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ANALYSIS OF 1,3-PROPANEDIOL PRODUCTION BY *KLEBSIELLA PNEUMONIAE* BLH-1 USING BAYESIAN STATISTICS

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ABSTRACT

The analysis of fermentative kinetic parameters enables the optimization of production by predicting the biochemical behavior of the microorganism, thereby enhancing the efficiency, economy, and scalability of the process for industrial applications. This study investigated the production of 1,3-propanediol by *Klebsiella pneumoniae* BLh-1 through the conversion of residual glycerol under oxygen-limited conditions in batch mode. Bayesian statistics via the Markov Chain Monte Carlo (MCMC) method was employed in the simulation, using the Metropolis-Hastings algorithm. The mathematical modeling considered the inhibitory effects through adaptations of the Monod and Luedeking-Piret equations. The average tolerance for residual glycerol was 104.97 g L^{-1}, and for the products, it was 31.24, 10.28, 4.97, and 33.07 g L^{-1} for 1,3-PDO, ethanol, acetate, and lactate, respectively. The microorganism exhibits characteristics of primary metabolites, with residual glycerol predominantly used for cell growth. Biomass, products, and substrate presented adjusted R^2 values above 83% and rRMSE values within acceptable limits, highlighting the biomass curve with a value of 0.09. In the hypothesis test, all state variables presented an F value greater than the critical F value (16.2582), considering $\alpha = 0.01$. Therefore, the results reinforce the quality and significance of the proposed model for the bioprocess.

Keywords: Modeling. Kinetic parameters. 1,3-Propanediol. Residual Glycerol. *Klebsiella pneumoniae.*

1 INTRODUCTION

1,3-Propanediol (1,3-PDO) is a chemical substance with extensive industrial applications, mainly in the production of polymers, pharmaceuticals, and solvents[1]. Typically, 1,3-PDO production is achieved via chemical routes using catalysts[2]. However, due to increasing environmental concerns and economic importance, researchers are exploring new production methods. The conversion of residual glycerol through microbial fermentation provides a sustainable and economical alternative for biorefineries, yielding 1,3-PDO, ethanol, acetate, and lactate[3].

Mathematical modeling combined with Bayesian techniques enables the investigation of experimentally unknown kinetic parameters. This facilitates process optimization and reduces experimental costs, both at laboratory and industrial scale[4,5]. The Markov Chain Monte Carlo method with the Metropolis-Hastings algorithm (MCMC-MH), based on Bayes' theorem, is a probabilistic technique that uses information from the prior probability distribution along with experimental measurements to estimate the posterior probability distribution[6]. This application allows better control of the bioprocess by enabling the understanding of physicochemical phenomena through microbial kinetic parameters, such as the inhibitory effects of the substrate and multiple products. It is worth noting that this control is a major challenge for biotechnological processes, making the process more efficient, economical, and scalable for the fermentation of 1,3-PDO[3,7].

Thus, this work aims to estimate the kinetic parameters to produce 1,3-PDO by *Klebsiella pneumoniae* BLh-1 in the conversion of residual glycerol into 1,3-PDO, ethanol, acetate, and lactate. A mathematical model adapted with the MCMC-MH technique is used to investigate bioprocess behavior in a batch bioreactor under oxygen-limited conditions.

2 MATERIAL & METHODS

The mathematical model of the bioconversion of residual glycerol by *K. pneumoniae* BLh-1 was developed based on the fermentative mass balance to obtain the differential equations for the concentration of biomass (dX/dt), substrate (dS/dt), and multiple products (dPi/dt), as described in Eqs (1)-(3). Bacterial death was disregarded in cell growth. Substrate consumption was accounted for cell growth and maintenance. The products investigated were 1,3-PDO, ethanol, acetate, and lactate. The primary and secondary metabolite effects were adapted from the Luedeking & Piret equation[8]. The specific microbial growth rate (μ) was adapted from the findings of Monod[9], considering the inhibitory effects of substrate concentrations and multiple products, as described in Eq. (4).

