frac{k_{cat1} C_E C_{AB}}{K_{m1}(1 + (C_{AN}/k_{AN}) + (C_{AOH}/k_{AOH})) + C_{AB}} \quad (2)$$

Rate of amoxicillin synthesis (ν_S):

$$\nu_S = \frac{k_{cat1} C_E C_{AB}}{K_{m1}(1 + (C_{AN}/k_{AN}) + (C_{AOH}/k_{AOH})) + C_{AB}} \times \frac{C_{NH}}{K_{EN} + C_{NH}} T_{max} \quad (3)$$

The kinetic parameters in Eqs. (1)–(3) were estimated using the algorithm of Marquardt [13], after a long series of initial-rates and batch assays [12]. Table 1 shows these parameters.

Table 1
Enzymic synthesis of amoxicillin

Parameter	
k_{cat1} (μmol/(IU min))	$(0.18 \pm 0.2) \times 10^{-1}$
k_{cat2} (μmol/(IU min))	$(0.33 \pm 0.3) \times 10^{-1}$
K_{m1} (mM)	7.91 ± 3.6
K_{m2} (mM)	12.5 ± 3.2
T_{max}	$(0.61 \pm 0.3) \times 10^{-1}$
K_{EN} (mM)	14.4 ± 2.1
k_{AB} (mM)	3.78 ± 0.7
k_{AN} (mM)	9.17 ± 2.1
k_{AOH} (mM)	10.9 ± 2.1
k_{NH} (mM)	62.0 ± 7.5

25 °C, pH 6.5: kinetic parameters of the simplified model (Eqs. (1)–(3)), with 95% confidence intervals [6]. Catalyst: penicillin G acylase immobilized on cross-linked agarose (6%); enzymic load: 30 IU/g_{gel} [12].

2.3.2. Hybrid-neural network model [5]

In this work, a feedforward neural network was used, trained by backpropagation, with information in one direction only. The topology of the neural networks used in this work is composed by four nodes in the input layer (substrates and products concentration) and one node in the output layer, related to the output variable (the kinetic rate), see Fig. 2. Only one hidden layer was used and the number of nodes in this layer was determined by trial and error.

Two feedforward NNs were trained to represent the rates of:

- amoxicillin net production (synthesis − hydrolysis) ⇒ $\nu_{AN} = \nu_s - \nu_{h2}$;
- *p*-hydroxyphenylglycine production (hydrolysis of the ester + hydrolysis of amoxicillin) ⇒ $\nu_{AOH} = \nu_{h1} + \nu_{h2}$.

The experimental data used to train the neural network were obtained during amoxicillin synthesis at pH 6.5 and 25 °C in a batch stirred tank reactor. During the learning phase, the only output variable was the rate of reaction. The training procedure used here alternates the backpropagation algorithm [14] with a random-search method, aiming at the minimization of the norm of the residues. For the training/validation of the NNs we have used in-house software, from the Process Simulation Group of DEQ/UFSCar, implemented in FORTRAN. One hidden layer with 20 neurons was used. The sigmoid was chosen as the transfer function within each node.

2.3.3. Mathematical model for the batch reactor

Rates of reaction (semi-empirical or neural network kinetic) were included in the batch reactor mass balances, as constitutive equations. Two mass balances unequivocally determine the system state (Eqs. (4) and (5)):

$$\frac{dC_{AN}}{dt} = \nu_{AN} \quad (4)$$

$$\frac{dC_{AOH}}{dt} = \nu_{AOH} \quad (5)$$

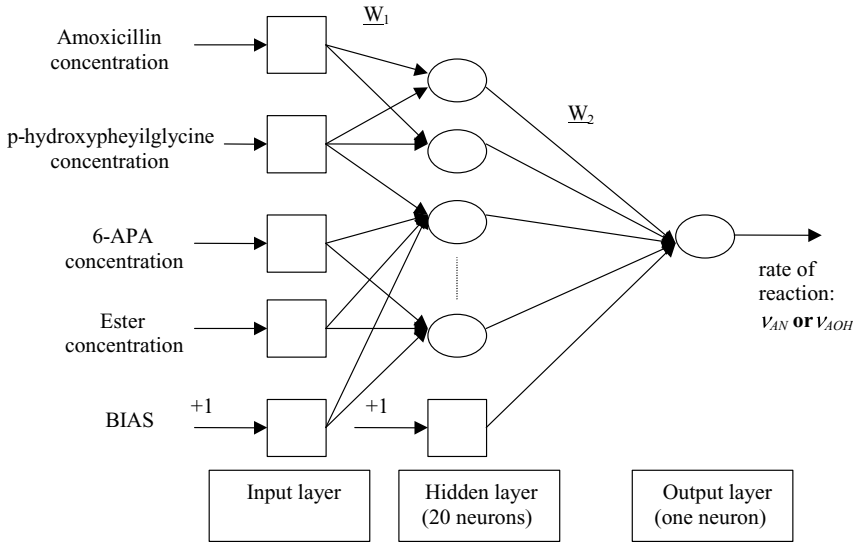

Fig. 2. Topology of the feedforward NN [5].

