

## BATCH ETHANOL FERMENTATION: THE CORRELATION BETWEEN THE FERMENTATION EFFICIENCY AND THE BIOMASS INITIAL CONCENTRATION DEPENDS ON WHAT IS CONSIDERED AS PRODUCED ETHANOL

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### SHORT COMMUNICATION

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#### ABSTRACT

Although numerous studies have examined many of the factors that affect the efficiency of batch ethanol fermentation, little attention has been paid to the influence of the biomass concentration on this efficiency. This paper shows that the influence of the biomass initial concentration on the fermentation efficiency depends on what is considered "produced ethanol". If only the ethanol present in the medium aqueous phase at fermentation completion is considered, the fermentation efficiency linearly decreases when the biomass initial concentration increases. If, however, the intracellular ethanol is also considered as produced ethanol, the fermentation efficiency is not affected by the biomass concentration.

**Key words:** ethanol fermentation efficiency, intracellular ethanol, biomass concentration

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Although numerous studies have examined many of the factors that affect the efficiency of batch ethanol fermentation, little attention has been paid to the influence of the biomass concentration on the above efficiency.

Falcone *et al.* (4) reported that the efficiency of batch ethanol fermentation of sugar-cane blackstrap molasses media decreased when the biomass initial concentration increased. No correlation between the fermentation efficiency and the biomass concentration, nor interpretation of the observed facts, however, were presented.

The main purpose of this paper is to show that the influence of the biomass initial concentration on the fermentation efficiency depends on the adopted definition of "produced ethanol", that is, depends on to consider also the intracellular ethanol as "produced" ethanol.

A process carried out with no cell recycle, due to its importance in laboratory-scale experiments and in some industrial fermentations, will initially be considered.

Compressed yeast (*Saccharomyces cerevisiae*) was used as inoculum in all the experiments. The fermentation media, prepared in volumetric flasks, were aqueous (distilled water)

solutions containing glucose (100.0, 150.0 and 200.0 g/L),  $\text{KH}_2\text{PO}_4$  (6.0 g/L), urea (2.5 g/L), yeast extract (2.5 g/L) and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (1.3 g/L). The experiments were carried out in 500-mL unstirred Erlenmeyer flasks connected to water-cooled reflux condensers (cooling water temperature, 5-10°C) in order to minimize evaporation/stripping losses. To each Erlenmeyer flask, containing 300 mL of fermentation medium, a calculated mass of compressed yeast was added in order to obtain the desired biomass initial concentration (~7 g/L, ~20 g/L and ~45 g/L, dry matter). The flasks were then incubated at  $32.0 \pm 0.5^\circ\text{C}$  until fermentation completion. The ethanol and glycerol concentrations were measured in the centrifuged (1,800 x g; 10 min) medium by the dichromate method (6) and by the enzymatic method proposed by Gattas *et al.* (5), respectively. The biomass concentrations were measured as follows: 5.0 mL of medium was filtered (Millipore membrane; pores diameter, 1.2  $\mu\text{m}$ ); the collected cells were washed (50 mL of distilled water) and then dried (105°C; 4 h) and weighed.

Considering only the ethanol present in the medium aqueous phase, the fermentation efficiency ( $\eta$ ) is defined by Equation 1, where  $M_S$  is the glucose initial mass,  $M_E$  is the ethanol final

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mass in the aqueous phase, and 0.511 is the stoichiometric ethanol yield factor.

$$\eta = \frac{M_E}{0.511 \cdot M_S} \cdot 100 \quad (\text{Eq. 1})$$

The values of  $M_S$  and  $M_E$  may be calculated by Equations 2 and 3, where  $S$ ,  $E$ ,  $V_{ai}$  and  $V_{af}$  are the glucose initial concentration, the ethanol final concentration, the initial volume of the aqueous phase (300 mL in all the experiments) and the final value of the aqueous phase volume, respectively.

$$M_S = S \cdot V_{ai} \quad (\text{Eq. 2})$$

$$M_E = E \cdot V_{af} \quad (\text{Eq. 3})$$

Otherwise, calling  $V$  the volume of the inoculated medium (practically constant during each test), Equations 4 and 5, where  $X_i$  and  $X_f$  are, respectively, the initial and the final concentrations of biomass (dry matter), and  $\sigma$  and  $\rho$  are, respectively, the fraction of the dry matter in the biomass and the microorganism density, may be written (1).

