Inclined Parallel Plate Sedimenter for Recycling of *Saccharomyces cerevisiae* Cells in Continuous Alcoholic Fermentation

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Foram realizados testes de um protótipo de sedimentador de placas paralelas inclinadas, com vistas à separação contínua de células de leveduras (*Saccharomyces cerevisiae*), de meios de fermentação alcoólica. Para vazões do efluente superior na faixa de 15 a 30 ml/min foi possível obter fluxos estáveis de um efluente clarificado, com teores médios de células na faixa de 0,2 a 1,0%, e tempos de residência variando na faixa de 17 até 77 minutos. Ficou, portanto, evidenciada a exeqüibilidade de utilização exclusiva de um sedimentador gravitacional como recurso para a reciclagem celular em fermentação alcoólica contínua, com eficiência equivalente à de sistemas bem mais sofisticados e caros.

A prototype of an inclined parallel plate sedimenter was tested for continuous separation of yeast cells *(Saccharomyces cerevisiae)* from alcoholic fermentation media. For overflow rates in the range of 15 to 30 ml/min, it was possible to obtain a stable flow of the clarified effluent with average cell contents of 0.2 to 1.0% and residence times of 17 to 77 minutes. Thus, the feasibility of the exclusive utilization of a gravitational sedimenter for cellular recycling in continuous alcoholic fermentation, with an efficiency equivalent to much more sophisticated and expensive systems, was demonstrated.

Key words: *continuous alcoholic fermentation, cell sedimentation, yeast separation, sedimenter,* Saccharomyces cerevisiae.

Introduction

Continuous alcoholic fermentation at high cellular density has great industrial interest because of the possibility of operation at high productivity compared to batch and fed-batch processes[1,2]. Continuous operation of yeast fermentation may be performed by perfusion processes, in which the cells are retained in the fermenter, or by cellular recycling, in which the cells are removed together with the medium, separated from the medium outside the fermenter and then recycled[3]. In any case, all continuous fermentation processes which have been developed still present certain limitations which restrict their application on a large scale. The most common limitations are the need for special strains (flocculants), the accumulation of toxic substances in the fermenter, with a consequent loss of viability and ethanol productivity, and, especially, the high cost of cellular recycling processes (centrifugation, filtration through membranes, etc.) in terms of installation, as well as operation and industrial maintenance.

Based on previous studies[4], it was possible to design a continuous alcoholic fermentation system with a double system of cellular recycling envolving sedimentation, followed by ultrafiltration to remove the cells which remain in the upper (clarified) effluent after sedimentation. In continuation of this work, it was decided to perfect the sedimenter prototype with the objective of eliminating the ultrafiltration step in the final clarification of the fermentation broth, permitting the operation of a continuous alcoholic fermentation system capable of associating simplicity of operation and maintenance with low operational cost.

The original prototype was modified and the results of the preliminary tests demonstrated the possibility of continuous extraction of clarified broth with a cell content of less than 0.5% [5-7], which is easily compensated by maintaining a low rate of cell growth in the fermenter. In the present work, the results of more detailed tests on the performance of the prototype for the separation of *Saccharomyces cerevisiae* cells from a conventional alcoholic fermentation medium containing 7-8% v/v ethanol is presented.

Experimental

Sedimenter Prototype for Cell Separation. The prototype, with inclined parallel plates, was constructed of transparent acrylic. Figure 1 shows the dimensions and func-

