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MATHEMATICAL MODELING AND ESTIMATION OF KINETIC PARAMETERS OF ACETIC ACID PRODUCTION BY *MOORELLA THERMOACETICA* IN A BATCH BIOREACTOR

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ABSTRACT

Acetic acid is economically important due to its versatile applications and its production potential from lignocellulosic biomass sugars. The development of kinetic models for the bioconversion dynamics of various sugars is crucial for the optimization of lignocellulosic biorefineries. This study estimated the kinetic parameters of batch bioreactor fermentation for the conversion of glucose, xylose, and their mixtures using the Markov Chain Monte Carlo method with the Metropolis-Hastings algorithm. The estimated concentrations were compared with experimental measurements from the literature, and the relative root mean square error (rRMSE) was calculated. The results showed greater yields in cell growth and product synthesis compared to literature values, as well as a lower affinity of the microorganism for glucose. The estimation results showed an excellent model fit for xylose (rRMSE < 10%) and a good fit for glucose and dual substrates (rRMSE < 15%), proving the efficiency of the mathematical model in simulating and estimating the analyzed parameters and its potential applicability to other bioprocesses.

Keywords: Bayesian statistics. Markov Chain Monte Carlo Method. Metropolis-Hastings. Lignocellulosic biomass. Computational Simulation.

1 INTRODUCTION

Acetic acid (acetate) is a chemical raw material of great importance that can be obtained directly from the sugars present in lignocellulosic biomass, with diverse applications such as in food, pharmaceuticals, textiles, silk, and films[1]. In nature, acetate plays a fundamental role as an intermediate in the anaerobic bacterial degradation of organic matter[2]. It is generated both as a product of the fermentation of various organic materials and through synthesis from CO_2 and/or other carbon precursors, being used as a food additive, antimicrobial recycling factor, acidulant, flavor enhancer, among others[2].

Bacteria that can produce acetate are commonly called acetogens, due to their ability to specifically catalyze this synthesis[3]. Among them, *Moorella thermoacetica* stands out as a strictly anaerobic, endospore-forming, and homoacetogenic bacterium that uses the acetyl-CoA pathway to reduce CO_2 to acetic acid or acetate. It demonstrates remarkable metabolic versatility, making it a promising thermophilic host for industrial biotechnology applications[4].

Mathematical modeling provides significant advantages in the analysis and optimization of biotechnological processes, enabling precise description of system behavior under different operational conditions and facilitating the understanding of underlying mechanisms in substrate bioconversion. Additionally, it allows for process performance prediction, identification of critical parameters, and optimization of operational conditions to maximize acetic acid production. This study estimates the kinetic parameters for the bioconversion of single and double substrates in acetic acid production by *Moorella thermoacetica* in a batch bioreactor, using Bayesian statistics with the Markov chain Monte Carlo (MCMC) method and the Metropolis-Hastings (MH) algorithm[5-8]. This approach provides a robust analysis of uncertainties associated with kinetic parameters, offering a more reliable and precise evaluation and optimization of the bioprocess.

2 MATERIALS & METHODS

The experimental measurements used in this work were obtained from literature[1], where strains of *Moorella thermoacetica* were selected and cultivated with glucose and xylose extract at a pH of 7.4 and a temperature of 58ºC to obtain acetate. The mathematical modeling consists of the mass balance represented by Equations (1-4). The specific growth rates of the biomass associated with the substrate (μ_{g1} and μ_{g2}) are described in Equations (5,6).

$$\frac{dX}{dt} = (\mu_{g1} + \mu_{g2})X - KdX \quad (1)$$

$$\frac{dS_1}{dt} = -\left(\frac{\mu_{g1}}{Y_{XS,1}}\right)X \quad (2)$$

$$\frac{dS_2}{dt} = -\left(\frac{\mu_{g2}}{Y_{XS,2}}\right)X \quad (3)$$

$$\frac{dP}{dt} = (\mu_{g1}Y_{PX,1} + \mu_{g2}Y_{PX,2})X \quad (4)$$

$$\mu_{g1} = \frac{\mu_{max,g1} S_1}{K_{S1} + S_1}\left(1 - \frac{P}{P_{max}}\right) \quad (5)$$

$$\mu_{g2} = \frac{\mu_{max,g2} S_2}{K_{S2} + S_2} \times \left(\frac{1}{1 + K_1 S_1}\right)\left(1 - \frac{P}{P_{max}}\right) \quad (6)$$

For this process, Bayesian statistics was used, Equation (7)[6,9], which allows information obtained from experimental data to be combined with assumptions about the model's parameters.

