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Modeling and optimization of acetic acid fermentation: A polynomial-based approach

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ABSTRACT

Vinegar production is a typical bioprocess in the scope of the agrifood industry. Its optimization requires careful modeling which has so far been addressed by using mainly unstructured first principles models. Because of the difficulties in obtaining these models, black box models, such as those used here, are becoming more frequently used. The polynomial models developed in this work, accurately reflect the effect of the major and typical operational variables used in industry for this process. Also, response surfaces were used to identify the optimum operating conditions with a view to maximizing the mean fermentation rate and productivity. The followed strategy has a huge industrial interest since yields a tool that does not only allow finding the best operational conditions depending on different criteria but also is useful for process control. As far as we know this is the first time that these variables have been correlated in this way.

1. Introduction

The optimization of acetic acid fermentation as a biotechnological process has been the subject of much study in recent times – particularly as regards vinegar production [1–6]. The complex interdependence of the variables influencing growth and activity in acetic acid bacteria [7–10] has led to the development of mathematical models for quantifying the relationships between the major variables. Most such models have a phenomenological or unstructured first principles basis [11–14] and use differential equations to balance substrate and product concentrations, and kinetic equations to define the influence of the different variables [15,16]. This approach has the advantage of being valid over broad ranges of operating conditions by virtue of its relying on physico–chemical properties of the processes concerned. However, it has the disadvantages that the obtained models are complex and that their kinetic equations have to be constructed from unknown parameters which must be estimated by applying optimization algorithms to experimental values [17]. Also, obtaining accurate, unambiguous estimates requires satisfying the structural and practical identifiability conditions [18–22]. The structural identifiability analysis condition only depends on the mathematical structure of the model equations, whereas the practical identifiability analysis condition additionally considers the amount of data used to estimate parameters and their quality. Checking that both conditions are fulfilled entails using computationally complex algorithms [23,24], which is an added disadvantage of first principles models.

On the other hand, black box models need not consider the physico–chemical principles behind the target process [25]. Rather, these models seek the simplest possible relationships between operational and process variables from experimental values obtained under different conditions. As a rule, black box models

Abbreviations: $(r_A)_{est}$, estimated mean acetic acid formation rate (g acetic acid (100 mL h) $^{-1}$); C, wine loading rate(L min^{-1}); E, ethanol concentration remaining at the time the reactor is unloaded (% (v/v)); V, percent unloaded volume (%); $(P_A)_{est}$, estimated acetic acid production (g acetic acid h^{-1}); $(EtOH_{mean})_{est}$, estimated mean ethanol concentration (% (v/v)); $(HAc_{mean})_{est}$, estimated mean acetic acid concentration (% (w/v)); $([Total\ cells]_{mean})_{est}$, estimated mean total cell concentration (cells mL^{-1}); $([Viable\ cells]_{mean})_{est}$, estimated mean viable cell concentration (cells mL^{-1}); $(V_{mean})_{est}$, estimated mean volume (L); HAc_{final}, acetic acid concentration at the time the reactor is unloaded (% (w/v)); t_{total}, total cycle duration (h); V_{mean}, mean volume (L).

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are easier to construct than phenomenological models and require no prior identifiability analysis, so they are more practical for process optimization and control. However, black box models are applicable over narrower operational ranges than phenomenological models because they constitute necessarily local approaches.

Polynomial models, which are among the most widely used black box models [26,27], allow operational and process variables to be correlated via linear or non-linear generalized polynomials of variable order, but usually first or second [28,29] – the latter tend to be more accurate and widely applicable by effect of their considering interactions between factors (operational variables). However, they require greater numbers of experimental data to fit coefficients; also, the number of experiments needed depends on the polynomial order, the number of factors and the number of levels (values) used to discretize each factor range. Experimental design is used to identify the factors most strongly influencing a process under specific experimental conditions, minimize the effects of uncontrolled factors (perturbations), isolate and assess the effect of each individual factor by statistical analysis [30] and rationalize (reduce) the number of experiments required [31]. Experimental design allows obtaining the simplest algebraic equations used to construct polynomial models.

The joint use of polynomial models and response surfaces provides a powerful tool for process optimization [1,6,32], as it facilitates identification of the optimum operating conditions of a process considering interactions between individual influential factors.

