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Kinetic modelling of a dark fermentation process using tequila vinasses as substrate for hydrogen production

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Abstract: In this work, a search methods strategy based on optimization algorithms is proposed for the kinetic modelling of a dark fermentation process using tequila stillage substrate for hydrogen production. Kinetic model based on the ADM1 approach is fitted to the experimental data to evaluate the dynamic behaviour of the metabolites produced and consumed during the mixed-culture fermentation. This batch fermentative process is based on mass balance analysis and correlated kinetic expressions following a Monod type dependence. In previous works, batch experiments were performed using different substrates to characterize the metabolic transformations involved in hydrogen production. Where, a kinetic model was developed and fitted to the obtained temporal profiles using Matlab and Excel solvers, allowing a satisfactory description of the metabolic pathways leading to hydrogen production. The aim of this research is to show the efficacy of the proposed optimization strategy to find the kinetic parameters of the model that will allow predictions on the behaviour of hydrogen production.

Keywords: Optimization algorithms, dark fermentation, mathematical model, biohydrogen.

1. INTRODUCTION

Biohydrogen production through Dark Fermentation (DF) processes from several organic substrates is sustainable because it presents a higher production, the recovery of high-added value by-products from carbohydrate-rich wastes and economic viability. Besides the conventional acetate and butyrate hydrogen-producing pathways, hydrogen production from lactate has been attracted more interest than at first expected. Lactic acid bacteria (LAB), the lactate producers par excellence, play an important role in the bioconversion of lactate into hydrogen, albeit there has been a matter of discussion on their function and effects upon the overall hydrogen production process efficiency. Since lactate-producing pathways result in a zero-hydrogen balance, the accumulation of lactate in the culture broth, which frequently occurs during acidogenesis, appears to be at first sight adverse for hydrogen production. Substrate consumption, excretion of bacteriocins and pH over acidification have been reported as the mechanisms promoted by the activity of LAB impairing hydrogen production (Noike et al., 2002). At this point it should be noted that under certain environmental conditions tipping the balance in favour of hydrogen production, the activity of LAB can be exploited for pH regulation, substrate hydrolysis, biomass retention, oxygen depletion and substrate detoxification (García-Depraect, León-Becerril, 2018). Such operational advantages seem to hing on the cross-feeding of lactate between LAB and lactate-consuming, hydrogen-producing bacteria through lactate-type fermentation. It has been shown that in batch experiments using complex substrates, the lactate-type fermentation consists of a primary fermentation in which reducing equivalents derived from carbohydrates are channeled mainly to lactate, acetate and biomass, and a secondary fermentation where the lactate and acetate produced are metabolyzed mainly in hydrogen and butyrate. However, notwithstanding that biohydrogen production from lactate has been ascertained as pivotal in several DF systems treating real complex substrates (including tequila vinasses (TV) (García-Depraect, León-

Becerril, 2018), cheese whey (Asunis et al., 2019), sugarcane vinasse (Fuess et al., 2018), among others), it is still a very poorly understood process. In Blanco et al. (2019), a kinetic model for dark fermentative biohydrogen production from synthetic cheese whey was fitted to the temporal profiles of total carbohydrates, lactate, acetate, butyrate, biomass and hydrogen gas. The relative average error indicated a satisfactory agreement between the measured and simulated values. The maximum biomass production was underestimated and the production of butyrate and hydrogen was overestimates. Despite of those fit errors, the kinetic model fulfilled its objective of providing insight into the predominant metabolic pathways involved in the mixed-culture fermentation of cheese whey. In Couto et al. (2020), a model based on ADM1 was proposed to describe sugarcane vinasse fermentation to produce hydrogen. The model was calibrated with data of the sugarcane vinasse processing at an initial concentration of 30kg·COD·m^3. The modelled soluble components that varies inside reactor are sugar, glycerol, butyrate, acetate, lactate and hydrogen. The model was cross validated in order to verify if it is adequate to describe this process under different conditions. The method was effective to find the minimal value of the objective function for the free parameters. However, the model did not present a good fit for all experimental data. Therefore, it is expected that the developed kinetic model would exhibit a limited predictive power for experiments performed under different conditions. In order to optimize the lactate-driven DF processes, this study aims to develop an optimization strategy to identify a mathematical model that describes the batch hydrogen production process through the lactate-type pathway.