$$V_{ai} = V \cdot \left( 1 - \frac{X_i}{\sigma \cdot \rho} \right) \quad (\text{Eq. 4})$$

$$V_{af} = V \cdot \left( 1 - \frac{X_f}{\sigma \cdot \rho} \right) \quad (\text{Eq. 5})$$

As  $V_{ai}$  is known (0.300L in all the tests), Equations 4 and 5 permit to calculate  $V$  and  $V_{af}$  in each experiment. The values of  $\sigma$  and  $\rho$  were, respectively, 0.300 (dry matter content of the biomass, 30.0%) and  $1.10 \cdot 10^3 \text{ g/L}$  (2). It is then possible to calculate  $M_S$  and  $M_E$  and, consequently,  $\eta$ . The absolute differences between  $V$  calculated by Equation 4 and the corresponding values of  $V$  calculated by the ratio mass/density are smaller than 0.5 mL.

Calling  $\eta_i$  the fermentation efficiency calculated considering also the intracellular ethanol at fermentation completion, Equation 6, where  $M_{Et}$  is the mass of intracellular ethanol plus the mass of ethanol in the aqueous phase, must be used.

$$\eta_i = \frac{M_{Et}}{0.511 \cdot M_S} \cdot 100 \quad (\text{Eq. 6})$$

The value of  $M_{Et}$  is calculated by Equation 7, since the intracellular ethanol concentration is equal to the concentration of ethanol in the aqueous phase (3,7).

$$M_{Et} = E \cdot V \quad (\text{Eq. 7})$$

Tables 1 and 2 show, respectively, the results of the experiments and the values calculated by Equations 1 to 7.

**Table 1.** Influence of the initial concentrations of glucose ( $S$ ) and biomass ( $X_i$ ) on the final concentrations of biomass ( $X_f$ ), ethanol ( $E$ ) and glycerol ( $G$ ).

Test number	$S$ (g/L)	$X_i$ (g/L)	$X_f$ (g/L)	$E$ (g/L)	$G$ (g/L)	$X_f - X_i$ (g/L)
1		7.4	14.0	38.5	4.4	6.6
2	100.0	21.7	31.8	36.8	4.6	10.1
3		45.1	54.4	34.0	3.8	9.3
4		6.8	15.3	57.9	4.7	8.5
5	150.0	21.3	28.5	55.3	4.4	7.2
6		45.5	54.9	50.8	4.2	9.4
7		7.5	15.0	82.1	5.6	7.5
8	200.0	19.1	26.1	79.1	5.2	7.0
9		46.5	57.8	72.1	5.5	11.3

**Table 2.** Values calculated from the results of Table 1 by Equations 1 to 7.

Test number	$M_S$ (g)	$V$ (L)	$V_{af}$ (L)	$M_E$ (g)	$M_{Et}$ (g)	$\eta$ (%)	$\eta_i$ (%)
1		0.307	0.294	11.3	11.8	73.7	77.0
2	30.0	0.321	0.290	10.7	11.8	70.0	77.0
3		0.347	0.290	9.9	11.8	64.6	77.0
4		0.306	0.292	16.9	17.7	73.5	77.0
5	45.0	0.321	0.293	16.2	17.8	70.5	77.4
6		0.348	0.290	14.7	17.7	63.9	77.0
7		0.307	0.293	24.1	25.2	78.6	82.2
8	60.0	0.318	0.293	23.2	25.1	75.7	81.9
9		0.349	0.288	20.8	25.2	67.8	82.2

$M_S$  = glucose initial mass.  $V$  = fermenting medium volume.  $V_{af}$  = aqueous phase final volume.  $M_E$  = final mass of ethanol disregarding the intracellular ethanol.  $M_{Et}$  = final mass of ethanol considering also the intracellular ethanol.  $\eta$  = fermentation efficiency calculated from  $M_E$  and  $M_S$ .  $\eta_i$  = fermentation efficiency calculated from  $M_{Et}$  and  $M_S$ .