$$\pi(\boldsymbol{P}|\boldsymbol{Y}) = \frac{\pi(\boldsymbol{P})\pi(\boldsymbol{Y}|\boldsymbol{P})}{\pi(\boldsymbol{Y})} \quad (7)$$

Where were π**(P|Y)** represents the posterior probability distribution; π(**P**) is the prior probability distribution; π(**Y|P**) is the likelihood function; π(**Y**) is the marginal probability density of the measurements, which acts as a normalization constant; **P** represents the vector of unknown parameters[6,9]. Thus, using the MCMC method to estimate kinetic parameters, values were obtained that closely align with experimental measurements[1]. These values enable the description, understanding, and prediction of bioconversion behavior. To validate the performance of the evaluated model, the relative root mean square error (rRMSE) was calculated, Equation (8), which facilitates the understanding of the discrepancy between experimental measurements and the mathematical model estimates.

$$rRMSE = \sqrt{\frac{\frac{1}{n}\sum_{i=1}^{n}\left(Y_i - \hat{Y}_i\right)^2}{\sum_{i=1}^{n}\left(\hat{Y}_i\right)^2}} \quad (8)$$

3 RESULTS & DISCUSSION

After applying Bayesian statistics, estimates of cell, glucose, xylose and acetate concentrations were obtained, in terms of the mean and the 99% credible interval (CI), as shown in Figure 1.