$$\frac{dX}{dt} = \mu X \tag{1}$$

$$\frac{dS}{dt} = -\left(\frac{1}{Y_{XS}}\frac{dX}{dt} + mX\right) \tag{2}$$

$$\frac{dPi}{dt} = \alpha_i \frac{dX}{dt} + \beta_i X \tag{3}$$

$$\mu = \mu_m \frac{S}{Ks + S}\left(1 - \frac{S}{S^*}\right)\left(1 - \frac{Pi}{Pi^*}\right) \tag{4}$$

where Y_{XS} is the yield coefficient of biomass by substrate (g/g), m is the cell maintenance rate (h^{-1}), α_i is the kinetic constant associated with primary metabolite formation (g/g), β_i is the kinetic constant associated with secondary metabolite formation (g/g),

μ_m is the maximum cell growth rate (h^{-1}), Ks is saturation constant (g/L), S^* is the inhibitory concentration of the substrate (g/L), Pi^* is the inhibitory concentration of the product i (g/L). Such variables were investigated. The experimental measurements and cultivation conditions were obtained from Rossi[10], which consisted of the fermentation of residual glycerol by *K. pneumoniae* BLh-1 under oxygen-limited conditions in a 2 L batch bioreactor at 37 °C, 300 rpm, pH 7.0, and 0.4 vvm of air.

The computational simulation was carried out based on the Bayesian structure of the posterior probability distribution, using Bayes' theorem, according to Eq. (5). The estimates of the kinetic parameters, using the MCMC-MH method, were carried out in terms of the mean and the 99% credible interval (CI)[5]. The square of the adjusted correlation coefficient (R^2adj) and the relative root mean squared error (rRMSE) were chosen to validate the model, described in Eq. (6)-(7)[11,12]. The hypothesis test was applied to verify the significance between the dependent variables (concentration of biomass, products and substrate) and the independent variable (time), as Gaudio[12], describing in Eq. (8)-(9): the value of F and degrees of freedom, respectively.

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

$$R^2adj = R^2 - \left(\frac{k-1}{n-k}\right)(1-R^2) \tag{6}$$

$$rRMSE = \frac{\sqrt{\frac{\sum_{i=1}^{n}(Y_m - Y_e)^2}{n_t}}}{\bar{Y}_m} \tag{7}$$

$$F = \frac{\frac{\sum_{i=1}^{n}(Y_e - \bar{Y}_m)^2}{k}}{\frac{\sum_{i=1}^{n}(Y_m - Y_e)^2}{n-k-1}} \tag{8}$$

$$GL = n - 1 \tag{9}$$

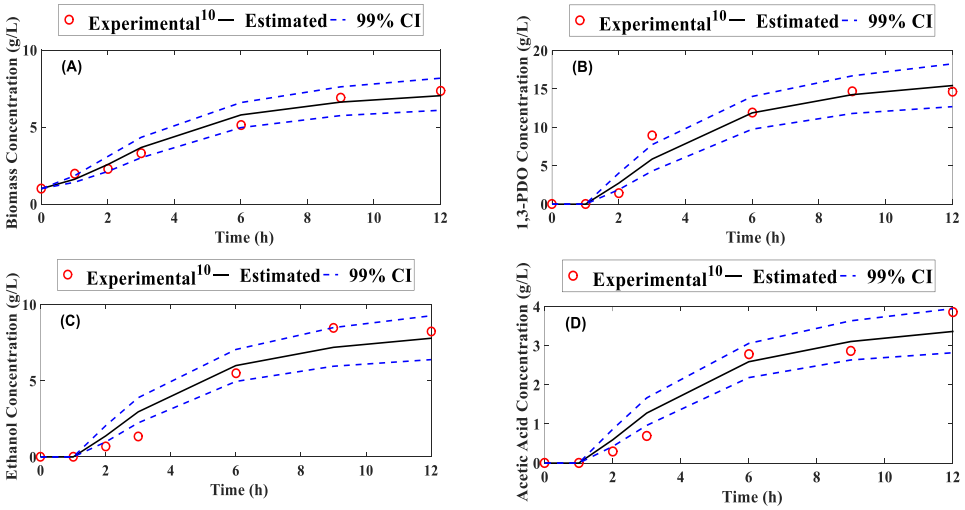
where π(**P**|**Y**) represents the posterior probability distribution; π(**P**) is the prior probability distribution; π(**Y**|**P**) is the likelihood function; π(**Y**) is the marginal probability density of the measurements, which acts as a normalization constant; **P** represents the vector of unknown parameters, **Y** is the vector of state variables of the mathematical model, R^2 is determination coefficient, k is number of independent variables, n is observed values, Y_m is the experimentally measured value, Y_e is the estimated value, $\bar{Y}_m$ is the average of the experimental value and n_t is total number of experimental measurements.