Reaction stoichiometry provides the concentrations of the remaining compounds. These ordinary differential equations could be easily solved by standard numerical methods, we have used Dassl subroutine [15].

2.3.4. Mathematical model for the semibatch reactor

Rates of reaction (semi-empirical or neural network kinetic) were included in the semibatch reactor mass balances, as constitutive equations. Six mass balances determine the system state (Eqs. (6)–(11)):

$$\frac{dC_{AN,l}}{dt} = \nu_{AN} \quad (6)$$

$$\frac{dC_{AOH,l}}{dt} = \nu_{AOH} \quad (7)$$

$$\frac{dC_{AB}}{dt} = -\nu_{AN} - \nu_{AOH} + F_{AB} \quad (8)$$

$$\frac{dC_{NH}}{dt} = -\nu_{AN} + F_{NH} \quad (9)$$

Substrates are added to the reactor in the form of powder, to avoid diluting the synthetic medium. Substrate were fed separately into the reaction when its concentration reached a minimum value, established at the beginning of the assay.

When antibiotic concentration reaches solubility

$$\frac{dC_{AN,l}}{dt} = 0 \quad \text{and} \quad \frac{dC_{AN,s}}{dt} = \nu_{AN} \quad (10)$$

When concentration of *p*-hydroxyphenylglycine reaches solubility

$$\frac{dC_{AOH,l}}{dt} = 0 \quad \text{and} \quad \frac{dC_{AOH,s}}{dt} = \nu_{AOH} \quad (11)$$

where l stands for liquid (soluble species) and s stands for solid (precipitated species). These ordinary differential equations were solved by standard numerical methods; we have also used Dassl subroutine [15].

2.3.5. Reactor performance indexes

Four parameters were defined to compare the reactors performance:

- Selectivity (S_{AN}) in this work is the rate of desired product (amoxicillin) per rate of undesired product (*p*-hydroxiphenylglicine):

$$S_{AN}\,(\%) = \frac{\nu_{AN}}{\nu_{AOH}} \times 100.$$

- Performance index with respect to 6-APA (I_{NH}) is the amount of the desired product formed (amoxicillin) per initial amount of 6-APA (substrate):

$$I_{NH}\,(\%) = \frac{C_{AN}}{C_{NH0}} \times 100.$$

- Global yield ($Y_{AN,AB}$) with respect to the ester substrate (AB), $Y_{AN,AB}$, is the amount of the desired product formed (amoxicillin) per initial amount of POHPGME:

$$Y_{AN,AB}\,(\%) = \frac{C_{AN}}{C_{AB0} - C_{AB}} \times 100.$$

- Amoxicillin productivity (P_{AN}) is the amount of the desired product formed (amoxicillin) per reaction time (t_{max}) to achieve the highest amoxicillin concentration:

$$P_{AN} = \frac{C_{AN}}{t_{max} E_0}$$

where E_0 is the reactor enzymatic load (IU/l).

3. Results and discussion

3.1. Batch reactor

Fig. 3 displays the obtained results of global yield with respect to ester during amoxicillin synthesis at pH 6.5

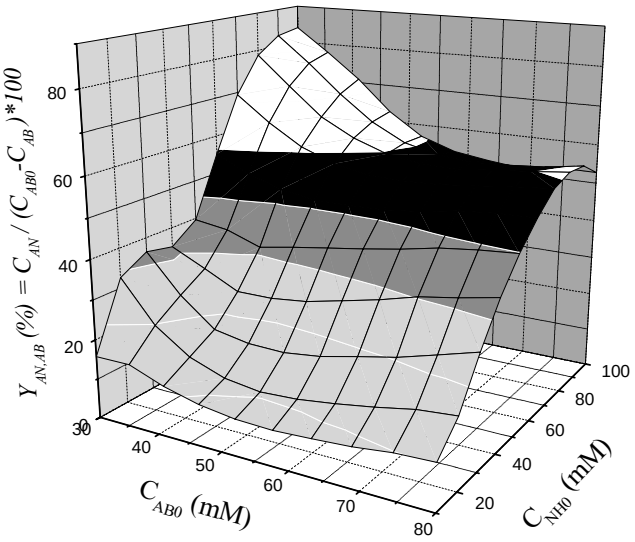

Fig. 3. Amoxicillin enzymatic synthesis, batch reactor: global yield with respect to AB ($Y_{AN,AB}$) at pH 6.5 and 25 °C.

