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Effect of temperature on sugarcane ethanol fermentation: Kinetic modeling and validation under very-high-gravity fermentation conditions

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ABSTRACT

In this work, a mechanistic model is developed to simulate the effect of temperature on *Saccharomyces cerevisiae* growth and ethanol production of batch fermentations. A wide temperature range is used to estimate the temperature-dependent kinetic parameters of the reaction kinetics. Because multi-parameter estimation problems are complex, an optimization-based procedure is used to determine the optimum parameter values. The calculated reaction rates are used to construct a mechanistic fed-batch model. Experimental data from several cycles of very-high-gravity (VHG) ethanol fermentation from sugarcane are used to validate the model. Acceptable predictions are achieved in terms of the residual standard deviation (RSD). In addition, a suitable fermentation temperature profile, nutrient supplementation and micro-aeration during cell treatment are essential factors to obtain a yield of up to 90%, with a productivity of 10.2 g/L h and an ethanol concentration of 120 g/L.

1. Introduction

Currently, ethanol fuel from sugarcane is used on a large scale as hydrous ethanol in vehicles powered by ethanol or gasoline, also referred to as flex-fuel vehicles. Additionally, this fuel is used as an anhydrous ethanol blend with gasoline. The ethanol industry in Brazil processes approximately 630 million tons of sugarcane to produce 35.4 million tons of sugar, 28.5 million m^3 of ethanol, and 32,300 GWh of electric power annually [1] and employs approximately 4.5 million people [2]. As a result, this industry continues to present challenges regarding the increasing concern about environmental impacts, primarily climate change effects and the reduced dependence on fossil fuel resources.

The ethanol industry predominantly uses fed-batch fermentation with cell recycling; 70–80% of distilleries utilize this mode of operation to produce ethanol [3]. In this configuration, 99.5% of the cells are reused in sequential fermentation (intensive recycling). The high cell density inside the bioreactors contributes to reducing the fermentation time to 6–11 h and to increasing the ethanol yield to 90–92%. The final ethanol concentration varies between 8 and 11°GL [4]. However, this current fermentation technology is limited in processing substrates at a concentration of up to 200 g/L TSAI (total sugars as invert in which the original sucrose is equivalent to 0.95 of the reducing sugars formed (glucose and fructose) and glucose and fructose originally present), representing the potential of obtaining an ethanol concentration of at least 11°GL. The increased amount of sugars and ethanol in conventional fermentation causes a decrease in the cell maintenance rate, thus reducing the percentage of cell viability and stuck fermentation [5].

One method to improve the current ethanol fermentation profitability is through process intensification. Very-high-gravity (VHG) technology is one type of improvement process aimed at obtaining high ethanol concentrations of up to 15°GL from moderately high sugar concentrations (>250 g/L) [6]. Furthermore, VHG technology enables reduction of the process water requirements, thus reducing the related distillation operational cost, vinasse generation and its treatment cost, resulting in significant energy savings [7].

Over the course of VHG fermentation, a cell encounters significant stress induced by osmotic pressure, which leads to variations in the fermentation kinetics. Furthermore, the level of ethanol in the late stage of the process depends strongly on the nutritional condi-

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tions for cell maintenance [8]. Another common source of kinetic fluctuation could be related to the variability of feedstock quality, which is caused by the quality of molasses (related to the level of exhaustion and sulfitation in the sugar production) and the quality of sugarcane juice, which may vary during the sugarcane season, depending on the variety, harvest period, climate conditions and juice extraction procedure [9].

Rossell et al. [10] recently developed a novel continuous VHG ethanol fermentation by enhancing the engineering design and operation process. Ethanol fermentation in multistage bioreactors with a well-defined temperature profile was considered in this technology. Higher temperatures were considered in the initial stages to maximize conversion, and lower temperatures were considered in the later stages to minimize the inhibition and cellular damage from the high ethanol concentration. In addition, a distributed carbon source feeding was considered in the first and second stages to avoid inhibition by high sugar concentrations. The process also included intracellular detoxification through a second centrifugation followed by a cell reactivation stage to promote membrane recovery and enzymatic restoration. The primary goal of this technology was to maintain active and viable cells for an entire harvest season, which is a critical condition to provide operating stability and a high rate of sugar conversion to ethanol.

The primary challenge in the VHG fermentation process is to determine the effect of temperature as well as high substrate and ethanol concentrations on cell growth kinetics, which clearly affect the performance of the fermentation process. Although different empirical studies were developed for improving VHG processes, they lack a systematic model-based approach. A realistic kinetic model is required for the design, optimization and control of the process.

Temperature is a crucial operating parameter due to its influence on the conversion of sugars to ethanol, which is an exothermic reaction, i.e., heat is released. In the case of conventional fermentation, a temperature control is required, often in the range of 32–35 °C [4]. For VHG fermentation, a quantitative understanding of the effect of temperature on substrate consumption and ethanol production rates must be investigated to define the most suitable operating conditions using the definition of optimum temperature profiles. Previous work [11] has addressed the effect of temperature on ethanol production from sugarcane by *S. cerevisiae*, resulting in the formulation of a mechanistic model, which includes terms for the cell, substrate and ethanol inhibitions. The results reported that the maximum specific growth rate increases as the temperature increases. After approximately 37 °C, the maximum specific growth rate begins to decrease. The maximum ethanol and cell concentrations, i.e., the concentrations at which cell growth ceases, are inversely related to the fermentation temperature. This model was validated during batch fermentation under conventional fermentation conditions.