3 RESULTS & DISCUSSION

In the simulation of 1,3-PDO production by *K. pneumoniae* BLh-1 under oxygen-limiting conditions, 17 kinetic parameters were estimated. Table 1 presents the values estimated by MCMC-MH, in terms of the mean and the 99% credible interval. Figure 1 illustrates the experimental measurements, obtained from Rossi[10], along with the estimates of the state variables of the mathematical model, which are the concentration of biomass, 1,3-PDO, ethanol, acetate, lactate and substrate, respectively. Table 2 represents the validation of the state variables, by adjusting experimental measurements and estimated values.

Table 1 Estimation of kinetic parameters for the production of 1,3-PDO.

Parameters	Unit	Mean	CI	Parameters	Unit	Mean	CI
μ_m	(h^{-1})	1.056	[0.684; 1.774]	α_{Pp}	$(g\ g^{-1})$	2.86	[2.27; 3.58]
S^*	$(g\ L^{-1})$	104.97	[69.23; 169.40]	α_{Pe}	$(g\ g^{-1})$	1.44	[1.15; 1.80]
Pp^*	$(g\ L^{-1})$	31.24	[17.24; 53.31]	α_{Pa}	$(g\ g^{-1})$	0.62	[0.50; 0.79]
Pe^*	$(g\ L^{-1})$	10.28	[6.97; 15.70]	α_{Pl}	$(g\ g^{-1})$	3.68	[2.69; 5.55]
Pa^*	$(g\ L^{-1})$	4.97	[3.33; 8.19]	β_{Pp}	(h^{-1})	7.70x10^{-15}	[6.46x10^{-16}; 2.97x10^{-14}]
Pl^*	$(g\ L^{-1})$	33.07	[16.09; 57.88]	β_{Pe}	(h^{-1})	7.83x10^{-09}	[7.52x10^{-10}; 2.75x10^{-09}]
K_S	$(g\ L^{-1})$	7.93x10^{-11}	[1.03x10^{-12}; 2.98x10^{-10}]	β_{Pa}	(h^{-1})	2.12x10^{-14}	[8.23x10^{-15}; 4.36x10^{-14}]
Y_{XS}	$(g\ g^{-1})$	0.102	[0.082; 0.128]	β_{Pl}	(h^{-1})	4.69x10^{-09}	[7.70x10^{-10}; 1.13x10^{-08}]
m	(h^{-1})	2.65x10^{-14}	[7.81x10^{-15}; 7.83x10^{-14}]				



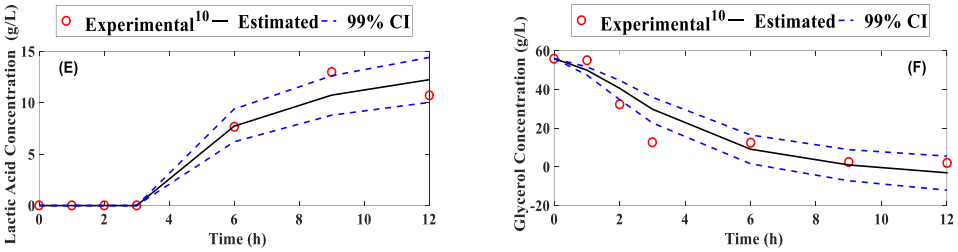


Figure 1 Estimation of state variables for experimental measures using MCMC-MH. Experimental points were obtained from Rossi[10]

Table 2 R^2adj and rRMSE of the models.