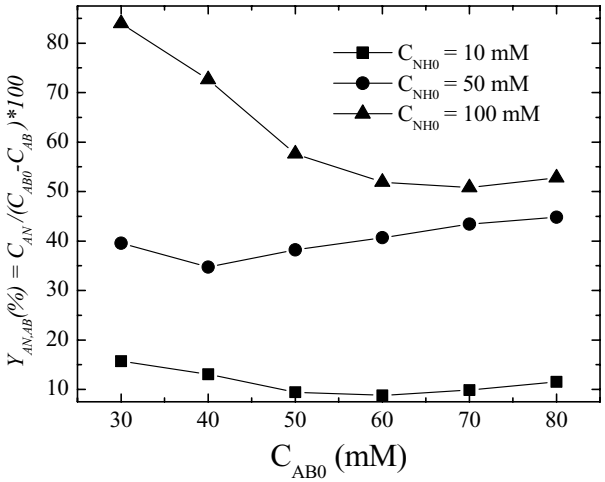

Fig. 5. Amoxicillin enzymatic synthesis, batch reactor: global yield with respect to AB at pH 6.5 and 25 °C. Different initial concentrations of POHPGME and three different initial concentrations of 6-APA.

and 25 °C, for different initial concentrations of substrate (POHPGME and 6-APA). These data were obtained using a hybrid-neural network model for a batch reactor. A point of maximum can be noticed for high initial concentration of 6-APA and low concentration of POHPGME. Therefore, this point is an optimal operation condition to the batch reactor.

Fig. 4 displays the global yield with respect to ester during amoxicillin synthesis at pH 6.5 and 25 °C, for different initial concentrations of 6-APA and three different initial concentrations of POHPGME. Fig. 5 pictures the global yield during amoxicillin synthesis at pH 6.5 and 25 °C, for different initial concentrations of POHPGME and three different initial concentrations of 6-APA.

It can be seen (Fig. 4) that the global yield increases with increasing 6-APA concentration up to a limit, when the increase of C_{NH0} causes a decrease in $Y_{AN,AB}$. This behavior may be caused by an inhibition effect of 6-APA in the rate of amoxicillin production (substrate inhibition). Such inhibitory effect was detected previously when studying the influence of initial concentrations of substrate in the amoxicillin synthesis [5].

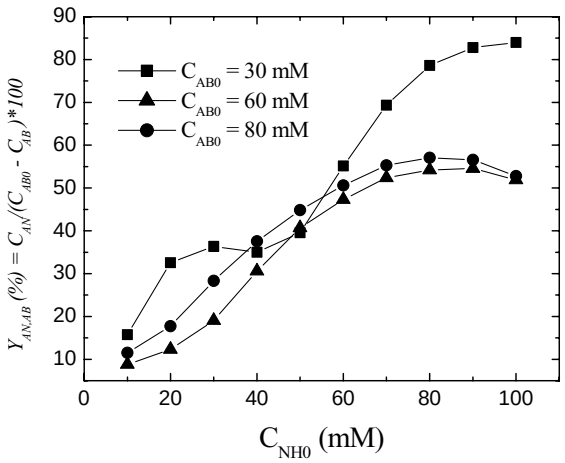

Fig. 4. Amoxicillin enzymatic synthesis, batch reactor: global yield with respect to AB at 25 °C and pH 6.5. Different initial concentrations of 6-APA and three different initial concentrations of POHPGME.

Observing the obtained results, it can be noticed that the point of maximum yield $Y_{AN,AB}$ is $C_{NH0} = 100$ and 30 mM. However, this might be a point of low productivity.

Fig. 6 displays the obtained results of performance index with respect to 6-APA during amoxicillin synthesis at pH 6.5 and 25 °C, for different initial concentrations of substrate (POHPGME and 6-APA). It can also be seen that high concentrations of 6-APA was not always favorable to the yield of synthesis.

Fig. 7 pictures amoxicillin productivity for different initial concentrations of 6-APA and POHPGME at 25 °C and pH 6.5. It can be seen that there is a point of maximum at high 6-APA and POHPGME concentrations. In other words, the highest amount of amoxicillin was produced at short times of reaction, when starting from high substrate concentration. This might be an interesting operational condition to the batch reactor since the duration of reaction is minimum.