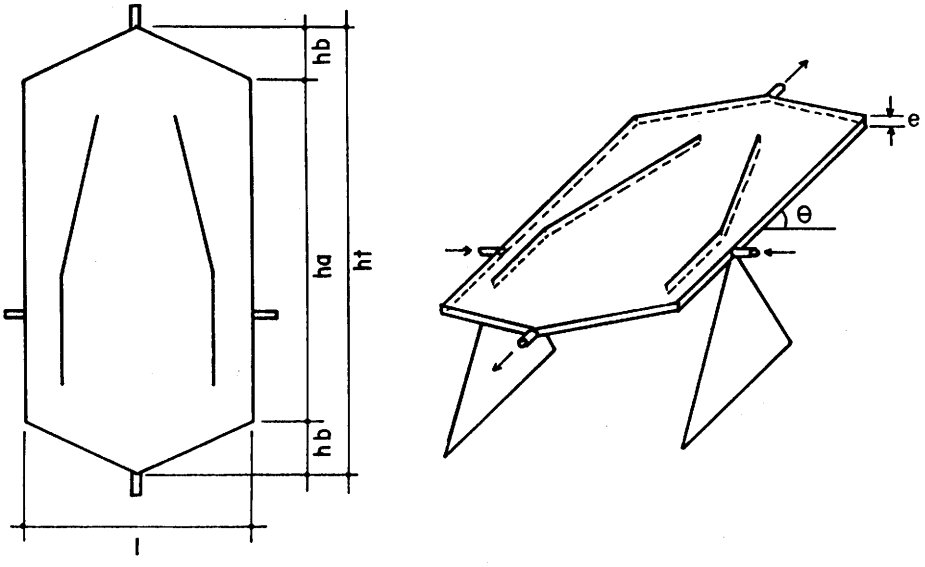

Figure 1. *Inclined parallel plate sedimenter prototype used in the tests. l=60 cm; ha=90 cm; hb=15 cm; ht=120 cm; e=10 mm; Θ = 20°.*

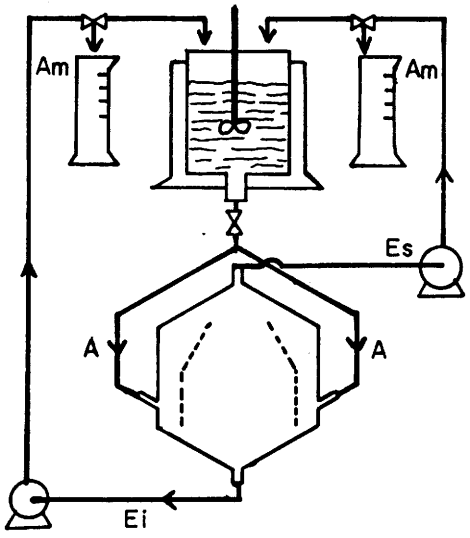

Figure 2. *Sedimenter assembly for continuous sedimentation tests of* Saccharomyces cerevisiae *cells. A = entrance points for fermentation medium; Es = outlet for upper effluent; Ei = outlet for lower effluent; Am = sampling points for tests of flow rate and concentration.*

tioning of the prototype. In Figure 2 is shown the assembly for sedimentation tests and in Figure 3, the operational scheme for continuous alcoholic fermentation with cellular recycling via an inclined parallel plate sedimenter.

The sedimentation tests were realized with the following materials:

Yeasts: fresh, pressed yeasts (Fleischmann), were obtained in the local commerce at most three days prior to use. The humidity of two samples was previously determined by drying in a vacuum oven at 70°C for 5 h[8]; the average humidity was 60%. Based on this value, the quantity of fresh yeast to be used in each fermentation was defined (210 g/l).

Fermentation Medium. Semi-synthetic medium constituted of sucrose (130 g/l), $(NH_4)_2SO_4$ (2.0 g/l), $MgSO_4.7H_2O$ (0.4 g/l), KH_2PO_4 (5.0 g/l), yeast extract (2.0 g/l) and chloranfenicol (100 mg/l).

Medium for Sedimentation Tests. Previously fermented broth, with an ethanol content of 7-8% v/v, substrate (sucrose) content below 0.2% w/v, biomass content of 17-21% v/v and a viability above 98%, was used. During the sedimentation tests, the pH was maintained between 3.0 and 3.6 and the temperature between 20 and 25°C. Each sedimentation test was preceded by a batch fermentation from 24 to 36 hours prior to the tests. This rest period permitted the natural exhaustion of the gas (the degasser is still being developed) without any apparent effect on cell size or performance. Preliminary studies showed that the cell size did not change significantly up to 50 hours after termination of batch fermentation and the fermentation capacity continued equivalent to that of fresh yeast after 24 to 36 hours. The range of variation in the biomass content was a result of variations in the humidity content of the fresh commercial yeast utilized in each fermentation (the yeast was acquired at different dates and locales) since the quantity of yeast used in each test was constant and defined on the basis of the preliminary determination of the average moisture content of only two samples (60% moisture). The alcohol content and the pH were maintained within the indicated ranges, with occasional adjustments when necessary.

Methods of Analyses. The biomass content was determined by centrifugation at 3000 rpm (15000 g)/10 min; the sugar content was determined gravimetrically, according to Fehling[9]; the ethanol content by distillation and by titration with sodium dichromate solution[10]; the biomass viability by dyeing with methylene blue and microscopy[11].