2. METHODOLOGY

Batch experiments were carried in a reactor with a working volume of 2 L. The fermentations were carried out in triplicate at 35 ± 1 °C. For start-up, the stirred tank reactor was filled with 10% v/v activated inoculum and 90% v/v tequila vinasse. The operational pH was fixed at 6.5 until the beginning of the acceleration phase in reference to the H_2 production. The pH was then shifted to 5.8 where it was kept constant thereafter. The stirring speed was maintained at 500 rpm. The biogas produced was measured using the µFlow biogas meter. Gas samples were collected to analyze the biogas composition using gas chromatography. Liquid samples were taken regularly for further analyses. Such fermentations successfully exhibited the lactate-type pathway, and were thoroughly characterized for hydrogen production, biomass growth, consumption of sugars, as well as the metabolic profile through time (García-Depraect, León-Becerril, 2018). The purpose of the model is to predict and explain the batch fermentative hydrogen production process via the lactate-type fermentation. The model network structure is presented in Fig. 1, which describes the consumption of carbohydrates, production of hydrogen and biomass, and accumulation of major soluble metabolic intermediates, i.e. lactate, acetate and butyrate. It is assumed that biomass growth only relies on the consumption of carbohydrates. Experimental data formerly obtained from García-Depraect, León-Becerril (2018) is used to model kinetic parameters.

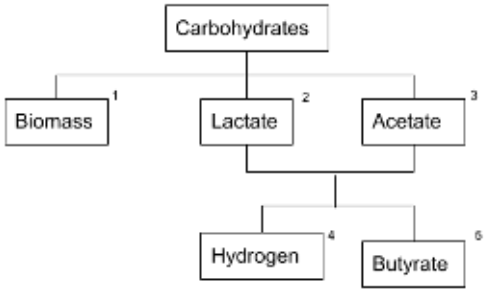

Fig. 1. Model network structure for hydrogen production via the lactate-type fermentation.

3. MATHEMATICAL MODELING

The mathematical model based on the ADM1 approach (Blanco et al., 2019) is developed following the model network structure presented in Fig. 1. The state variables are carbohydrates (X_1) and the fermentation products in the experiments: biomass (X_2), lactate (X_3), acetate (X_4), butyrate (X_5) and biohydrogen (X_6). All state variables are expressed in terms of g of COD per liter of reaction volume. The biomass is quantified as VSS and is assumed to represent the total microbial consortium, capable of conducting all of the metabolic pathways proposed in the model. The carbohydrate concentration is converted in terms of COD considering that lactose is the only carbohydrate present in the tequila stillage substrate. The nonlinear mathematical model is described by six differential equations where the consumption rates follow Monod-type kinetics. The model parameters are described in Table 1.

$$\frac{dX_1}{dt} = -X_2 \cdot I \cdot k_C \cdot \frac{X_1}{X_1+KS_C} \quad (1)$$

$$\frac{dX_2}{dt} = -Y \cdot \left(\frac{dX_1}{dt}\right) - k_d \cdot X_2 \quad (2)$$

$$\frac{dX_3}{dt} = -(1-Y) \cdot f_{C-A}\left(\frac{dX_1}{dt}\right) - X_2 \cdot I \cdot k_L \cdot \frac{X_3}{X_3+KS_L} \quad (3)$$

$$\frac{dX_4}{dt} = -(1-Y) \cdot f_{C-A}\left(\frac{dX_1}{dt}\right) - f_{L-A} \cdot X_2 \cdot I \cdot k_L \cdot \frac{X_3}{X_3+KS_L} \quad (4)$$

$$\frac{dX_5}{dt} = f_{L-B} \cdot X_2 \cdot I \cdot k_L \cdot \frac{X_3}{X_3+KS_L} \quad (5)$$

$$\frac{dX_6}{dt} = -(1-Y) \cdot f_{C-H}\left(\frac{dX_1}{dt}\right) + f_{L-H} \cdot X_2 \cdot I \cdot k_L \cdot \frac{X_3}{X_3+KS_L} \quad (6)$$

where I is an empirical function to model low-pH inhibition proposed by IWA (Batstone et al. 2002), as described in (7).

$$I = \begin{cases} e^{\left(-3\cdot\left(\frac{pH-pH_{UL}}{pH_{UL}-pH_{LL}}\right)^2\right)}, & if\ pH<pH_{UL} \\ 1, & if\ pH > pH_{UL} \end{cases} \quad (7)$$

in which pH_{UL} is the upper limit for pH inhibition, above which microorganisms are not inhibited, and pH_{LL} is the lower limit for pH inhibition, at which the microorganisms are completely inhibited. As mentioned before, pH was maintained at 5.8 during the hydrogen production phase. Nevertheless, variations around the pH setpoint are considered due to lag time in the control devices in order to evaluate effects of pH variations on microbial metabolism. It is assumed that the production rates of hydrogen and butyrate follow a Monod-type dependence on the concentration of lactate only, considering that acetate is not the limiting substrate in that reaction.

