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SELECTION OF MODELS APPLIED TO THE SYNTHESIS OF AMOXICILLIN IN AN ENZYME REACTOR

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ABSTRACT

Amoxicillin is a semi-synthetic antibiotic of the penicillin class, widely used for treating bacterial infections. Its industrial production involves complex chemical processes that generate pollutants. A more sustainable alternative is enzymatic synthesis, developed by the pharmaceutical industry to obtain semi-synthetic antibiotics, including amoxicillin. Four semi-empirical models were evaluated using the Markov chain Monte Carlo (MCMC) method with the Metropolis-Hastings algorithm to estimate the kinetic parameters and select the best-suited model for the experimental data. The metric chosen for selection was the relative root mean square error (rRMSE). The mathematical model that considers the inhibitory effects caused by the substrates (ester and 6-APA), as well as by the products (amoxicillin) and by-products (POHPG) formed by the synthesis and hydrolysis reactions of the ester and amoxicillin, showed the best fit to the experimental data, considering a 99% credible interval. The model was validated and the metrics were efficient for selecting the best kinetic model.

Keywords: Amoxicillin, Enzymatic reactor, Mathematical modeling, Markov chain Monte Carlo method, Enzymatic models.

1 INTRODUCTION

Amoxicillin is a β-lactam antibiotic that belongs to the penicillin group. It has a broad spectrum of action against harmful bacteria in the human body[1]. This compound has advantages due to its low toxicity, strong antimicrobial activity, and good clinical efficiency[2]. Amoxicillin is commonly produced through the chemical route, which has drawbacks such as high process costs, generation of pollutants, and adverse conditions like extreme temperatures[3]. A promising alternative for the pharmaceutical industry is the biotechnological production of amoxicillin. Enzymatic synthesis is particularly noteworthy due to its low use of aggressive solvents, such as carcinogens or pollutants[4]. The bioprocess is the result of the reaction between the ester (p-hydroxyphenylglycine methyl ester) and 6-APA (6-amino penicillanic acid), catalyzed by PGA (penicillin G acylase), an enzyme with transferase activity[5]. This reaction produces amoxicillin and methanol.

Bayesian statistics is a data analysis approach based on Bayes theorem. It involves updating available knowledge about parameters in a statistical model with information from observed data[6]. The Markov chain Monte Carlo (MCMC) method is used within Bayesian statistics to estimate an unknown a priori distribution and obtain a known a posteriori distribution from samples generated by an iterative process[7]. To implement MCMC, the Metropolis-Hastings (MH) algorithm was used. This algorithm uses the accept/reject principle, generating values from an auxiliary distribution and accepting them with a defined probability when observing random samples from this distribution. The use of the MH algorithm is a common approach in MCMC simulations[8].

This study evaluated four semi-empirical models to produce amoxicillin in a bioreactor catalyzed by penicillin G acylase (PGA) immobilized in glycerol-agarose. The MCMC method with the Metropolis-Hastings algorithm was used to estimate the kinetic parameters and select the best-suited model for the experimental data.

2 MATERIAL & METHODS

The substrate and bioproduct concentrations were measured experimentally, and the reaction time was determined based on literature[5]. The mathematical model, described in literature[5], assumes that antibiotic synthesis only occurs after 6-APA binds to the acyl-enzyme complex, the rate of complex formation is not affected by the presence of 6-APA, and the process takes place in a batch enzymatic bioreactor. The mathematical model in Table 1 represents the rates of ester consumption (V_{AB}), amoxicillin synthesis (V_S), amoxicillin hydrolysis (V_{AN}), and by-product production (V_{AOH}).

Table 1 Modelos cinéticos semiempiricos[5].

Models	Ester consumption rate (V_{AB})	Antibiotic hydrolysis rate (V_{AN}):	Synthesis rate (V_S):	Synthesis rate POHPG (V_{AOH}):
1	$V_{AB}=\frac{Kcat_1 \cdot C_{AB} \cdot C_{EZ}}{Km_1+C_{AB}}$	$V_{AN}=\frac{Kcat_2 \cdot C_{AN} \cdot C_{EZ}}{Km_2+C_{AN}}$	$V_S=V_{AB}*T_{MAX}*(\frac{C_{NH}}{K_{EN}+C_{NH}})$	$V_{AOH}=V_{AB}-V_S+V_{AN}$
2	$V_{AB}=\frac{Kcat_1 \cdot C_{AB} \cdot C_{EZ}}{Km_1 \cdot (1+\frac{C_{AN}}{k_{AN}})+C_{AB}}$	$V_{AN}=\frac{Kcat_2 \cdot C_{AN} \cdot C_{EZ}}{Km_2 \cdot (1+\frac{C_{AB}}{k_{AB}})+C_{AN}}$	$V_S=V_{AB}*T_{MAX}*(\frac{C_{NH}}{K_{EN}+C_{NH}})$	$V_{AOH}=V_{AB}-V_S+V_{AN}$

