PERFORMANCE EVALUATION OF THE MICROORGANISM RESPONSIBLE FOR INDUSTRIAL ALCOHOL FERMENTATION - REDUCED FERMENTATION METHODOLOGY

Lorena M. F. Leite[1]*, Telma T. Franco[1], Silvio R. Andrietta[2]

[1] *Department of Process Engineering, Faculty of Chemical Engineering, Universidade Estadual de Campinas, Campinas, Brasil*
[2] *Andrietta Soluções em Bioprocessos, Campinas, Brasil.*
** Corresponding author's email address: l222813@dac.unicamp.br*

ABSTRACT

Optimizing industrial fermentation processes is essential to increase economic viability. This study demonstrates the effectiveness of the Reduced Fermentation Methodology (RFM) in evaluating the performance of yeast strains in industrial sugarcane must alcoholic fermentation processes. The RFM allows for a detailed analysis of mass balance and fermentation kinetics, providing accurate data on fermentative yield and yeast productivity throughout the harvest season. The results confirm that RFM is a robust tool for monitoring and improving the operational efficiency of industrial fermentation processes.

Keywords: Alcoholic fermentation, Reduced Fermentation Methodology, Kinetics, Mass Balance, Fermentative Yield

1 INTRODUCTION

Alcoholic fermentation by *Saccharomyces cerevisiae* occurs through a series of metabolic reactions. Due to the enzymatic nature of these reactions, various factors can influence fermentative yield and consequently industrial efficiency. In processes with cell recycling, changes in yield and productivity are common due to variations in the inoculum population throughout the harvest season. Adaptation to environmental conditions and the entry of wild yeasts and other contaminating microorganisms through the raw material can also cause alterations. Therefore, it is unlikely that a yeast strain initially used as inoculum in a fermentation with cell recycling will be fully preserved until the end of the harvest. The yeast strains that remain in the process play a crucial role in fermentative yield. A strain can be considered inadequate not only when it shows deficiencies in ethanol production, cell multiplication, or high production of by-products, but also when it has a slow metabolism, resulting in longer alcoholic fermentation and consequently reducing productivity. Often, industrial efficiency and fermentative yield calculated within the industries are inconsistent or inaccurate, leading to incorrect interpretations regarding necessary corrective actions and improvements. The speed of sugar metabolism into ethanol can be affected by various factors, some of which are directly related to the fermentative medium, such as the presence of inhibitory substances, bacterial contamination, and the absence of essential nutrients. Understanding the effects on fermentative kinetics and obtaining reliable fermentative yield values are essential to assess the performance of the industrial fermentation process. This study aims to evaluate the Reduced Fermentation Methodology (RFM) as an alternative for assessing the performance of yeast strains through mass balance in industrial processes at different periods of the harvest.

2 MATERIAL & METHODS

The proposed methodology involves conducting batch laboratory-scale alcoholic fermentation assays to evaluate fermentative performance related to fermentative yield and productivity through mass balance. The following parameters are considered to determine the good performance of the fermentation process using this methodology: a) Ethanol yield (YP/S), given by the mass of ethanol produced from a given mass of TRS consumed; b) Cell yield ($Y_{X/S}$), given by the amount of cells produced from a given mass of TRS consumed; c) Fermentative yield (RY), given by the percentage ratio of the obtained $Y_{P/S}$ to the theoretical $Y_{P/S}$ (0.511 g $_{ethanol}$/g $_{TRS}$); d) Maximum conversion rate (R_{max}), given by the highest value of CO_2 mass produced per hour; e) Maximum specific conversion rate (SR_{max}), given by the highest value of CO_2 mass produced per hour relative to the cell concentration in the fermentation medium; f) Specific ethanol production (SP), given by the mass of ethanol produced per hour relative to the cell concentration in the fermentation medium.

