MATHEMATICAL MODELING OF THE KINETICS OF BIOETHANOL PRODUCTION BY *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Bioethanol is a fuel of great economic and environmental importance for Brazil, being the subject of extensive research on its bioconversion. In this study, the fermentative kinetic parameters for bioethanol production by *Saccharomyces cerevisiae* NP 01 were estimated using sweet sorghum juice as substrate in a batch bioreactor. The mathematical modeling was performed using the Markov chain Monte Carlo method with the Metropolis-Hastings algorithm. The estimates fit the experimental data, considering a 99% credible interval, showing excellent fit for biomass growth, substrate consumption and for ethanol production, validated according to the metric of relative root mean square error, with values below 10%. These results significantly contributed to a better understanding of the bioprocess.

Keywords: Bioethanol. Kinetic parameters. Modeling. Markov chain Monte Carlo method. *Saccharomyces cerevisiae*.

1 INTRODUCTION

Bioethanol is currently one of the main biofuels under research due to its wide industrial applicability, especially in the automotive and alcoholic beverages sectors[1,2]. The production of bioethanol is predominantly carried out from sugarcane and corn. However, several studies are being conducted to explore other feedstock sources, such as sweet sorghum juice (SSJ), due to the advantages offered by sweet sorghum (*Sorghum bicolor* L. Moench). This crop requires fewer fertilizers for production, has a shorter production cycle, and consumes less water[3]. Like sugarcane and corn, sweet sorghum uses glucose as its main carbon source[4]. It is typically produced through the fermentation of sugars by traditional or genetically modified yeasts, such as *Saccharomyces cerevisiae*. This bioprocess involves the conversion of glucose by yeast, under anaerobic conditions, to form ethanol[5,6].

Despite the significant advantages offered by bioethanol production through SSJ, some challenges are encountered, such as low sugar yield and ethanol production in comparison to sugarcane[4]. which make it difficult to expand on an industrial scale. Mathematical modeling can minimize these issues by employing the Markov chain Monte Carlo method with the Metropolis-Hastings algorithm (MCMC-MH), enabling the estimation of unknown kinetic parameters and optimization of the targeted process. This probabilistic technique is based on applying prior distributions to obtain estimated parameters from posterior distributions, according to Bayes' theorem. By utilizing this technique, it is possible to predict experimentally unknown values and improve yield[7,8,9].

Therefore, this study aims to estimate the kinetic parameters of ethanol production through the conversion of SSJ by *Saccharomyces cerevisiae* NP, as previously described in the literature[5], using an adapted mathematical model and applying MCMC-MH to investigate the kinetic behavior of the bioprocess in a batch bioreactor.

2 MATERIAL & METHODS

Experimental data on the bioconversion process of SSJ into bioethanol in a batch reactor, using *Saccharomyces cerevisiae* NP 01, were obtained from the literature[5] and were mathematically modeled using adapted differential equations from the literature[10] for cell growth, substrate consumption, and product generation. A mass balance was performed through the interaction of biomass (X), substrate (S), and product (P) concentrations, according to the Eqs. (2-4).

$$\mu = \frac{\mu_{max} S}{K_S + S}\left(1 - \frac{X}{X_{inb}}\right)\left(1 - \frac{S}{S_{inb}}\right)\left(1 - \frac{P}{P_{inb}}\right) \quad (1)$$

$$\frac{dX}{dt} = \mu X \quad (2)$$

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (3)$$

$$\frac{dS}{dt} = -\left(\frac{1}{Y_{XS}}\frac{dX}{dt} + mX\right) \tag{4}$$

The microbial growth model disregards cell death and the effects of primary and secondary metabolites were adapted from the Luedeking-Piret equation[11]. Substrate consumption was considered for both cell growth and maintenance, with ethanol being the considered product. The specific microbial growth rate (μ) was adapted from the Monod equation[12], accounting for inhibitory effects of substrate and product concentrations, as described in Eq. (1). The experimental measurements used in this work were obtained by SALAKKAM et al. (2023)[5]. Computational simulation was conducted using Bayesian statistics of the posterior probability distribution, Eq. (5). For the MCMC-MH method, a 99% credible interval (CI) was considered[9].

