PREDICTING ALCOHOLIC FERMENTATION OUTCOMES WITH DYNAMIC METABOLIC FLUX BALANCE ANALYSIS

Gabriel B. Silva[1], Homero A. Neto[1], Thais S. Milessi[2] & Felipe. F. Furlan[2]

[1] *Universidade Federal de São Carlos, Programa de Pós-graduação em Engenharia Química, São Carlos - SP, Brasil.*
[2] *2Universidade Federal de São Carlos, Departamento de Engenharia Química, São Carlos - SP, Brasil.*
** Corresponding author's email address: furlan@ufscar.br*

ABSTRACT

The sugar and alcohol industry uses highly developed ethanol production processes, but the complexity of the process can lead to low predictability in fermentations, causing problems in subsequent stages. Tools that make it possible to predict the behavior of fermenters in adverse situations are extremely useful. In this context, Dynamic Flux Balance Analysis (dFBA) is a promising tool, allowing yields to be estimated based only on substrate consumption kinetics, without the need for extensive experimental data. This study evaluated the effectiveness of dFBA integrated with kinetic models to predict yields in batch fermentations. Experimental data was obtained from two batch fermentations using *Saccharomyces cerevisiae* in stirred tank bioreactor using molasse as substrate to reproduce industrial conditions. The kinetic model was adjusted to predict cell, substrate and ethanol concentrations over time. The simulation with dFBA showed good accuracy, with a mean deviation of 1.09 $g.L^{-1}$ in ethanol concentration, and 0.80 $g.L^{-1}$ for cell concentration. These results highlight the potential of dFBA to predict results and optimize fermentation processes.

Keywords: Flux Balance Analysis; Batch fermentation; *Saccharomyces cerevisiae*; Optimization.

1 INTRODUCTION

Sugarcane plays a very important role within the food and energy context in Brazil. With an annual processing of 705 million tons of sugarcane[1], the Brazilian sugar-alcohol sector has more than 340 active production units, making Brazil the world's largest producer of sugar, with 46 million tons per year, and the second largest producer of ethanol, with 24.9 billion liters[1].

Most Brazilian mills use the Mellet-Boinot process to produce ethanol [2]. As this is a process that often has complex configurations, mixing continuous and batch stages, the predictability of fermentations is an important factor in plant efficiency. However, accurately predicting product and cell yields during this process can be challenging due to the complexity of metabolic interactions and variations in experimental conditions. Traditionally, yields are obtained through experiments, but these methods can be limited in their ability to predict results under conditions other than those tested.

A promising approach to overcome these limitations is dFBA, which consists of a mathematical modeling technique that uses information about an organism's metabolic pathways to predict metabolic flows [3] and, consequently, product and cell yields. This technique makes it possible to simulate different cultivation conditions, offering a valuable tool for optimizing fermentation processes. Recent studies prove the effectiveness of using dFBA for various applications. Oliveira et al. [4] developed a predictive control for fermentation using *Saccharomyces cerevisiae* based on the use of dynamic flux balance analysis (dFBA), employing the Yeast8 model to create an economic parameter-based predictive control tool. Moreno-Paz et al.[5] uses dFBA to predict yeast growth in response to different industrial conditions.

For this work, a batch fermenter model was developed to represent the concentrations of ethanol, substrate, and cells over time in the fermentation of molasses under industrial conditions. This model was integrated with dFBA to allow the calculation of the yields. Subsequently, two batches were conducted in a stirred tank reactor to adjust the model for one of them and predict the concentration behavior over time for the other, using both the model adjusted with experimental yields and the model with predicted yields from dFBA.

2 MATERIAL & METHODS

The experimental data was obtained from simple batch fermentations carried out in a 5 L stirred tank bioreactor with medium recirculation and agitation. Two fermentations were carried out (B1 and B2), using the yeast *Saccharomyces cerevisiae* baker's yeast, with an initial cell concentration of 15 g/L and a Total Reducing Sugars (TRS) concentration of 150 $g.L^{-1}$. Samples were taken at 30-minute intervals and cell concentrations were quantified by UV-VIS spectrophotometry at 600 nanometers using optical density. Substrate and ethanol concentrations were determined by high-performance liquid chromatography (HPLC) on a Waters e2695 chromatograph with a RezexTM ROA-Organic acid H+ ion exclusion column[8].

The modeling of the fermentation process was developed in Python, using the Scipy library, based on mass and energy balances applied exclusively to the fermenter. The model was adjusted so that the kinetics are based on glucose consumption, as shown equations 1-3.

$$\frac{dS}{dt} = -\mu_S * X \quad (1)$$

$$\frac{dX}{dt} = \mu_S * X * Y_{XS} \quad (2)$$

$$\frac{dP}{dt} = \mu_S * X * Y_{PS} \quad (3)$$

Where *X* is the cell concentration (g.L^{-1}), *S* is the substrate concentration (g.L^{-1}), *P* is the product concentration (g.L^{-1}), Y_{XS} is the cell yield per substrate consumed and Y_{PS} is the product yield per substrate consumed. μ_S is the substrate specific consumption rate (h^{-1}), which was adapted from the model proposed by Mesquita[6], where the cell growth rate is transformed into the speed of glucose consumption through the experimental yield term (Eq. 4).

$$\mu_S = \frac{1}{Y_{XS}} \frac{\mu_{max} S}{K_S + S} \left(1 - \frac{P}{P_M}\right) \exp\left(-\frac{S}{S_M}\right) \quad (4)$$

Where, μ_{max} is the maximum specific cell growth rate(h^{-1}), K_S is the saturation constant (g.L^{-1}), S_M is the substrate inhibition constant (g.L^{-1}), S_M is the product inhibition constant (g.L^{-1}).

