DETERMINATION OF THE REACTION VOLUME PROFILE OF A BATCH ALCOHOLIC FERMENTATION REACTOR

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The amount of fermented material contained in the bioreactors for ethanol production is one of the factors considered in the calculation of fermentative yield. Therefore, determining this volume must be carried out with care and precision to minimize deviations that, when applied on an industrial scale, may lead to inconsistencies in this parameter's value. In order to evaluate the profile of volume variation in an alcoholic fermentation bioreactor, laboratory tests were conducted under operational conditions similar to those used in industry. The amount of carbon dioxide released and the density of the fermentative medium were used to calculate the volumetric balance of the bioreactors every hour during the process. The results indicated that the variation in the bioreactor volume was not significant throughout the process, as the mass loss caused by carbon dioxide production was compensated by the reduction in the density of the reaction medium due to the decrease in Total Reducing Sugars concentration and the increase in ethanol concentration. The observed error in determining the volume of the medium, through the volumetric balance of the fermenters, was approximately 0.23%.

Keywords: 1. Industrial Process. 2. Volumetric Balance. 3. Alcoholic Fermentation

1 INTRODUCTION

The industrial processes for ethanol production in Brazil, the second-largest global producer, predominantly involve cell recycling. In this method, yeast cells from a fermentation cycle are separated by centrifugation, treated with acid, and reintroduced into the bioreactors for a new fermentation cycle.

The evaluation of the performance of this process involves calculating the fermentative yield, a parameter that can be determined in different ways. One of these approaches uses the final volume of fermented wine to determine the volume of must fed into the fermenter, subtracting from this volume the volume of diluted and treated yeast cream transferred to the fermenter. The precise determination of this volume is crucial as it is used in calculating the mass of total reducing sugars supplied to the fermenter.

However, the alcoholic fermentation process presents various factors that can interfere with the volumetric balance of the fermenter, resulting in errors in determining the volume of must by the method of direct measurement of the fermented wine. These factors include the significant release of carbon dioxide during the fermentation process and the variation in the density of the fermentation medium due to the consumption of substrate by yeast cells and the increase in ethanol concentration, a product of the bioconversion of sugars. Therefore, it is necessary to evaluate the effect of these factors on the variation in reaction volume in the fermenters and determine if this observed difference significantly impacts the fermentative yield values obtained by this method. With this goal in mind, experiments were conducted in 5-liter batch bioreactors to estimate the extent of the variation in reaction volume throughout the fermentation process and verify if the observed difference affects fermentative yield values[3].

2 MATERIAL & METHODS

The inoculum was prepared from commercially obtained lyophilized yeast with a final concentration of 75 g/L. A synthetic fermentation medium was used, composed of 28 g of yeast extract, 25 g of ammonium chloride, 25 g of potassium dihydrogen phosphate, 4.8 g of magnesium sulfate heptahydrate, and 5.0 g of potassium chloride. The experiments were conducted in 5-liter benchtop bioreactors, adding 800 g of inoculum and 3,200 g of culture medium. The total fermentation time was 10 hours, and samples were collected, centrifuged, and analyzed every hour. The analyses included: i) Supernatant: Concentration of sucrose, glucose, fructose, and ethanol by liquid chromatography; ii) Precipitate: Cell concentration by removing soluble solids, drying, and weighing.

Table 1 presents the equations and stoichiometric reactions used in the elaboration of the volumetric balance of the bioreactor. The variables are defined as follows: AC: Accumulation of Mass Variation in Process; MA_{SA}: Mass Exiting the Process; MA_{CO_2}: Mass of Carbon Dioxide Produced; MA_{ET}: Mass of Ethanol Produced; ρ: Density of the Fermentation Medium; V: Volume of the Bioreactor Reaction; BX: Total Soluble Solids Concentration (brix); S: Sucrose Concentration in the Medium; G: Glucose Concentration; F: Fructose Concentration; $SSNS_M$: Concentration of Non-Sugar Soluble Solids in the Medium (% by weight); BX_M: Concentration of Total Soluble Solids in the Medium (% by weight); TRSM – Concentration of Total Reducing Sugars in the Medium (g/L); ρ_M: Density of the Medium (g/mL); BX_i: Concentration of Total Soluble Solids in the Medium at Time i (% by weight); TRS_i: Concentration of Total Reducing Sugars in the Medium at Time i (g/L); ρ_i: Density of the Medium at Time i (g/mL); i: Fermentation Time (i = 0, ..., n); P: Ethanol Concentration in the Fermenting Medium (g/L); ρ_{ET100}: Density of 100% Ethanol (g/mL); TRS_M - Total Reducing Sugars Concentration in the Medium (g/L); TRS_i - Total Reducing Sugars Concentration in the Medium at Time i (g/L).