3	$V_{AB}=\frac{Kcat_1 \cdot C_{AB} \cdot C_{EZ}}{Km_1 \cdot (1+\frac{C_{AN}}{k_{AN}}+\frac{C_{AOH}}{k_{AOH}})+C_{AB}}$	$V_{AN}=\frac{Kcat_2 \cdot C_{AN} \cdot C_{EZ}}{Km_2 \cdot (1+\frac{C_{AN}}{k_{AN}}+\frac{C_{AOH}}{k_{AOH}})+C_{AN}}$	$V_S=V_{AB}*T_{MAX}*(\frac{C_{NH}}{K_{EN}+C_{NH}})$	$V_{AOH}=V_{AB}-V_S+V_{AN}$
4	$V_{AB}=\frac{Kcat_1 \cdot C_{AB} \cdot C_{EZ}}{Km_1 \cdot (1+\frac{C_{AN}}{k_{AN}}+\frac{C_{AOH}}{k_{AOH}})+C_{AB}}$	$V_{AN}=\frac{Kcat_2 \cdot C_{AN} \cdot C_{EZ}}{Km_2 \cdot (1+\frac{C_{AB}}{k_{AB}}+\frac{C_{NH}}{k_{NH}}+\frac{C_{AOH}}{k_{AOH}})+C_{AN}}$	$V_S=V_{AB}*T_{MAX}*(\frac{C_{NH}}{K_{EN}+C_{NH}})$	$V_{AOH}=V_{AB}-V_S+V_{AN}$

Description: Model 1, without inhibition; model 2, with the inhibitors ester (POHPGME; shown here as AB) and amoxicillin (AN); model 3, inhibitors amoxicillin and POHPG (AOH); model 4, inhibitors ester, amoxicillin, 6-APA (NH) and POHPG. Ci indicates the concentration of compound i; EZ, enzyme; kAB, inhibition constant (POHPGME); kAN, inhibition constant (amoxicillin); kAOH, inhibition constant (POHPG); kNH, inhibition constant (6-APA); Ken, 6-APA adsorption constant; Tmax , maximum conversion ratio of the complex acyl-enzyme-nucleus into product; V_{AB}, rate of POHPGME consumption (mM/min); V_{AN}, rate of amoxicillin production (mM/min); V_S , rate of amoxicillin synthesis (mM/min). Km_1, Michaelis-Menten constant for the hydrolysis of amoxicillin; Km_2, Michaelis-Menten constant for ester consumption; $kcat_1$, catalytic constant for the hydrolysis of amoxicillin; $kcat_2$, catalytic constant for ester consumption.

To represent the dynamics of the state variables in the bioreactor, four ordinary differential equations were selected to represent the concentrations of ester (C_{AB}), amoxicillin (C_{AN}), 6-APA (C_{NH}), and POHPG (C_{AOH}), as shown in Table 2.

Table 2 EDOs for synthesis process[5].

State variables	Ester (C_{AB})	Amoxicillin (C_{AN})	6-APA (C_{NH})	POHPG (C_{AOH})
EDOs	$\frac{dC_{AB}}{dt}=-V_{AB}$	$\frac{dC_{AN}}{dt}=V_S-V_{AN}$	$\frac{dC_{NH}}{dt}=V_{AN}-V_S$	$\frac{dC_{AOH}}{dt}=V_{AOH}$

The posterior probability distribution was obtained using Bayesian statistics, using MCMC with the Metropolis-Hastings algorithm. The parameter estimates were based on the mean and a 99% credible interval. The best model was selected using the relative root mean square error (rRMSE).

$$rRMSE=\frac{\sqrt{\sum_{i=1}^{n}(Y_m-Y_e)^2}}{\frac{n_t}{\bar{Y}_m}}$$

where Y_m is the experimentally measured value, Y_e is the estimated value, $\bar{Y}_m$ is the mean of the experimentally measured value, and n_t is the total number of measurements.

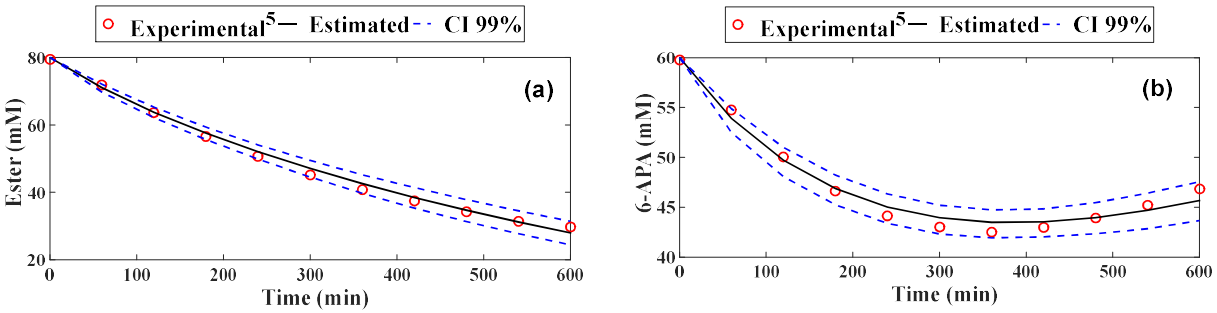
3 RESULTS & DISCUSSION

The rRMSE results are presented in Table 3. It is observed that the smallest errors were for the concentration of ester, amoxicillin, and 6-APA in model 4. On the other hand, model 2 presents the lowest error value for the concentration of POHPG. Model 4 better represents the experimental data for the amoxicillin production process. The rRMSE values for all state variables in model 4 are below 10%, which classifies it as excellent[8].