Two alcoholic fermentation assays were conducted with substrate and inoculum from different industries. Similar initial concentrations of sugar and cells were used in both assays. The substrate, composed of sucrose, glucose, and fructose, was derived from sugarcane juice and molasses. However, each assay used *Saccharomyces cerevisiae* inoculum from the cell recycling of the respective industrial process, hydrated and treated with sulfuric acid. The inoculum was transferred to Erlenmeyer flasks and incubated at 34°C for 10 hours. Each flask was weighed at one-hour intervals on a semi-analytical balance for 10 hours. The assays were performed in triplicate. The gradual reduction in mass characteristic of fermentation refers to the release of CO_2 during the process. For kinetic analysis purposes, the mass of CO_2 produced, which is directly linked to ethanol production, was considered. Sugar concentrations were analyzed in the substrate and fermented material (after 10 hours of fermentation) by

liquid chromatography. Ethanol and cell concentrations were determined in the inoculum and fermented material (after 10 hours) by liquid chromatography and gravimetry for dry mass determination.

3 RESULTS & DISCUSSION

This section presents the results obtained from the laboratory-scale alcoholic fermentation assays, followed by a detailed data analysis. The results include measurements of ethanol, cell, and sugar concentrations, as well as CO_2 conversion profiles and kinetic analysis of the fermentation. Tables 1 and 2 present the results obtained from the quantitative laboratory analyses, while Figures 1 and 2 illustrate the CO_2 conversion profiles (g CO_2/h) and the mass of CO_2 produced (g) obtained for the three repetitions of each assay.

Table 1 Laboratory Analysis Results.

Assay	Material	Concentration					
		Ethanol (°GL)	Cell (g/L)	Sucrose (g/L)	Glucose (g/L)	Fructose (g/L)	TRS (g/L)
Assay 1	Yeast	4,41	32,00	-	-	-	-
	Substrate	-	-	15,66	1,58	1,88	19,12
	Fermented broth (Time = 10)	8,75	12,33	0,00	0,00	0,13	0,013
Assay 2	Yeast	4,43	30,00	-	-	-	-
	Substrate	-	-	15,52	1,61	1,56	19,51
	Fermented broth (Time = 10)	9,20	10,00	0,00	0,02	0,32	0,34

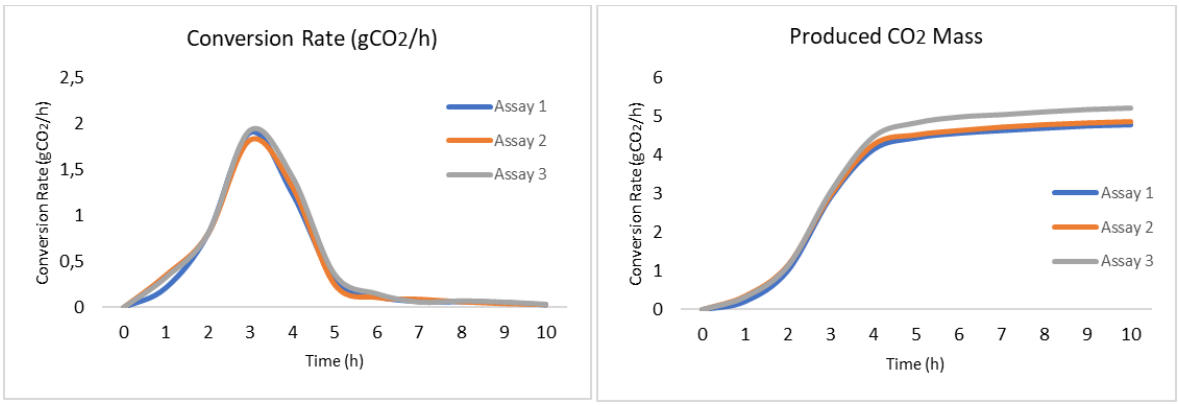

Figure 1 Profile of CO_2 Conversion Rate and Produced CO_2 Mass for Company 1's assay.

