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Characterization of an Immobilized Yeast Cells Fluidized-Bed Bioreactor for Ethanol Fermentation

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ABSTRACT

The operational characterization of a fluidized-bed bioreactor for ethanol fermentation using Ca-alginate immobilized yeast cells is described. An additional air stream is supplied to the fermenter to ensure and maintain satisfactory fluidization behavior of beads and to avoid slug formation. The influence of physical properties such as bead density and liquid density on the fluidization quality and stability are discussed.

Index Entries: Immobilization; fluidized-bed; ethanol fermentation; *Saccharomyces cerevisiae;* Ca-alginate.

INTRODUCTION

In recent years, the renewed interest in the production of fuels and solvents by fermentation has led to the development of new technologies to improve the results obtained with conventional fermentation processes. Important efforts have focused on ethanol production, and various systems for continuous fermentation have been studied (*1,2*). Among them, immobilized cell technology is particularly relevant and a great number of papers on that topic have appeared in the literature (*3*). The most popular type of continuous bioreactor used in this case is the tubular packed-bed and, to a lower extent, the fluidized-bed fermenter. The former is easy to operate, but gas accumulation inside the reactor and bead compactation (factors limiting the reactor efficiency and its operational stability) have

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been reported as its main drawbacks. The fluidized-bed is a useful way to overcome such problems and to obtain an increase in ethanol productivity in the reactor (*4–6*). Nevertheless, it is a more complex system, as it involves three different phases: solid (immobilized cell beads), liquid (fermentation medium), and gas (CO_2 produced by fermentation and, in some cases, an additional gas stream used to promote and maintain fluidization).

Although several studies have described the performance of these bioreactors for ethanol fermentation, a more basic description of the fluidization phenomena and the factors affecting it is reduced to a few cases (*7*). Moreover, the extensive work carried out on this type of reactor in classical Chemical Engineering (*8*) is not directly applicable to bioreactors owing to the important differences in particles properties and sizes. In the present work, the fluidization of small Ca-alginate beads entrapping yeast cells is characterized, and the influence of various physical parameters (i.e., bead and liquid density) on the quality of fluidization and operational stability of the fermenter is studied.

MATERIAL AND METHODS

Microorganisms

The yeast strain *Saccharomyces cerevisiae* IFI-256 (kindly provided by T. Benítez, Departamento de Genética, Universidad de Sevilla) was used in this work.

Cell Immobilization

Yeast cells were immobilized by entrapment in Ca-alginate beads (Protanal LF-10/60, Protan, Norway) according to the procedure described in the literature (*9*). For the present application, small diameter beads are required to improve fluidization and its production is attained using the method described by Vorlop et al. (*10*) with a few variations. In the equipment schematized in Fig. 1, a suspension containing Ca-alginate 2% (w/v) and cells is pumped through an hypodermic needle into a 2% (w/v) $CaCl_2$ solution. An air stream flowing concentrically to the needle breaks the liquid stream into small droplets whose diameter is regulated by the air flowrate used, as the frequency of bead production is determined by the liquid flowrate. The bead diameter used in the experiments described below was 0.75 mm.

Fermentation Medium

The composition of the medium used in the fermentation experiments was (in g/L): glucose, 50–200; yeast extract, 2; NH_4Cl, 1.3; $MgSO_4$, 0.82; KH_2PO_4, 2; sodium citrate, 1.1; and citric acid, 1.5 (pH 4.0).

