
EVALUATION OF EXTRACTIVE BATCH ETHANOL FERMENTATION AT HIGH TEMPERATURE WITH CO₂ STRIPPING

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ABSTRACT

In the present work, it was evaluated the removal of ethanol during an extractive batch fermentation at high temperature with CO₂ stripping. A conventional batch fermentation (40 °C) (without ethanol removal) was performed as control experiment and, subsequently, an extractive fermentation at 40 °C with ethanol removal by CO₂ stripping was carried out. It was verified an increase in the ethanol productivity of 41.4% compared to the conventional fermentation. Thus, the ethanol removal from the fermentative broth at 40 °C showed as an alternative to decrease the inhibitory effect of ethanol on yeast.

1. INTRODUCTION

In Brazil distilleries, ethanol is produced by fermentation of sugars, mainly sucrose, from sugarcane juice. However, the final ethanol concentration in fermentation broth is limited due to the effect of ethanol inhibition on the yeast. This process achieves ethanol concentrations in the fermentation broth close to 8–11% (v·v⁻¹) for fermentations at temperatures in the range of 32–35°C (WHEALS et al. 1999; AMORIM et al. 2011). Another problem faced by distilleries is related to the increase of fermentation temperature broth during the process. In some regions of high temperature, there is the challenge to maintain the temperature of the fermentation medium during the process. An alternative to overcome these two difficulties in the ethanol production process would be to use the extractive fermentation process with CO₂ stripping in fermentations using thermotolerant yeasts. The stripping process is favored if carried out at higher temperatures. The use of higher temperatures facilitates the process control. In this context, the objective of the present work was to evaluate the removal of ethanol during an extractive batch fermentation at high temperature with CO₂ stripping.

2. MATERIALS AND METHODS

2.1. Conventional Ethanol Batch Fermentation and Extractive Ethanol Batch Fermentations

Firstly, a conventional batch fermentation (CF) was performed as control experiment. Subsequently, an extractive fermentation with CO₂ was performed. The fermentations were performed with commercial lyophilized *Saccharomyces cerevisiae* (Y-904, AB Brasil, Pederneira, SP, Brazil) with an initial concentration of 10 g.L⁻¹ (in dry basis). The composition of the culture medium was (g.L⁻¹): sucrose (180.0), KH₂PO₄ (5.6), MgSO₄.7H₂O (1.4), yeast extract (6.8), and urea (5.32). The batch ethanol fermentations were conducted in a bubble column bioreactor (2 L). The temperature was maintained at 40 °C using a thermostatic bath. The extractive fermentation (EF) with ethanol removal by CO₂ stripping initiated after 3 h of fermentation with a specific gas flow rate (ϕ) of 2.5 vvm. Cell concentration was measured by dry weight (after 24 h at 80 °C). Concentrations of sucrose, glucose, fructose, and ethanol were determined by HPLC (Waters, U.S.A.) equipped with a refractive index detector and a Sugar-Pak I column (300 × 6.5 mm, 10 μ m, Waters) maintained at 80 °C.

2.2. Mathematical Modeling of Extractive and Conventional Batch Fermentations

According to Sonego et al. (2014), the mathematical model of the extractive batch fermentation (equations 1-4) utilize mass balance equations for cells, substrate, and ethanol, considering the removal of ethanol and water by the CO₂ stream, as well as changes in the broth volume (V).

$$\frac{dCx}{dt} = \mu \cdot Cx - Cx \cdot \frac{1}{V} \cdot \frac{dV}{dt} \quad (1)$$

$$\frac{dCs}{dt} = -\frac{1}{Y_{X/S}} \cdot \mu \cdot Cx - Cs \cdot \frac{1}{V} \cdot \frac{dV}{dt} \quad (2)$$

$$\frac{dCE}{dt} = \frac{Y_{E/S}}{Y_{X/S}} \cdot \mu \cdot Cx - CE \cdot \frac{1}{V} \cdot \frac{dV}{dt} - k_E \cdot CE \quad (3)$$

$$\frac{dV}{dt} = -\frac{(k_E \cdot CE + k_w \cdot (\rho_w - CE)) \cdot V}{\rho_w} \quad (4)$$

where Cx is the cell concentration (g.L⁻¹), μ is the specific cell growth rate (h⁻¹), Cs is the limiting substrate concentration (g.L⁻¹), CE is the ethanol concentration (g.L⁻¹), Y_{X/S} is the cell yield coefficient (g_X·g_S⁻¹), and Y_{E/S} is the ethanol yield coefficient (g_E·g_S⁻¹), V is the broth volume (L), k_E is the removal rate constant for ethanol (h⁻¹), k_w is the removal rate constant for water (h⁻¹), and ρ_w is the specific mass of water (g.L⁻¹).

The hybrid Andrews–Levenspiel kinetic model was used to represent the cell growth (Equation 5).

$$\mu = \mu_{max} \cdot \frac{C_S}{\left(K_S + C_S + \frac{C_S^2}{K_{IS}}\right)} \cdot \left(1 - \frac{C_E}{C_{E_{max}}}\right)^n \quad (5)$$

where μ_{max} is the maximum specific cell growth rate (h^{-1}), K_S is the saturation constant ($\text{g}\cdot\text{L}^{-1}$), K_{IS} is the substrate inhibition constant ($\text{g}\cdot\text{L}^{-1}$), $C_{E_{max}}$ is the maximum concentration of ethanol after which cell growth ceased, and n is a dimensionless constant.