Table 3 rRMSE of the models.

State variables	Molde 1 (%)	Model 2 (%)	Model 3 (%)	Model 4 (%)
Ester	7.32	2.82	2.64	2.39
Amoxicillin	12.50	7.62	9.30	6.11
6-APA	3.23	1.92	2.30	1.48
POHPG	12.82	2.73	6.10	2.75

As shown in Figure 1, all the experimental data fall within the 99% credible interval. This implies that the substrate consumption and product synthesis dynamics can be accurately represented by the estimated parameter values (Table 4) under the proposed operating conditions: pH 6.5, 25°C, 1g of 30 i.u/mL derivative, 80 mM ester, and 60 mM 6-APA5. After selecting the mathematical model based on rRMSE, various analyses can be performed. For example, it is possible to examine the inhibitory effects of substrates (6-APA and ester) and products during the enzymatic synthesis of amoxicillin[5].



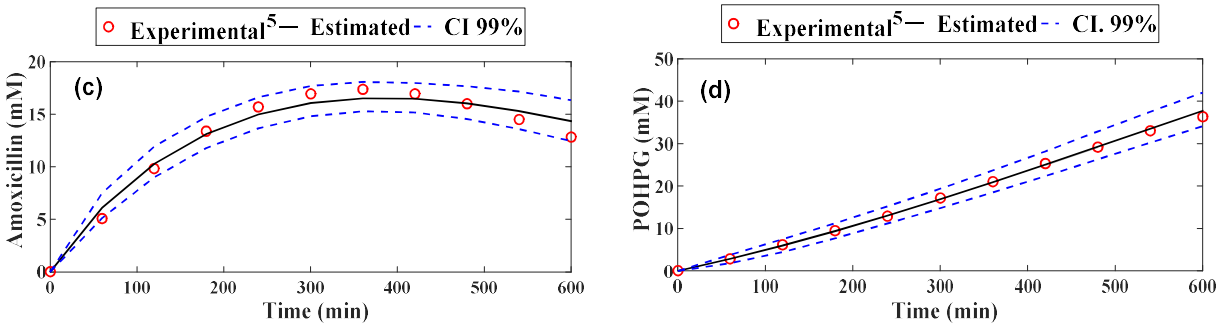


Figure 1 Experimental measurements and estimated.

Table 4 shows the parameter estimates, including averages and 99% credible interval. The results indicate that PGA has an affinity for the ester, with an average estimated value of Km1 of 5.45. In contrast, amoxicillin has a higher affinity, with an average estimated value of Km2 of 1.69. This relationship reflects the interaction of the substrate with the enzyme's active site. Amoxicillin has a greater affinity due to its intermediate product status and the reaction equilibrium favouring hydrolysis of the product. The parameter Tmax indicates the maximum rate of conversion from the acyl-enzyme complex to the product, amoxicillin. The estimated value points to a new conversion rate of 0.82, meaning that amoxicillin is synthesized 82% of the time after complex formation. The estimated Ken parameter of 7.95 suggests an increase in 6-APA adsorption on the active site of the PGA, resulting from the decrease in the Ken value and leading to a higher process yield.

Table 4 Estimation of kinetic parameters.

Parament	Initial estimate[5]	Mean value	Lower value	Higher value
kcat1 (μmol/i.u. min)	0.18	0.18	0.15	0.23
kcat2 (μmol/i.u. min)	0.33	0.39	0.28	0.66
Km1 (mM)	7.91	5.45	4.11	6.94
Km2 (mM)	12.5	1.69	1.43	2.05
Tmax (Dimensionless)	0.61	0.82	0.72	0.96
Ken (mM)	14.4	7.95	5.65	10.41
kAB (mM)	3.78	0.68	0.43	1.07
kAN (mM)	9.17	1.99	1.18	3.29
kAOH (mM)	10.9	9.86	6.28	13.27
kNH (mM)	62.04	9.76	6.70	14.47

4 CONCLUSION

To represent the synthesis of amoxicillin in an enzymatic reactor, different semi-empirical models were evaluated. Model 4 fitted better to the experimental data, indicating that the hydrolysis of amoxicillin and the ester is competitively inhibited by the ester and amoxicillin, respectively. The hydrolysis of amoxicillin is also competitively inhibited by 6-APA, while the hydrolysis of the ester and the antibiotic is competitively inhibited by POHPG. The model performed excellently (rRMSE < 10%) to describe the enzymatic synthesis of amoxicillin under the experimental conditions of the bioreactor.

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