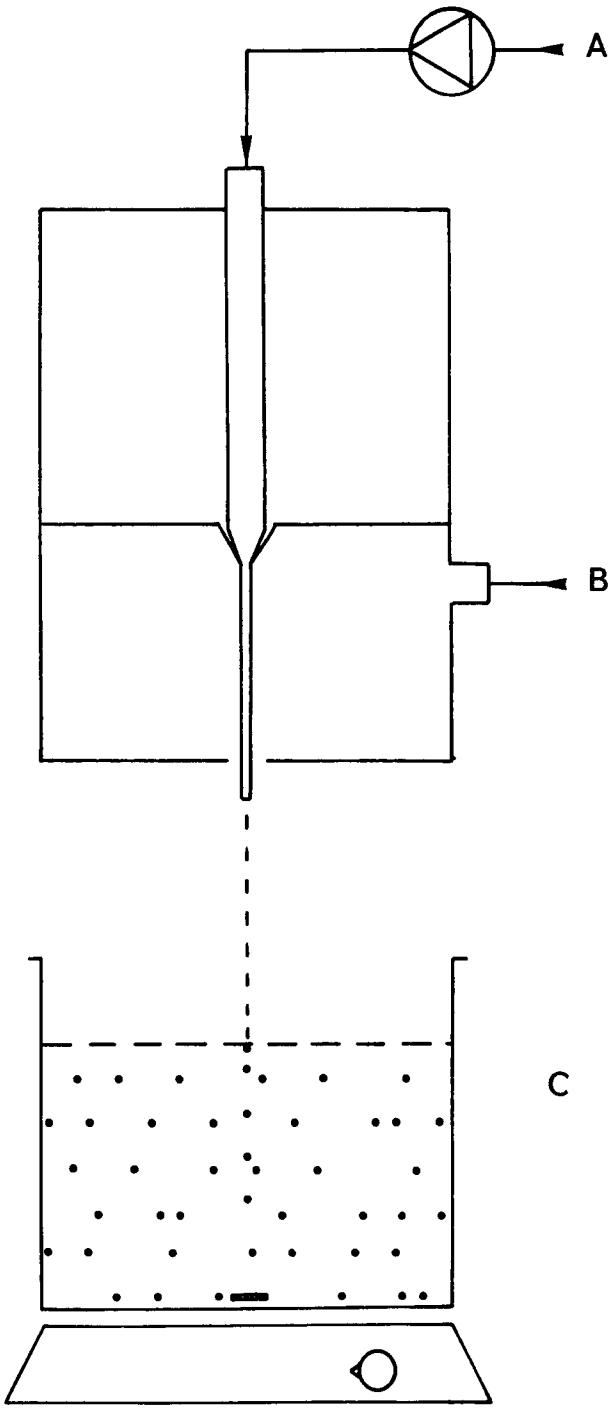


Fig. 1. Immobilization equipment for small size beads production. A, Ca-alginate and yeast cells suspension. B, compressed air, C, gel formation in $CaCl_2$ solution.

Continuous Fermentation

In Fig. 2, a scheme of the continuous fluidized-bed fermenter is shown. The tubular reactor was 2.54 cm in diameter and 54 cm long, with an expansion section on the top to allow bead sedimentation. Air was sparged in fine bubbles through a 5 μm pore sintered glass disc placed in the fermenter bottom. A pressure stabilizer was used in the air line to avoid fluctuations in the supply. Liquid product was removed from the fermenter by means of a peristaltic pump and an additional sedimentation chamber was used to collect beads that could occasionally be removed from the expansion section. The total fermenter volume was 270 mL and it was filled with 100 mL of beads.

Analytical Methods

Glucose and ethanol were measured by HPLC as previously reported (*11*).

Cell Concentration and Viability

Free and immobilized cell concentrations were measured by direct microscopic observation, as already described (*12*), and methylene blue method was used to determine viability (*13*).

Bead Density

Bead density was estimated by means of a pyknometer, removing previously the superficial water using filter paper.

RESULTS AND DISCUSSION

Preliminary Tests for Fluidized-Bed Operation

Various continuous fermentation runs with different sugar concentrations in the feed and flowrates (without using any additional gas stream) were run in order to test the hydrodynamic behavior of the bioreactor. It was found that liquid flowrate (which in the present case is limited to relatively low values, around 100–200 mL/h, especially if high percentage of sugar consumption has to be attained) and CO_2 gas produced by fermentation, were not enough to support steady fluidization of the bed. Instead, CO_2 gas accumulation in the bed resulted in the formation of big bubbles that were eliminated in the form of slugs, thus producing a very unstable reactor performance. A picture of the bed during the evolution of an slug is shown in Fig. 3.