If global yield and amoxicillin productivity were compared, it can be seen there were two possible sets of operational conditions:

(a) low POHPGME concentration (30 mM) and high 6-APA concentration (100 mM) ⇒ point of highest global yield;
(b) high POHPGME concentration (80 mM) and high 6-APA concentration (80 mM) ⇒ point of highest productivity.

Notice that the point of highest $Y_{AN,AB}$ was not the one of highest productivity. The point of highest $Y_{AN,AB}$ was indeed the point of minimum productivity. Therefore, the choice of initial operational conditions for the batch

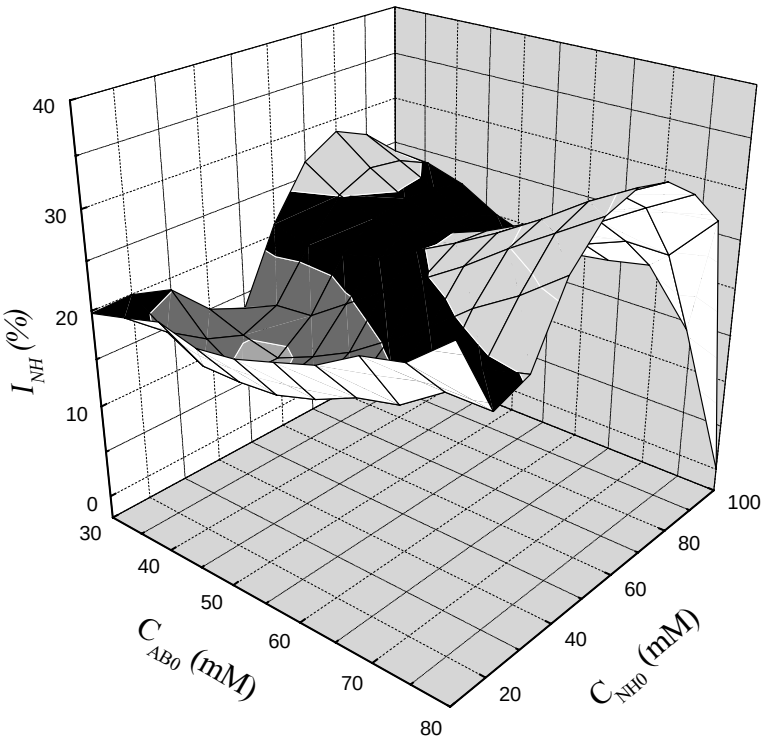

Fig. 6. Amoxicillin enzymatic synthesis, batch reactor: performance index (I_{NH}) at pH 6.5 and 25 °C.

production of amoxicillin is connected to an economical evaluation.

The obtained results suggest that a semibatch reactor, with slow feed of substrates, may provide a better performance. Fogler [16] suggest the use of this type of reactor when undesired substrate hydrolysis takes place, what is the case of amoxicillin synthesis.

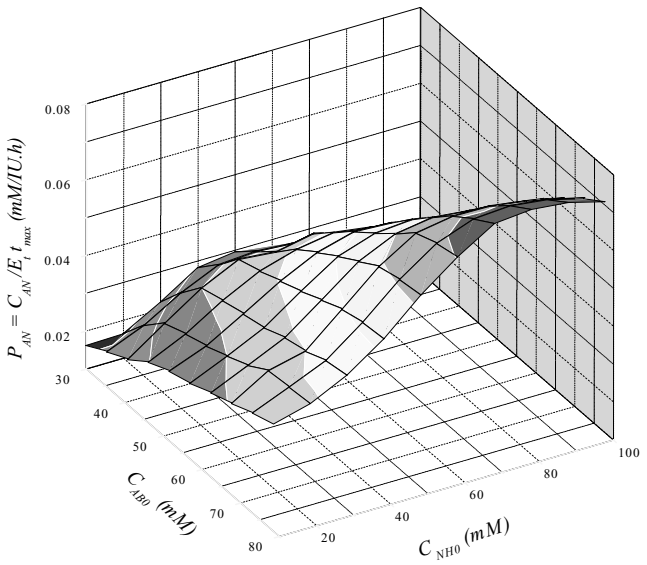

Fig. 7. Amoxicillin enzymatic synthesis, batch reactor: reactor productivity at pH 6.5 and 25 °C.

3.2. Semibatch reactor: model validation

Fig. 8 illustrates the quality of fit for validation experiments. It can be observed that a hybrid-neural network model for a semibatch reactor was capable of representing the experimental data.