Tests of Continuous Cell Sedimentation. During the operational tests, biomass contents of the original broth (fed to the sedimenter) and the upper and lower effluents were periodically determined by measuring the amount of sediment deposited when centrifuged for 10 min at 15000 g.

Experimental Method. Once the sedimenter was filled, the removal of the upper and lower effluents was initiated at previously fixed flow rates, these effluents being recy-

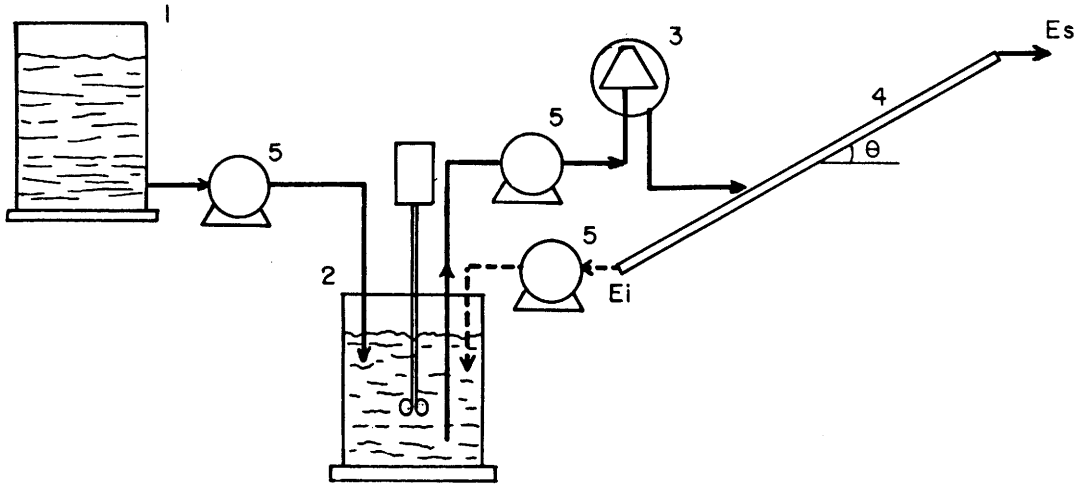

Figure 3. *Flow sheet for continuous alcoholic fermentation with cellular recycling through an inclined parallel plate sedimenter. 1) Substrate reservoir; 2) Fermenter; 3) Degasser; 4) Sedimenter; 5) Lower effluent recycled to the fermenter; 6) Cell poor overflow sent to still.*

cled to the broth container (Figure 2). After about 2 h, the removal of the aliquots from the upper and lower effluents for determination of flow rate and biomass concentration was begun. The system was considered in equilibrium when at least three successive results were obtained which did not differ from one another by more than 5% and for which $Q_A.[A] = Q_U[U] + Q_O[O]$, where Q_A, Q_U and Q_O represent the flow rates at the inlet and the lower and upper outlets, respectively, and [A], [U] and [O] represent the biomass concentrations in the inflow and in the underflow (lower effluent) and overflow (upper effluent), respectively. The results presented in Tables 1 and 2 correspond to the average values from two distinct experiments (realized on separate days), representing, therefore, the means from at least six determinations.

Since the flow rates of the upper and lower effluents were not identical, the separation efficiencies were evaluated via two distinct parameters:

a) Relationships between the concentrations of the upper and lower effluents and that of the fed broth.

$$C_{CU} = [U]/[A] \quad \text{and} \quad C_{DO} = [A]/[O]$$

where C_{CU} = concentration coefficient of the biomass in the underflow relative to that at the inlet and C_{DO} = dilution coefficient of the biomass in the overflow.

b) Relationship of the volume of biomass recycled in the lower effluent to the total volume of biomass fed to the sedimenter during a certain period.

$$T_{UR} = \frac{Q_U[U]}{Q_O[O] + Q_U[U]}$$

where T_{UR} = rate of recycling of the biomass, permitting evaluation of the volume of biomass that returns to the reactor via the lower effluent relative to the total biomass volume extracted from the sedimenter during the same period.