As a consequence of the previous experiments, the use of an additional airstream to promote and maintain fluidization was considered. It was observed a significant improvement in the quality of fluidization, as well

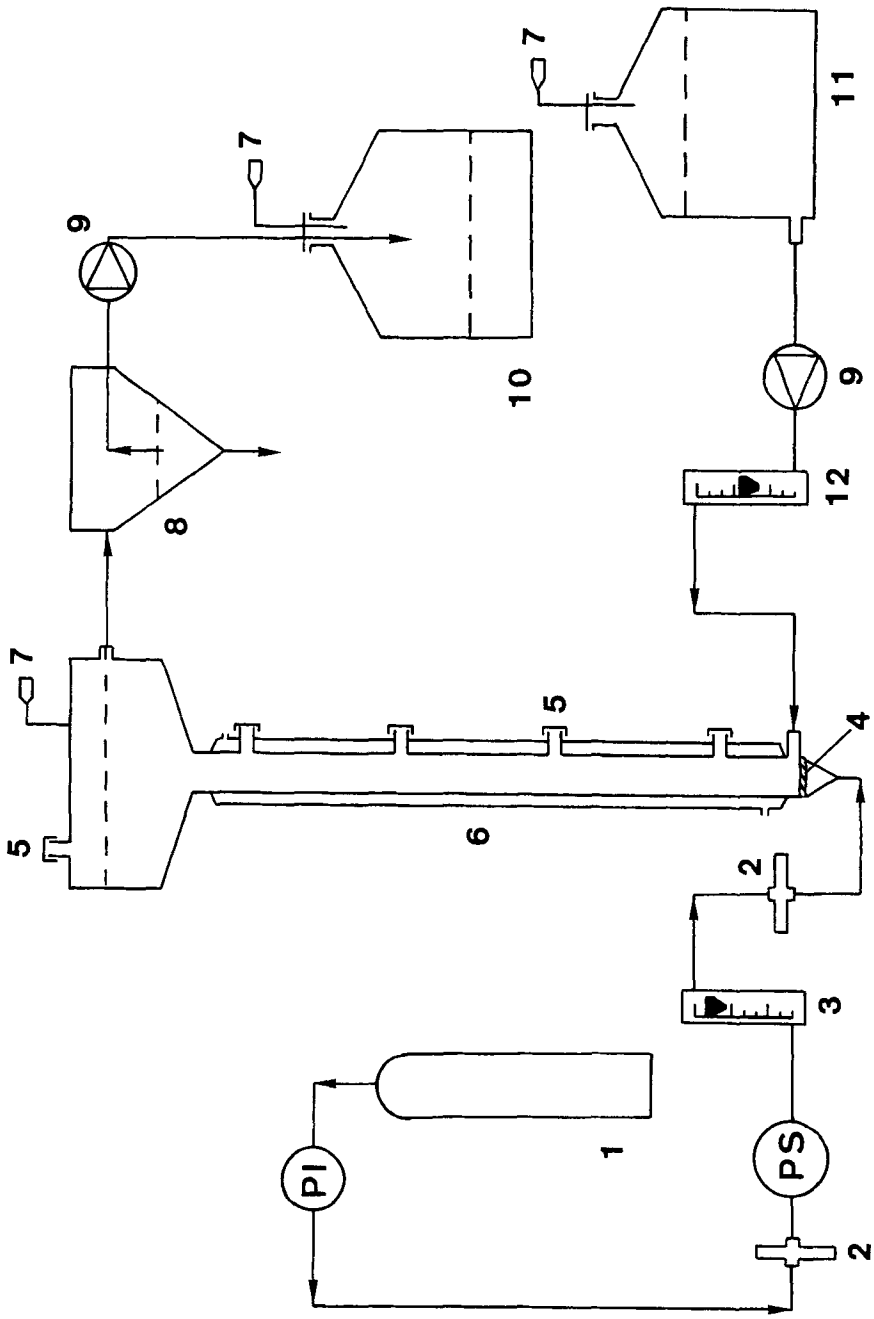


Fig. 2. Experimental setup for continuous fermentation: 1. compressed air cylinder, 2. sterile filter, 3. air rotameter, 4. sintered glass disc, 5. sample port, 6. jacketed tubular reactor with expansion section, 7. sterile connection to atmosphere, 8. settler, 9. peristaltic pump, 10. effluent tank, 11. medium tank, and 12. liquid rotameter.