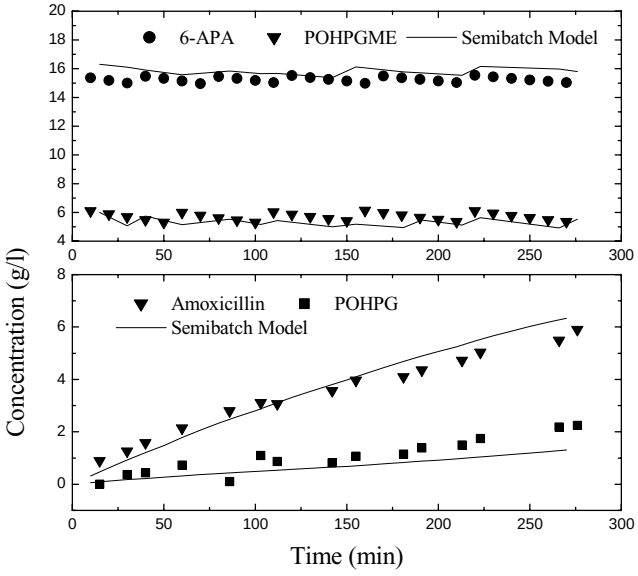

Fig. 8. Validation test of the hybrid-neural network model for a semibatch reactor: amoxicillin synthesis at pH 6.5 and 25 °C with powder substrate feed.

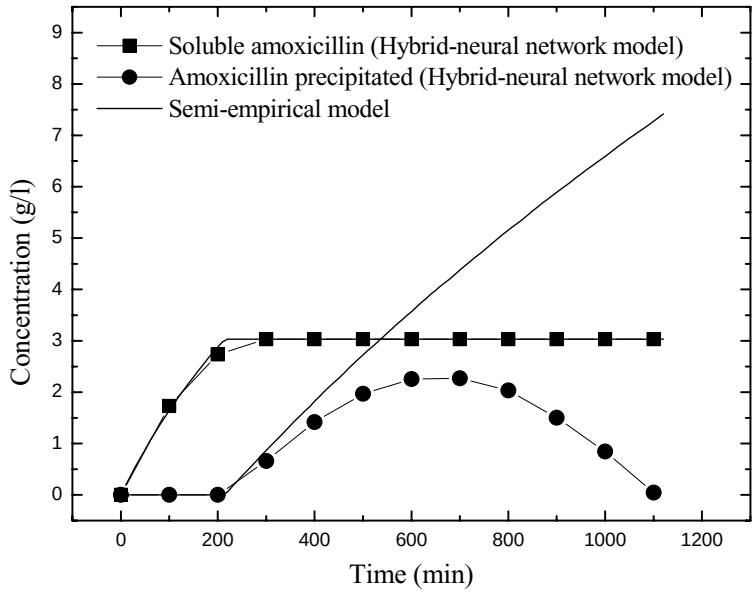

Fig. 9. Semibatch model validation test: amoxicillin synthesis at pH 6.5, 25 °C.

The hybrid-neural network model, however, failed in representing long reaction times. Fig. 9 shows the simulated results for amoxicillin synthesis at 25 °C and pH 6.5, comparing the hybrid-neural network model and the semi-empirical model for the semibatch reactor.

It can be noticed that when reaction achieves 10 h, the hybrid model indicated a consumption of the formed antibiotic, what was not expected (Fig. 9). This behavior is probable caused by the lack of information during the learning phase of the neural network, used to represent the kinetics. The unexpected pattern was not observed when the semi-empirical kinetic model was used to simulate amoxicillin synthesis at the same operational conditions, reinforcing the hypothesis of problems during the learning phase of the neural network kinetic model. New batch experiments of amoxicillin synthesis were performed, starting the reaction with different concentrations of substrates and products. The neural network was trained again and new simulation results are pictured in Fig. 10.

It can be noticed that the simulated data no longer indicates the antibiotic consumption. For the same operational conditions, the semi-empirical semibatch model and the hybrid-neural network semibatch model showed almost the same behavior to amoxicillin synthesis at pH 6.5 and 25 °C (Fig. 10).

3.3. Semibatch reactor: simulation results

Based on the results obtained in this work and in previous works [5,6], the hybrid-neural network semibatch model