After determmining the biomass concentration of the overflow and underflow corresponding to each pair of effluent rates, the medium was completely removed to a single container where it was homogenized and the biomass concentration of an aliquot was determined. Except for very low underflow rates which resulted in an accumulation of cells at the bottom of the sedimenter, the concentration of cells in the broth was the same as that at the inlet. Thus, there was no accumulation of cells in the sedimenter and one can consider that V/Q_A represents the average residence times of the cell population.

Results

Table 1 shows the principal experimental points obtained, while Table 2 shows the separation efficiency parameters and the residence times corresponding to each experimental point indicated in Table 1. Figure 4 shows the variation in the degree of recycling of the biomass as a function of the flow rate of the upper effluent, maintaining constant the average flow rates for the lower effluent.

Discussion

Table 1 shows that it is possible to obtain a continuous, practically cell-free overflow at flow rates near 15 ml/min (900 ml/h), while varying the flow rate at the lower outlet in the range of 61 to 186 ml/min. When the flow rate of the lower effluent was increased to 350 ml/min, the concentration of biomass in the overflow increased to 2.1%. An increase in the rate of overflow resulted in a concomitant increase in the biomass content (up to 7-12%). A variation in flow rate of the lower effluent in the range of 60 to 180 ml/min hardly affected the biomass content of the overflow. However, a significant increase in this content occurred when the inflow rate was exaggerated (365-400 ml/min). Under the conditions tested, the biomass concentration of the inflow varied from 17.0 to 20.0%, that of the overflow from 0.2 to 11.6% and that of the lower effluent from 18.0 to 27.7%. Thus, an effective separation occurred in all cases.

Table 2 shows that the best separation efficiency parameters correspond to flow rates of 16.4 ml/min and 60.6 ml/min for the upper and lower effluents, respectively. For these rates, 99.8% of the biomass fed to the sedimenter (16.4% v/v) was recycled. The concentration coefficient of the lower effluent was 1.4; the overflow was nearly cell free (dilution coefficient = 91). Under these operational conditions, the residence time was 80.5 minutes. An increase in the underflow rate to 186 ml/min per-

Table 1. *Equilibrium values for the upper and lower effluent concentrations at pre-fixed inlet biomass concentrations and inlet and upper and lower effluent flow rates.*

Q_A (ml/min)	[A] (% v/v)	Q_O (ml/min)	[O] (% v/v)	Q_U (ml/min)	[U] (% v/v)
77.0	18.8	16.4	0.2	60.6	23.8
200.6	20.0	14.9	0.4	185.7	21.6
365.4	20.7	15.1	2.1	350.3	21.5
92.5	20.0	28.7	1.5	63.8	27.7
212.4	20.9	29.8	1.1	182.6	24.1
399.1	19.3	31.4	7.8	367.7	19.6
101.0	18.0	39.0	3.4	62.0	27.1
226.6	19.2	44.2	3.9	182.4	21.8
393.3	18.2	46.5	10.1	346.8	19.6
122.0	17.0	60.0	7.5	62.0	26.6
246.4	18.7	56.1	9.5	190.3	22.3
400.7	17.1	53.9	11.6	346.8	18.0

Average standard deviations: Q_A3.7; [A] 0.7; Q_O 1.0; [O]0.5; Q_U4.1; [U] 0.8.

mitted a decrease in the average residence time to 30.9 minutes without detriment to the level of recycling. Even with a lower effluent flow rate of 350 ml/min, it was possible to obtain a high degree of recycling of biomass (99.6%). However, this level does not reflect the increase in the biomass content of the overflow, which, in this case, was over 2.1%. The level of recycling of the biomass tended to decrease with an increase in overflow rate; for Q_O = 30.0 ml/min, the average level of recycling was 97.7%; for Q_O = 43.2 ml/min, it was 94% and for Q_O = 56.7 ml/min, the average was 85.9%. In any case, the increase in flow rate of the lower effluent was reflected in a reduction in the concentration coefficient. The level of recycling of the biomass was greatly reduced when the system was operated with a mean overflow rate of 57 ml/min. However, even so, the concentration coefficient of the biomass in the lower effluent was maintained at 1.5 for lower effluent flow rates of 60 ml/min and 1.2 for flow rates of 90 ml/min, these situations corresponding to residence times of 52 and 25 minutes, respectively. These facts, plus the low sensitivity of the overflow concentration to variations in the lower effluent flow rate in the range of 60 to 180 ml/min, demonstrated that the length of the prototype was exaggerated relative to the system to

