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MODELING OF INDUSTRIAL BUBBLE COLUMN BIOREACTORS FOR FARNESENE PRODUCTION BY ENGINEERED YEASTS

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ABSTRACT

Semi-empirical mathematical models, based on an analogy with the classical model of the mass-spring system, were developed to describe the dynamic behavior of two key variables of an industrial bioprocess of farnesene production by engineered yeasts, in bubble column bioreactors, operated in fed-batch mode. The proposed models reproduced well the trend of oscillatory behavior experimentally exhibited by the modeled variables in all the performed fed batches, with three parameters of the models being estimated with good precision and a fourth parameter with reasonably acceptable precision. Moreover, the mathematical models were validated by statistical tests, enabling the use of the proposed models for further optimization studies of the bioprocess.

Keywords: Semi-empirical. Mathematical modeling. Dynamic behavior. Oscillations. Fed-batch operation.

1 INTRODUCTION

Bubble columns reactors feature a high liquid to gas volume ratio, which is indicated when conducting rather slow reactions in the liquid phase (case of bioreactions) as large reaction volumes can be processed.[1] Furthermore, they have other advantages such as a simple operation without any moving parts, excellent mixing, high heat and mass transfer rates, low catalyst attrition rates and the flexibility to operate at a wide range of residence time.

Fermentation is a biological process that requires the use of microorganisms such as yeast, bacteria, or fungi. Under certain environmental conditions, some microorganisms may not have efficient fermentative activity, but genetic engineering of these microorganisms can lead to improvements in their efficiencies, as is the case of yeasts that were genetically modified to adapt to processing conditions for farnesene production. The various industrial applications of the farnesene has increased its production.

Farnesene is produced in an industrial unit by aerobic bioprocess, using sucrose from sugarcane as a substrate source, and engineered yeasts, in six bubble column reactors with 200 m^3 volume, operated in a fed-batch mode. The addition of substrate in these bioreactors is a critical operational issue due to the possibility of occurrence of under- or over-feeding, with consequent negative impacts on the performance of the bioprocess.

In order to a better control of the bioreactors feeding, the main objective of this work was to develop mathematical models to describe the oscillatory behavior of two key variables of the bioprocess (concentration of acetaldehyde and ethanol), which indicate the deviation of the metabolic route from the production of farnesene to that of ethanol. Due to the high dynamic complexity of the bioprocess, a semi-empirical approach, based on an analogy with the dynamic behavior of a spring-mass system, was used to develop the mathematical models, which were statistically validated by usual specific tests.[1]

2 MATERIAL & METHODS

The bubble column bioreactors used in the industrial plant of farnesene production are equipped with internal air bubblers, external recirculation system, comprising a centrifugal pump and a plate-heat exchanger for homogenization and temperature control, pH and oxygen sensors, ammonia addition system for pH control, air filtration system, exhaust system of gases for controlling the pressure at the top of the bioreactors and system of substrate addition. Sucrose-based medium, prepared from sugar cane syrup and supplemented with micronutrients (vitamins, metals and salts), as well as an engineered strain of *Saccharomyces cerevisiae* are employed in the bioprocess. The bioreactors are operated in fed-batch mode, under intermittent pulses of substrate carried out through automatic adjustment of the feed flow, aiming to indirectly control the ethanol concentration in the medium by control of the amount of substrate made available.[2]

Due to this operational complexity of system , the most important response variables in this stage of the bioprocess (concentration of acetaldehyde and ethanol) exhibited oscillatory dynamic behavior, whose mathematical modeling, according to a phenomenological approach, becomes complicated because, in addition to effects of different natures, including kinetic, hydrodynamics and heat/mass transfer effects, the oscillations in the state variables are not free, but rather forced due to the pulses of substrate and to the action of other control systems on the bioprocess such as those of pH, temperature and pressure in the head space of the bioreactors. Thus, a semi-empirical approach, based on the classical model of the mass-spring system, was used to develop oscillatory mathematical models to describe the temporal behavior of these specific variables, which represent the concentrations of the participating species in the ethanol formation reaction in deviation from that of farnesene.

Analysis of the experimental data referring to the modeled variables revealed that the temporal-behavior trends of these variables can be described by the solution of the ordinary differential equation representative of the dynamic behavior of a system of mass m and spring of elastic constant k, subject to a driven-force $F = F_0 cos(\theta t)$, Equation (1), in which y is the displacement from the equilibrium position:

$$my'' + ky = F_0 cos(\theta t)\,;\; y(0) = 0\,, \qquad y'(0) = 0 \tag{1}$$

If $\theta \neq \omega$ and $\omega = \sqrt{k/m}$, the solution of Equation (1) is given by the Equation (2):

$$y(t) = \frac{f_0}{(\omega^2 - \theta^2)}\left(cos(\theta t) - cos(\omega t)\right);\; f_0 = F_0/m \tag{2}$$