2.3 Model Fitting and Numerical Procedure

The kinetic parameters were estimated using an optimization algorithm based on the genetic algorithm (GA) together with the Runge–Kutta algorithm for numerical solving of the differential equations that represented the model. The criterion used for the best fitting and parameter optimization was minimization of the sum of squared residuals.

3. RESULTS AND DISCUSSION

3.1 Conventional Batch Fermentation - Model Fitting and Kinetic Parameter Estimation

The parameters k_E and k_W were obtained from stripping experiments with CO_2 performed using a hydroalcoholic solution (data not shown). The kinetic parameters for the current model were estimated from the batch experiments. The estimated values were: $\mu_{max}=0.147 \text{ h}^{-1}$, $K_S=27.19 \text{ g}\cdot\text{L}^{-1}$, $K_{IS}=67.23 \text{ g}\cdot\text{L}^{-1}$, $C_{E_{max}}=63.58 \text{ g}\cdot\text{L}^{-1}$ e $n=0.88$. The cell and ethanol yield coefficients, $Y_{X/S}=0.016 \text{ g}_X\cdot\text{g}_S^{-1}$ and $Y_{E/S}=0.46 \text{ g}_E\cdot\text{g}_S^{-1}$, were calculated using the experimental C_X , C_S , and C_E data obtained in the conventional batch fermentations. Figure 1 illustrates the excellent fit of the model to the experimental data.

3.2. Extractive Batch Ethanol Fermentations - Experimental and Simulation

Figure 1-A shows the results for conventional batch fermentation. In this fermentation it was verified a high sugar concentration after 12 h of fermentation ($39,98 \text{ g}\cdot\text{L}^{-1}$). At this time, the ethanol concentration was $59,76 \text{ g}\cdot\text{L}^{-1}$. This result was due to a strong effect of ethanol inhibition which is more intense at high temperature, resulting in a decrease in the substrate consumption rate for this assay.

It can be seen that in the extractive fermentation (Figure 1-B) the substrate consumption rate increased after the beginning of ethanol stripping compared to the conventional fermentation. Consequently, lower ethanol concentration in the fermentation medium leads to a reduction in ethanol inhibition. Thus, substrate was totally consumed after 11h of fermentation. Compared to conventional fermentation (ethanol productivity of $4.98 \text{ g}_E\cdot\text{L}^{-1}\cdot\text{h}^{-1}$), extractive fermentation presented a 41.4% increase in the ethanol productivity. In the extractive fermentation, ethanol productivity ($7.04 \text{ g}_E\cdot\text{L}^{-1}\cdot\text{h}^{-1}$)

was calculated considering the initial substrate concentration ($168.5 \text{ g}\cdot\text{L}^{-1}$) and the ethanol/substrate yield ($Y_{E/S}$) of $0.46 \text{ gE}\cdot\text{gS}^{-1}$.

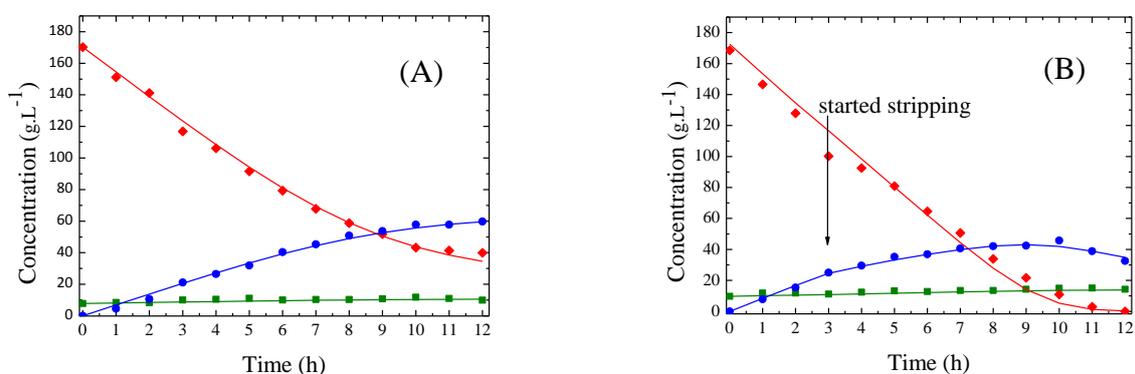


Figure 1. Comparative plots of experimental and simulated (—) data for C_x (■), C_s (◆), e C_E (●): (A) conventional fermentation; (B) extractive fermentation with CO_2 (FE- CO_2).

4. CONCLUSIONS

The obtained results showed that the removal of the ethanol by stripping with CO_2 was able to minimize the effect of ethanol inhibition on the yeast, which resulted in an increase in the substrate consumption rate and consequently an increase in ethanol productivity (approximately 41%). Thus, the CO_2 stripping technique was a promising approach to be used in high temperature fermentation.

5. ACKNOWLEDGMENTS

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