Initially, the global system balance was determined following the theory of mass conservation, and since it is not a nuclear reaction, there is no production or consumption of mass in the system. The mass of the inoculum and fermentation medium was added to the reactor at time zero, considering that the mass input into the bioreactor during fermentation is zero. During fermentation, the production and release of CO_2 occur, considered as the mass exiting the bioreactor. Thus, the equation for the global balance can be written as Equation 1.

Through the stoichiometric transformation of glucose into ethanol, considering the molecular weights of carbon dioxide (88 g/mol) and ethanol (92 g/mol), it is established that the production of CO_2 is related to the production of ethanol by the factor of 0.9565 gCO_2/gethanol. Therefore, the production of carbon dioxide can be described by Equation 2. The infinitesimal variation of mass in the bioreactor over time is described by Equation 3. Thus, the global mass balance of the bioreactor providing the mass variation over time is described by Equation 4.

The density of the fermentation medium varies with the concentration of sugars and ethanol in the medium. Equation 5 presents the relationship between the concentration of soluble solids and density, derived from a linear regression of data from the table of density of sugary solutions[1]. The concentration of total reducing sugars (TRS) can be stoichiometrically calculated according to Equation 6. The concentration of non-sugar soluble solids (SSNS) in the fermentation medium is given by the difference between the concentration of total soluble solids and the concentration of total reducing sugars, as shown in Equation 7.

Considering the small variation in the concentration of SSNS during fermentation, the concentration of total soluble solids in relation to the concentration of total reducing sugars is determined by Equation 8. Substituting the values of BXi into Equation 5, the value of the density of the medium in terms of the concentration of total reducing sugars (TRS) in the medium (ρ') is determined. The variation in the density of the fermentation medium in relation to the concentration of ethanol (ρ'') is shown in Equation 9, obtained through linear regression of the table relating ethanol concentration (%v/v) to the density of the medium[2]. Considering both factors, the density of the fermentation medium can be calculated according to Equation 10. The mass of ethanol produced during fermentation can be calculated using the variation in ethanol concentration and the volume of the bioreactor throughout fermentation.

Table 1 Equations and Reactions for the Volumetric Balance of the Bioreactor.

N° Equation	Equation	N° Equation	Equation
Equation1	$AC = -MA_{SA} = -MA_{CO_2}$	Equation 6	$TRS = \frac{S}{0,95} + G + F$
Equation 2	$MA_{CO_2} = 0,9565\ MA_{ET}$	Equation 7	$SSNS_M = BX_M - \frac{TRS_M}{\rho_M x10}$
Equation 3	$AC = \frac{dMA}{dt} = \frac{d(\rho V)}{dt}$	Equation 8	$BX_i = \frac{TRS_i}{\rho_i x10} + SSNS_M$
Equation 4	$\frac{d(\rho V)}{dt} = 0,9565 x MA_{ET}$	Equation 9	$\rho'' = -0,0011\ x\ \frac{P}{\rho_{ET100}\ x\ 10} + 0,9979$
Equation 5	$\rho' = (0,0043\ x\ BX + 0,9974)x1000$	Equation 10	$\rho = \rho'\ x\ \rho''$

The concentration of non-sugar soluble solids (SSNA) was calculated taking into account the brix and the concentration of reducing sugars (TRS) in the fermentation medium, using Equation 7.

