European J Appl Microbiol Biotechnol (1981) 13:96–101

European Journal of Applied Microbiology and Biotechnology

A Horizontal Packed-Bed Bioreactor to Reduce CO_2 Gas Holdup in the Continuous Production of Ethanol by Immobilized Yeast Cells

Takeshi Shiotani* and Tsuneo Yamané

Department of Chemical Engineering, Faculty of Engineering, Kansai University, Suita, Osaka 564, Japan

Summary. CO_2 gas, evolved during alcohol fermentation using immobilized yeast, causes several undesirable problems in a packed-bed bioreactor installed vertically as it increases the dead space and causes hydrostatic pressure. In order to reduce this "CO_2 gas phase effect" which lowers the efficiency of ethanol production, a shallow, horizontal packed-bed bioreactor has been developed with a free space above the gel bed. The horizontal packed-bed bioreactor was 1.5 times more productive than the vertical packed-bed bioreactor when operated continuously. Yeast cells immobilized in calcium alginate gel reached a steady state much quicker than those immobilized in polyacrylamide gels. In the horizontal packed-bed bioreactors, calcium alginate gel was also superior to polyacrylamide gel with respect to ethanol productivity. The profiles of both glucose and ethanol concentrations against axial sampling sites suggested that the horizontal packed-bed bioreactor was similar to a plug flow reactor. The mean gel size gradually increased upstream (1.9 mm to 3.3 mm). With the economic production of ethanol in view, the published data on different continuous alcohol production processes have been compared by plotting their productivities (y-coordinate) against the ethanol concentrations in the effluents (x-coordinate) for the dilution rate or space velocity at which the yield of ethanol from glucose was 95%. The horizontal packed-bed bioreactor has a very high performance which makes this bioreactor promising for the economic production of ethanol.

1. Introduction

A number of papers about the continuous production of ethanol from sugar using immobilized but growing yeast cells with the aim of economically obtaining liquid energy and chemical resources from renewable biomass have been published recently (Wada et al. 1980; 1981; Moo Young et al. 1980; Ghose and Bandyopadyay 1980; Grote et al. 1980; Arcuri et al. 1980; Sitton and Graddy 1980; Kierstan and Bucke 1977; Navarro and Durand 1977). The immobilized growing microbial cell system is superior to conventional cell suspension systems both in terms of volumetric productivity of ethanol (abbreviated to productivity in this paper) and effluent ethanol concentration. Many different support materials have been reported for cell immobilization, for instance; brick, ceramic, wood chips, calcium alginate gel, κ-carrageenan gel etc. However, the bioreactors used until now have been restricted to the vertical packed-bed type. The use of packed-bed bioreactors seems reasonable because particulate catalysts which are packed in reactors have high specific interfacial areas of solid-liquid contact and also because the velocity of liquid creeping over the static solid particles substantially alleviates the film resistance of mass transfer. If the material used for cell immobilization is selected carefully, cell holdup within the packed-bed column can be considerably increased.

Carbon dioxide gas evolution is an essential feature of ethanol production by fermentation. The stoichiometric equation shows that CO_2 is released equimolarly with ethanol in the course of alcohol fermentation (Yamané and Shiotani 1981). The rate of CO_2 evolution is almost proportional to the rate of ethanol formation since cell growth is not significant in the immobilized growing cell system. As a typical example, when a 20% glucose solution is supplied to a vertical packed-bed bioreactor and the yield of ethanol from glucose is assumed to be 95%,

* On leave from The Institute of Production Technology, Kanegafuchi Chemical Industries Co., Ltd., Takasago, Hyogo 676, Japan