For better applicability of the proposed model, it was reparametrized as follows:

$$C_i(t) = P_1\left(cos(P_2 t) - cos(P_3 t)\right) \tag{3}$$

In Equation (3): $C_i(t) = C_A\ (Concentration\ of\ Acetaldehyde)\ or\ C_E\ (Concentration\ of\ Ethanol),\ P_1 = \frac{f_0}{(\omega^2 - \theta^2)}, P_2 = \theta, P_3 = \omega.$

The parameters of the model were estimated by nonlinear regression, minimizing the sum of squares of the residuals between the experimental values and the values calculated by the model, according to Marquardt's algorithm. To achieve this, the mathematical model was adjusted to the experimental data of C_A and C_E obtained in 9 fed-batches, using the OriginPro 8 software. Validation of the adjustments were performed by using the statistical tests of Fischer (*F*) and of the *p*-value. Preliminary model adjustments for both variables proved to be poor (data not shown), which required a reformulation of the model by including a fourth parameter (P_4) in order to improve its predictive capacity, according to Equation (4). The P_4 parameter represents a base value around which the term $P_1\left(cos(P_2 t) - cos(P_3 t)\right) + P_4$ makes the predicted values to oscillate.

$$y(t) = P_1\left(cos(P_2 t) - cos(P_3 t)\right) + P_4 \tag{4}$$

Due to the parameters P_1, P_2, P_3 and P_4 coming from a semi-empirical approach, they incorporate several aspects involved in the dynamic behavior of the bioprocess and, for this reason, they were estimated for each fed-batch carried out, aiming to incorporate variations resulting from different conditions prevailing in the bioreactors.

3 RESULTS & DISCUSSION

The inclusion of the additional parameter (P_4) in the model formulation proved to be appropriate, resulting in good parameter estimates and good model adjustments for both variables, as shown in Tables 1 and 2, and in Figure 1. It can be observed that the standard deviations of the parameters are small in relation to the estimated values of the parameters themselves, with emphasis on the parameters referring the arguments of the trigonometric terms (P_2 and P_3), which present the smallest relative deviations. Furthermore, it is observed that the P_4 parameter is also estimated with good precision. However, the largest standard deviations are observed for the P_1 parameter, which is attributed to the strong interaction (correlation) of this parameter, mainly with the angular parameters P_2 and P_3. Thus, during the parameter estimation step, P_2 and P_3 have their estimates privileged while P_1 has its estimate impaired.

Figure 1 shows illustrative graphs of the adjustments of the proposed model to experimental data on the concentration of acetaldehyde (C_A) and ethanol (C_E). It can be observed that the proposed model describes well the trend of oscillatory behavior of the modeled variables, which is quantitatively proven by the high calculated values of the *F* ratio (F_c) presented in Tables 1 and 2, which were much higher than the tabulated value (F_t), which in all cases was 2.60. Furthermore, according to the *p* statistics data presented in Tables 1 and 2, it can be seen that, for all adjustments made, the calculated value of *p* was lower than the value adopted for the significance level of the test (α=0.05), also confirming, the validity of the models for all fed-batches carried out.

Table 1 Parameter estimates and statistical indicators of the quality of the model fit to the acetaldehyde concentration data in each fed-batch

FED-BATCH	P_1 (ppm)	P_2 (h^{-1})	P_3 (h^{-1})	P_4 (ppm)	F_C	p
PBF-001	22.181 ± 11.685	0.826 ± 0.006	0.579 ± 0.005	966.75 ± 11.33	1821.90	0.00
PBF-089	32.359 ± 8.440	1.039 ± 0.003	0.710 ± 0.003	836.76 ± 8.07	2688.82	0.00
PBF-106	16.690 ± 8.053	0.806 ± 0.005	0.281 ± 0.005	836.37 ± 7.93	2786.11	0.00
PBF-117	38.055 ± 11.441	0.791 ± 0.003	0.345 ± 0.003	1074.30 ± 11.17	2315.39	0.00
PBF-118	26.058 ± 11.463	0.800 ± 0.005	0.501 ± 0.005	1119.48 ± 11.16	2516.25	0.00
PBF-119	-95.891 ± 13.415	0.814 ± 0.002	0.451 ± 0.001	1328.32 ± 13.11	2583.75	0.00
PBF-127	-32.438 ± 13.637	0.803 ± 0.004	0.299 ± 0.004	798.27 ± 13.27	908.33	0.00
PBF-129	28.897 ± 11.839	0.788 ± 0.004	0.301 ± 0.004	970.83 ± 11.67	1735.49	0.00
PBF-130	-33.125 ± 18.100	0.803 ± 0.007	0.276 ± 0.006	1167.30 ± 17.66	1093.68	0.00

Table 2 Parameter estimates and statistical indicators of the quality of the model fit to the ethanol concentration data in each fed-batch