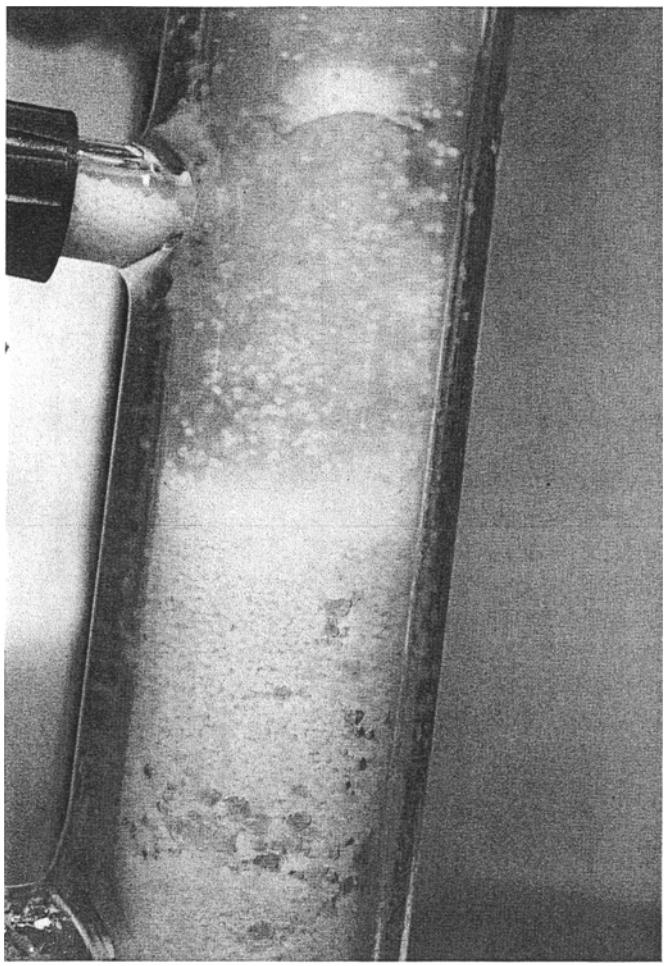


Fig. 3. Photograph of the bioreactor during the evolution of a gas slug.

as absence of gas accumulation in the reactor and slug formation. In the presence of an ethanol concentration higher than 10 g/L (a usual fact in ethanol fermentation), coalescence of gas bubbles was completely avoided and an uniform stream of very small bubbles was obtained throughout the reactor. In Fig. 4 a picture of the bed steadily fluidized is presented. The continuous fermentation results in the three-phase fluidized-bed are summarized in Table 1. One interesting aspect to mention is the absence of internal glucose and ethanol concentration profiles in the fermenter, which indicates that its behavior is that of a well-mixed reactor.

However, in spite of the improvements just described, some stability problems were still present in the fluidized-bed operation. It was found that after a period of time operating with satisfactory fluidization of the bed (usually 2–3 d), a compaction of beads in the reactor bottom was produced, causing uneven distribution of gas bubbles from the sparger,