4. OPTIMIZATION STRATEGY

The nonlinear model is composed of four kinetic parameters and eight stoichiometric coefficients. Due to the high nonlinearity of the system, search methods strategy based on optimization algorithms is implemented to find optimal values. Three methods are proposed: Genetic Algorithm -Trust Region Algorithm (GA-RA), Simplex Search Method (SSM) and Pattern Search algorithm (PSA) (Kochenderfer, Wheeler, 2019). The objective of the optimization strategy is to minimize the cost function composed of the Mean Squared Errors (MSE) of the measured and simulated state values along the simulation time horizon. The optimization strategy is implemented in Matlab ™ and the flowchart is shown in Table 2.

Table 1. Model parameters

Variable	*Meaning*	*Unit*
Y	Biomass yield on carbohydrates	g COD g COD^{-1}
K_d	First- order biomass decay coefficient	h^{-1}
K_c	Maximum carbohydrate consumption rate	g COD g COD^{-1} h^{-1}
K_{SC}	Half- saturation constant for carbohydrate consumption	g COD h^{-1}
K_{SL}	Half- saturation constant for lactate uptake	g COD h^{-1}
K_L	Maximum lactate uptake rate	g COD g COD^{-1} h^{-1}
I	Low- pH inhibition function	pH
f_{lb}	Butyrate stoichiometric coefficient	g COD g COD^{-1} h^{-1}
f_{ca}	Acetate stoichiometric coefficient	g COD g COD^{-1} h^{-1}
f_{ch}	Hydrogen stoichiometric coefficient	g COD g COD^{-1} h^{-1}
f_{la}	Acetate stoichiometric coefficient	g COD g COD^{-1} h^{-1}
f_{lh}	Hydrogen stoichiometric coefficient	g COD g COD^{-1} h^{-1}
f_{cl}	carbohydrates stoichiometric coefficient	g COD g COD^{-1} h^{-1}

Table 2. Optimization algorithms

GA-RA

1. Establishment of the objective function and initial conditions
2. Creation of the initial population
3. Evaluation of each individual
4. If the stopping criterion is satisfied → **End** and then execute the TR algorithm
   a. Find the quadratic subproblem
   b. Set the initial parameters
   c. Find the unconstrained minimum of the subproblem
   d. Obtain the newton step δ_k
   e. Compute $x_{k+1} = x_k + \delta_k$
   f. Evaluate gradient
   g. If gradient < Ɛ → **Stop**
   h. If gradient > Ɛ, repeat step c
5. If the stopping criterion is not satisfied create a new population using reproduction, combination and mutation and repeat **step 3**.

SSM

1. Establishment of the objective function and initial conditions
2. Make the initial table
3. Select locally the column with the minimum negative value of the objective row
4. Select globally the minimum
5. Update variables
6. Select locally the row with the minimum ratio
7. Select globally the minimum
8. Update values
9. Update the pivot row
10. Update the remaining constraint rows
11. Update the objective row
12. If the stopping criterion is satisfied → **End**
13. If the stopping criterion is not satisfied repeat **step 3**

PSA

1. Establishment of the objective function and initial conditions
2. Set mesh parameters and iterations
3. Set the starting point
4. Construct pattern vectors and mesh points
5. Calculate objective function
6. If the stopping criterion is satisfied → **End**
7. If the stopping criterion is not satisfied but the poll is successful, then, expand mesh size and repeat **step 3.**
8. If the poll is not successful, then, contract mesh size and repeat **step 3**.

5. RESULTS

Each searching algorithm is implemented separately without restrictions and the stopping criterion is the minimum value of the objective function. The developed algorithms are programmed to iteratively compute successive approximations to the solution of the problem. Initial values for the model parameters were selected real and positives in a neighborhood close to the estimated values. The algorithms convergence, iterations number and the minimum function value is displayed in Fig. 2. Where the GA-RA algorithm converged to the minimum function value of 31.13 after 470 iterations, the SSM converged to the minimum function value of 8.85 after 810 iterations and the PSA converged to the minimum function value of 22.14 after 798 iterations. In order to know the algorithms efficiency, a MSE comparison of each variable state is presented in Table 3. The best fit found by the SSM algorithm is presented in Figures 3 to 8. As can be seen, a satisfactory agreement between the measured and simulated values was obtained, as indicated by the MSE reported. Despite the uncertainties introduced by measurement errors, unmeasured disturbances and the undefined mixed-culture fermentation of a complex substrate, lactate and acetate consumption leading to the production of butyrate and hydrogen, the SSM algorithm is efficient to fit the model to measured values with a minimum estimation error.