The substrate consumption rate is then inserted into the dFBA model as a boundary condition through the substrate assimilation reaction, making it possible to estimate the other internal balances to maximize cell growth and obtain the flows of cell products and by-products. With these flows, yields are calculated in relation to substrate consumption, allowing for a detailed and dynamic analysis of the fermentation process. The dFBA model used in this work was IMM904, which represents the metabolism of the yeast *Saccharomyces cerevisiae* with 1577 reactions and 1226 metabolites. To simulate an anaerobic environment, the oxygen flow was set to zero, and other nutrients were added to represent a complex environment, as described by Lino *et al.*[7], such as sugarcane molasse.

The model without the use of dFBA only considers the equations described by Mesquita[6], which quantifies cell growth and uses the yields (Y_{XS} and Y_{PS}) calculated experimentally.

3 RESULTS & DISCUSSION

The kinetic model parameters (Equation 4) were estimated using data from fermentation B1, obtaining the following values: μ_{max} = 0.2464 h^{-1}, K_S = 7.0186 g.L^{-1}, S_M = 1568.8 g.L^{-1} and P_M = 150.26 g.L^{-1}. Additionally, the initial real concentrations of cells, sugars, and ethanol in both experiments were estimated, with values of *X* = 13.42 g.L^{-1}, *S* = 127.40 g.L^{-1}, and *P* = 5.80 g.L^{-1} for B1 and *X* = 13.69 g.L^{-1}, *S* = 110.05 g.L^{-1}, and *P* = 3.56 g.L^{-1} for B2.

Using the estimated initial values for B1 and the experimental one for B2, it was possible to simulate the fermentations using both the estimated yields (model A) and the yields predicted by FBA (model B). For this, at each time iteration, the value corresponding to the glucose consumption flux was passed to the metabolic flux model, previously configured for anaerobic conditions, as described by Lino[7], allowing the quantification of all cellular fluxes, which enables the calculation of yields. With the obtained values, the variations in cell and product concentration were defined. Figure 1 presents the data showing the behavior of cell, ethanol and sugar concentrations over time for the experimental data, simulated with estimated yields and with yields calculated using dFBA, for both batches.

The adjusted kinetic model was able to accurately predict the behavior of cell, substrate, and ethanol concentrations over time in both batches, including the final fermentation time. The simulation using FBA proved to be very effective in predicting fermentation behavior. The ethanol concentration showed a mean square deviation of 1.10 g.L^{-1} for the model A and 1.09 g.L^{-1} for model B for the B2 fermentation. For cell production, the deviation was 0.54 g.L^{-1} (model A) and 0.96 g.L^{-1} (model B). For B1, the deviations were 0.85 g.L^{-1} (model A) and 1.15 g.L^{-1} (model B) for ethanol and 0.44 g.L^{-1} (model A) and 0.80 g.L^{-1} (model B) for cells. This shows that the prediction capacity of both models are similar. This accuracy demonstrates the potential use of dFBA to predict cell behavior in different experimental conditions, opening possibilities for its use in process optimization applications.

It is worth noting that the deviation can still be reduced by better defining the anaerobic conditions of the process. This is possible by identifying and quantifying substances present in the molasse, which is a complex compound rich in nutrients[9], which can be defined within the boundary conditions of the metabolic model, similar to what was done by Lino[7], but for the specific medium used in the experiment.