Table 2. *Separation efficiencies and residence times for distinct operational conditions.*

Operational Conditions						
Q_O (ml/min)	Mean Q_O (ml/min)	Q_U (ml/min)	T_{UR} (% v/v)	C_{CU}	C_{DO}	Residence Times (min)
16.4		60.6	99.8	1.4	91.0	80.5
14.9	15.5	185.7	99.8	1.1	50.0	30.9
15.1		350.3	99.6	1.0	9.9	17.0
28.7		63.8	97.6	1.4	13.3	67.0
29.8	30.0	182.6	99.3	1.2	19.0	29.2
31.4		367.7	96.7	1.0	2.5	15.5
39.0		62.0	92.7	1.5	5.3	61.4
44.2	43.2	182.4	95.8	1.1	4.9	27.4
46.5		346.8	93.5	1.1	1.8	15.8
60.0		60.0	78.0	1.5	2.3	50.8
56.1	56.7	190.3	88.8	1.2	2.0	25.2
53.9		346.8	90.9	1.1	1.5	15.5

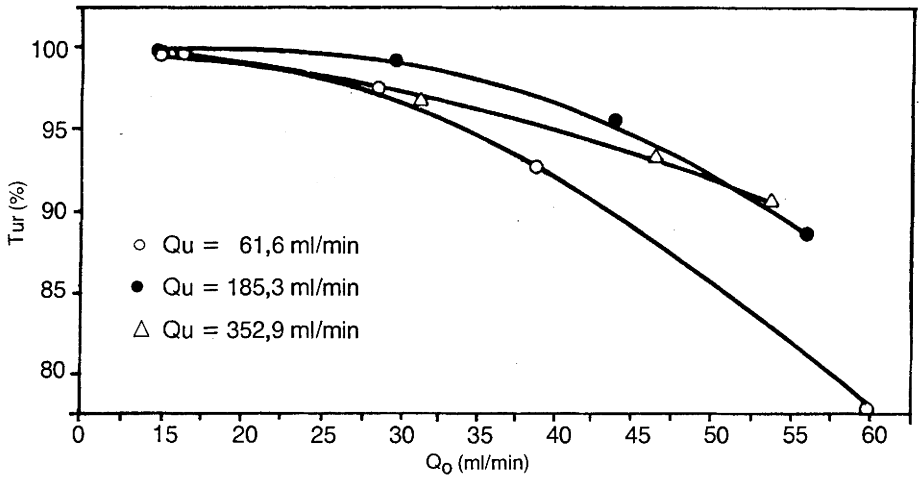

Figure 4. *Percent of biomass recycled in the upper effluent relative to the total biomass fed to the sedimenter, maintaining constant the lower effluent flow rate.*

be treated (yeast cells in alcoholic fermentation broth).

Figure 4 shows that the level of recycling of the biomass is more strongly affected by a variation in the overflow rate (in the range of 15 to 60 ml/min) than by a variation in the flow rate of the lower effluent in the range of 62 to 353 ml/min. In general, the degree of recycling decreased with an increase in overflow rate for a given mean underflow rate. On the other hand, when the overflow rate was maintained constant, an increase in flow rate of the lower effluent was reflected in an increase in the level of recycling of biomass, at least for underflow rates from 62 to 185 ml/min. For very high underflow rates (353 ml/min), the operational conditions were more difficult to control and an anomalous tendency was observed. In addition, in the range tested, the level of recycling is more strongly affected by the flow rate than by the concentration of the lower effluent. Therefore, it is possible to increase the degree of recycling of the biomass concurrently with a reduction in the mean residence time in the sedimenter.

The results obtained permitted the deduction of the following equation:

$$T_{ur} = -7.44 \times 10^{-5}(Q_U)^2 + 4.12(Q_U)^{0.4639}(Q_O)^{2.5} - 8.89 \times 10^{-2}(Q_O)^{1.65} + 2.62\,(Q_O)^{0.65} + 0.885\,(Q_O) + 89.69$$

Using the above equation, it is possible to predict the level of recycling of the biomass which corresponds to any pair of values of Q_O and Q_U, within the range tested, with a maximum error of 1.3%.