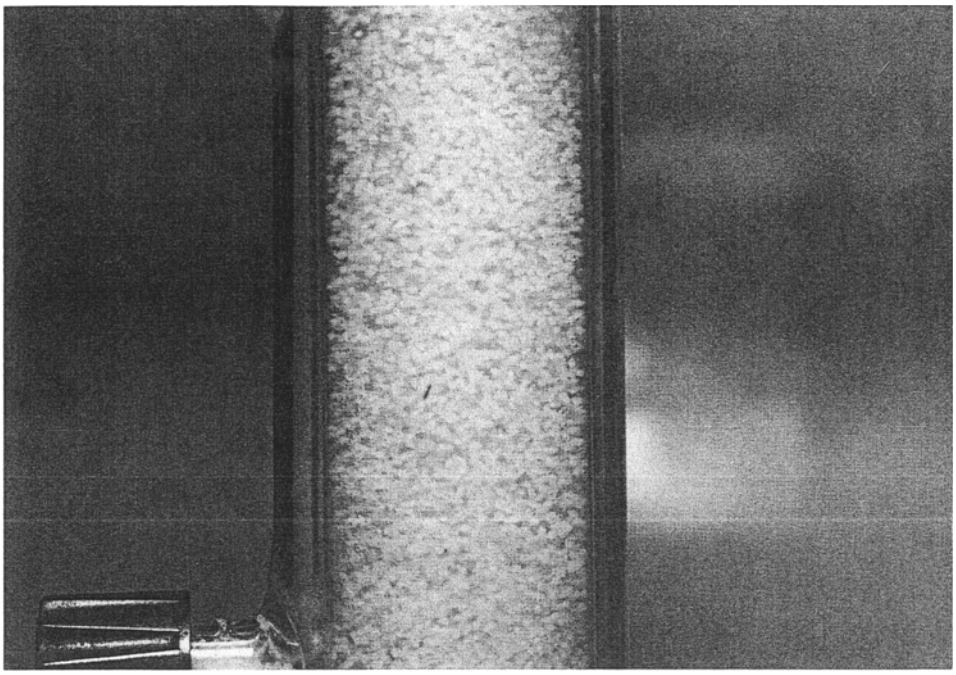


Fig. 4. Photograph of the bioreactor during steady and uniform fluidization.

Table 1
Continuous Fermentation Results in the Three-Phase Fluidized-Bed Bioreactor[a]

S_0, g/L	Residence time, h	Percent glucose consumption	Ethanol, g/L	Productivity, g/L h
100	3.85	100.0	42.8	11.1
100	2.70	100.0	41.4	15.3
100	1.80	92.1	39.3	21.8
100	0.77	51.9	22.0	28.6
100	0.54	48.5	20.5	38.0
46	2.70	100.0	19.0	7.0
146	2.70	95.6	61.4	22.7
185	2.70	68.8	56.5	20.9

[a]Total reactor volume has been used in productivity calculations.

channeling, loss of fluidization uniformity, and, eventually, the apparition of gas slugs. It was also noticed that this fact happened more easily when low sugar concentrations were used in the feed. Both observations seem to indicate that two physical properties, liquid and solid density, could have a significant influence on the fluidization quality in the bioreactor. So, new experiments were carried out to study this relationship.

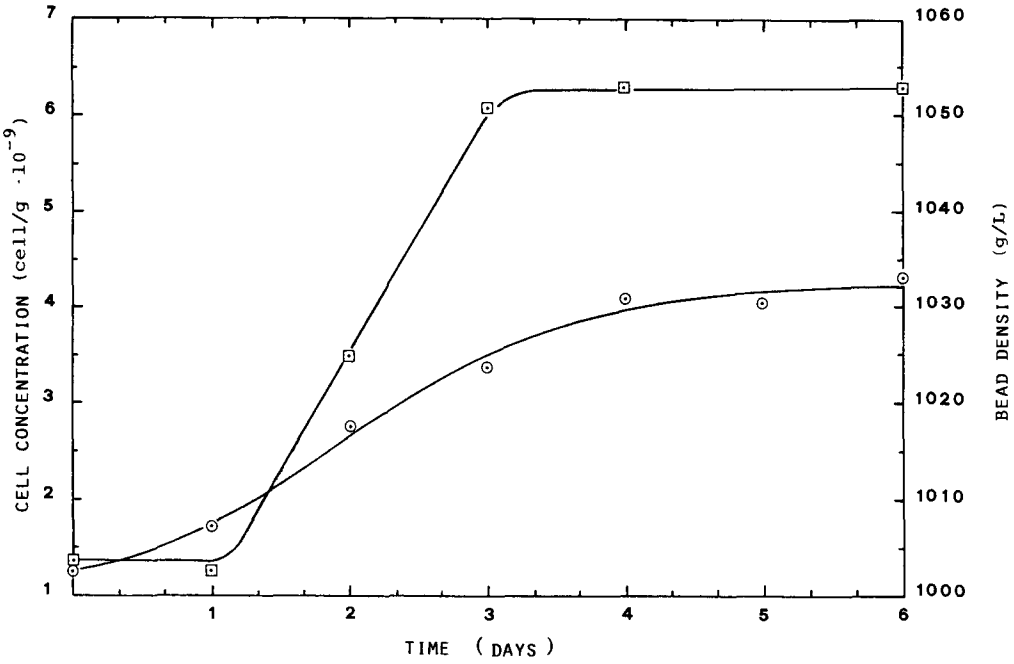


Fig. 5. Variation of bead density (⊡) and cell concentration (⊙) inside the beads with incubation time in fermentation medium.

