I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM • XV SHEB • XV ENZITEC FLORIANÓPOLIS • 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
BIOPROCESS ENGINEERING

MATHEMATICAL MODELING OF THE CELL GROWTH STAGE IN AN INDUSTRIAL BIOPROCESS FOR FARNESENE PRODUCTION

Samuel C. Oliveira* & Fabiano A. Souza

Postgraduate Program in Biomaterials and Bioprocess Engineering (PPG-EBB)
School of Pharmaceutical Sciences (FCF)/São Paulo State University (UNESP), Araraquara-SP, Brazil
** Corresponding author's email address: samuel.oliveira@unesp.br*

ABSTRACT

A simple, non-segregated and unstructured mathematical model based on the Monod equation, without terms of inhibition and of limitation by oxygen, was developed to describe the microbial growth in one of the initial stages of the farnesene production by genetically modified yeasts. An analysis of the effects of the pressure applied in the fermenters was carried out on the biomass yield and on the parameters. The model, which consisted of two equations in transient state (batch regime), satisfactorily described the trends of the modeled variables for batches carried out under different experimental conditions of initial concentrations of cells and substrate.

Keywords: Cell growth. Batch-wise. Modeling. Kinetics. Parameter estimation.

1 INTRODUCTION

Several technologies have been developed to obtain renewable products, including those that use genetically modified microorganisms, which are used for the bioprocessing of sugars derived from biomass, generating intermediate compounds that can later be transformed into various final products. Among these is the farnesene production using an engineered industrial strain of *Saccharomyces cerevisiae*.[1] Farnesene is a hydrocarbon that can be used as a precursor in numerous applications, from the synthesis of special products, including some fine chemical through high-performance fuels, to its use as a solvent free of VOCs (Volatile Organic Compounds), with a very promising perspective of market increase.[1]

The farnesene production bioprocess in the industrial unit of the DSM (Dutch State Mines) Company at Brotas/SP comprises two stages, the first being cell multiplication and the second being farnesene production. The cell multiplication stage is subdivided into three phases: A, B and C. Phase A consists of cell multiplication at laboratory scale while phase B is the first phase of cell multiplication at industrial scale, being carried out in initial fermenters (IFs). Phase C is the second phase of cell multiplication at industrial scale, which is carried out in seed fermenters. During a certain period of operation of the industrial plant, target values determined in bench tests for the concentrations of cells and substrate were not reached in phase C, resulting in lower production of farnesene in the main fermenters. In fact, the problem started in the initial fermenters and cascaded to the seed fermenters. Thus, seed train intensification has received considerable attention aiming at to generate and accumulate biomass in sufficient quantity to inoculate the main fermenters and to allow the farnesene production in an optimized manner.

The objective of this work is to propose a mathematical model that represents well the behavior of the main variables of the microbial growth in the IFs, aiming at future simulation studies regarding this stage of the industrial bioprocess of farnesene production.

2 MATERIAL & METHODS

The experimental data on cell concentration, expressed as optical density (OD), and substrate (sucrose) concentration used in the modeling were provided by the industrial unit, referring to 18 batches carried out under different initial concentrations of cells and substrate, 8 of them at 1.0 bar and 10 at 0.5 bar in the IFs. The biomass and substrate concentrations were determined by spectrophotometric and enzymatic methods, respectively.

The mathematical model proposed to describe the dynamic behavior of cell (X) and substrate concentrations (S) is non-segregated, unstructured, does not consider inhibitory effects of any kind, nor oxygen limitation, and is represented by the following mass balance equations:

$$\frac{dX}{dt} = \mu_X X = \left(\frac{\mu_{max} S}{K_S + S}\right) X \tag{1}$$

$$\frac{dS}{dt} = -\mu_S X = -\frac{\mu_X}{Y_{X/S}} X = -\left(\frac{1}{Y_{X/S}}\right)\left(\frac{\mu_{max} S}{K_S + S}\right) X \tag{2}$$

In Equations (1) and (2), μ_X and μ_S are the specific rates of cell growth and substrate consumption, respectively, μ_{max} is the maximum specific growth rate, K_S is the Monod saturation constant, and $Y_{X/S}$ is the biomass yield per substrate consumed.