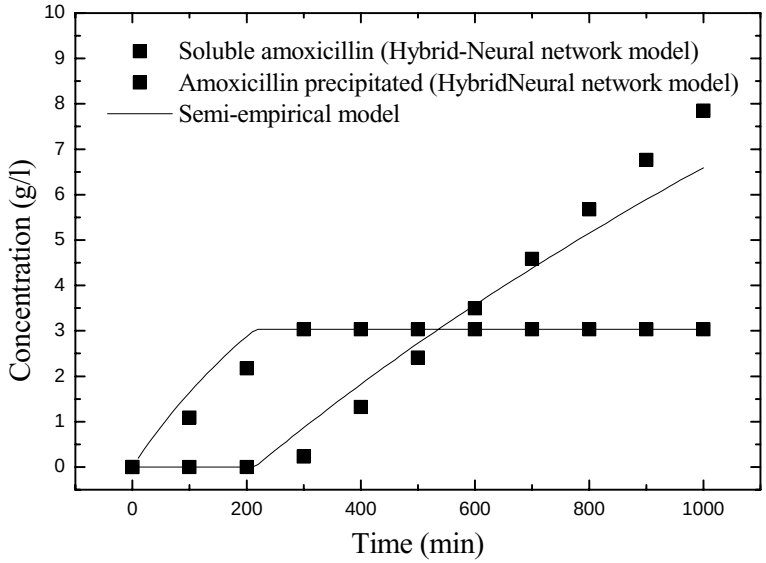

Fig. 10. Hybrid-neural network semibatch model validation test: amoxicillin synthesis at pH 6.5, 25 °C.

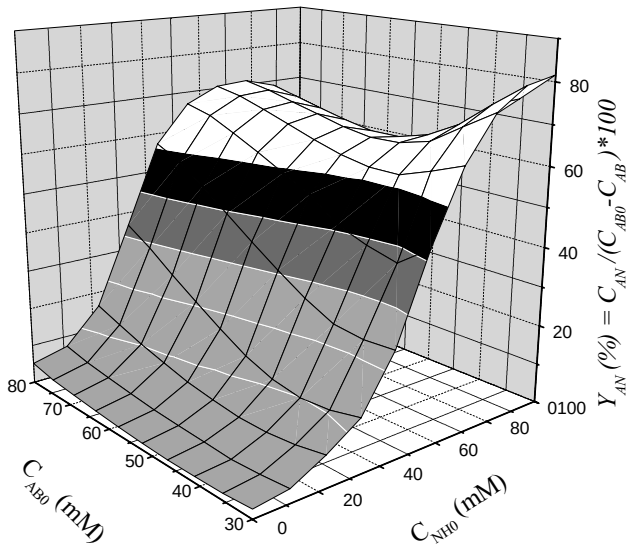

Fig. 11. Amoxicillin enzymatic synthesis, fed-batch reactor: global yield with respect to AB ($Y_{AN,AB}$) at pH 6.5 and 25 °C.

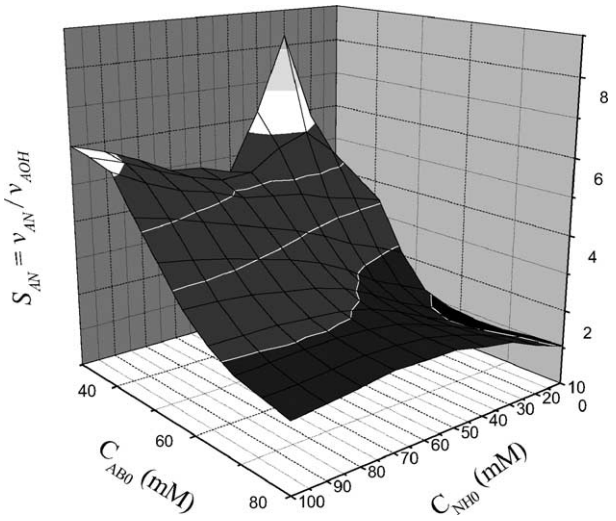

Fig. 13. Amoxicillin enzymatic synthesis, fed-batch reactor: local selectivity (S_{AN}) at pH 6.5 and 25 °C.

was used to look for optimal operational conditions. Fig. 11 displays the simulating results of global yield with respect to ester during amoxicillin synthesis at pH 6.5 and 25 °C, for different initial concentrations of substrate (POHPGME and 6-APA). As already expected, a point of maximum could be noticed for high initial concentration of 6-APA and low concentration of POHPGME.

Fig. 12 displays the obtained results of performance index with respect to 6-APA during amoxicillin synthesis at pH 6.5 and 25 °C, for different initial concentrations of substrate (POHPGME and 6-APA). It can also be seen that high concentrations of 6-APA was not always favorable to the yield of synthesis.

Fig. 13 displays the obtained results of local selectivity (S_{AN}) during amoxicillin synthesis at pH 6.5 and 25 °C, for different initial concentrations of substrate (POHPGME and 6-APA). It can be seen that selectivity decreases with increasing ester concentration. That is to say, high selectivity was obtained when concentrations of POHPGME were low. It can also be noticed that the effect of 6-APA concentration on the selectivity is more significant when low concentrations of ester were used.