Microbial Growth and Bead Density

The variation of beads density in relationship with microbial proliferation inside them was investigated. 120 mL of immobilized cell beads with an initially low cell concentration were incubated in a shaked flask containing 600 mL of fermentation medium of 100 g/L glucose concentration. The medium was replaced daily by fresh material. Cell concentration and bead density were measured over time, and the results obtained are plotted in Fig. 5. As it can be seen, density increases as a consequence of cell growth inside the beads, until steady-state is reached. It should be pointed out that although these variations are not high in absolute figures (50 g/L in the whole experiment), they could have relevant impact on the bed hydrodynamics, as the driving force to expand a bed with a fluid moving upwards is proportionally dependent on the difference in densities between solid and liquid (*14*), and this difference changes from 10 g/L at the beginning of the experiment to 50 g/L at the end, a possible cause of bed destabilization.

Liquid Density and Fluidization

An experiment to investigate the influence of liquid density on fluidization behavior at constant bead density was carried out as follows. The bed was initially operated at a high liquid feed rate, consequently, glucose concentration was high throughout the fermenter and therefore

liquid density was also high. Then the liquid flowrate was set to its usual value (100 mL/h), thus enabling fermentation of a high percentage of the sugar feed to the reactor. As a consequence of sugar consumption and ethanol formation, liquid density decreased as the bioreactor approached the steady state causing a progressive destabilization of the bed, with increasing gas channeling and solid and liquid backmixing owing to the bed contraction. Also, the gas flowrate needed to keep bed expansion increased as glucose concentration decreased. Figure 6 shows the variations already described. During this experiment, cell concentration and bead density are assumed constant, because beads previously incubated were used and also because of the short time required for it, 6 hours. It is then evidenced that liquid density has an important role in fluidized-bed fermenter operation.