0171-1741/81/0013/0096/$01.20

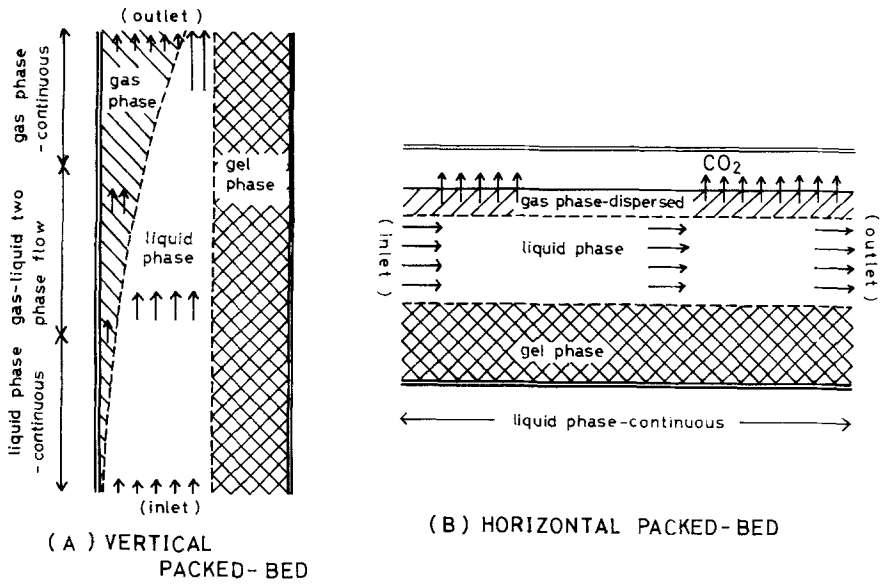

Fig. 1.
Schematic sketch of vertical and horizontal packed-bed bioreactors

the volume of CO_2 produced per volume of effluent is 52:1 at 30 °C and 1 atm. The CO_2 gas holdup near the outlet is thus very high compared with liquid holdup. This gas holdup produces a dead space, decreasing the solid-liquid contact area which may lead to reduced glucose conversion. Vertical packed-bed bioreactors do not have any open part other than the outlet and therefore the evolved CO_2 gas bubbles produced in the biochemical reaction must flow toward the outlet. In an upflow type, packed-bed bioreactor a gas bubble flows only when its pressure exceeds the hydrostatic pressure of the packed-bed. All of the immobilized growing cells suffer from the combined hydrostatic pressure drop and gas bubble pressure. If the immobilization material is composed of an elastic gel whose mechanical strength is relatively poor, the excess pressure caused by the CO_2 gas phase deforms the gel pellets and they tend to stick to each other which results in closure of the liquid path so that both the liquid and gas flows become nonuniform. At worst, the gel pellets are abraded or ruptured. All these undesirable effects of evolved CO_2 gas on the performance of packed-bed bioreactors are referred to as "CO_2 gas phase effect" in this paper. As both the conversion and the effluent ethanol concentration increase, the CO_2 gas phase effect becomes more serious. In fact, we have often encountered the CO_2 gas phase effect in experiments on continuous ethanol production using yeast-containing polyacrylamide gels in a vertical packed-bed column.

It is therefore important that when ethanol is produced by pellets of gel-immobilized growing cells packed in a column, evolved CO_2 gas should be exhausted, as promptly and as much as possible, from the gel-bed and that mutual adhesion of the gel pellets should be avoided to eliminate channeling of liquid flow. To reduce the CO_2 gas phase effect we have now desgined a shallow, horizontal packed-bed bioreactor which has a free space above it. In this paper this new bioreactor will be compared with a conventional vertical packed-bed bioreactor with respect to productivity and effluent ethanol concentration.

Bioreactors

Two types of bioreactors were used. One was a vertical column with thermostat jacket (26 mm internal diameter and 400 mm long during the steady state of continuous operation, Pharmacia column K26/40). The other was a horizontal packed-bed bioreactor specially designed for this study. It consisted of a slender box of transparent acrylic resin (30 mm broad, 300 mm long and 50 mm high) with a thermostat jacket covering the bottom and both sides. Inlet and outlet pipes were connected to the ends. The gel-bed was about 25 mm high (working volume was 230 ml in the steady state of continuous operation), leaving 220 ml of free space above the gel-bed in the bioreactor. To avoid downstream movement of the gel during startup, the bioreactor was sectioned vertically every 50 mm by No. 32 stainless steel wire mesh. A flat cover above the free space had several holes for releasing CO_2 gas.

The two bioreactors is shown schematically in Fig. 1, in which each of the three phases (gel, liquid and CO_2 gas) is lumped together into one imaginal part illustrated with different outline. In the vertical packed-bed bioreactor (A), as the CO_2 gas holdup increases downstream, the liquid holdup decreases inversely. In the inlet region the liquid phase is continuous and the gas phase is dispersed with the gel phase, but in the outlet region the phase relationship is reversed. Since the liquid phase narrows, the actual velocity of its flow must increase with the increasing axial distance