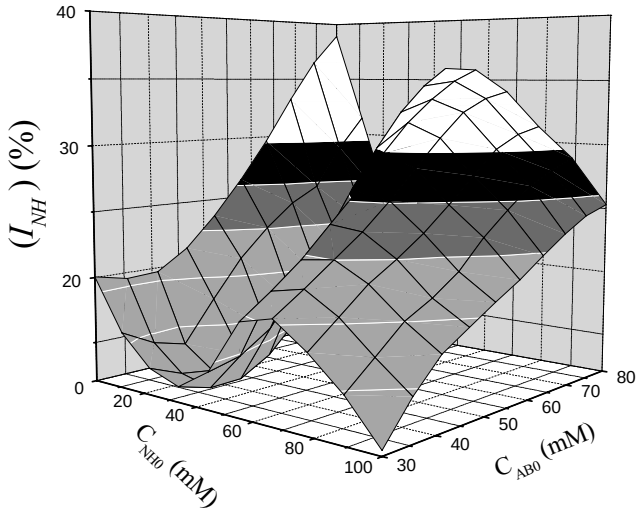

Fig. 12. Amoxicillin enzymatic synthesis, fed-batch reactor: performance index (6-APA) at pH 6.5 and 25 °C.

If local selectivity, performance index and global yield were compared, it can be seen there were two possible sets of operational conditions:

(a) low POHPGME concentration (30 mM) and high 6-APA concentration (80–100 mM) ⇒ point of highest global yield, high selectivity and medium performance index;
(b) high POHPGME concentration (80 mM) and medium (60 mM) 6-APA concentration ⇒ point of highest performance index with respect to 6-APA and highest local selectivity.

Notice that the point of highest local selectivity and highest performance index was not the one of highest global yield. Therefore, the choice of initial operational conditions for the semibatch production of amoxicillin is also connected to an economical evaluation.

Figs. 14 and 15 picture the local selectivity time course and the productivity time course, respectively. As reaction time increases, it can be notice that the local selectivity initially increases up to a limit, when it starts to decrease. Similar pattern is also observed for amoxicillin productivity.

3.4. Semibatch reactor versus batch reactor for amoxicillin synthesis

Table 2 compares the best values of global yield and performance index obtained to both reactors studied in this work for the production of amoxicillin. Fig. 15 shows the

Table 2
Global yield $Y_{AN,AB}$ and performance index I_{NH} for batch or semibatch production of amoxicillin at 25 °C and pH 6.5

Parameter	Batch reactor	Semibatch reactor
Global yield, $Y_{AN,AB}$ (%)	82.0	82.0
Performance index, I_{NH} (%)	30.0	38.0

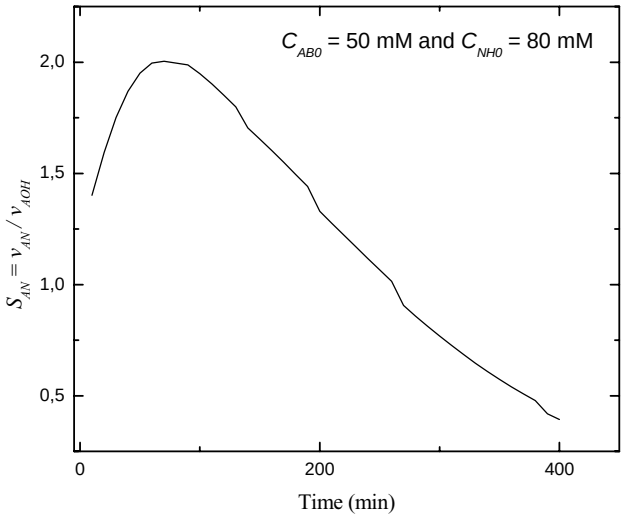

Fig. 14. Amoxicillin enzymatic synthesis, fed-batch reactor at 25 °C and pH 6.5: local selectivity time course.

amount of amoxicillin produced per volume of a batch and a semibatch reactor.

It can be observed that, although, selectivity and yields of amoxicillin are quite similar to both reactors, the amount of amoxicillin produced per volume of reactor is higher to the semibatch reactor. The semibatch reactor presented also another advantage: while the ester substrate was fed to the reactor, there was no antibiotic hydrolysis (Fig. 16).