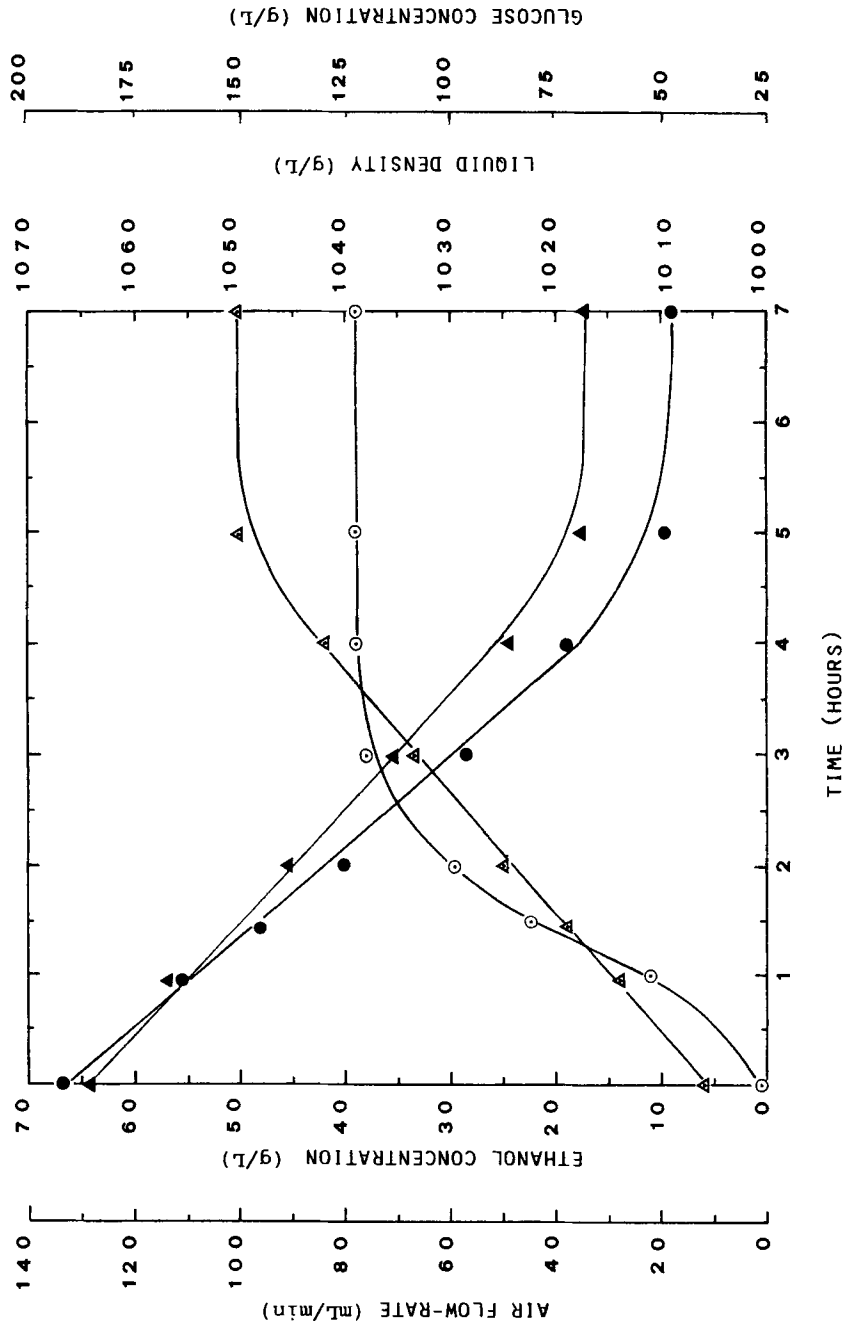
Operational Stability

Based on the previous observations, a new experiment was carried out to determine the influence of both liquid and solid densities on the fluidized-bed stability for a reasonably long period of operation. In this case, liquid density was increased by the addition to the medium of a sugar nonmetabolizable by the yeast strain: 150 g/L lactose was used. The bioreactor was filled with beads at low initial cell concentration and up to the fifth day, in which steady state was reached, the increase in the difference of densities between solid and liquid resulted in an increase of the minimum gas flowrate necessary to fluidize the bed. At steady state, the bed behavior was found more satisfactory than in the case of no lactose addition, and the reactor could work correctly for ten days, when it was stopped. The results obtained in this experiment are presented in Fig. 7.

We conclude that the addition of lactose overcomes the effects of glucose exhaustion on liquid density and provides a density level that can ensure good fluidization for long fermentation periods. At the end of the experiment, 60% of the beads were taken out of the fermenter and exposed to a 5 g/L $CuSO_4$ solution to kill the cells. After that, beads were replaced in the reactor, and it was observed that the increase in glucose concentration caused by the lower living cells present was reflected in a lower minimum gas flowrate needed for fluidization, thus corraboraiting the previous observations.

CONCLUSIONS

Satisfactory fluidized-bed operation for ethanol production using immobilized yeast cells is not possible with the low liquid flowrates needed to attain complete sugar conversion and the CO_2 produced by fermentation, which additionally accumulates forming slugs. In this work, a possible solution to this problem, consisting in the use of an additional gas stream, has been investigated. In this case, it has been shown that the in-


GLUCOSE CONCENTRATION (g/L)
200
175
150
125
100
75
50
25
LIQUID DENSITY (g/L)
1070
1060
1050
1040
1030
1020
1010
1000
TIME (HOURS)
0
1
2
3
4
5
6
7
ETHANOL CONCENTRATION (g/L)
70
60
50
40
30
20
10
0
AIR FLOW-RATE (mL/min)
140
120
100
80
60
40
20
0

