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MATHEMATICAL MODELING OF AMOXICILLIN SYNTHESIS AT HIGH ESTER CONCENTRATION

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ABSTRACT

This study investigates the enzymatic synthesis of amoxicillin at high ester concentration through mathematical modeling and parameter estimation. A kinetic model based on the mechanism of penicillin G acylase (PGA), adapted for immobilized PGA, was utilized. The reactions were described by differential equations, implemented in Matlab, and compared with existing experimental data from the literature. Parameter estimation was conducted using the Monte Carlo Markov Chain (MCMC) method, employing the Metropolis-Hastings algorithm and a Gaussian probability distribution. The results demonstrated that the experimental data were within the 99% credible interval, indicating the model's consistency. The estimated kinetic parameters showed low rRMSE values, ranging from 4.09% to 9.24%, suggesting a low deviation between the model predictions and the experimental data. It was concluded that the enzymatic synthesis of amoxicillin at high ester concentration can be accurately simulated through mathematical approaches, with MCMC proving effective in predicting experimental data. This study confirms the feasibility of using mathematical models to enhance the understanding of the production process of β-lactam antibiotics.

Keywords: Penicillin G Acylase. Parameter Estimation. Markov Chain Monte Carlo.

1 INTRODUCTION

Amoxicillin is a semi-synthetic antibiotic effective in the treatment of bacterial infections, which is attributed to the β-lactam ring in its structure.[1] Amoxicillin is traditionally synthesized through chemical conversions, involving extreme operating conditions and the use of toxic solvents. The enzymatic synthesis of amoxicillin, catalyzed by penicillin G acylase (PGA), is a sustainable and efficient approach compared to traditional synthesis, as it operates under mild and selective conditions, avoiding the use of aggressive chemicals and extreme temperatures.[2] The process involves the reaction of hydroxyphenylglycine methyl ester (PHPGME) with 6-aminopenicillanic acid (6-APA). PGA catalyzes three reactions: (1) the production of Amoxicillin; (2) PHPGME hydrolysis to p-hydroxyphenyl glycine (PHPG) and methanol; and (3) amoxicillin hydrolysis to 6-APA and PHPG.[3] Figure 1 schematizes the reactions involved in the enzymatic synthesis of amoxicillin catalyzed by PGA.

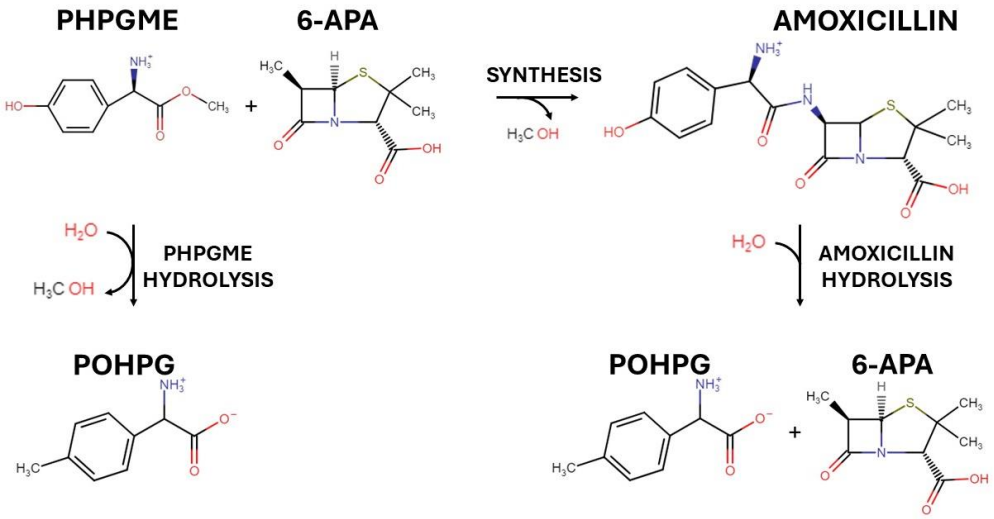

Figure 1 Enzymatic synthesis of amoxicillin catalyzed by PGA.

Mathematical modeling and simulation are useful tools to improve understanding of the production processes of β-lactam antibiotics. Researchers[4–9] have developed mathematical models for the enzymatic synthesis of β-lactam antibiotics. However, it was observed that these models had limitations in describing the synthesis at high ester concentrations. In this study, the enzymatic synthesis of amoxicillin at high ester concentration was investigated through modeling and parameter estimation.