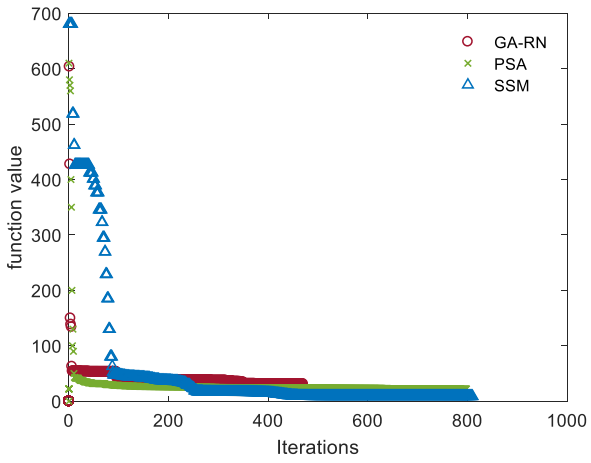

Fig 2. Objective function minimization

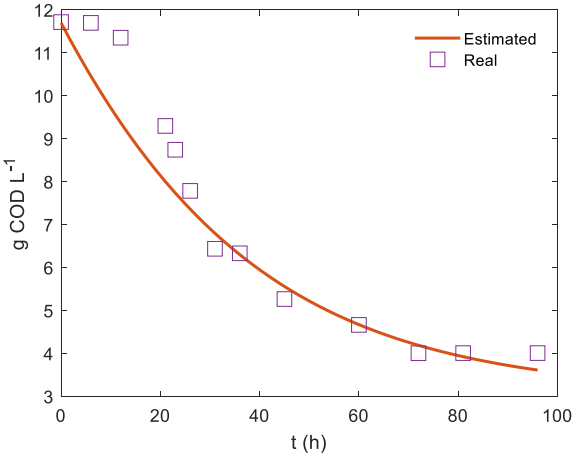

Fig 3. Carbohydrates

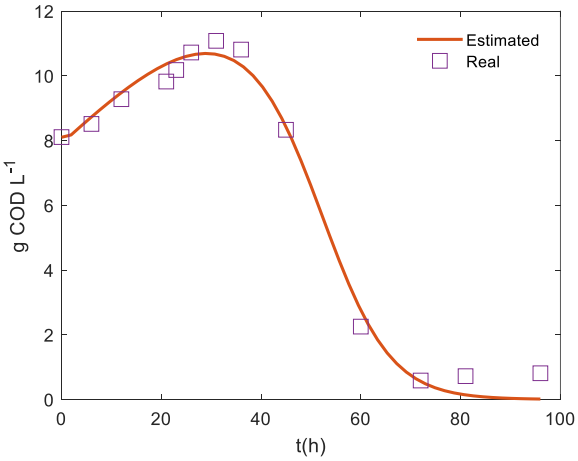

Fig 4. Lactate

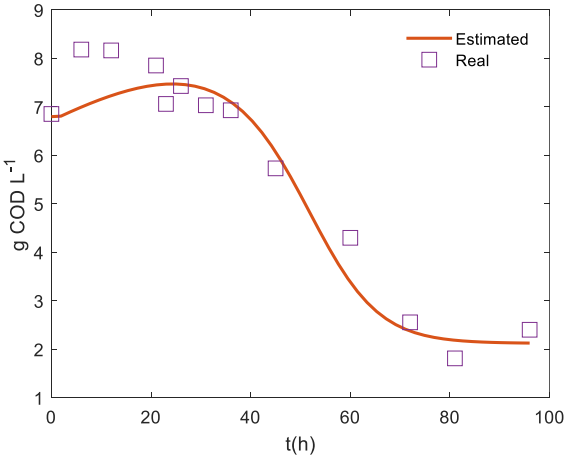

Fig 5. Acetate

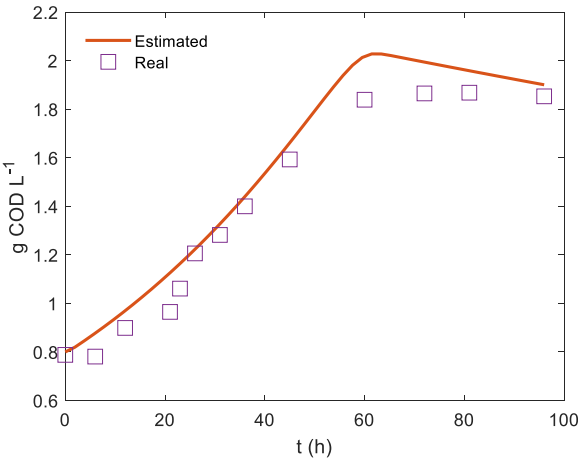

Fig 6. Biomass

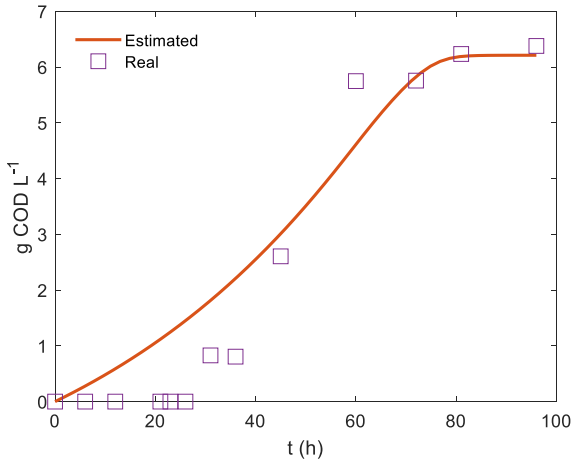

Fig 7. Butyrate

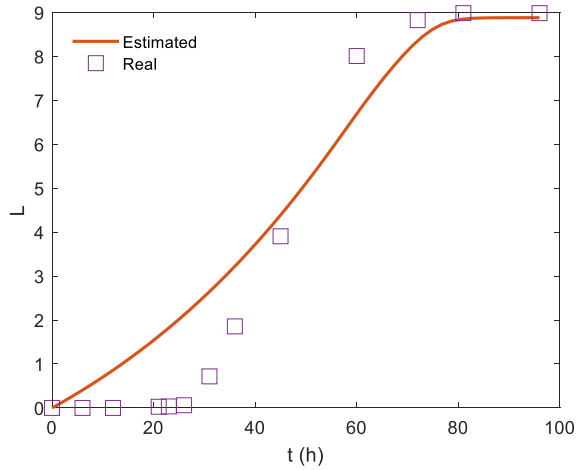

Fig 8. Hydrogen production

Table 3. Model parameters

Variable	MSE		
	GA-RA	SSM	PSA
X_1	2.2131	0.6706	11.8235
X_2	0.0095	0.0091	0.1160
X_3	0.3307	0.1646	1.1858
X_4	0.3031	0.2564	0.2902
X_5	1.0786	0.7512	1.1020
X_6	3.3517	1.4401	2.2794

The mathematical model presented an inferior fit for the carbohydrates, butyrate and hydrogen production. The carbohydrates were underestimated, which can be attributed to the model limitations to estimate slow consumption at the beginning of the fermentation. Butyrate and hydrogen production were overestimated during the first fifty hours due to a relationship with the carbohydrates behaviour. In addition, the reduced mathematical model does not represent the variety of bacteria or the lag times of the process. However, the estimation strategy results are acceptable for a good approximation of the real data that will allow predictions about the behaviour of hydrogen production. The mathematical model could be further extended and improved by adding experimentally measured stoichiometric parameters. In addition, this model could be useful for optimal control design to maximize the hydrogen production in presence of disturbances.

6. CONCLUSIONS

In this work a nonlinear kinetic model for a dark fermentation process fits the data measured in a batch process using the search methods strategy. The measured data correspond to carbohydrates, biomass, lactate, acetate, butyrate and hydrogen production. Three search methods based on optimization algorithms are implemented to find the kinetic and stoichiometric parameters of the model. An objective function is constructed with mean squared error of the measured and the simulated states, the algorithms goal is to minimize this objective function. The algorithms convergence, iterations number and the minimum function value were analyzed, where the SSM had the best convergence with a minimum function value of 8.85 after 810 iterations. The fitted model delivers a satisfactory description of the hydrogen production process, providing

understanding into the main transformation pathways leading to hydrogen production. As future work, a robust model representing bacterial communities capable of producing hydrogen from lactate and acetate may be suitable to optimal control implementation for long-term hydrogen production in a continuous system.

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