Conclusion

Bearing in mind the objective of coupling the sedimenter to the continuous alcoholic fermentation system, it would be of interest to combine the high efficiency of volumetric biomass recycling (over 99%) with the shorter residence time possible (of the order of 10-20 minutes). The short residence time is necessary because, while the cells are present in the sedimenter, they are subjected to aggressive physiological conditions (alcohol content above 7% and lack of sugar and oxygen) which are potentially detrimental to the metabolic activity of the cells and the permeability of the cytoplasmic membrane to ethanol[12-15]. Also, the permanence of the cells in the sedimenter is a factor which contributes to the decrease in the overall productivity of the process, since the fraction of cells which remain outside the fermenter correspond to inactive cells (they are not producing ethanol).

The prototype tested permitted operation with a residence time of 17 minutes and a separation efficiency of 99.6%. However, the recycling rate of the lower effluent which led to this residence time (350 ml/min) is considered excessively high for coupling to a continuous fermentation system whose rate of feeding of the medium to be fermented should be equal to the rate of removal of the upper effluent from the sedimenter (15-16 ml/min). It is desirable that the flow rate of the lower effluent be as close as possible to the overflow rate as a criterion of operational practicality, as well as to avoid an increase in energy consumption which is not reflected in an increase in overflow rate.

The results presented above permit one to arrive at the following conclusions: 1) It is perfectly possible to effect the continuous separation of yeast cells from the alcoholic fermentation broth using an inclined parallel plate gravitational sedimenter which is very simple to make and to operate. A continuous flow of fermented, clarified broth, nearly free of cells, and another continuous flow of cell-rich broth which can be recycled to the fermenter in a continuous alcoholic fermentation system were obtained. 2) The optimization of the prototype tested requires the reduction in the residence time and in the outflow of the lower effluent without loss of the separation capacity obtained in the upper effluent (flow rates of 15-30 ml/min, with a cell content of 0.2 to 1%. This objective should be reached by the reduction of the longitudinal dimension of the sedimenter and the introduction of internal alettes to guarantee that the efficiency of the cell separation is maintained[16]. These studies are under way and should be published in the near future. The optimized prototype is expected to present a separation efficiency above 99% for equal upper and lower effluent flow rates and a residence time on the order of 10-15 minutes.

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References

1. G.E. Guidoboni, *Enz. Microbiol. Technol.* **6**, 194-200 (1984).
2. A.B.R.A. Maia, P. Strehaiano and G. Goma, *Rev. Latinoam. Microbiol.* **29**, 175-82 (1987).
3. G. Goma and G. Durand, *8th. Int. Biotech. Symp. Proceedings*, Paris, Soc. Frencaise de Microbiologie (1988).
4. A.B.R.A. Maia, A.M.D. Ramos, J.A.N.Raim, L.J. de Paiva and R. Abramof, *Química Nova* **12**, 303-306 (1989).
5. A.B.R.A. Maia, M.P.S. Rocha, R.L. Alves, A.S. Albuquerque, C.A. Ferreira, M.A. Martins and H.I. Andrade, *XXIX Congr. Bras. Química,* São Paulo, Assoc. Bras. Química, (Oct., 1989).
6. A.B.R.A. Maia and D.L. Nelson, *Proc. APBioChEC'90,* 619-24 (April 22-25, 1990).
7. A.B.R.A. Maia, Patent Pending, Brazil, INPI Nº 004036 (1989).
8. *Off. Meth. Anal. Assoc. Off. Anal. Chem.* 12th Ed. (AOAC, Washington, 1975), p.191-564.
9. *Normas Analíticas do IALUTZ,* D.D.E. Rebocho, Ed. (IALUTZ, São Paulo, 1976), p. 277.
10. M.A. Joslyn, Alcoholometry. In: *Methods in food Analysis,* 2nd Ed., M.A. Joslyn, Ed (Academic Press, New York, 1970).
11. J.S. Pierce, In: *Recommended Methods of Analysis* (The Institute of Brewing, London, 1977).
12. D.S. Thomas and A.H. Rose, *Arch. Microbiol.* **122**, 49-55 (1979).
13. J.M. Navarro, *Cell Mol. Biol.* **26**, 241-6 (1980).
14. D. Blanchet and D. Ballerini, *Rev. Inst. Franc. Pétrole* **42**, 375-83 (1987).
15. N. Van Uden, *Actas Simp. Iberoam. Catal.* **1**, 183-96 (1984).
16. K.W. Pearce, *3rd. Congr. European Federation of Chem. Engineering. Symp. on Interaction between Fluids and Particles,* Nº 26 (1962)