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The Influence of Physical Properties on the Operation of a Three-Phase Fluidized-Bed Fermentor with Yeast Cells Immobilized in Ca-Alginate

P. BÉJAR, C. CASAS, F. GÒDIA, AND C. SOLÀ

Unitat d'Enginyeria Química, Universitat Autònoma de Barcelona, Bellaterra, 08193 Barcelona, Spain

ABSTRACT

A three-phase fluidized-bed bioreactor with Ca-alginate immobilized yeast cells in which an external air stream is introduced to promote and maintain fluidization was studied with respect to its characterization and air-flow optimization. The fluidization behavior is very strongly dependent on the physical properties of both liquid and solid phases (liquid viscosity, density, surface tension, and solid density). Satisfactory or unsatisfactory fluidization behavior could be determined in a graph on the basis of two parameters calculated with the values of the physical properties. A correlation on the minimum air-flow rate necessary to obtain acceptable fluidization of the immobilized yeast beads was developed using experimental data obtained in two fermentors with different L/D ratios.

Index Entries: Immobilization; ethanol fermentation; Ca-alginate; fluidized-bed; physical properties; viscosity of medium; density effects in fluidized bioreactor; yeast.

NOMENCLATURE

v_L	liquid velocity (m/s)
σ_L	liquid surface tension (kg/s^2)
μ_L	liquid viscosity (kg/m s)
ϱ_L	liquid density (kg/m^3)

*Author to whom all correspondence and reprint requests should be addressed.

ϱ_s	solid density (kg/m^3)
d_p	bead diameter (m)
S	cross-section of the fermentor (m^2)
ϕ_c	fermentor diameter (m)
m_G	mass air-flow rate (kg/s)
m_L	mass liquid-flow rate (kg/s)
m_{CO_2}	mass flow rate of CO_2 generated by fermentation (kg/s)
Q_L	volumetric liquid flow rate (mL/h)
Q_G	volumetric air-flow rate (mL/min)

INTRODUCTION

Interest has developed in continuous columnar bioreactors with immobilized cells in relation to fermentation processes, such as ethanol production, because of the high productivities obtained and other well-known features (*1–3*). Fluidized-bed bioreactors, which are used to a relatively lower extent, present various advantages: The mass-transfer between the fermentation broth and the solid beads is improved, since relatively small particles can be used, the gas generated by fermentation does not accumulate in the bioreactor, the supply of a gas stream (i.e., N_2 in an anaerobic fermentation, air in an aerobic one) is easy, and finally, the efficiency of the beads and the overall productivity are also improved with respect to other immobilized cell fermentors, such as packed-bed (*4,5*).

Although fluidized-bed reactors have been extensively used in chemical processes (*6–8*), the results obtained are not directly applicable to bioreactors. This is mainly owing to the difference in particle properties and also to the fact that the velocity of the liquid phase in most biological systems is comparatively insignificant (unless a recirculation loop is provided) because of the high residence time needed to achieve a complete conversion of the substrate. The approach used here to achieve good fluidization behavior is to supply an additional air flow from the bottom of the reactor. No liquid recirculation is used.

The main focus of the present work has been the characterization of the operation of a three-phase fluidized-bed with Ca-alginate entrapped yeast cells with measurement of the minimum air-flow rate needed for satisfactory and stable fluidization for many experimental runs at different conditions. As has been reported (*9–11*), fluidization behavior has a strong dependence on the value of physical properties of the fermentation medium and solid beads, which in this case change in the fermentation as steady state is reached. A methodology has been established in order to determine whether or not a set of experimental conditions would ensure a stable fluidization. Finally, a correlation has been developed to calculate the minimum air-flow rate needed to promote and maintain fluidization from the values of the physical properties of liquid and beads in the fermentor.

METHODS

Microorganisms

The strain *Saccharomyces cerevisiae* IFI-256 (*12*) (kindly provided by T. Benitez, Departamento de Genética, Universidad de Sevilla) was used.

Cell Immobilization

Yeast cells were immobilized by entrapment in Ca-alginate (Protanal Lf 10/60, Protan, Norway), using a double-flux needle to control bead diameter (0.75 mm beads were used throughout the work), according to the described procedure (*11*).

Fermentation Medium

The fermentation medium had the following composition (in g/L): glucose 25–150: 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).