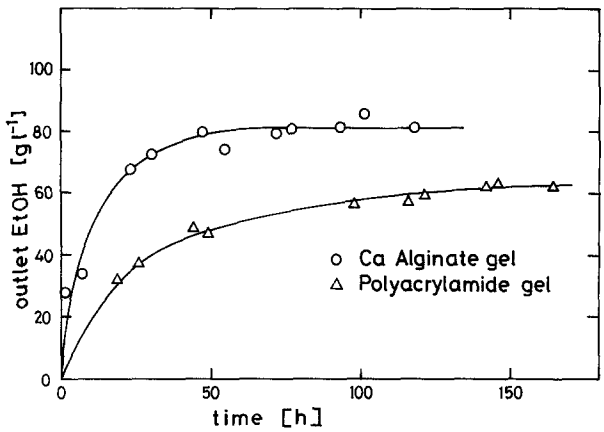

Fig. 2. Start of continuous alcohol fermentation by yeast, immobilized in either calcium alginate or polyacrylamide gel, in the horizontal packed-bed bioreactor. Space velocity were 0.55 h^{-1} for calcium alginate gel and 0.56 h^{-1} for polyacrylamide gel

of the run. Conversely, in the horizontal packed-bed bioreactor (B), if its gel-bed is not too deep, the CO_2 gas is released upwards to the free space by its buoyancy. The liquid which travels horizontally, can be expected to be in continuous phase throughout its path. Thus, the CO_2 gas phase effect should be reduced substantially even if not completely eliminated.

Materials and Methods

Microorganism and Feed Medium

Baker's yeast was kindly donated by the baker's yeast factory of Kanegafuchi Chemical Industries, Co., Ltd., Takasago, Hyogo Prefecture. It had been cultivated in molasses media and was used after washing and several centrifugations. The composition of the medium fed to the bioreactor was: 19.6% glucose, 0.5% yeast extract, 0.25% NH_4Cl, 0.1% NaCl, 0.55% K_2HPO_4, 0.025% $MgSO_4 \cdot 7H_2O$ and 0.15% $CaCl_2 \cdot 2H_2O$. The pH was adjusted to 5.0 with H_2SO_4 solution prior to sterilization.

Immobilization Procedures

The cells were immobilized by entrapping in two kinds of gel, polyacrylamide or calcium alginate. For immobilization in polyacrylamide gel, a method similar to that of Chibata et al. (1974) was used except with 0.5 g wet cell/ml saline solution. The polymerized sheet was cut into 2 mm cubes according to Yamané et al. (1979).

For immobilization in calcium alginate gel, a slurry was made by mixing 18 ml of concentrated cell suspension (0.67 g wet cells/ml) with 77 ml of a 1.3% solution of sodium alginate (Nakarai Chemicals Co., Ltd., the viscosity of a 1% solution is 1,000 cp at 25 °C.). The slurry was then forced with a syringe through a needle (0.2 mm internal diameter) into 1 l of 0.5% $CaCl_2$ solution. The average diameter of the spherical gels was about 1.5 mm after an overnight incubation in the medium.

Experimental Procedure

The entrapped cells were incubated overnight statically and batchwise in 500 ml of the feed medium and then were packed into the bioreactor. The sterilized feed medium, stored in a refrigerator, was supplied continuously to the bioreactor by a flanger type pump (Nikkiso Co. Ltd., model SK). The bioreactor was maintained at 30 °C by circulating water, from a thermostatically controlled water bath, in the jacket. Samples for the determination of ethanol, glucose and cell concentrations were taken from the effluent every morning and evening. They were clarified by centrifugation and the supernatants were stored in a refrigerator before analysis of the ethanol and glucose concentrations. At the same time, the effluent flow rate was calculated from the amount of effluent collected in a bottle during a sample interval.

Analytical Procedure

Ethanol was determined by gas chromatography (Shimazu Co., Ltd., model GC6A) with a dual column-dual detector system using n-propanol as the internal standard. The glass columns (3 m long and internal diameter 3 mm) packed with PEG 1000 (80–100 mesh) were used with a flame ionization detector. Glucose was determined by the method of Bertrand. The cell concentration of the effluent was determined turbidometrically using a calibration curve of cell concentration [g dry cell l^{-1}] against turbidity at 500 nm.

Results and Discussion

Startup

It was observed that ethanol in the effluent increased gradually early during the continuous operation, as shown in Fig. 2, and that at the same time the volume of the gel-bed increased to some extent. Clearly the entrapped cells grew in both the polyacrylamide and calcium alginate gels until a steady state was reached just as in the κ-carrageenan gel (Wada et al. 1980). Growth of yeast in a polyacrylamide gel has also recently been reported (Siess and Divies 1981). Besides, it was observed that the gel size increased during this period. When calcium alginate gels were used, ethanol in the effluent increased more rapidly than when polyacrylamide gels were used. A steady state was reached in a shorter time with calcium alginate gel than polyacrylamide gel. It seems that growth of cells in the gel depends on various factors such as the initial concentration of active cells in the gel, the nutritional content of the feed medium, mechanical strength of the gel, size of the gel etc.. When steady state was attained, the gels stopped "growing" and the cell growth in the gel balanced dynamically with leakage of the cells from the gel.