2 MATERIAL & METHODS

The mathematical model is based on the kinetics and mechanism of PGA, developed by McDonald et al.[9] To perform parameter estimation, the model was changed to represent amoxicillin synthesis catalyzed by immobilized PGA. The kinetic model is described in equations 1-4, where E is the total concentration of immobilized PGA.

$$\frac{d[AMOX]}{dt} = V_{AMOX} = \frac{E}{k_3K_N + k_4C_{NH} + k_5C_{NH}} \frac{k_2k_4C_{AB}C_{NH}}{K_S} - \frac{k_{-4}C_{AN}(k_3K_N + k_5C_{NH})}{K_P} \quad (1)$$

$$\frac{d[POHPG]}{dt} = V_{POHPG} = \frac{E(k_3K_N + k_5C_{NH})}{k_3K_N + k_4C_{NH} + k_5C_{NH}} \frac{k_2C_{AB}}{K_S} - \frac{k_{-4}C_{AN}}{K_P} \quad (2)$$

$$\frac{\mathrm{d}[\mathrm{PHPGME}]}{dt} = V_{PHPGME} = -(V_{AMOX} + V_{POHPG}) \quad (3)$$

$$\frac{\mathrm{d}[6-\mathrm{APA}]}{dt} = V_{6-APA} = -(V_{AMOX}) \quad (4)$$

The model was implemented in Matlab and its solutions were compared with experimental data of Gonçalves[4], who studied the production of amoxicillin through the reaction of POHPGME and 6-APA catalyzed by PGA immobilized in glyoxyl-agarose, at 25°C, pH 6.5 and 3 mL of 30 UI/mL of biocatalyst.

The estimation of the model parameters was carried out using the Markov Chain Monte Carlo (MCMC) method. This method is a statistical technique used to generate samples through an iterative process.[10] The implementation of the MCMC method used the Metropolis-Hastings algorithm.[11-13] Figure 2 illustrates this iterative process. The vector of initial estimate parameters is defined as P^1. Here, i represents the iteration counter of the Markov chain, and j indicates the acceptance criterion for the proposed distributions. The number of states in the Markov chain, denoted as N, was set to 10^5, and the relative standard deviation, w, was 0.6. The iteration counter i serves as the algorithm's stopping criterion and is incremented to $i+1$ at the end of each iteration until the number of states in the chain is reached.

During each iteration, a random number u is generated from a uniform distribution $U(0,1)$. Subsequently, a new candidate parameter vector is proposed through a Gaussian transition kernel. The acceptance or rejection of this vector depends on its comparison with the Hastings ratio, RH. The Hastings ratio is the ratio between the probability of the new proposed parameter vector and the probability of the current parameter vector. If $u \leq RH$, the vector is accepted; otherwise, it is rejected. After acceptance, the j counter is incremented, and the posterior probability distribution is updated. If the vector is rejected, there will be no update.[10-13]