Fermentors

The experiments were run in two tubular fermentors with different L/D ratios. Their diameters were 2.54 and 5 cm, and their length 55 cm. Both had an expansion section at the top, to facilitate the return of the beads to the tubular section. Air was introduced at the bottom of the reactors, sparging it through a 5-μm pore size sintered glass disk. Four ports distributed along the fermentors were employed for liquid and bead sampling. The total reactor volumes were 270 and 1080 mL, and were loaded with 100 and 230 mL of beads, respectively. In the steady state, yeast concentration in the beads was $4\text{–}6\times10^9$ cell/mL. Cells were enumerated under the microscope in a Burker-type chamber.

Analytical Methods

Glucose and ethanol were measured by HPLC using a Bio-Rad Aminex HPX 87H column.

Physical Properties

Bead and liquid densities were measured using a pycnometer. Liquid viscosity was determined in a Cannon-Fenske viscosimeter. For liquid surface tension measurements, a stalakmometer was employed.

RESULTS

Factors Affecting Fluidization Behavior

In some preliminary fluidization experiments (*11*), it was found that the limited liquid flow rate that could be used in the fermentor to attain near complete conversion of the glucose in the feed (without using a recirculation loop) and the CO_2 gas generated by the fermentation were not sufficient to promote and maintain bead fluidization. The driving force supplied by these two phases was unable to offset the bed weight, thus resulting in the compaction of the gel beads and formation of CO_2 slugs. This situation is described as nonsatisfactory fluidization behavior.

One way to overcome the previously mentioned problems is by means of the introduction of an air stream in the form of small bubbles through the base of the fermentor. In this manner, the necessary driving force to achieve a satisfactory fluidization was attained in most cases. Several continuous experiments with different glucose concentrations and feed-flow rates were run in the two fermentors. In every experiment, measurement of the physical properties at the steady state was performed. The minimum air-flow rate necessary to keep the bed fluidized was also evaluated in each experiment. This determination was performed visually, and corresponded to the minimum flow at which homogeneous distribution of the three phases in the fermentor was observed and steady operation was maintained. Under this limit of air-flow rate, bed compaction and slug formation appeared.

In spite of the improvements already mentioned, under some circumstances, the operation of the fermentors was still not steady; an uneven distribution of the sparged gas was observed with channeling and eventually formation of slugs. This situation happened more easily when low sugar and high ethanol concentration (thus, low liquid densities) were present in the fermentor as well as high cell load in the beads (high solid density). These facts seem to indicate that the difference between densities of liquid and solid beads plays a key role in the determination of the fluidization behavior. Another factor affecting fluidization is the decrease in the liquid surface tension caused by increasing the ethanol concentration in it, reducing the coalescence of gas bubbles, and as a consequence, the formation of slugs.

The previous observations led to a more systematic study of the influence of physical properties on the hydrodynamics of the fluidized bed. The two following parameters, both derived from those used by Baker to characterize two-phase flow in pipes (*13*), were defined to characterize the fluidization quality:

$$B_x = [(\mu_L^2 / \sigma_L (\rho_s - \rho_L)^{1/2})]^{1/2}$$
$$B_y = \{m_L / (\rho_s - \rho_L)^{1/2} m_{CO_2}^{1/2}]\}^{1/2} \quad (1)$$

