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Batch ethanol fermentation of molasses: a correlation between the time necessary to complete the fermentation and the initial concentrations of sugar and yeast cells

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Batch fermentations of sugar-cane blackstrap molasses to ethanol, using pressed yeast as inoculum, demonstrated an exponential relationship between the time necessary to complete the fermentation and the initial concentrations of sugar and yeast cells. The parameters of the derived exponential equations depended on the experimental conditions.

Key words: Ethanol, fermentation, modelling, molasses, yeast.

The time (t) necessary to complete the batch fermentation of sugar-cane blackstrap molasses to ethanol has been correlated to the initial concentrations of sugar (S_0) and yeast cells (X_0) by equation (1) with correlation coefficient (r) (Queiroz 1982):

$$t = 0.773\ (S_0/X_0) \qquad (r = 0.92) \qquad (1)$$

This equation was obtained from results of 45 batch tests carried out under the following experimental conditions (Queiroz 1982): temperature (T) = 30°C; S_0 = 91 to 226 g/l; initial concentration of urea in the mash (N_0) = 0.5 and 1.0 g/l.

Equation (2), representing the correlation between the experimental values of t (t_e) (Queiroz 1982) and the calculated values of t from equation (1) (t_c), clearly shows that equation (1) cannot be considered a good correlation between t and S_0/X_0. The relative differences between t_c and t_e varied from 0% to 52% (with a mean of 15 ± 11%) (Queiroz 1982).

$$t_c = 1.42 + 0.75\ t_e \qquad (r = 0.90) \qquad (2)$$

The differences between t_e and t_c are a consequence of the corresponding fermentation tests being carried out with mashes having very different values of S_0 and N_0.

In spite of its practical applications, equation (1) can only be considered a rough approximation of the influence of S_0/X_0 on t. The main purpose of the present paper is to show that there is an exponential relationship between t and S_0/X_0, and that the parameters depend on the experimental conditions.

Materials and Methods

Fermentations

Sugar-cane blackstrap molasses [60% w/w total reducing sugars calculated as glucose (TRS)] was dissolved in tap water to obtain a TRS concentration of about 500 g/l. Pressed yeast (28.9% to 30.0% dry matter) was suspended in tap water to give approximately 60 g dry matter/l. Molasses, yeast suspension and tap water were mixed in varying proportions and added in 100-ml lots to 250-ml Erlenmeyer flasks. Three drops of anti-foam (5 ml of ICI Silcolapse 5000—a silicone oil based material—in 100 ml distilled water) were added to each flask. Flasks were incubated in a rotary shaker (200 rev/min) at 30 or 36°C. Experiments were also carried out supplementing the mash with 1.0 g yeast extract/l, 1.0 g $NH_4H_2PO_4$/l and 0.25 g $MgSO_4.7H_2O$/l.

Analyses

The end of fermentation, and consequently the value of t, was determined by measuring the variation in the weight of the flask containing the fermenting medium (Borzani & Falcone 1953). This weighing method permits the determination of t because, at the

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end of the fermentation, even if evaporation is not avoided, the slope of the weight vs time curve clearly changes and a straight line is then obtained due only to evaporation losses.

TRS concentrations were measured by the dinitrosalicylic acid method (Miller 1959). The dry matter content of the pressed yeast was determined by drying the pressed yeast at 105°C to constant weight.

Results

Figures 1 to 3 present the experimental results. The curves can be represented by the empirical equations (3) to (10):

$$t = 1.418\ (S_0/X_0)^{0.7017} \quad (3)$$

$$t = 1.778\ (S_0/X_0)^{0.7139} \quad (4)$$

$$t = 1.096\ (S_0/X_0)^{0.7317} \quad (5)$$

$$t = 1633\ (S_0/X_0)^{0.6916} \quad (6)$$

$$t = 2.241\ (S_0/X_0)^{0.4189} \quad (7)$$

$$t = 2.956\ (S_0/X_0)^{0.4102} \quad (8)$$

$$t = 2.089\ (S_0/X_0)^{0.3935} \quad (9)$$

$$t = 2.764\ (S_0/X_0)^{0.4120} \quad (10)$$

The correlation between the values of t calculated (t_c) by equations (3) to (10) and the experimental values of t (t_e) is given by equation (11). Figure 4 shows the probability plot of the differences $t_c - t_e$.

$$t_c = -0.028 + 1.003t_e \qquad (r = 0.993) \quad (11)$$

Equation (12) may be proposed as a general representation of the correlation between t and S_0/X_0:

$$t = K(S_0/X_0)^{\alpha} \qquad (0 < \alpha < 1) \quad (12)$$

The influence of the experimental conditions on the values of empirical constants K and α is presented in Table 1.

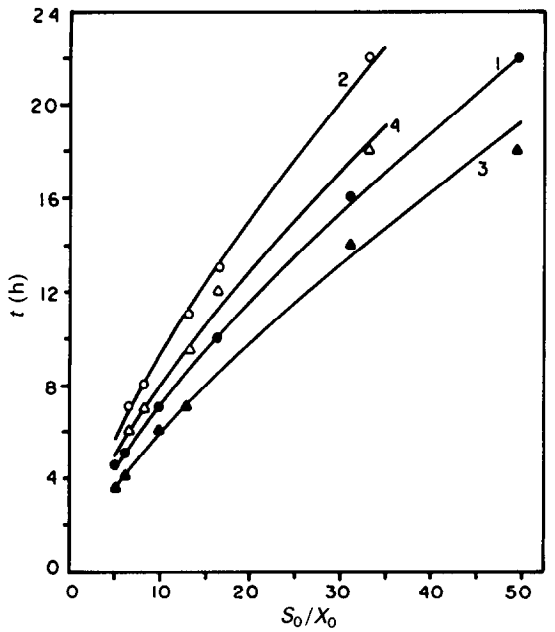

Figure 1. Influence of the initial concentrations of TRS (S_0) and yeast cells (X_0) on the time necessary to complete the fermentation (t). Experiments carried out with no nutrient addition. Curve 1 (●): $T = 30°C$; $S_0 = 148.8$ g/l; equation (3). Curve 2 (○): $T = 30°C$; $S_0 = 198.4$ g/l; equation (4). Curve 3 (▲): $T = 36°C$; $S_0 = 148.8$ g/l; equation (5). Curve 4 (△): $T = 36°C$; $S_0 = 198.4$ g/l; equation (6).

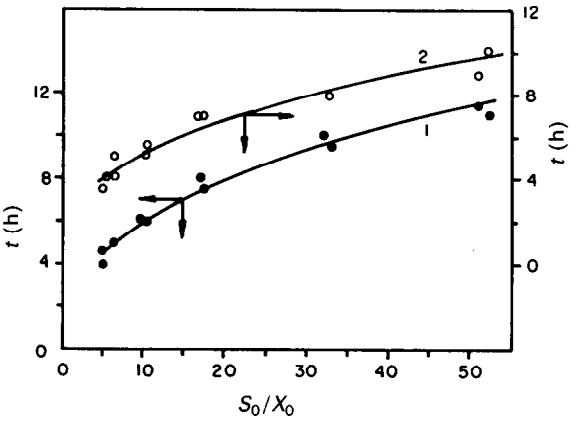

Figure 2. Influence of the initial concentrations of TRS (S_0) and yeast cells (X_0) on the time necessary to complete the fermentation (t). Experiments carried out with nutrient addition. Curve 1 (●): $T = 30°C$; $S_0 = 150.1$ g/l; equation (7). Curve 2 (○): $T = 36°C$; $S_0 = 150.1$ g/l; equation (9).

Discussion

Equations (3) to (10) led to better calculated values of t than equation (1). Other empirical equations, in place of equation

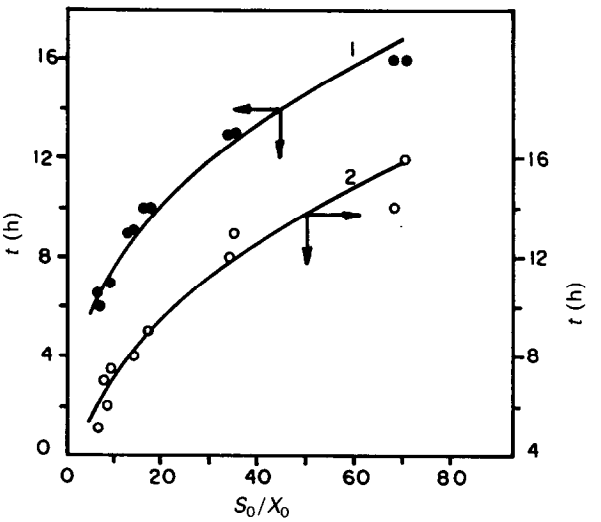

Figure 3. Influence of the initial concentrations of TRS (S_0) and yeast cells (X_0) on the time necessary to complete the fermentation (t). Experiments carried out with nutrient addition. Curve 1 (●): $T = 30°C$; $S_0 = 200.5$ g/l; equation (8). Curve 2 (○): $T = 36°C$; $S_0 = 200.5$ g/l; equation (10).

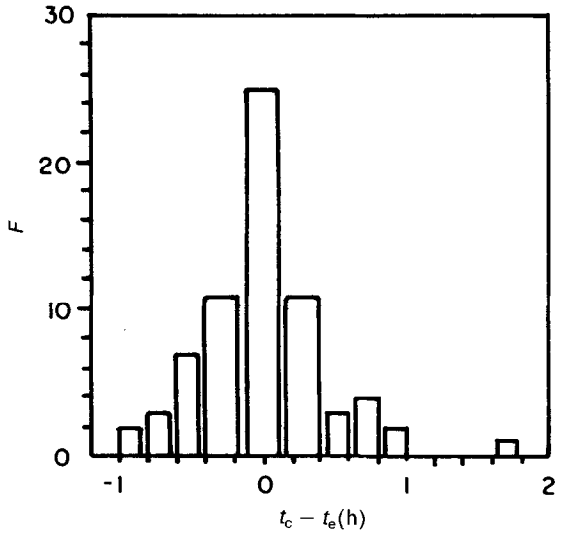

Figure 4. Absolute frequency (F) of the difference $t_c - t_c$.

(12), could be proposed to correlate t and S_0/X_0. If we consider, for instance, the fermentation tests represented by curve 1 in Figure 1 and those represented by curve 1 in Figure 2, the empirical equations (13) and (14) could, respectively, be proposed to correlate t and S_0/X_0:

$$t = 2.95 + 0.396(S_0/X_0) \quad (13)$$

$$t = 12.72\left(\frac{S_0/X_0}{10.39 + (S_0/X_0)}\right) \quad (14)$$

If equation (13) is adopted to represent the correlation between t and S_0/X_0, we conclude that when $S_0 = 0$, that is when no sugar exists in the medium, the calculated value of t is 2.95 h, while the expected value is zero. However, if equation (14) is applied, we conclude that when $X_0 = 0$, that is when no yeast cells exist in the medium, the calculated value of t is 12.72 h, while the expected value is ∞. In this respect, equation (12) is more accurate than either equation (13) or (14).

Table 1 shows that the value of α seems to be affected only by the addition of nutrients to the fermentation medium. When no nutrients were added to the mash, the average value of α was 0.71 (SD = 0.02), while 0.41 was the average value of α (SD = 0.01) when nutrients were added to the medium. In each of the above cases, α was practically unaffected by the variations of S_0 and T.

Table 1. Influence of the experimental conditions on the values of K and α [see equation (12)].

Fermentation medium	S_0 (g/l)	T = 30°C		T = 36°C	
		K (h)	α	K (h)	α
Without nutrient addition	148.8	1.418	0.7017	1.096	0.7317
	198.4	1.778	0.7139	1.633	0.6916
With nutrient addition	150.1	2.241	0.4189	2.089	0.3935
	200.5	2.956	0.4102	2.764	0.4120

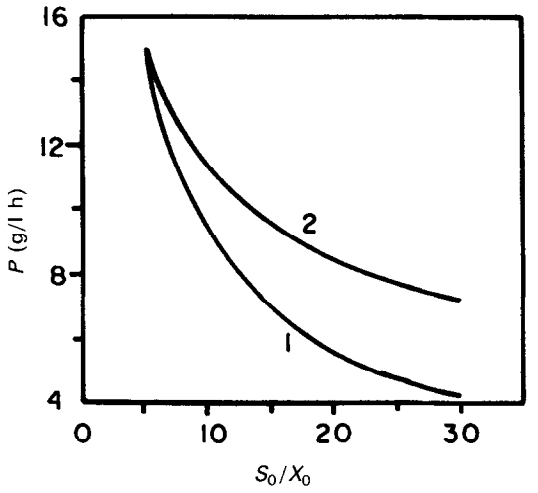

Figure 5. Influence of the initial concentrations of TRS (S_0) and yeast cells (X_0) on the ethanol productivity (P). Curve 1: no nutrients added to the mash. Curve 2: nutrients added to the mash. The values of P were calculated by equation (16) assuming $S_0 \approx 150$ g/l and $T = 30$°C.

Table 1 also shows how the experimental conditions affected the value of K. When nutrients were added to the fermentation medium, increasing of K as high as 90.6% was observed ($T = 30$°C; $S_0 \approx 150$ g/l). The influence of S_0 on K was also significant: when S_0 increased from about 150 g/l to approximately 200 g/l, K increased up to 49.0% ($T = 36$°C; no nutrients added to the mash). Finally, the influence of the temperature on K was not strong: when T decreased from 36°C to 30°C, K increased up to 29.4% ($S_0 \approx 150$ g/l; no nutrients added to the medium).

Equation (12) permits the ethanol productivities under different experimental conditions to be evaluated and compared. The ethanol concentration in the completely fermented medium (E) is:

$$E = 0.511\eta S_0 \quad (15)$$

where η is the ethanol yield (fraction of theoretical value). Combining equations (12) and (15) we may calculate the ethanol productivity (P):

$$P = E/t = 0.511\eta S_0/K(S_0/X_0)^{\alpha} \quad (16)$$

Considering that the average value of η, in our tests, was 0.87 (SD = 0.02), equation (16) and Table 1 permit P to be calculated under the experimental conditions described in this paper. Figure 5 presents, for example, the influence of S_0/X_0 on P when $S_0 \approx 150$ g/l and $T = 30$°C. Similar curves correlate P and S_0/X_0 for the other values of S_0 and T. Figure 5 clearly shows that the addition of nutrients did not affect P when $S_0/X_0 = 5$ (or $X \approx 30$ g/l), but strongly affected the ethanol productivity when $S_0/X_0 = 30$ (or $X_0 \approx 5$ g/l). In

this last case, the nutrient addition increased the value of P by 66.9%. These results were expected because, when the initial cell concentration is relatively high, the quantities of nutrients present as cell components are sufficient to assure maximum microbial activity. The situation is completely different when X_0 is relatively low. The above influence of S_0/X_0 on P would certainly not be observed if starved cells were used as inoculum.

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