State variables	R^2adj	rRMSE	F value (α = 0.01)	Significant
Biomass	0.9464	0.09	192.1810	True
1,3-PDO	0.9101	0.18	109.1718	True
Ethanol	0.8310	0.25	65.5673	True
Acetate	0.9346	0.22	81.0925	True
Lactate	0.9813	0.23	127.4936	True
Residual glycerol	0.9626	0.31	40.6145	True

The results demonstrated that the microorganism exhibits a strong affinity for consuming residual glycerol, as evidenced by the nearly zero estimated Ks value. Pan[4] also showed a low Ks of 0.28 mmol/L, reinforcing that the mathematical model aligns well with the bioprocess. Additionally, due to the high affinity of *K. pneumoniae* BLh-1 for glycerol, a high maximum microbial growth rate (μ_m) was observed. The mathematical model for biomass exhibited an rRMSE of 0.09, indicating an excellent fit between the experimental data and the estimates obtained by MCMC[13]. This is illustrated in Figure 1, where all experimental measurements fall within the 99% credible interval.

The estimated inhibitory concentrations of substrate (S^*) and multiple products (Pi^*) on microbial growth aligned well with Rossi[10] experimental measurements, being of the same order of magnitude. In comparison to Silva[14], the estimated values also demonstrated physical coherence for the bioprocess in a batch bioreactor. Additionally, these parameters provide insights into the microorganism's tolerance levels for each investigated concentration. K. pneumoniae BLh-1 exhibited greater tolerance to the concentrations of substrate (S^*), lactate (Pl^*) and 1,3-PDO (Pp^*) at values of 104.97 $g\ L^{-1}$ [69.23; 169.40], 33.07 $g\ L^{-1}$ [16.09; 57.88] e 31.24 $g\ L^{-1}$ [17.24; 53.31], respectively.

The estimated metabolic effects of the organism indicated that the microorganism exhibited primary characteristics associated with cell growth, justified by the βi values tending to zero. The 1,3-PDO curve showed good rRMSE fits (0.18), while the other products were considered regular, according to Zhou[13]. Residual glycerol was primarily used for microbial growth (Y_{XS}) rather than for cellular maintenance (m), with the estimated values of m tending to zero. In addition to rRMSE, which evaluates the magnitude of the prediction error relative to the observed values, considering the randomness of the MCMC, adjusted R^2 (R^2adj) to assess the variability of the estimated experimental data and ensure the quality of the fermentation model's fit to the biomass, products, and substrate equations. All state variables showed good fits, with reliability greater than 83%. In hypothesis tests, all F values were greater than the critical F value (16.2582), considering α = 0.01, indicating that the regression model was statistically significant.

4 CONCLUSION

In this study, the fermentative kinetic parameters for 1,3-PDO production with *K. pneumoniae* BLh-1 in a batch bioreactor were estimated. The developed mathematical model represents the bioprocess under limited oxygen conditions. The inhibitory effects of the substrate and products and the metabolic effects were analyzed, presenting values of 104.97 $g\ L^{-1}$ for residual glycerol and 31.24 $g\ L^{-1}$ for 1,3-PDO. The microorganism demonstrated affinity for the substrate, influencing the growth rate. The state variables fitted well to the model, with variability and reliability above 83% in adjusted R^2 and low prediction errors by MCMC-MH, with biomass and 1,3-PDO at 0.09 and 0.18, respectively. In hypothesis tests, all F values were higher than the critical value (16.2582), reinforcing the quality and significance of the model.

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