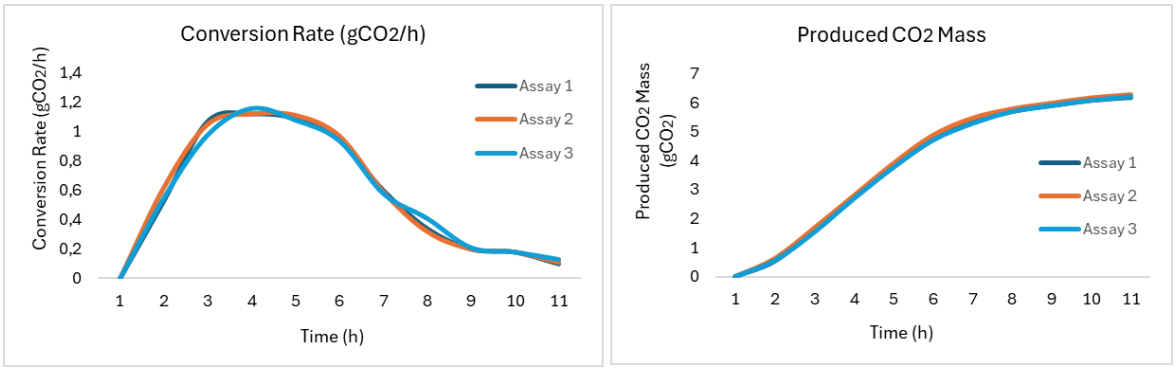

Figure 2 Profile of CO_2 Conversion Rate and Produced CO_2 Mass for Company 2's Assay.

Table 2 Kinetic and Mass Balance Results

Assays	$Y_{P/S}$ (gEt/gTRS)	$Y_{X/S}$ (gDM/gTRS)	RY (%)	R_{max} (gCO_2/h)	SR_{max} ($gCO_2/h.gDM$)	SP (gEt/hxgDM)
Assay 1	0,457	0,035	89,31	1,88	0,649	0,352
Assay 2	0,462	0,030	90,40	1,14	0,465	0,242

- Productivity Evaluation

Figures 1 and 2 show that the kinetic behavior presented in Assays 1 and 2 by the inoculum in the respective industrial fermentation media was distinct, with maximum conversion rates (R_{max}) ranging from 1.14 g CO_2/h to 1.88 g CO_2/h. This indicates a variation in the fermentative process behavior between the two assays, where Assay 1 showed a higher conversion rate compared to Assay 2, possibly due to the higher fermentation rate of the yeast strains present or better quality of the raw material used. The maximum conversion rate is related to ethanol productivity; thus, this methodology is useful for evaluating the productivity of the fermentation medium and industrial inoculum set, in addition to being important for defining the necessary fermentation time for the total conversion of sugars present in the fermentation medium.

- Yield Evaluation

The performance related to the yield of the cells used as inoculum in a process can be obtained by evaluating the fermentative yield calculated by the mass of TRS consumed and the mass of ethanol produced. Evaluating the data in Table 2, it is verified that the inoculum used in Assay 2 presented a higher fermentative yield than that used in Assay 1, with values of 90.40% and 89.31%, respectively. Thus, despite its lower speed, the inoculum used in the fermentation of Assay 2 demonstrated better performance for ethanol production per mass of TRS consumed.

The productivity and yield evaluations showed that the Reduced Fermentation Methodology (RFM) is capable of determining the performance characteristics of the strains present in the industrial inoculum when grown in their respective industrial media.

4 CONCLUSION

The use of RFM for evaluating the performance of the fermentative process (fermentation medium and industrial inoculum set) proved to be adequate, being capable of identifying conversion rate characteristics and fermentative yield. This makes its use interesting for monitoring the performance of fermentation processes throughout the harvest season of an industrial unit and can be especially important when conventional performance determination methods do not provide results that adequately represent the process conditions.

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