FED-BATCH	P_1 (ppm)	P_2 (h^{-1})	P_3 (h^{-1})	P_4 (ppm)	F_C	p
PBF-001	-389.76 ± 60.30	0.792 ± 0.002	0.425 ± 0.002	3020.80 ± 58.80	673.11	0.00
PBF-089	-317.34 ± 81.50	0.994 ± 0.003	0.503 ± 0.003	3162.91 ± 78.74	408.18	0.00
PBF-106	323.12 ± 56.32	0.809 ± 0.002	0.498 ± 0.002	3225.41 ± 55.24	862.66	0.00
PBF-117	476.68 ± 72.73	0.757 ± 0.002	0.314 ± 0.002	3482.14 ± 71.34	607.26	0.00
PBF-118	-239.46 ± 76.53	0.803 ± 0.003	0.370 ± 0.003	3417.75 ± 75.08	522.67	0.00
PBF-119	-165.64 ± 72.28	0.919 ± 0.005	1.036 ± 0.005	3307.68 ± 68.12	590.52	0.00
PBF-127	252.48 ± 49.70	0.821 ± 0.002	0.413 ± 0.002	2792.13 ± 48.47	838.57	0.00
PBF-129	-313.10 ± 65.16	0.801 ± 0.002	0.318 ± 0.002	3010.61 ± 63.90	562.90	0.00
PBF-130	-164.26 ± 73.61	0.801 ± 0.005	0.400 ± 0.005	3511.92 ± 71.57	604.12	0.00

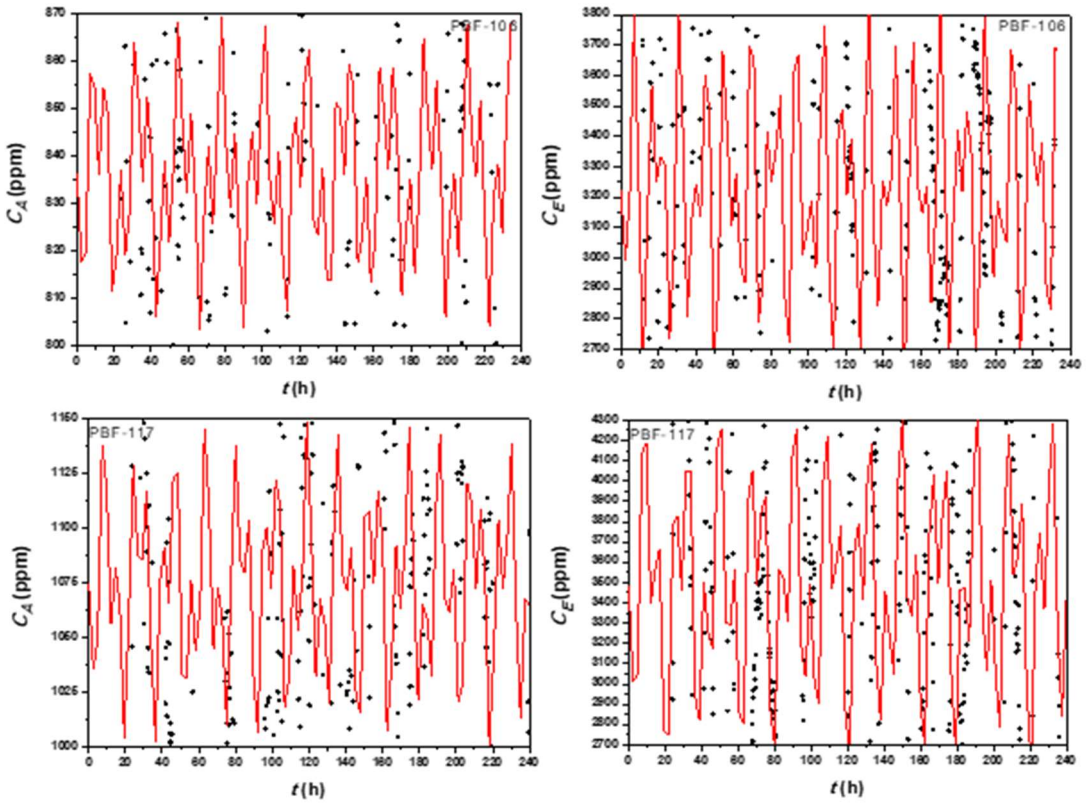

Figure 1 Graphs of model fit to experimental data from some fed-batches

4 CONCLUSION

The analogy between the oscillations that occur in the dynamics of a mass-spring system and those observed in a farnesene production bioprocess allowed the proposition of semi-empirical mathematical models that satisfactorily describe the temporal behavior of two key variables of yeast metabolism, which can be used to perform optimization studies on the addition substrate system currently used in the industrial plant, aiming at a better control of the ethanol concentration in the fermentative process.

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