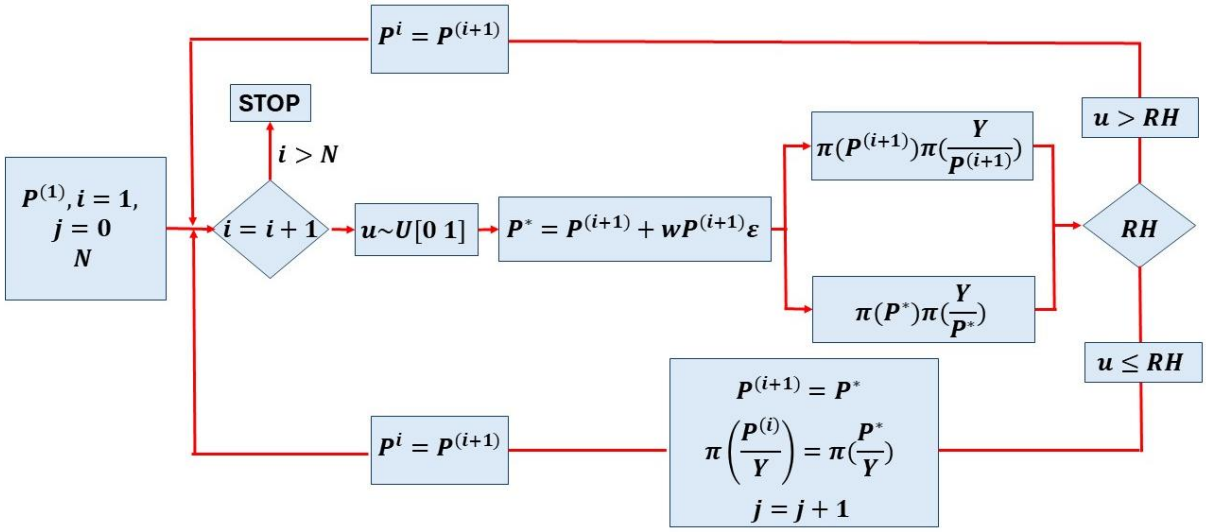

Figure 1 Iterative process used to estimate kinetic parameters.

The MCMC was evaluated using the relative root mean square error (rRMSE), represented by Eq. (5), as a performance measure, providing an indication of the relative accuracy of the kinetic model in relation to the experimental data.

$$rRMSE = \sqrt{\frac{\frac{1}{n}\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2}{\sum_{i=1}^{n}(\hat{Y}_i)^2}} \quad (5)$$

3 RESULTS & DISCUSSION

Figure 2 compares the experimental measurements and those estimated by the MCMC method for amoxicillin synthesis, ester consumption, 6-APA consumption and POHPG synthesis, with a 99% credible interval (CI). For all state variables studied, the experimental measures are within the credible interval, indicating that the experimental data are consistent with the predictions of the mathematical model within a 99% uncertainty level.

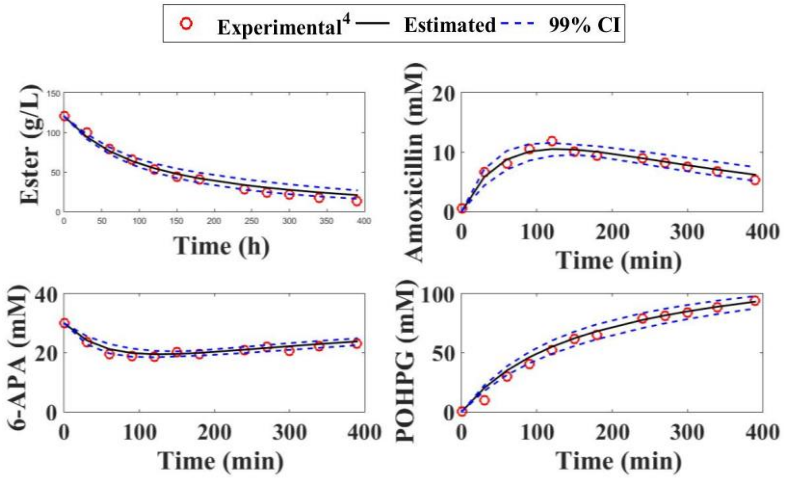

Figure 1 Experimental measurements and estimates by the MCMC method.

Table 1 presents the values of the kinetic parameters estimated by the MCMC method, in relation to the mean and the 99% credible interval.

Table 1 Estimation of kinetic parameters.

Parameter	Mean value	Lower value	Higher value
K_2 (s^{-1})	0.03	0.02	0.03
K_3 (s^{-1})	0.96	0.61	1.66
K_4 (s^{-1})	0.07	0.05	0.08
K_{-4} (s^{-1})	0.01	0.00	0.00
K_5 (s^{-1})	0.15	0.12	0.21
K_S (mol/L)	3.19	2.59	3.89
K_P (mol/L)	0.24	0.19	0.31
K_N (mol/L)	1.65	1.07	2.47

The rRMSE results show that the MCMC method presented excellent fit for all state variables, with rRMSE values below 10%[14], being 9.24% for the ester, 8.31% for amoxicillin, 4.09 % for 6-APA and 6.84% for POHPG. This confirms that there is low deviation between the model predictions and the experimental data studied.

4 CONCLUSION

The study demonstrates that the enzymatic synthesis of amoxicillin at high ester concentration can be accurately simulated using mathematical approaches. By employing the kinetic model, it was possible to obtain a robust representation of the synthesis process. MCMC, implemented with the Metropolis-Hastings algorithm, proved to be effective in predicting experimental data, as evidenced by the low rRMSE values.

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