Table 1 clearly shows that the influence of  $X_i$  on  $\eta$  is not a consequence of the produced quantities of biomass ( $X_f - X_i$ ) and/or glycerol. Otherwise, Table 2 shows that, for each medium composition, the total mass of ethanol (intracellular plus extra cellular ethanol) at fermentation completion was not affected by the biomass concentration. The influence of  $X_i$  on  $\eta$  seems then to be due to the fact that  $X_f$  increases when  $X_i$  increases; consequently, the mass of intracellular ethanol increases and

the mass of ethanol in the medium aqueous phase diminishes leading to a lower value of  $\eta$ .

It seems advisable to correlate  $\eta$  and  $X_i$ . Combining Equations 1 to 7, Equation 8 was obtained.

$$\frac{\eta}{\eta_i} = 1 - \frac{X_f}{\sigma \cdot \rho} \quad (\text{Eq. 8})$$

Otherwise, Equation 9 (8), where  $\alpha$  and  $\beta$  are empirical constants that depend on the experimental conditions, correlates  $X_f$  and  $X_i$ .

$$X_f = \alpha + \beta \cdot X_i \quad (\text{Eq. 9})$$

Equations 8 and 9 lead to Equation 10, that is,  $\eta$  linearly decreases when  $X_i$  increases.

$$\eta = \eta_i \cdot \left( 1 - \frac{\alpha}{\sigma \cdot \rho} \right) - \frac{\eta_i \cdot \beta}{\sigma \cdot \rho} \cdot X_i \quad (\text{Eq. 10})$$

From the values of  $X_i$  and  $X_f$  of Table 1, Equation 11 was obtained.

$$X_i = 6.89 + 1.067 \cdot X_f \quad (\text{Eq. 11})$$

$(r = 0.998)$

In this case, the values of  $\alpha$  and  $\beta$  (Eq. 9) are 6.89 g/L and 1.067, respectively. Equation 10, remembering that  $\sigma\rho = 330$  g/L, leads then to Equations 12 (when  $S = 100.0$  g/L;  $\eta_i = 77.0\%$ ), 13 (when  $S = 150.0$  g/L;  $\eta_i = 77.1\%$ ) and 14 (when  $S = 200.0$  g/L;  $\eta_i = 82.1\%$ ).

$$\eta = 75.39 - 0.2439 \cdot X_i \quad (\text{Eq. 12})$$

$$\eta = 75.49 - 0.2493 \cdot X_i \quad (\text{Eq. 13})$$

$$\eta = 80.38 - 0.2654 \cdot X_i \quad (\text{Eq. 14})$$

The absolute differences between  $\eta$  calculated by Equations 12 to 14 and the corresponding values of  $\eta$  of Table 2 varied from 0.1% to 0.4% (average, 0.22%; standard deviation, 0.10%)

If the fermentation process involves the recycle of cells, as the process developed by Les Usines de Melle for the industrial production of ethanol, the inoculum of the fermentation medium is the biomass that was separated (usually by centrifugation) from a previous completed fermentation. In other words, the yeast cells of the inoculum already contain ethanol. In this case, in spite of the fact that some new cells are frequently produced, the influence of the initial biomass concentration on the fermentation efficiency is very probably insignificant.

All things considered, it is indispensable to inform which method (Eq. 1 or Eq. 6) was used to calculate the fermentation

efficiency, mainly when the fermentation process does not involve the recycle of cells.

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## RESUMO

### Fermentação alcoólica descontínua: a correlação entre o rendimento da fermentação e a concentração inicial de biomassa depende do que se considere etanol produzido

Embora muitos estudos tenham avaliado muitos dos fatores que afetam a eficiência da fermentação etanólica, a influência da concentração da biomassa na eficiência do processo tem recebido pouca atenção. Esse trabalho mostra que a influência da concentração inicial de biomassa depende do que se considera "etanol produzido". Se, terminada a fermentação, considera-se como etanol produzido apenas aquele existente na fase aquosa do meio, o rendimento da fermentação decresce linearmente quando a concentração inicial de biomassa aumenta. Entretanto, se o etanol intracelular também é considerado, a concentração da biomassa não afeta o rendimento da fermentação.

**Palavras-chave:** rendimento da fermentação alcoólica, etanol intracelular, concentração de biomassa

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