This study investigated the effect of temperature on ethanol production from sugarcane. For this purpose, a mechanistic fermentation model was developed, with kinetic parameters determined using a model-based optimization algorithm. The model was validated through experiments, including fed-batch fermentation with cell recycling, operating under VHG conditions. The proposed methodology drives the systematic development of an industrially reliable mathematical model for VHG fermentation.

2. Material and methods

2.1. Microorganism

This study used an unclassified *Saccharomyces cerevisiae* strain cultivated in the Bioprocess Development Laboratory at CTBE, provided by the Faculty of Food Engineering/State University of Campinas and originally obtained from the Santa Adélia sugarcane mill. The stock culture was maintained in YPD (10 g/L yeast extract, 20 g/L peptone and 20 g/L dextrose) at −80 °C with 30% (v/v) glycerol.

2.2. Batch fermentations

A stock culture was activated in liquid YPD medium at 33 °C under agitation (250 rpm) for 24 h on an Innova 44 orbital shaker (New Brunswick, NJ, USA). Then, a sample was streaked onto YPD agar plates, incubated at 33 °C for 48 h, and stored at 5 °C. The inoculum for all fermentations was prepared by transferring three loops from agar plates to a new semi-synthetic liquid medium. The medium consisted of 2.30 g/L urea, 6.60 g/L K_2SO_4, 3.0 g/L KH_2PO_4, 0.50 g/L $MgSO_4{\cdot}7H_2O$, 1.0 g/L $CaCl_2{\cdot}2H_20$, 5.0 g/L yeast extract, 25.44 mg/L trace elements (as detailed in Basso [12]), 3.0 ppm thiamine and 80.0 g/L TSAI. Then, the cells were incubated at 33 °C under agitation (250 rpm) for 12 h on the Innova 44 orbital shaker. Commercial crystal sugar was used as the source of TSAI. After the inoculation period was complete, the cells were recovered using a Beckman Avanti J-26 XP centrifuge (JLA-16.250 rotor, 5509 × *g*, 10 °C, 15 min). The cells were diluted with sterilized potable water, and this suspension was inoculated in a 7.5 L Bioflo 115 bioreactor (New Brunswick, NJ, USA). The initial cell concentration was approximately 1 g/L. The propagation was conducted in fed-batch mode at 33 °C and aerated with the carbon source limited to 18 g TSAI/L to minimize ethanol production through the Crabtree effect [13]. The agitation and airflow were controlled by dissolved oxygen (DO) control in cascade mode, with the dissolved O_2 concentration maintained above 25% air saturation. The propagation medium was the same as that used for the inoculum, with 180 g TSAI/L. After all sugars were consumed, to recover cells, the fermented medium was centrifuged using a Beckman Avanti J-26 XP centrifuge (JLA-9.1000 rotor, 5509 × *g*, 10 °C, 15 min). The cells were re-suspended with sterilized potable water and refrigerated at 5 °C until inoculation for further fermentations. The fermentation medium for the cell propagation was similar to the semi-synthetic medium described above. The salt and nutrient requirements were varied according to the initial cell density. All batch fermentations were performed in a 3 L Bioflo 115 bioreactor (New Brunswick, NJ, USA) under agitation at 200 rpm with a working volume of 2 L. The experiments were performed using the initial cell and substrate concentrations and at the temperatures shown in Table 1.

2.3. VHG fed-batch fermentation with cell recycling

The inoculum and cell propagation were conducted according to the aforementioned batch fermentation. The inoculum medium contained 5.0 g/L urea, 1.1 g/L $(NH_4)_2$ HPO_4 (DAP), 1.0 g/ L $MgSO_4{\cdot}7H_2O$, 5.0 ppm $ZnSO_4{\cdot}7H_2O$, 3.0 ppm thiamine, 5.0 g/L yeast extract and 80.0 g/L TSAI. The propagation medium contained 5.0 g/L urea, 1.1 g/L DAP, 1.0 g/L $MgSO_4{\cdot}7H_2O$, 5.0 ppm $ZnSO_4{\cdot}7H_2O$ and 150.0 g/L TSAI. The TSAI source consisted of 79% (w/w) from sugarcane juice and 21% (w/w) from sugarcane molasses. The expected final concentration was 400 g TSAI/L. Physicochemical treatment, referred to as clarification, was performed to reduce the salt concentration, which influences the fermentation performance due to the elevated osmotic pressure. Substrate clarification was performed with the addition of 85% of 0.4 mL/L phosphoric acid and lime at a concentration of 8°Be until pH 6.4 was reached, followed by heating at 95 °C and the addition of 4 ppm non-ionic polymer [14]. Several minutes of rest were required for flake formation and decantation. These conditions allowed the drag of soluble impurities. Thus, a clarified substrate with high quality, minimal turbidity and low calcium concentration was produced. After clarification