The kinetic parameters (μ_{max}; K_S) were estimated from the experimental data provided by the industrial unit, using the Marquardt method to minimize the sum of squared residuals (*SSR*) between the experimental and calculated values of each state variable, according to Equation (3), in which the calculated values were obtained by numerical integration of Equations (1) and (2), through the 4th-order Runge-Kutta-Gill method.[1]

$$SSR = \sum_{i=1}^{N}\left(X_{exp.,i} - X_{calc.,i}\right)^2 + \sum_{i=1}^{N}\left(S_{exp.,i} - S_{calc.,i}\right)^2 \quad (3)$$

Two different sets of parameters were estimated, one for each pressure (*P*) applied to the IFs (0.5 and 1.0 bar). The IFs were initially operated at 1.0 bar and to parameter estimation in this condition were selected experimental data from eight batches, codified as Batch 01-08, wich were carried out with different initial concentrations of cells and substrate (X_0; S_0). Subsequently, to evaluate the effect of pressure on cell growth, the pressure was changed to 0.5 bar and the experimental data from 10 batches (Batch 11-20) in this new operating condition, also performed with different X_0 and S_0 were submitted to a new parameter estimation.

3 RESULTS & DISCUSSION

Two average values of $Y_{X/S}$ were calculated for the different batches carried out at the pressure used in the IFs, obtaining $Y_{X/S}$=0.48 g/g for *P*=1.0 bar and $Y_{X/S}$=0.64 g/g for *P*=0.5 bar (see Table 1), indicating that the reduction in operating pressure in the IFs favored microbial growth. The pressure effect on aerobic bioprocesses is contradictory in the literature, with positive and negative reports, that is, if on one hand the increase in pressure favors oxygenation of the medium, on the other hand, it disfavors the release of gases that can be toxic to the metabolism, like CO_2 is for yeasts.[2]

Table 1 Calculated value of $Y_{X/S}$ for each batch

P (bar)	Batch	X_0 (OD)	S_0 (g/L)	X_f (OD)	S_f (g/L)	$Y_{X/S}$ (g/g)
1.0	01	0.11	50.6	30.8	0.17	0.61
1.0	02	0.12	52.7	28.8	0.05	0.54
1.0	03	0.09	54.0	31.2	3.91	0.62
1.0	04	0.10	56.6	21.5	0.14	0.38
1.0	05	0.10	51.1	27.1	0.06	0.53
1.0	06	0.09	63.8	23.0	4.37	0.39
1.0	07	0.20	74.9	27.9	3.00	0.39
1.0	08	0.10	75.3	28.8	3.10	0.40
0.5	11	0.24	65.4	48.2	3.34	0.77
0.5	12	0.25	59.7	43.0	0.10	0.72
0.5	13	0.27	67.6	48.4	0.07	0.71
0.5	14	0.17	74.5	42.5	1.94	0.58
0.5	15	0.18	77.8	53.4	1.97	0.70
0.5	16	0.29	79.5	44.2	0.00	0.55
0.5	17	0.16	76.1	40.1	3.90	0.55
0.5	18	0.36	75.4	52.0	0.29	0.69
0.5	19	0.10	70.9	38.0	0.31	0.54
0.5	20	0.14	74.0	42.3	0.27	0.57

Table 2 presents the sets of parameter values, their respective standard deviations, *SSR*, *N* (number of experimental points) and the *MSR* (Mean Squared Residual) for each operating pressure applied at the IFs. Considering that the kinetic parameters were estimated with 24 and 30 data points at 1.0 bar and 0.5 bar, respectively, the μ_{max} parameter was estimated with good precision, as the standard deviations of the estimates are sufficiently smaller than the parameter values themselves, which did not occur to K_S parameter, whose estimates are poor.

Table 2 Estimated values of the kinetic parameters of the mathematical model for each pressure applied at the IFs

P (bar)	μ_{max} (h^{-1})	K_S (g/L)	*SSR*	*N*	*MSR=SSR/N*
1.0	0.109 ± 0.006	1 ± 3	1892	24	78.83
0.5	0.146 ± 0.017	24 ± 10	2264	30	75.47

To check whether the estimated values of each parameter at 1 bar are statistically different from those estimated at 0.5 bar, the Tukey test was applied at a confidence level of 95% and was concluded that values are, indeed, different.[1]

The estimated values of μ_{max} are much lower than others reported in the literature for the growth of *S. cerevisiae* on glucose, which demonstrates that μ_{max} has the potential to be increased, making microbial growth in IFs faster.