3 RESULTS & DISCUSSION

The concentration of non-sugar soluble solids (NSSS) was determined by calculation, taking into account the Brix and the concentration of reducing sugars (RS) in the fermentation medium. Using Equation 7, the value corrected for dilution due to the presence of the inoculum resulted in 2.53% (w/w). The results of the volume variation and other data for each studied interval are then presented in Table 1.

Upon analyzing the data presented in Table 2, it is observed that the reaction mass of the bioreactor decreased from 4,303.77 g at the beginning of the assay to 4,000.47 g at the end. The density (ρ) also recorded a reduction from 1.08 g/mL to 1.00 g/mL, while the reaction volume varied from 4,000 mL to 4,009.25 mL.

Table 2 Hourly results of the evaluated parameters in the bioreactor

Time	TRS (g/L)	Ethanol (g/L)	Brix (%p/p)	Ethanol conc. (%v/v)	Density due to TRS (g/mL)	Density due to ethanol (g/mL)	Actual density (g/mL)	Cass of CO2 produced (g)	Reactor mass (g)	Reactor volume (mL)
0	171,19	0,97	18,407	0,123	1,078	0,998	1,076	0,00	4303,77	4000,00
1	163,65	6,89	17,742	0,872	1,075	0,997	1,072	22,64	4281,13	3992,83
2	139,30	17,94	15,524	2,271	1,066	0,995	1,061	42,21	4238,92	3995,01
3	109,11	31,66	12,815	4,007	1,054	0,993	1,047	52,41	4186,51	3996,88
4	78,76	44,93	10,051	5,687	1,042	0,992	1,034	50,74	4135,77	4000,90
5	54,96	55,73	7,849	7,055	1,033	0,990	1,023	41,36	4094,41	4003,28
6	35,40	63,53	5,993	8,042	1,025	0,989	1,014	29,86	4064,55	4009,42
7	22,35	71,05	4,737	8,994	1,020	0,988	1,007	28,84	4035,71	4006,29
8	12,82	76,06	3,805	9,628	1,016	0,987	1,003	19,19	4016,52	4005,81
9	6,45	79,48	3,176	10,061	1,013	0,987	1,000	13,11	4003,41	4005,34
10	2,66	80,25	2,799	10,158	1,011	0,987	0,998	2,94	4000,47	4009,25

These results indicate that the loss of mass from the reaction medium of the bioreactor was compensated by the decrease in density, influenced by the variation in the concentration of reducing sugars (TRS) and ethanol. Based on the data from Table 1, it is estimated that the error in determining the fermentative yield due to the expansion of the reaction medium is only 0.23%. Figure 1 illustrates the variation in volume over the fermentation time.

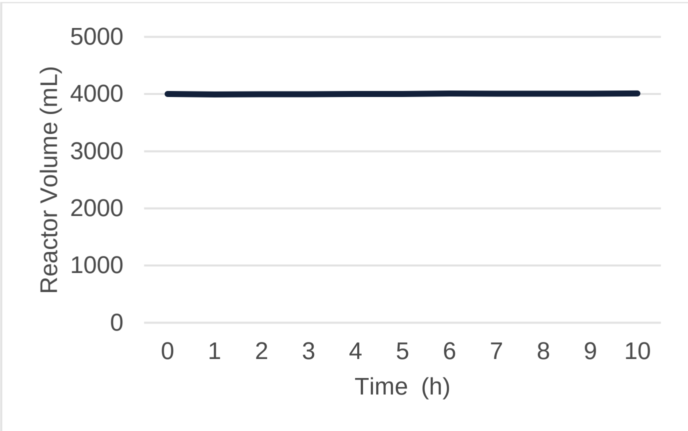

Figure 1 Volume Profile of the Reaction Medium Over Fermentation Time

It can be observed in the volume profile over time, as depicted in Figure 1, that the reaction volume remained practically constant throughout the fermentation process.

4 CONCLUSION

In fermentative processes where the final ethanol concentration reaches values around 80 g/L, the variation in the reaction volume, i.e., the crude wine, would be approximately 9.25 mL in a total of 4,000 mL. This represents an expansion of 0.23%. Thus, the contribution of bed expansion to the error in the fermentative yield calculation using the volumetric balance method in industrial fermenters can be considered insignificant and, therefore, disregarded.

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