$$\pi(\mathbf{P}|\mathbf{Y}) = \frac{\pi(\mathbf{P})\pi(\mathbf{Y}|\mathbf{P})}{\pi(\mathbf{Y})} \tag{5}$$

The relative root mean square error (rRMSE) was chosen for model validation, as described in Eq. (6)[13].

$$rRMSE = \frac{\sqrt{\frac{\sum_{i=1}^{n}(\mathbf{Y_m} - \mathbf{Y_e})^2}{n_t}}}{\overline{\mathbf{Y_m}}} \tag{6}$$

3 RESULTS & DISCUSSION

The experimental measurements obtained by SALAKKAM et al. (2023)[5] were used to estimate the parameters of the sweet sorghum juice conversion process into ethanol. Figure 1 shows ethanol production, biomass growth, and substrate consumption. It is observed that the experimental data are within the 99% credible interval, demonstrating a good fit of the mathematical model and indicating that the MCMC-MH method was effective in estimating.

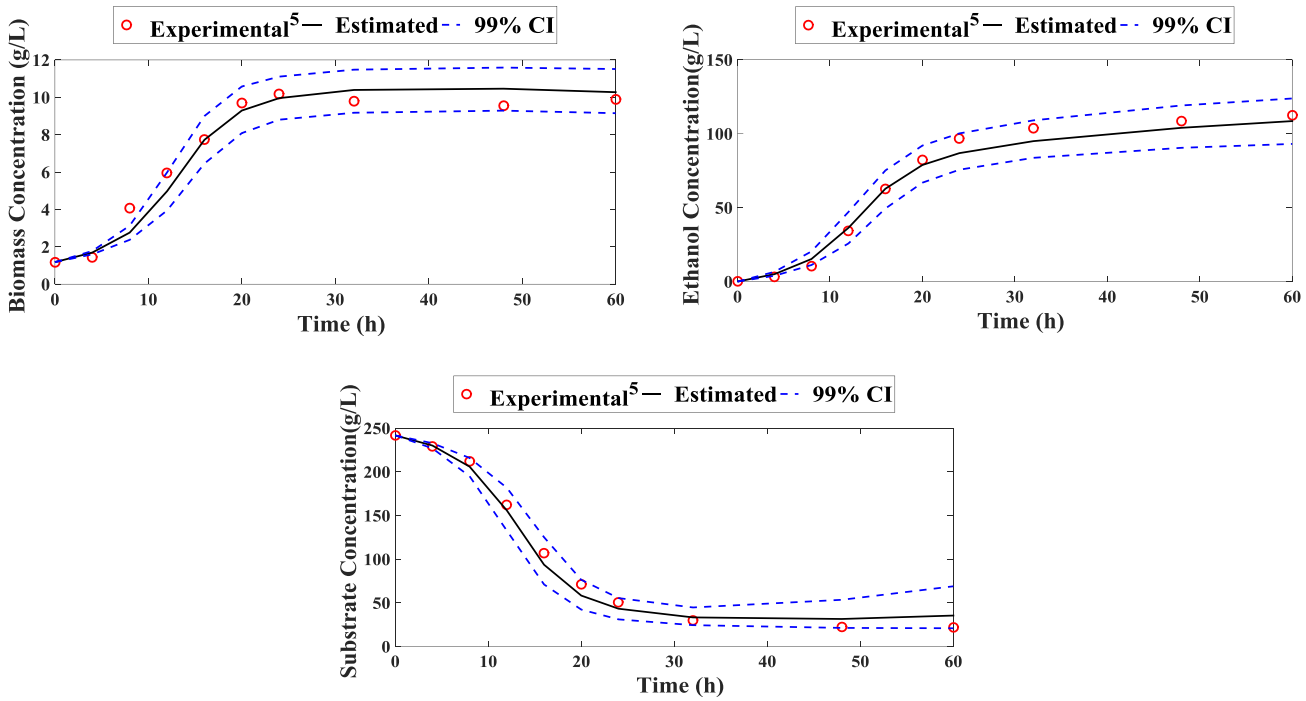

Figure 1: Comparison of the model prediction (solid lines), experimental data (markers), and credible interval (dashed lines) for biomass, ethanol and substrate concentrations.