Regarding the effects of pressure on the kinetic parameters, the value of μ_{max} at 0.5 bar is approximately 34% higher than that estimated at 1.0 bar, indicating that the pressure reduction in the IFs favors microbial growth, thus reducing the negative effects of this gas on cellular metabolism. The inhibition of several enzymes from *S. cerevisiae* for partial pressures of CO_2 above 0.5 bar has already been reported.[2]

The value of K_S for 0.5 bar was higher than values reported in the literature for the growth of *S. cerevisiae*.[1] This result can be attributed to the following factors, values of K_S that appear to have no influence on the profiles predicted by the model and problems with correlation between K_S and μ_{max}, which would negatively affect the K_S estimation.

Figure 1 presents illustrative graphs of proposed model fit to experimental data from some batches, showing a satisfactory description of the temporal profiles of the modeled state variables (X and S), with this good pattern of adjustment being representative for the other batches carried out. The curves regarding model predictions were obtained for the initial conditions imposed in each batch, using the values of parameters estimated with all data points available at each pressure.

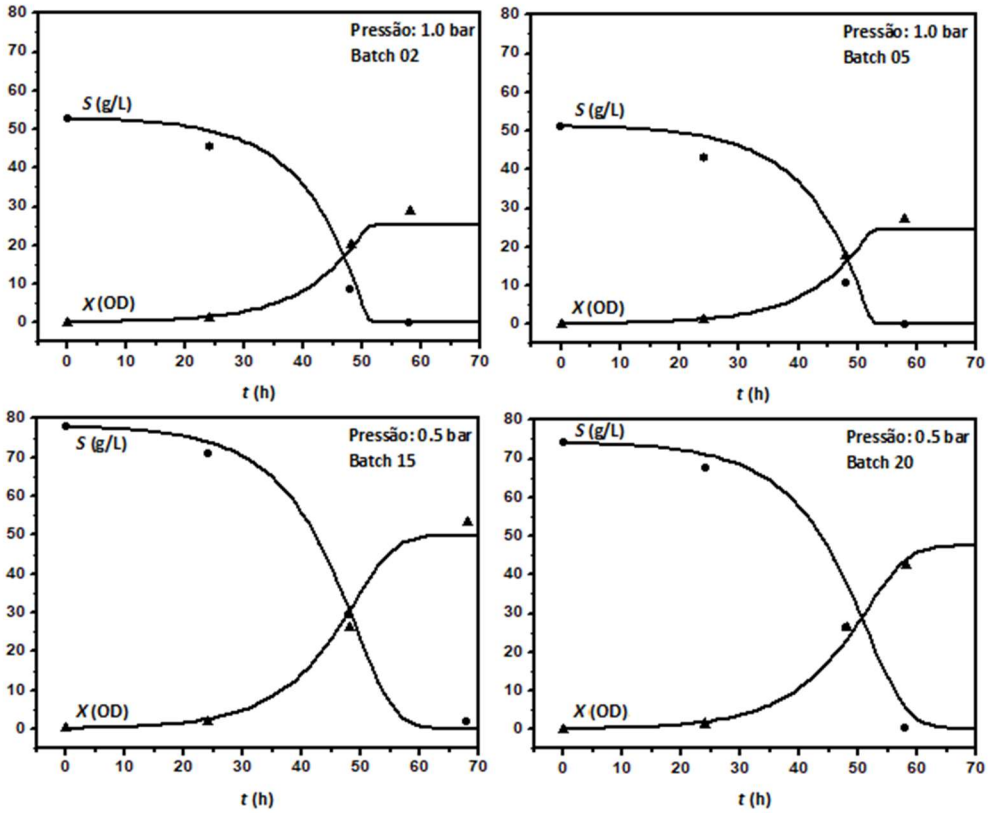

Figure 1 Graphs of the model fit to experimental data from some batches

4 CONCLUSION

A simple mathematical model, containing two kinetic parameters and a biomass yield factor, was developed and applied to describe the cell growth stage in an industrial bioprocess for farnesene production. The model predictions agreed well with the experimental data for the two variables considered in modeling, cell and substrate concentration, enabling the developed model to be used for simulation studies aimed at optimizing cell growth at this stage of the bioprocess.

REFERENCES

[1] SOUZA, F. A. 2020. Modelagem, simulação e ajuste de condições operacionais da etapa de crescimento celular do bioprocesso industrial de produção de farneseno. Dissertação (Mestrado em Engenharia de Biomateriais e Bioprocessos) - Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista.

[2] CARBULONI, R. 2020. Detecção, diagnóstico e proposta de soluções baseadas em modelos matemáticos para falhas na etapa de propagação do inóculo do bioprocesso industrial de produção de farneseno. Dissertação (Mestrado em Engenharia de Biomateriais e Bioprocessos) - Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista.