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Influence of the Reactor Shape on the Kinetics of Ethanol Production in Laboratory-Scale Batch Fermentation Tests Carried out in Unstirred Vessels

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ABSTRACT

The influence of the shape of laboratory-scale unstirred reactors on the kinetics of ethanol production in batch ethanol fermentation was studied. Two reactors were used: a 1-L glass measuring cylinder and a 2-L Erlenmeyer flask. The volume of inoculated medium in each reactor was 1,000 mL. The above influence was affected by the ratio between the initial yeast cells concentration (X_0: ~7 g/L, ~14 g/L, and ~21 g/L, dry matter) and the initial glucose concentration (S_0: ~100 g/L, ~150 g/L and ~200 g/L). When X_0/S_0 increased from 0.038 to 0.219 the influence of the reactor shape decreased, and was not observed when $X_0/S_0 = 0.22$ to 0.24. The reactor shape practically did not affect the ethanol yield, the final yeast cells concentration and both the viability and the morphology of the cells in tests carried out at the same value of X_0/S_0.

Key words: Batch ethanol fermentation; ethanol production kinetics; reactor shape; unstirred reactor

INTRODUCTION

Due to the difficulty to obtain useful information on the kinetics of fermentation processes from reactors that have spatially nonuniform conditions, it is desirable to study kinetics in reactors that are well mixed (Bailey and Ollis, 1986).

Laboratory-scale fermentation tests, however, are frequently carried out in small unstirred reactors. When gases are produced (e.g. ethanol fermentation, anaerobic treatment of wastewater, acetone-butanol fermentation), the ascending gas bubbles, depending on the experimental conditions, may significantly affect the degree of homogeneity of the fermenting medium and, consequently, the kinetic behavior of the microbial cells. Among the above conditions, the reactor shape must be considered.

Sung and Dague (1995), studying the anaerobic treatment of wastewaters in unstirred reactors, concluded that the performance of the reactors with a ratio height/diameter between 1.8 and 5.6 was better than that of reactors with the above ratio equal to 0.61 to 0.93. Only one paper was found studying the influence of the shape of unstirred reactors on batch ethanol fermentation. In the above paper, Gómez et al. (1981) reported that the reactor shape significantly affected the time to attain complete fermentation of media prepared from blackstrap molasses. No information was found reporting the influence of the reactor shape on batch acetone-butanol fermentation.

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Such facts clearly show that the reactor shape must be considered mainly to compare the kinetic results obtained in different experiments.

The aim of this work was to study the influence of the shape of laboratory-scale unstirred reactors on the kinetics of ethanol fermentation.

MATERIALS AND METHODS

Baker's compressed yeast (*Sacharomyces cerevisiae*) was used as inoculum in all the experiments. Tests were carried out with initial yeast concentrations (dry matter) approximately equal to 7 g/L, 14 g/L and 21 g/L.

The fermentation media were prepared dissolving D-glucose, KH_2PO_4, urea, yeast extract and $MgSO_4.7H_2O$ in distilled water. The initial glucose concentrations of the inoculated media were approximately equal to 100 g/L, 150 g/L and 200 g/L. The initial concentrations of the other nutrients (% of the initial glucose concentration) were: KH_2PO_4, 4.00; urea, 1.75; yeast extract, 1.75; $MgSO_4.7H_2O$, 0.75. The initial pH of the inoculated media was 4.5. No antifoam was used, since very small quantities of foam were produced.

The reactors used in this work were 1-L glass measuring cylinders and 2-L Erlenmeyer flasks. With the only purpose to assure the same evaporation losses in both reactors, the cover schematically represented in Fig. 1 was placed on the cylinder. The above reactor shapes were chosen because they are the most frequently used to carry out ethanol fermentation laboratory-scale experiments. Each reactor received 1,000 mL of inoculated media, leading to *H/D* (*H* = height of the medium layer; *D* = average reactor diameter) equal to 6.3 and 0.46 in the cylinder and in the Erlenmeyer flask, respectively. The tests were carried out at 32±1ºC. The rectors were not stirred and no air was supplied.

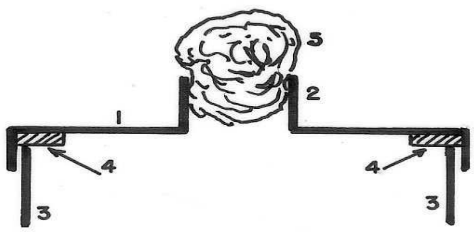

Figure 1 - Schematic representation of the cover placed on the cylinder. 1: metallic cover. 2: cover neck dimensionally equal to the Erlenmeyer flask neck. 3: reactor wall. 4: rubber gasket. 5: cotton wool plug (4.5g).

The DNS method (Miller, 1959), dichromate method (Joslyn, 1970) and the method described by Borzani et al. (1977) were used to measure the concentrations of glucose, ethanol and yeast cells, respectively. The yeast cells viability was measured by the methylene blue staining method (Vairo, 1961).

The relative ethanol yield (η) and the ethanol productivity (*P*) were calculated by Equations [1] and [2], respectively, where E_p is the concentration of produced ethanol, S_0 is the initial glucose concentration, t_f is the time to attain complete fermentation and 0.511 is the stoichiometric ratio between ethanol and glucose.

$$\eta = \frac{100E_p}{0.511S_0} \qquad [1]$$

$$P = \frac{E_p}{t_f} \qquad [2]$$

The ethanol production rates (*dE/dt*) were calculated by the numerical differentiation method (Sinclair and Cantero, 1990).

RESULTS

Fig. 2, where *E* is the ethanol concentration at time *t*, shows the results obtained in a typical experiment.

Table 1, where X_0 and X_f are the initial and the final yeast cells concentrations (dry matter) respectively, shows the results obtained in our tests. The final concentrations of glucose were smaller than 1% of S_0.

Figs. (3), (4) and (5) present the influence of both X_0/S_0 and the reactor shape on the ethanol production rates.

The yeast cells viability at the end of the experiments carried out in the cylinder and in the Erlenmeyer flask varied from 77% to 85% and from 83% to 89%; respectively. The cells viability of the inocula was 97-99%. The yeast cells morphology was not affected by the reactor shape.

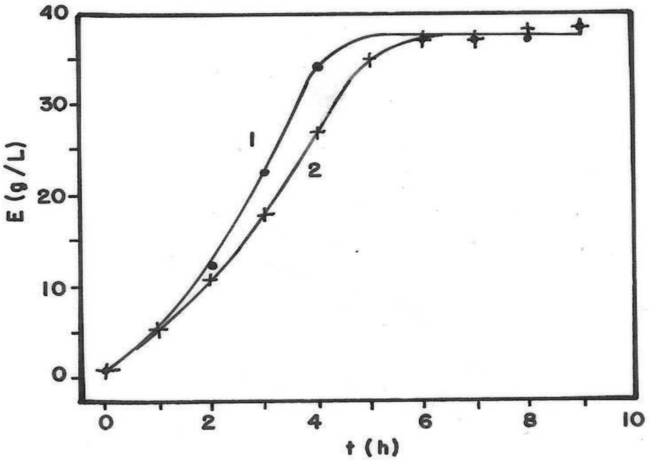

Figure 2 - Ethanol concentrations (*E*) during tests carried out using the same medium (S_0 = 96.8 g/L; X_0/S_0 = 0.062) in a 1-L glass measuring cylinder (Curve 1; Test N°1) and in a 2-L Erlenmeyer flask (Curve 2; Test N°2). See Table 1.

Table 1 - Results obtained in all the tests.

Test N°	Reactor (*)	S_0 (g/L)	X_0 (g/L)	X_f (g/L)	X_0/S_0	t_f (h)	E_p (g/L)	*P* (g/L.h)	η (%)
1	Cyl.	96.8	6.0	14.2	0.062	5.0	36.5	7.3	73.8
2	Erl.	96.8	6.0	14.7	0.062	6.0	36.5	6.1	73.8
3	Cyl.	97.0	13.5	20.1	0.139	3.5	34.2	9.8	69.0
4	Erl.	97.0	13.5	20.6	0.139	4.0	33.9	8.5	68.4
5	Cyl.	97.0	21.2	23.2	0.219	2.5	31.6	12.6	63.7
6	Erl.	97.0	21.2	26.2	0.219	2.5	31.6	12.6	63.7
7	Cyl.	156.8	7.6	13.5	0.048	9.0	60.5	6.7	75.5
8	Erl.	156.8	7.6	11.9	0.048	11.0	58.1	5.3	72.5
9	Cyl.	148.7	14.5	21.2	0.097	4.0	58.0	14.5	76.3
10	Erl.	148.7	14.5	19.0	0.097	5.0	57.7	11.5	75.9
11	Cyl.	149.2	21.3	30.2	0.143	4.0	57.2	14.3	75.0
12	Erl.	149.2	21.3	29.4	0.143	5.0	58.7	11.7	77.0
13	Cyl.	198.6	7.6	9.6	0.038	10.0	80.6	8.1	79.4
14	Erl.	198.6	7.6	11.9	0.038	13.0	80.2	6.2	79.0
15	Cyl.	187.2	13.8	21.2	0.074	7.0	79.0	11.3	82.6
16	Erl.	187.2	13.8	16.6	0.074	9.0	78.4	8.7	82.0
17	Cyl.	198.2	21.4	23.7	0.108	6.0	73.2	12.2	72.3
18	Erl.	198.2	21.4	24.1	0.108	7.0	74.4	10.6	73.5

(*) Cyl.: Cylinder Erl.: Erlenmeyer flask

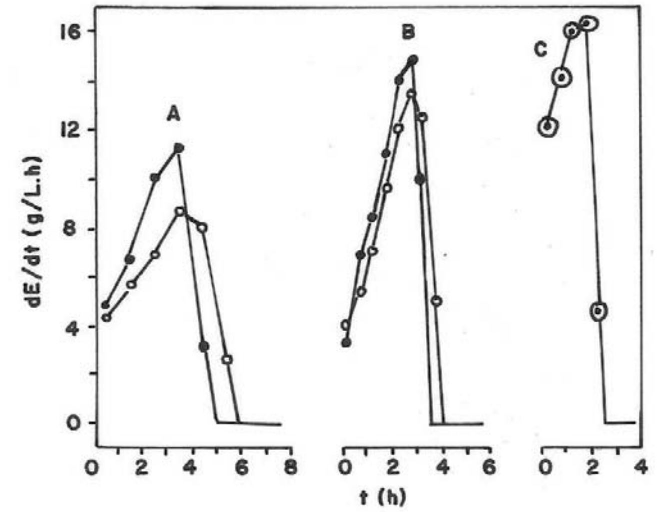

Figure 3 - Influence of both the reactor shape (Cylinder, •; Erlenmeyer flask, o) and X_0/S_0 on the ethanol production rate (*dE/dt*) when $S_0 \cong 100$ g/L. A: tests nº 1 and 2 ($X_0/S_0 = 0.062$). B: tests nº 3 and 4 ($X_0/S_0 = 0.139$). C: tests nº 5 and 6 ($X_0/S_0 = 0.219$). See Table 1.

DISCUSSION

During batch ethanol fermentation experiments carried out in unstirred reactors, the frequency of the contacts between the yeast cells and the molecules of the substances dissolved in the medium, depends on both the CO_2 production rates (mainly affected by the concentrations of glucose and yeast cells) and the velocities of the ascending CO_2 bubbles (affected by both the CO_2 production rates and the reactor shape).

When media with the same composition are fermented in the cylinder and in the Erlenmeyer flask, the CO_2 will be produced at the same rate at the beginning of the test, but the velocities of the ascending gas bubbles in the cylinder will be greater than in the Erlenmeyer flask. If the CO_2 production rate is sufficiently high, the yeast cells will be maintained in suspension in both reactors leading to practically the same kinetic results (see Fig. 3,C).

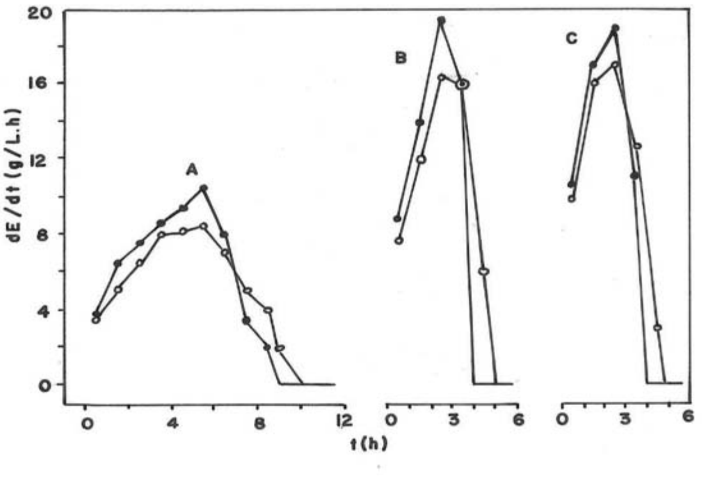

Figure 4 - Influence of both the reactor shape (Cylinder, •; Erlenmeyer flask, o) and X_0/S_0 on the ethanol production rate (*dE/dt*) when $S_0 \cong 150$ g/L. A: tests nº 7 and 8 ($X_0/S_0 = 0.048$). B: tests nº 9 and 10 ($X_0/S_0 = 0.097$) . C: tests nº 11 and 12 ($X_0/S_0 = 0.143$). See Table 1.

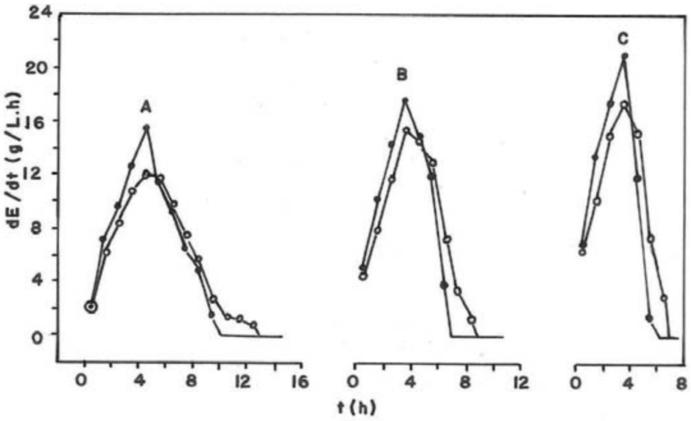

Figure 5 - Influence of both the reactor shape (Cylinder, •; Erlenmeyer flask, o) and X_0/S_0 on the ethanol production rate (dE/dt) when $S_0 \cong 200$ g/L. A: tests nº 13 and 14 ($X_0/S_0 = 0.038$). B: tests nº 15 and 16 ($X_0/S_0 = 0.074$). C: tests nº 17 and 18 ($X_0/S_0 = 0.108$). See Table 1.

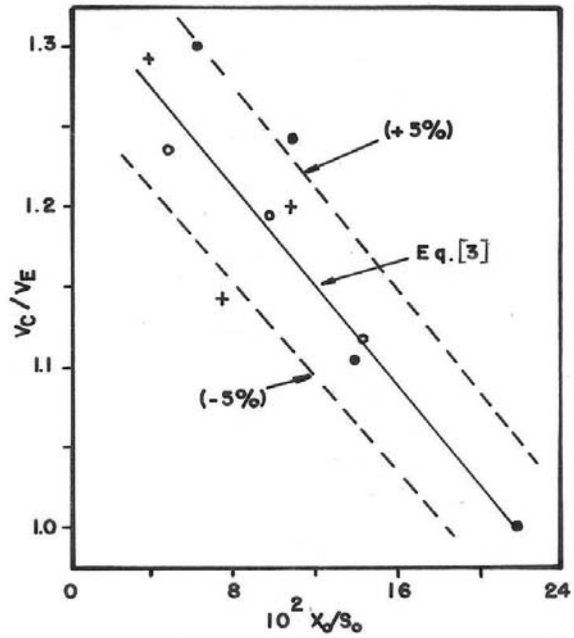

Figure 6 - Influence of X_0/S_0 on V_C/V_E. Initial glucose concentration: ~ 100 g/L (•), ~150 g/L (o) and ~ 200 g/L (+).

If, however, the CO_2 production rate is relatively small, the yeast cells will tend to settle faster in the Erlenmeyer flask than in the cylinder. In this last case, the fermentation will be more rapid in the cylinder leading to shorter fermentation times and greater ethanol productivities (See Appendix).

Otherwise, it must be pointed out that a batch fermentation, even in well mixed reactors, is not a steady-state process, that is, the glucose and cells concentrations, as well as the CO_2 production rate, the number, diameters and velocities of the ascending CO_2 bubbles (whose measurement is presently unfeasible), vary from time to time. Consequently, it is very difficult (perhaps impossible) to propose theoretical correlations between the obtained kinetic results and the actual experimental conditions, since such conditions change as fermentation proceeds. Borzani et al. (1993), however, reported that the time necessary to complete the batch ethanol fermentation may be calculated by $K(S_O/X_O)^{\alpha}$, where K $(K>1)$ and α $(0<\alpha<1)$ depend on the experimental conditions. In other words, the above time decreases, and consequently the CO_2 production rate increases, when X_0/S_0 increases. Based on the above fact, the ratio X_0/S_0 was assumed as a reference parameter to discuss the experimental results, leading to several empirical and practical consequences.

Fig. 6 where V_C and V_E are, respectively, the maxima values of dE/dt in tests carried out in the cylinder and in the Erlenmeyer flask for the same value of X_0/S_0, show how X_0/S_0 affects V_C/V_E. Equation [3] is also represented in Fig. 6 (r = correlation coefficient).

$$\frac{V_C}{V_E} = 1.33 - 1.425\frac{X_0}{S_0} \qquad [3]$$

$$(r = -0.882)$$

Calling t_{fC} and t_{fE}, respectively, the complete fermentation times in tests carried out in the cylinder and in the Erlenmeyer flask for the same value of X_0/S_0, Figs. 7 and 8 show, respectively, the correlation between t_{fE} and t_{fC} (see Equation [4]) and the correlation between t_{fE} - t_{fC} and X_0/S_0 (see Equation [5]).

$$t_{fE} = -0.62 + 1.335 t_{fC} \qquad [4]$$

$$(r = 0.996)$$

$$t_{fE} - t_{f_c} = 1.02\frac{0.219 - (X_0/S_0)}{0.021 + (X_0/S_0)} \qquad [5]$$

$$(r = 0.985)$$

Fig. 9, where E_{pC} and E_{pE} are, respectively, the concentrations of produced ethanol in tests carried out in the cylinder and in the Erlenmeyer flask for the same value of S_0/X_0, clearly shows that the shape of the flask practically did not affect the quantity of produced ethanol. Obviously, the same conclusion was reached regarding the relative ethanol yield.

Calling P_C and P_E the ethanol productivities in tests carried out in the cylinder and in the Erlenmeyer flask, respectively, for the same value of S_0/X_0, Fig. 10 shows the correlation between P_C/P_E and S_0/X_0 (see equation [6]).

$$\frac{P_C}{P_E} = 1.356 - 1.457\frac{X_0}{S_0} \qquad [6]$$

$$(r = -0.864)$$

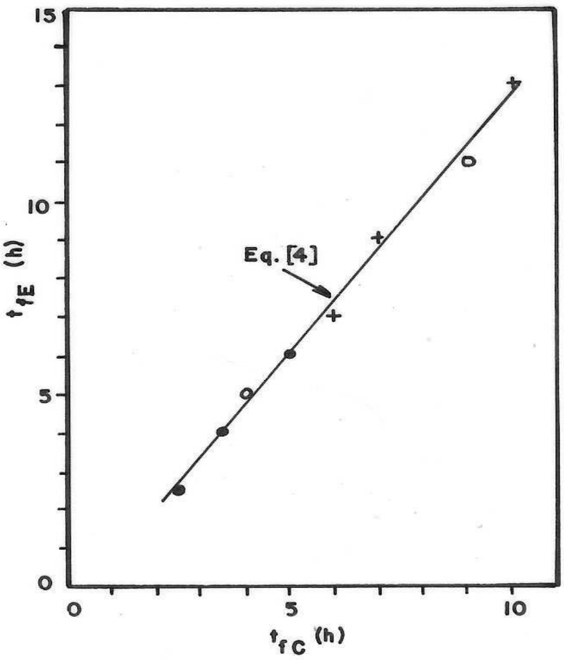

Figure 7 - Correlation between t_{fE} and t_{fC}. Initial glucose concentration: ~100 g/L (•), ~150 g/L (o) and ~200 g/L (+).

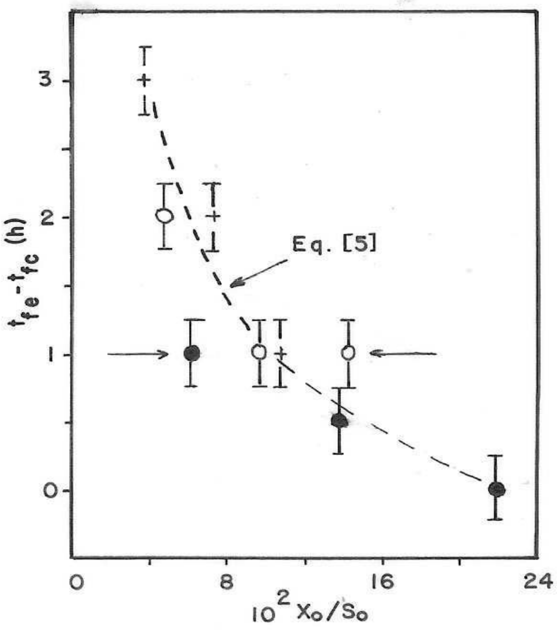

Figure 8 - Influence of X_0/S_0 on $t_{fE} - t_{fC}$. Initial glucose concentration: ~100 g/L (•), ~150 g/L (o) and ~200 g/L (+). The points indicated by arrows were not considered to obtain Equation [5].

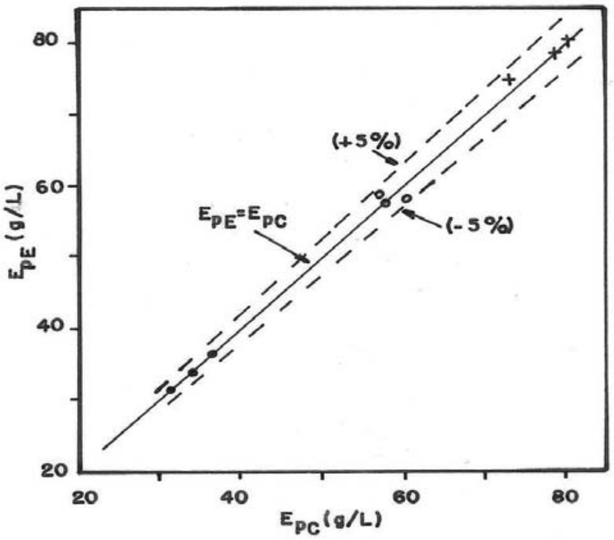

Figure 9 - Correlation between E_{pE} and E_{pC}. Initial glucose concentration: ~100 g/L (•), ~150 g/L (o) and ~200 g/L (+).

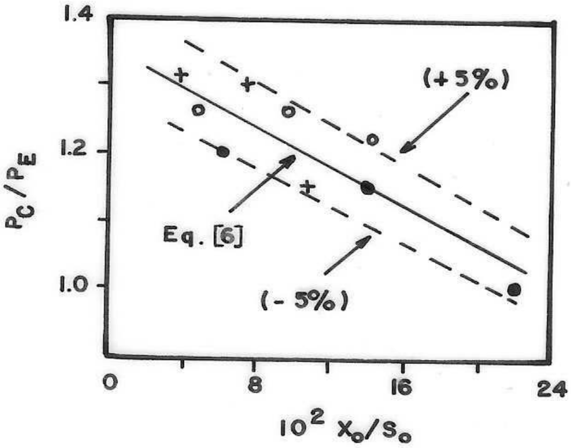

Figure 10 - Influence of X_0/S_0 on P_C/P_E. Initial glucose concentration: ~100 g/L (•), ~150 g/L (o) and ~200 g/L (+).

Fig. 11, where X_{fC} and X_{fE} are, respectively, the final yeast cells concentrations in tests carried out in the cylinder and in the Erlenmeyer flask for the same value of X_0/S_0, seems to indicate that there is no systematic and significant influence of the reactor shape on the yeast cells final concentrations.

Figs. 3 to 8 and 10 clearly show that the influence of the reactor shape on the kinetics of the ethanol production decreased when X_0/S_0 increased. In the particular case of the tests carried out with $S_0 \cong 100$ g/L, the above influence disappeared when $X_0/S_0=0.219$ (see Fig. 3).

Otherwise, the empirical Equations [3] and [6] permit to calculate X_0/S_0 when $V_c=V_E$ and $P_c = P_E$, respectively, i.e., the values of X_0/S_0 corresponding to an experimental condition where the reactor shape would not affect the process kinetics. The above values of X_0/S_0 calculated by Equations [3] and [6] were 0.234 and 0.244, respectively.

Table 2 - Results obtained in the additional tests.

S_0 (g/L)	X_0 (g/L)	X_0/S_0	***Reactor*** (*)	t_f (h)	E_p (g/L)	P (g/L.h)	η (%)
150	32.0	0.213	Cyl.	2.5	58.6	23.4	76.4
			Erl.	2.7	60.5	22.4	78.9
200	43.2	0.216	Cyl.	3.5	56.1	19.1	65.4
			Erl.	4.0	56.4	17.1	66.9

(*) Cyl.: Cylinder Erl: Erlenmeyer flask

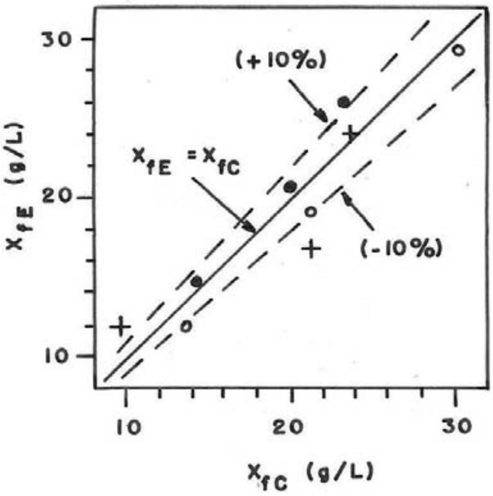

Figure 11 - Correlation between X_{fE} and X_{fC}. Initial glucose concentration:~100 g/L (•), ~150 g/L (o) and ~200 g/L(+).

The average of the X_0/S_0 values cited above (0.219, 0.234 and 0.244) is 0.232 (standard deviation, 0.013).

Assuming X_0/S_0 = 0.232, Equation [5] leads to $t_{fE} - t_{fC} = -3.1$ min, i.e., considering the uncertainties associated with the evaluations of t_{fE} and t_{fC}, $t_{fE} = t_{fC}$.

We may then conclude that the reactor shape affected the ethanol production kinetics when X_0/S_0 was smaller than 0.22 to 0.24.

Considering that with S_0 approximately equal to 150 g/L and 200 g/L the maximum values of X_0/S_0 were 0.143 and 0.108, respectively, two sets of additional tests were carried out with the only purpose to confirm the conclusions cited above. The results obtained in such tests (see Table 2) are in accordance with the results presented in Figs. 7 to 10.

The evolved CO_2 was not measured. Otherwise, considering that the mass of produced CO_2 is practically proportional to the mass of produced ethanol, the influence of X_0/S_0 on the CO_2 productivity would be represented by an Equation similar to Equation [6].

It seems advisable to point out that the results presented in this paper could be important mainly when the purpose is to compare kinetic parameters calculated from different batch ethanol experiments carried out in unstirred reactors. In this case, depending on the value of X_0/S_0, the reactor shape could significantly affect the values of the above parameters. Otherwise, the cells morphology, cells viability, quantity of the produced cells and the relative ethanol yield were pratically not affected by the reactor shape.

Obviously, the equations presented in this paper cannot be applied to laboratory-scale tests carried out under other experimental conditions and also to industrial reactors, since in this case the fed-batch technique is used.

APPENDIX

The CO_2 production rates and the velocities of the ascending CO_2 bubbles were previously assumed as the causes of the obtained kinetic results (see DISCUSSION and Figs. 3 to 5). The calculation (or the measument) of the velocities of the ascending CO_2 bubbles, however, is presently impossible.

Otherwise, when the calculation of the actual flow velocities is unfeasable (e.g. the flow of gases through a porous membrane and the flow of liquids through a fixed bed), the superficial velocities are usually considered as the flow parameters. The superficial velocities of the evolved CO_2 were then calculated.

The above calculation was carried out considering the following values: a) volume of fermenting medium, 1L (see MATERIALS AND METHODS); b) ratio between the mass of produced CO_2 and the mass of produced ethanol, 0.955 g/g; c) specific volume of the CO_2 at the experimental conditions (temperature, 32°C; atmospheric pressure, $9.33 \cdot 10^4$Pa), 617 mL/g; d) cylinder diameter,

5.9 cm; e) Erlenmeyer flask average diameter (considering only the portion containing the fermenting medium), 14.0 cm.

Equations [1-A] and [2-A] permitted to calculate the superficial velocity (measured in cm/min) of the evolved CO_2 at time *t* (*R*) and the average superficial velocity of the evolved CO_2 (R_{av}), respectively.

$$R = \frac{12.5}{D^2} \cdot \frac{dE}{dt} \qquad [1\text{-A}]$$

$$R_{av} = \frac{12.5}{D^2} \cdot \frac{E_p}{t_f} \qquad [2\text{-A}]$$

The values of *R* were calculated at $t = 0.5\ h$, since the produced CO_2 evolves after saturating the fermenting medium. Table 1-A shows the calculated values o *R* at $t = 0.5\ h$ and R_{av}.

Table 1-A - Calculated values of the superficial velocity at $t = 0.5\ h$ (*R*) and of the average superficial velocity (R_{av}) of the evolved CO2.

Test N°	***R* (at *t = 0.5h*) (cm/min)**	**R_{av} (cm/min)**
1	1.76	2.62
2	0.28	0.39
3	1.44	3.51
4	0.24	0.54
5	4.31	4.54
6	0.76	0.81
7	1.29	2.41
8	0.22	0.34
9	3.16	5.21
10	0.45	0.74
11	3.84	5.14
12	0.64	0.75
13	0.79	2.69
14	0.14	0.39
15	1.83	4.05
16	0.30	0.56
17	2.48	4.38
18	0.38	0.68

Figs. 3 to 5 show that only Tests N°5 (cylinder) and N°6 (Erlenmeyer flask), carried out using the same inoculated medium (see Table1), led to identical results. It is then possible to conclude that $R = 0.76$ cm/min (at $t = 0.5\ h$), or $R_{av} = 0.81$ cm/min, were sufficiently high to maintain all the yeast cells suspended in the medium.

Otherwise, Table 1-A also shows that: a) all the values *R* (at $t = 0.5\ h$) and all the values of R_{av} of the tests carried out in cylinders were higher then, respectively, 0.76 cm/min and 0.81 cm/min; b) excluding Test N° 6, the values R (at $t = 0.5\ h$) and R_{av} of the tests carried out in Erlenmeyer flasks are smaller than 0.76 cm/min and 0.81 cm/min, respectively.

The above results permit to say that the assumption presented at the DISCUSSION is acceptable.

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of Douglas Dalla Justina and Renato Piplovic.

NOMENCLATURE

dE/dt	ethanol production rate (g/L.h)
D	reactor internal diameter (cm)
E	ethanol concentration (g/L)
E_p	produced ethanol concentration (g/L) E_{pC} value of E_p in test carried out in the cylinder (g/L)
E_{pE}	value of E_p in test carried out in the Erlenmeyer flask (g/L)
H	height of the medium layer (cm)
P	ethanol productivity (g/L.h)
P_C	value of P in test carried out in the cylinder (g/L.h)
P_E	value of P in test carried out in the Erlenmeyer flask (g/L.h)
r	correlation coefficient
R	superficial velocity of the evolved CO_2 (cm/min)
R_{av}	average superficial velocity of the evolved CO_2 (cm/min)
S_0	initial glucose concentration (g/L)
t	time (h)
t_f	value of t to attain complete fermentation (h)
t_{fC}	value of t_f in test carried out in the cylinder (h)
t_{fE}	value of t_f in test carried out in the Erlenmeyer flask (h)
V_C	maximum value of dE/dt in test carried out in the cylinder (g/L.h)
V_E	maximum value of dE/dt in test carried out in the Erlenmeyer flask (g/L.h)
X_0	initial yeast cells concentration (dry matter) (g/L)
X_f	final yeast cells concentration (dry matter) (g/L)
X_{fC}	value of X_f in test carried out in the cylinder (g/L)
X_{fE}	value of X_f in test carried out in the Erlenmeyer flask (g/L)
η	relative ethanol yield (%)

RESUMO

Estudou-se a influência da geometria de reatores não agitados na cinética de produção de etanol em experimentos de fermentação alcoólica realizados por processo descontínuo em escala de laboratório. Foram utilizados dois reatores: um cilindro graduado de 1 L e um frasco de Erlenmeyer de 2 L. Cada reator operou com 1000 mL de meio inoculado. A influência da geometria do reator foi afetada pela relação entre a concentração inicial de levedura (X_0: ~7 g/L, ~14 g/L, e ~21 g/L, matéria seca) e a concentração inicial de glicose (S_0: ~100 g/L, ~150 g/L e ~200 g/L). A influência da geometria do reator diminuiu quando X_0/S_0 aumentou de 0,038 a 0,219, tornando-se nula para X_0/S_0 = 0,22 a 0,24. A geometria do reator praticamente não afetou tanto o rendimento em etanol e a concentração final de levedura quanto a viabilidade e a morfologia das células em experimentos com o mesmo valor de X_0/S_0.

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