For the proposed model, nine parameters and their respective credible intervals were estimated, as presented in Table 1. These parameters include the maximum specific growth rate (μ_{max}), biomass inhibitory concentration for cell growth (X_{inib}), substrate inhibitory concentration for cell growth (S_{inib}), product inhibitory concentration for cell growth (P_{inib}), substrate saturation constant (K_s), growth associated product formation rate (α), non-growth associated product formation rate (β), biomass yield per substrate (Y_{Xs}), and cell maintenance (m).

Upon analyzing the estimated parameters in Table 1, a significant affinity between the substrate and yeast can be observed, evidenced by the low value of K_s close to zero[12]. Additionally, the process of cell growth, substrate consumption, and ethanol production exhibits slow kinetics, as indicated by the low value of μ_{max}. The model also indicates a low value of cell maintenance, a characteristic observed in *Saccharomyces cerevisiae* yeast as reported in the literature[5].

The parameter estimates reveal significant inhibition values for biomass, product, and substrate. The parameter distribution suggests a focus on the substrate curve, indicating that the microorganism concentrated substrate consumption for cell growth, with minimal utilization for cell maintenance. This is consistent with the experimental values observed in the literature for the yields Y_{xs} and m[5].

Table 1 Kinetic parameters estimated with 99% confidence interval and calculation of the rRMSE

Parameters	Unit value	Mean	CI 99%
μ_{max}	h^{-1}	0.0011	[0.0010; 0.0014]
X_{inib}	g/L	0.140	[0.106; 0.189]
S_{inib}	g/L	24.156	[16.419; 29.97]
P_{inib}	g/L	106.923	[88.253; 126.868]
K_s	g/L	0.0698	[0.054; 0.0814]
α	g/g	9.190	[7,701; 11.017]
β	g/g	0.049	[0.0371; 0.062]
Y_{Xs}	g/g	0.044	[0.038; 0.050]
m	h^{-1}	0.0013	[0.0009; 0.0018]
rRMSE X (Biomass concentration)	**rRMSE P (Ethanol concentration)**		**rRMSE S (Substrate concentation)**
9.41%	8.15%		7.55%

In addition to the parameters, Table 1 also presents the rRMSE results, indicating an excellent fit of the model to the biomass growth curve, substrate consumption curve and for the ethanol production curve, with rRMSE values below 10%. These results confirm the effectiveness of the proposed model in representing and predicting the sweet sorghum juice conversion process into ethanol.

4 CONCLUSION

In this study, mathematical modeling was conducted to estimate fermentative kinetic parameters for bioethanol production from SSJ using *Saccharomyces cerevisiae* NP 01 in a batch bioreactor. The proposed model underwent investigation through the application of the MCMC-MH method, with experimental measurements within the 99% credible interval. The obtained estimates of the kinetic parameters align with the evaluated process, showcasing the model's effectiveness in representing the bioprocess. Data and modeling indicate that SSJ is a promising feedstock for bioethanol production, owing to its significant substrate-yeast affinity despite exhibiting slow kinetics. Furthermore, the rRMSE results were deemed excellent (< 10%), further supporting the accuracy of the mathematical model and the analyzed kinetic parameters in simulating the bioprocess.

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ACKNOWLEDGEMENTS

The authors thank the Federal University of Pará (UFPA) and the Graduate Program in Biotechnology (PPGBIOTEC/UFPA).