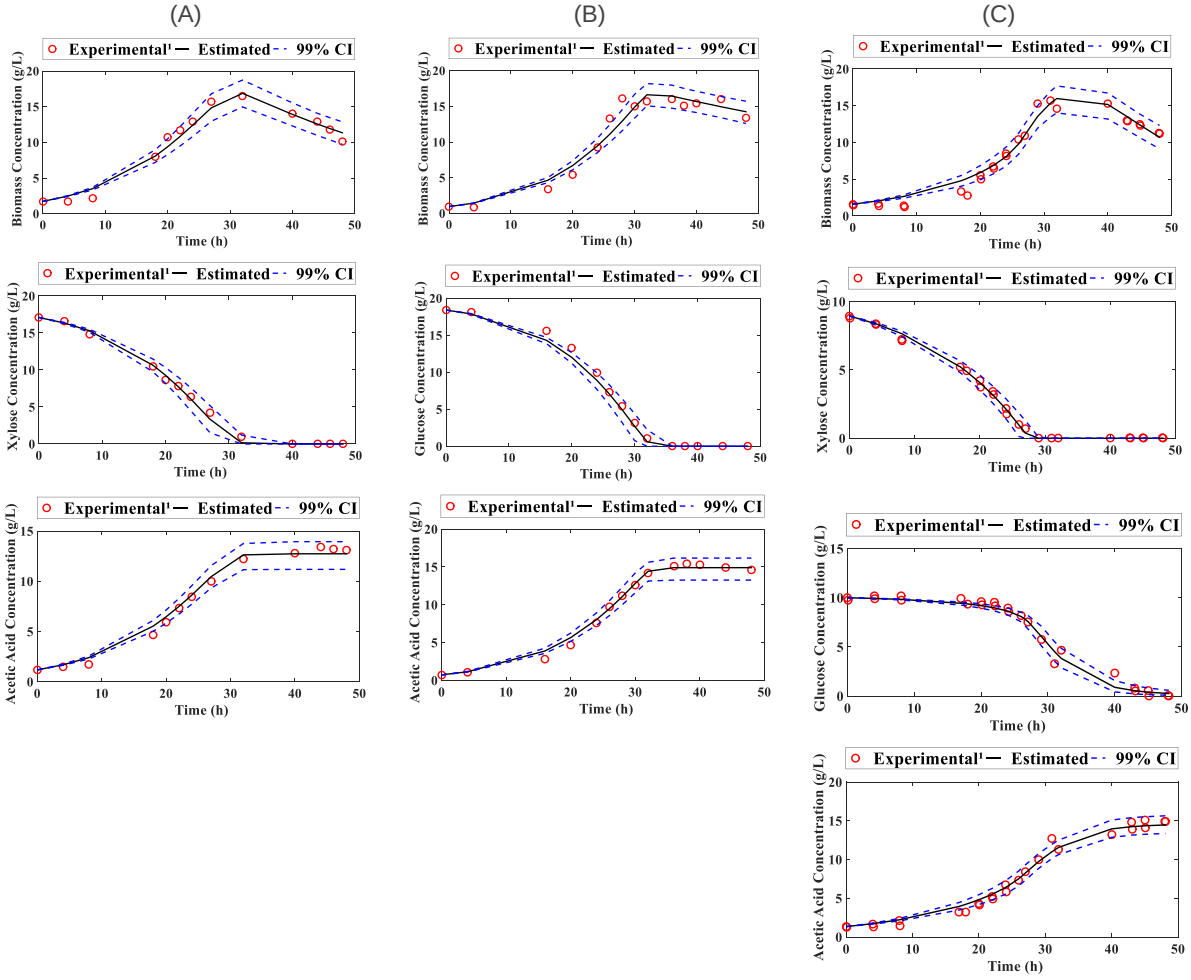

Figure 1 Comparison of model prediction and experimental data for batch culture of *M. thermoacetica* (A) in xylose, (B) in glucose and (C) in xylose/glucose.

Figure 1 shows *Moorella thermoacetica* preference for xylose, since in column (C) glucose consumption starts close to the end of xylose. The results also show a good agreement between the estimated concentrations and experimental measurements, which are within the 99% credible interval, showing the efficiency of the simulation via the MCMC method with the Metropolis-Hastings algorithm. For the single-substrate models, 5 parameters were estimated, including the maximum specific growth rate (μ_{max}),

substrate saturation constant (K_S), cell death rate constant (K_d), cell growth yield from substrate (Y_{XS}) and cell product yield (Y_{PX}). For the dual substrate model, the substrate inhibition constant (K_1) was added. The value of P_{max} was kept constant for all fermentations.

The results presented in Table 1 indicate that glucose saturation constant (K_{S2}) is almost 8 times greater than that of xylose in fermentation with a single substrate, suggesting a lower affinity of the microorganism for glucose. This observation is supported by data from dual-substrate fermentation. However, both glucose and xylose showed higher values for (Y_{PX}), with productivity and yield exceeding the literature baseline[1] for single fermentation, and superior only for glucose in dual fermentation.

The Y_{XS} values for xylose and glucose were higher than the data found in the literature, with xylose[10] varying between 0.133 - 0.058 g/L and glucose[11] 0.21 g/L, considering an initial substrate concentration of 17.1 g/L and 18.4 g/L, respectively. For the model fit, the rRMSE values were excellent for fermentation with xylose due to rRMSE < 10% and good for glucose and for the double substrate (10% < rRMSE < 15%).

Table 1 Estimation of kinetic parameters in terms of mean and 99% credible interval, and calculation of rRMSE.

Parameters	Xylose	Glucose	Xylose/Glucose
$\mu_{max,g1}$ (h^{-1})	0.13 (0.12; 0.14)	-	0.09 (0,08; 0,10)
$\mu_{max,g2}$ (h^{-1})	-	0.12 (0,11; 0,13)	0.29 (0,24; 0,33)
K_{S1} $(g\ L^{-1})$	0.02 (0,02; 0,03)	-	0.06 (0,04; 0,08)
K_{S2} $(g\ L^{-1})$	-	0.15 (0,09; 0,20)	9.27 (6,86; 10,98)
$Y_{XS,1}$ $(g\ g^{-1})$	1.47 (1,30; 1,68)	-	1.23 (1,06; 1,38)
$Y_{XS,2}$ $(g\ g^{-1})$	-	1.02 (0,88; 1,23)	1.44 (1,16; 1,76)
$Y_{PX,1}$ $(g\ g^{-1})$	0.46 (0,40; 0,54)	-	0.36 (0,29; 0,46)
$Y_{PX,2}$ $(g\ g^{-1})$	-	0.76 (0,63; 0,88)	0.68 (0,50; 0,87)
K_d (h^{-1})	0.03 (0,03; 0,04)	0.01 (0,01; 0,02)	0.04 (0,03; 0,05)
K_1 $(g\ L^{-1})$	-	-	1.06 (0,77; 1,45)
P_{max} $(g\ L^{-1})$	48.00	48.00	48.00
Parameters rRMSE (%)	**Xylose (%)**	**Glucose (%)**	**Xylose/Glucose (%)**
X	7.7	11.4	12.7
S_1	6.7	-	6.4
S_2	-	9.1	8.0
P	4.8	4.6	7.7

4 CONCLUSION

The modeling and simulation of the data using MCMC proved to be efficient in representing the experimental measurements and in estimating the parameters evaluated. As a result, the model studied was able to predict the dynamics of bioconversion in a satisfactory manner and has the potential to be used to optimize the fermentation of lignocellulosic sugars to acetic acid by *Moorella thermoacetica*, due to its advantages in precise parameter estimation and realistic data representation. This demonstrates that the modeling can be adapted to different conditions and substrate types, making it versatile and applicable to a wide range of industrial processes.

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