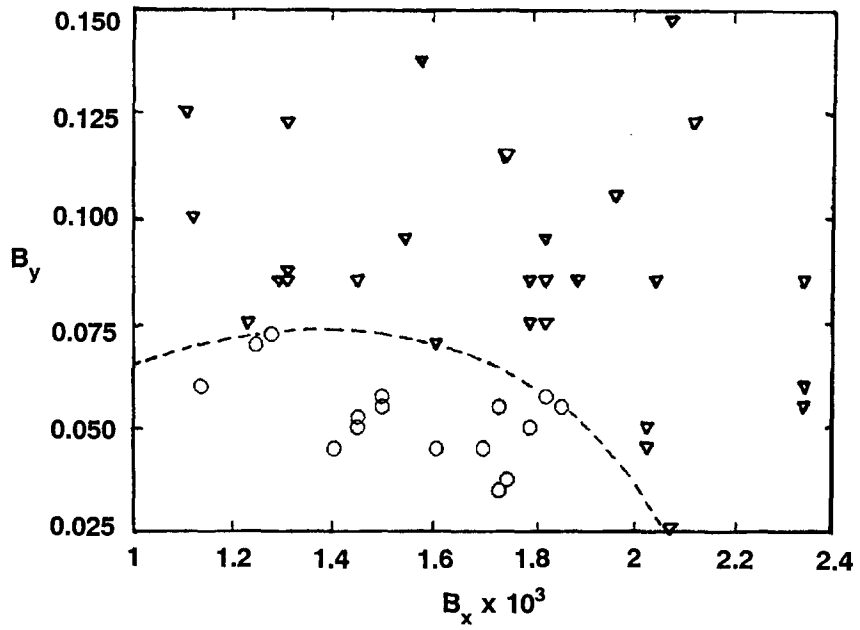

Fig. 1. Fluidization chart. Representation of parameters B_x and B_y calculated for the runs made in the fluidized-bed fermentor. ∇, Runs with satisfactory fluidization. ○, Runs with unsatisfactory fluidization.

where all the symbols are defined in the nomenclature section. It should be mentined that the Q_{CO_2} generated in the fermentor was calculated from the values of glucose and ethanol concentrations, the liquid flow rate, and the stoichiometry of the fermentation. The two parameters were calculated for all the experimental runs and represented in a graph (*see* Fig. 1). The points with satisfactory or unsatisfactory fluidization appeared concentrated in two zones that can be clearly distinguished. The calculation of the coordinates of the flow-region chart, B_x and B_y, from data obtained in a steady-state run always gives an intersection point situated in the upper region. On the other hand, the intersection point corresponding to a run with stability probblems in the fermentor is found in the lower region. Although the border between these two regions is drawn as a line in Fig. 1, it has broader transition zones and was not precisely determined.

In order to increase the range of definition of this chart, the physical properties of the liquid phase were modified by the addition to the fermentation medium of a nonmetabolizable sugar, lactose. As a consequence, liquid density and viscosity attained different steady-state values, depending on the lactose concentration, as well as the glucose concentration in the feed and the amount of consumption. Table 1 summarizes the illustrative data obtained from some representative runs carried out in both fermentors, all of them presenting satisfactory fluidization behavior. The fermentations were run, in most of the cases, at almost complete glucose

Table 1
Summary of Chemical and Physical Data Obtained
from Some Representative Experiments Showing Satisfactory Fluidization Behavior

Glucose feed g/L	Lactose g/L	EtOH g/L	Q_L mL/h	Q_G mL/min	ϱ_L g/mL	ϱ_s g/mL	μ_L cp	σ_L kg/s^2	% sugar consumption	Ferment. vol, mL
25	100	9.7	77	38	1.02	1.07	0.92	0.075	100	270
10	50	4.1	150	50	1.025	1.08	0.91	0.085	100	270
25	175	11.0	150	4	1.083	1.087	1.40	0.075	100	270
135	175	41.0	35	16	1.067	1.084	1.40	0.070	74	270
100	150	40.2	30	7.5	1.043	1.106	1.37	0.065	100	270
100	75	31.2	95	8	1.038	1.105	1.14	0.064	76	270
100	50	41.5	161	90	1.017	1.083	1.05	0.068	100	1080
50	100	21.0	179	42	1.030	1.090	1.07	0.076	100	1080
100	100	42.5	180	55	1.045	1.088	1.14	0.066	100	1080
50	100	20.7	205	65	1.040	1.096	1.04	0.072	100	1080

consumption. Typical fermentation yields for yeast were found: 0.42 g of ethanol/g glucose, very close to the yields for the same strain in batch fermentation. The degree of mixing in the fermentor was high, presenting a liquid flux pattern very close to a CSTR, as indicated by the absence of glucose and ethanol concentration profiles along the fermentors. The effect of lactose concentration was quite notable, since it influenced directly the difference in densities between the beads and the liquid, which is a crucial parameter here. In fact, experimental runs showing unsatsifactory fluidization in the absence of lactose were driven to good fluidization behavior when it was added. Satisfactory fluidization was possible in the absence of lactose only in a limited range of experimental conditions, as the presence of some unconverted glucose in the medium (therefore, relatively high medium density). The high osmolality reached in some of the experiments in Table 1 should be pointed out. Although it had no apparent effect on the activity of the yeast strain used, it could have serious effects on other microorganisms.