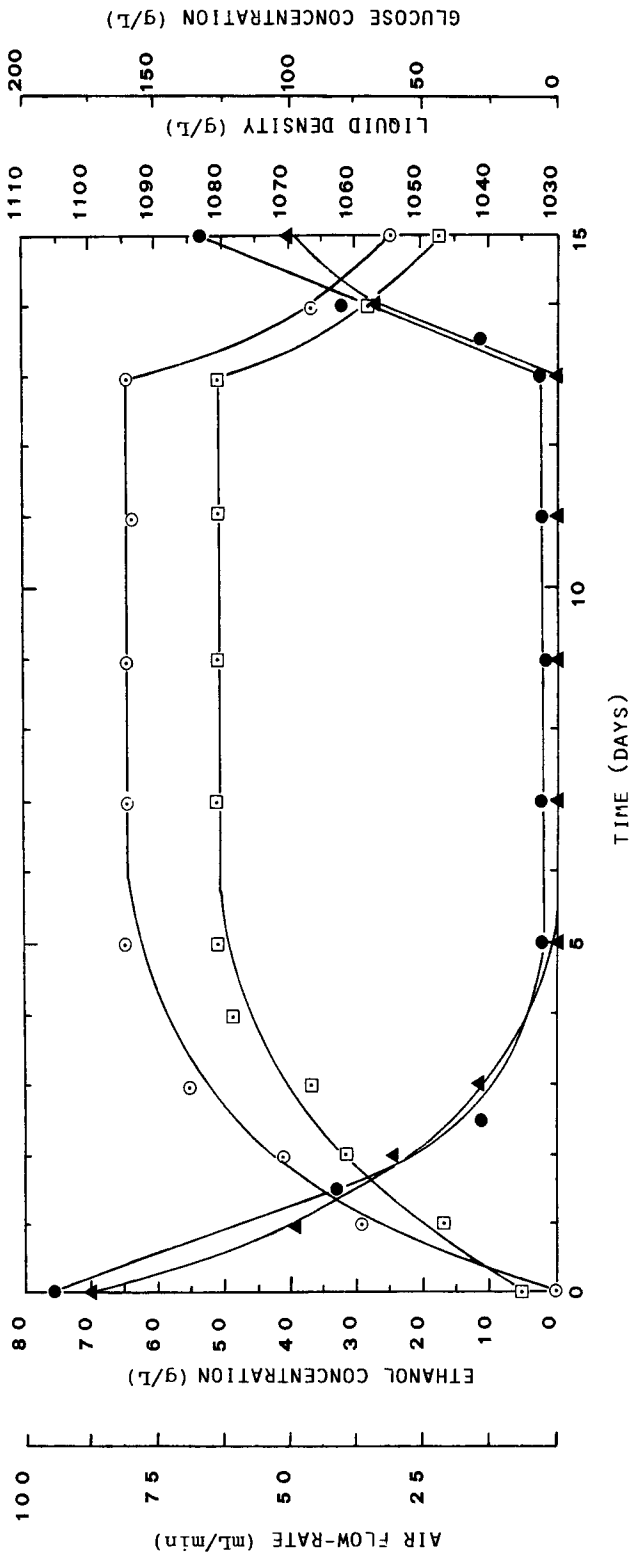


Fig. 7. Operational stability of the fluidized-bed in the presence of 150 g/L lactose in the fermentation medium. Glucose concentration (▲), ethanol concentration (⊙), liquid density (●), air flow-rate (⊡). At day 13, 60% of the yeast in the beads were killed by contact with $CuSO_4$ and quickly replaced into the fermenter.

fluence of physical properties, such as liquid and bead densities, appears to play a key role in the bed hydrodynamics. From the point of view of efficiency, high cell load into beads and maximum glucose consumption are desired. However, these facts have a negative influence on fluidization, because bead weight increases and liquid density decreases. Thus, higher gas flowrates have to be employed, and problems of distribution and bed compaction are found. To avoid this operational drawback, the addition of an inert sugar like lactose has proven to be useful. Although this is not an interesting alternative from the economical point of view, it can be a way to determine optimum operation conditions for this type of fermenter. Further work is currently underway to establish the influence of other physical properties to have a more general criterion to predict the operational behavior of a three-phase fluidized-bed bioreactor.

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