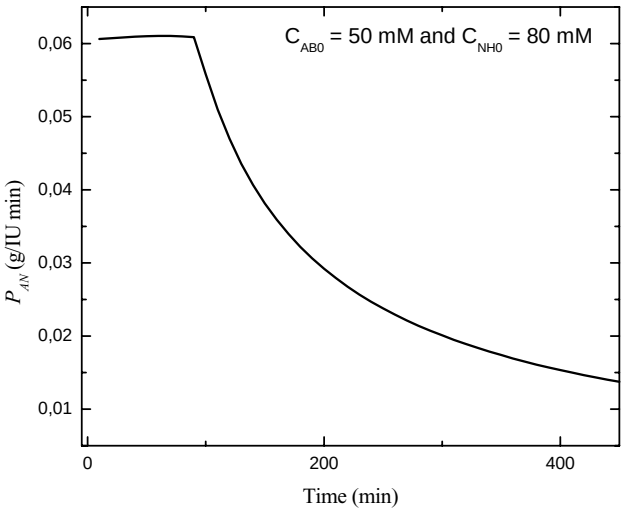

Fig. 15. Amoxicillin enzymatic synthesis, fed-batch reactor at 25 °C and pH 6.5: productivity time course.

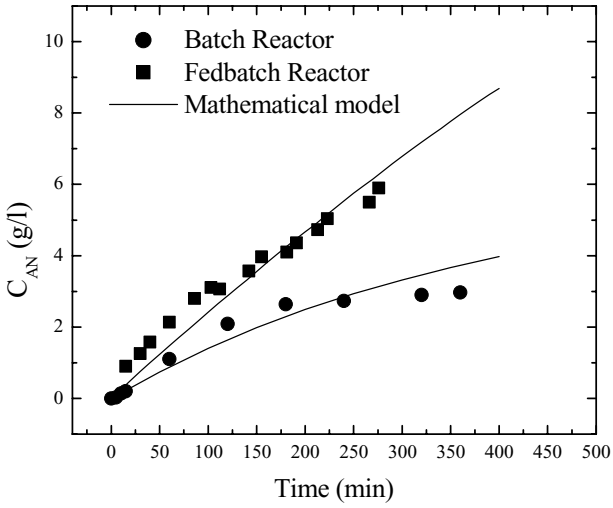

Fig. 16. Amount of amoxicillin produced per volume of a batch and a semibatch reactor at pH 6.5 and 25 °C.

4. Conclusion

A hybrid-NN model was able to capture the complex kinetics of the enzymatic synthesis of amoxicillin. Simulated productivity and yield surfaces indicate that cost/profit objective functions are not unimodal. Optimum reactor operational strategies should be put forth with care. Simulations for a batch reactor indicated that there are two sets of optimal operational conditions, if we consider global yield and productivity. The point of highest $Y_{AN,AB}$ is not the one of highest productivity. The point of highest $Y_{AN,AB}$ is indeed the point of minimum productivity. The hybrid-neural network semibatch model is able to represent experimental data, when the set of experimental data, used in the learning phase, are obtained from batch assays in the presence of different initial concentration of substrates and products. Simulations for the semibatch reactor indicated there are different sets of optimal operational conditions, if we consider local selectivity a performance index with respect to 6-APA consumption and yield. Notice that the point of highest local selectivity (S_{AN}) was not the one of highest global yield. Therefore, the choice of initial operational conditions for the batch or semibatch production of amoxicillin is connected to an economical evaluation. Last but not least, the performance of batch and semibatch reactors were compared on the point of optimal global yield and it was observed that the semibatch reactor had a better performance than the batch reactor, when considering amount of amoxicillin produced per volume of reactor. As a role, the semi-continuous operation of the integrated reactor (reaction + crystallization) provided better results than the batch mode.

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Appendix A. Nomenclature

AB	*p*-hydroxyphenylglycine methyl ester
AN	amoxicillin
AOH	*p*-hydroxyphenylglycine
6-APA	6-aminopenicillanic acid
C_i	concentration of *i*
E	enzyme
F_{AB}	feeding of solid POHPGME per reactor volume (mM/min)
F_{NH}	feeding of solid 6-APA per reactor volume (mM/min)
IU	international unit
k_{cat}	kinetic rate constant, mechanistic model
k_i	inhibition constant
K_{EN}	6-APA adsorption constant
NN	neural network
PAA	phenylacetic acid
PDAB	*p*-dimetilaminobenzaldehide
PGA	penicillin G acylase
POHPGME	*p*-hydroxyphenylglycine methyl ester
T_{max}	maximum conversion ratio of the complex acyl-enzyme-nucleus into product
ν_{AB}	rate of POHPGME consumption (mM/min)
ν_{AN}	rate of amoxicillin production (mM/min)
ν_{AOH}	rate of POHPG production (mM/min)
ν_{h1}	rate of POHPGME hydrolysis (mM/min)
ν_{h2}	rate of amoxicillin hydrolysis (mM/min)
ν_S	rate of amoxicillin synthesis (mM/min)
W	vector of weights of the NN
X	fraction of enzyme saturated with 6-APA

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