Empirical Correlation on the Air-Flow Rate

A correlation to estimate the minimum mass gas-flow rate, m_G (kg/s), needed to achieve and maintain fluidization has been studied. By applying a dimensional analysis to the physical data previously identified as the key factors affecting fluidization, the following correlation was obtained:

$$m_G = e^{-28.0} (Re_P)^{1.42} We^{-0.71} (\rho_s - \rho_L / \rho_L)^{0.63} (S / d_p^2)^{1.26} \quad (2)$$

where

$$Re_P = (\rho_L v_L d_p / \mu_L)$$

$$W_e = (\rho_L v_L^2 \phi_c / \sigma_L) \quad (3)$$

and all the symbols are described in the nomenclature section.

The parameters of the correlation were obtained by multiple linear regression of all the data obtained in all the experimental runs (up to 30) that had presented satisfactory fluidization, with a normalized SD of 0.21. Figure 2 shows the good correlation that exists between the experimental air-flow rates and those calculated using the correlation. It should be emphasized that the correlation was built on the data of two particular fermenters, and its extension to other types was not explored.

DISCUSSION

The approach followed in the characterization of the operation of a three-phase fluidized-bed with immobilized yeast cells in which an air stream is used provides two different tools that can be useful in its design.

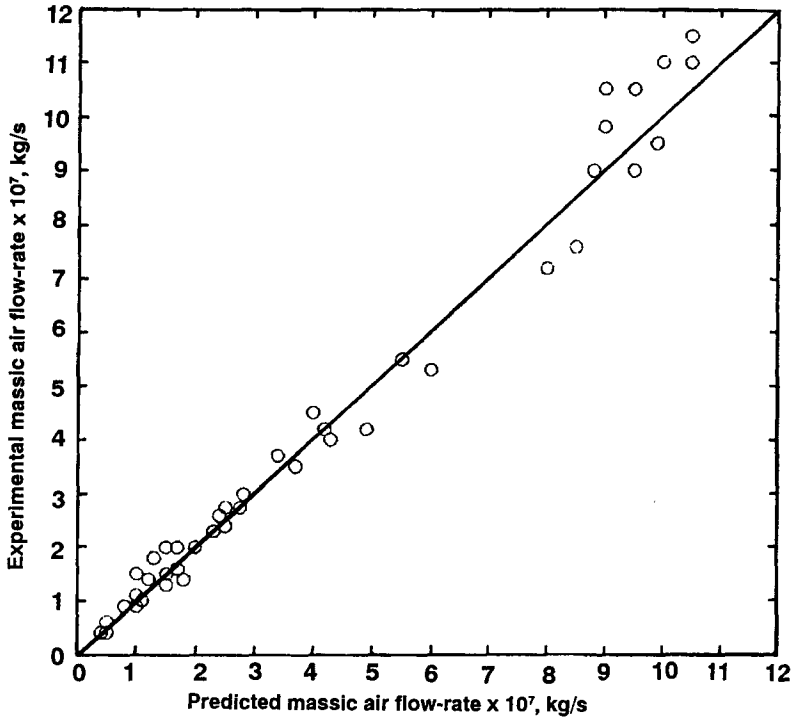

Fig. 2. Air-flow rates needed to keep good fluidization: experimental values vs those calculated with the correlation developed in the text.

They are based in the values of the physical properties of beads and liquid medium. First, the calculation of the parameters B_x and B_y will indicate whether or not a good fluidization behavior can be attained. Second, the use of the empirical correlation developed here will give the air-flow rate needed to reach a smooth operation of the fermenter.

The procedure of lactose addition in the feed medium to evaluate the effect of changes of physical properties on fluidization was used here because it is easy to carry out under laboratory conditions. From the process point of view, it is clearly unpractical, and other possibilities should be regarded. The modification of bead density is particularly promising and can be attained in two different ways: changing the structure of the entrapment matrix to make its density lower and using a different microorganism with a lower density than yeast. The second possibility is a reason in favor of the use of *Zymomonas mobilis*, which would also provide some other advantages, such as high fermentation rates and high ethanol yields. In fact, in some preliminary experiments with *Zymomonas mobilis* NRRL 14023 (*14*), it has been seen that, under exact operational conditions and without any lactose addition, the gas (N_2 in this case) flow rates necessary for good fluidization are significantly lower than when yeast beads were employed. Other authors have also reported the operation of fluidized-bed fermentors with immobilized *Z. mobilis* without any exter-

nal gas addition (*5*). A further challenge for the correlation described here should be the use of data obtained with this second microorganism. Further understanding of three-phase fluidized-bed bioreactors should be attained to improve their design, especially regarding their potential use in biotechnological processes, in particular in those were a gas phase has to be eliminated, or it is needed for oxygen supply or its removal.

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