In this work, we exploited the advantages of these models to construct quadratic polynomials for the process variables of acetic acid fermentation. To this end, we used three different factors, namely: the ethanol concentration remaining in the reactor at the time it was unloaded, the percent unloaded volume and the reactor loading rate, which are the three operational variables most widely used by industry. As far as we know, this is the first time that these variables have been correlated in this way. The ensuing models were used to optimize the process via the response surfaces of the variables and the results compared with those of previously reported first principles models.

2. Material and methods

2.1. Raw material or substrate

The acetification substrate was white wine from the Montilla–Moriles region, a protected designation of origin in southern Spain [33]. The wine had an initial ethanol concentration of 11.7 ± 0.3%(v/v) and an acidity of 0.2% (w/v).

2.2. Microorganisms

The inoculum used consisted of 3 L of fermentation broth from an industrial tank in full operation (Deoleo S.A., Córdoba, Spain).

2.3. Fermentation conditions

Experiments were conducted on a fully automated 8 L Frings reactor (Heinrich Frings GmbH & Co., KG, Bonn, Germany), details of which can be found in previous works [14,24,34–38]. The reactor was operated in a semi-continuous mode to facilitate assessment of the influence of the ethanol concentration at the time it was unloaded, the mean unloaded volume and the wine loading rate on the fermentation rate and acetic acid production. A constant temperature of 31 °C was used to mimic industrial conditions.

The ethanol concentration at the time of unloading ranged from 0.5 to 3.5% (v/v), the mean unloaded volume from 25 to 75% of the final working volume from 2 to 6 L and the loading rate from 0.01 to 0.06 L min^{-1}. The air flow rate at the time the reactor reached its final volume (8 L) was 7.5 L (h L medium)$^{-1}$.

Table 1
Control factors used in the Box–Behnken experimental plan and their levels.

Factor	Code	Level		
		(−1)	(0)	(+1)
Ethanol at unloading time, (%) (v/v)	*E*	0.5	2	3.5
Unloaded volume, (%)	*V*	25	50	75
Loading rate, L min^{-1}	*C*	0.01	0.035	0.06

The bioreactor was fully equipped to operate in an automated manner, so it was loaded, unloaded and monitored via appropriate computer software. This methodology afforded a high operational reproducibility and exhaustive recording of data.

For estimating the mean acetification rate, the method proposed elsewhere [35], using the variation of the ethanol concentration over the fermentation cycle, was used.

2.4. Experimental design

We used a central composite design (viz., a Box–Behnken design) to simultaneously examine the influence of all factors and reduce the number of experiments needed as far as possible. A total of 15 different sets experimental conditions were needed to characterize the 3 variables considered (see Tables 1 and 2). In any case, a huge experimental labor has been carried out; Table 2 shows the number of useful replications for each set of experimental conditions (a total number of 176). Additionally, each time the operational conditions were modified, a variable number of adaptation cycles can be necessary until get repetitive results.

2.5. Analytical methods

Volume was measured by means of an EJA 110 differential pressure probe (Yokogawa Electric Corporation, Tokyo, Japan).

The ethanol concentration was monitored in a continuous manner by using an Alkosens® probe and an Acetomat® transducer (Heinrich Frings GmbH & Co., KG, Bonn, Germany). The probe was calibrated by determining ethanol with an alcohol meter [39] in media previously obtained by steam distillation.

Acetic acid concentrations were measured by acid–base titration [39] .

Total cell concentrations were determined by direct counting in a Neubauer chamber using a light microscope, and viable cell concentrations similarly but using a LIVE/DEAD BacLight bacterial viability kit and the fluorescence unit of the microscope.

2.6. Mathematical methods

The optimum values of the operational variables were determined by establishing the Karush–Kuhn–Tucker (KKT) conditions [40,41] to be fulfilled by the optimum points of a non-linear restricted optimization problem. The problems addressed here were defined as follows:

$$\begin{aligned} &\text{Max } f(x_1, x_2, \ldots, x_n) \\ &\text{s.t. } g_1(x_1, x_2, \ldots, x_n) \le 0 \\ &g_2(x_1, x_2, \ldots, x_n) \le 0 \\ &g_3(x_1, x_2, \ldots, x_n) \le 0 \\ &\ldots\ldots\ldots \\ &g_m(x_1, x_2, \ldots, x_n) \le 0 \end{aligned} \quad (1)$$