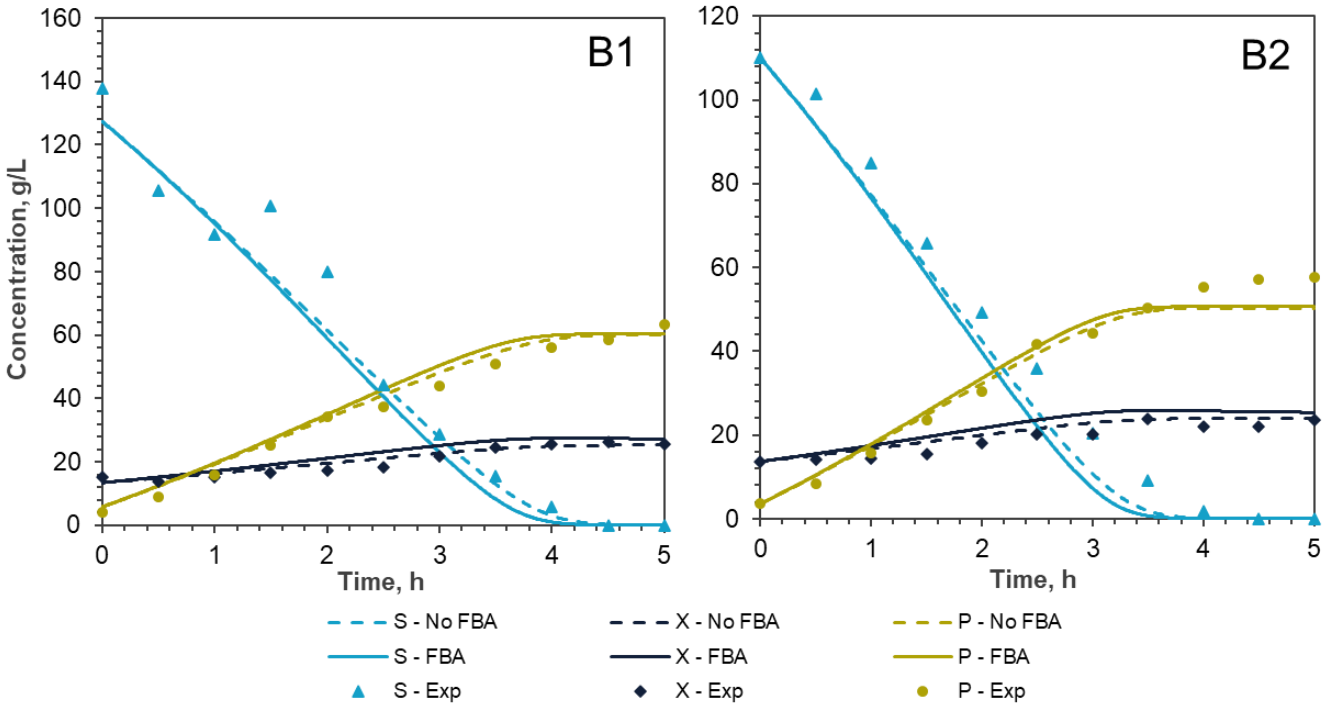

Figure 1 Cell (X), substrate (S) and ethanol (P) concentrations over time for experimental data (dots), simulated with experimental yields (dashed) and simulated with yields calculated by FBA (solid line). B1 represents the adjusted batch and B2 the predicted batch.

4 CONCLUSION

The simulation using the dFBA model demonstrated reasonable accuracy, with an average deviation of only 1.15 $g.L^{-1}$ in predicting the final ethanol yield under the same conditions as the adjustment, and 1.09 $g.L^{-1}$ for a different cultivation scenario, compared to 0.86 $g.L^{-1}$ and 1.10 $g.L^{-1}$ without FBA. For cell concentration, the average deviation for dFBA model was 0.80 $g.L^{-1}$ for B1 and 0.96 $g.L^{-1}$ for B2. These results highlight the potential of dFBA as a powerful tool for predicting metabolic outcomes under conditions different from experimental data, as well as its applicability in optimizing fermentation processes. The integration of FBA with kinetic models offers a robust and accurate approach to predicting yields in fermentation processes.

REFERENCES

1. USDA. Published April 18, 2024. Accessed June 7, 2024. https://fas.usda.gov/data/brazil-sugar-annual-10
2. BASSO LC, BASSO TO, ROCHA SN, BASSO LC, BASSO TO, ROCHA SN. In: *Biofuel Production - Recent Developments and Prospects*. IntechOpen; 2011. doi:10.5772/17047
3. DEHKORDI MB, PISHVAIE MR, VAFA E. *Computers & Chemical Engineering*. 2023;179:108444. doi:10.1016/j.compchemeng.2023.108444
4. DE OLIVEIRA RD, GUEDES MN, MATIAS J, LE ROUX GAC. *Ind Eng Chem Res*. 2021;60(40):14464-14475. doi:10.1021/acs.iecr.1c01242
5. MORENO-PAZ S, SCHMITZ J, MARTINS DOS SANTOS VAP, SUAREZ-DIEZ M. *Microbial Biotechnology*. 2022;15(5):1434-1445. doi:10.1111/1751-7915.13995
6. MESQUITA TJB. Doctoral thesis. Universidade Federal de São Carlos; 2021.
7. SENNE DE OLIVEIRA LINO F, BAJIC D, VILA JCC, SÁNCHEZ A, SOMMER MOA. *Nat Commun*. 2021;12(1):1498. doi:10.1038/s41467-021-21844-7
8. SANDRI JP, RAMOS MDN, PEREZ CL, MESQUITA TJB, ZANGIROLAMI TC, MILESSI TS. *Biochemical Engineering Journal*. 2023;191:108777. doi:10.1016/j.bej.2022.108777
9. PALMONARI A, CAVALLINI D, SNIFFEN CJ, ET AL. *Journal of Dairy Science*. 2020;103(7):6244-6249. doi:10.3168/jds.2019-17644

ACKNOWLEDGEMENTS

This work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Funding Code 001, process nº 2024/02416-4, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and the Financier of Studies and Projects (FINEP) in partnership with the company Fermentec.