Comparison of Vertical and Horizonatl Paked-Beds

The performance of vertical and horizontal packed-bed bioreactors, containing the same amounts of immobilized cells in polyacrylamide gel, was compared with respect

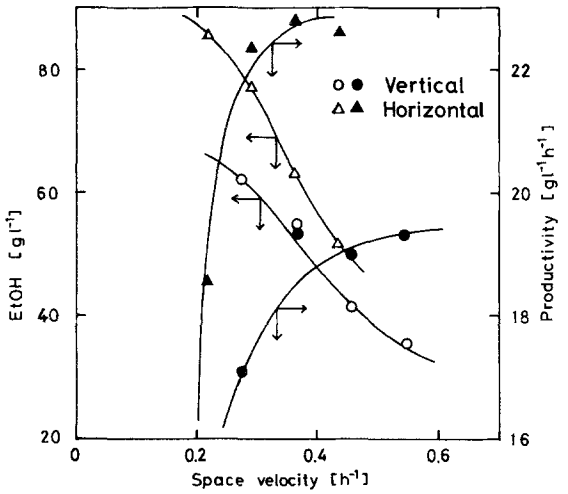

Fig. 3. Comparison of a vertical with the horizontal packed-bed bioreactor (using polyacrylamide gel)

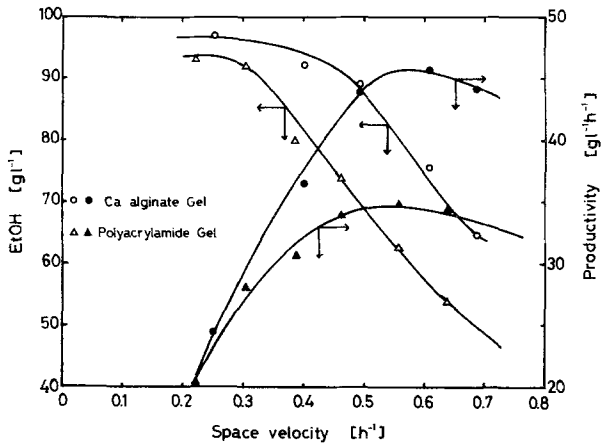

Fig. 4. Comparison of polyacrylamide gel with calcium alginate gel in the horizontal packed-bed bioreactor

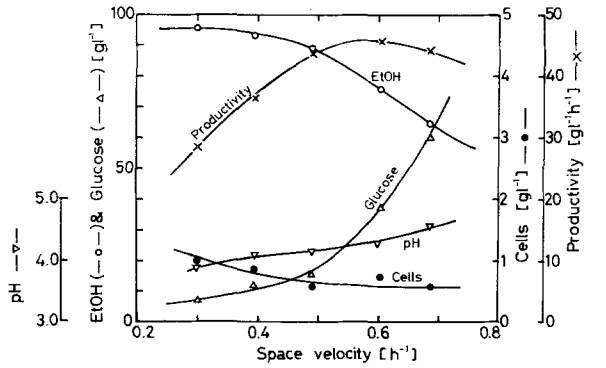

Fig. 5. Dependence of various variables of the effluent on the space velocity in the horizontal packed-bed bioreactor (using calcium alginate gel)

to changes in effluent ethanol concentration and the productivity changes with space velocity (Fig. 3). The productivity ($gl^{-1}h^{-1}$) was calculated from the ethanol concentration in the effluent, multiplied by the feed rate of the effluent and divided by the steady state bed volume (i.e., working volume). The space velocity (h^{-1}) is defined as the ratio of the medium feed rate to the steady state gel bed volume in a packed-bed reactor. It is the reciprocal of the space time.

The productivity of the horizontal packed-bed was 1.5 times greater than that of the vertical packed-bed bioreactor. The reduced productivity of the vertical packed-bed bioreactor is mostly due to the "CO_2 gas phase effect" since the amount of immobilized cells packed in the two types of the bioreactors was the same. Apart from its lower productivity, the gas holdup in the vertical packed-bed bioreactor sometimes increased abnormally so as to squash the gel beads. The scale up of vertical packed-bed bioreactor required for the continuous production of ethanol would, in the our opinion, be difficult because of the CO_2 gas phase effect. The horizontal packed-bed bioreactor operated much easier than the vertical one. CO_2 gas was released upwards from all over the free surface which looked as if it was boiling.

Performance of the Horizontal Packed-Bed Bioreactor

Two kinds of gel were compared in the horizontal packed-bed bioreactor in terms of their effect on the ethanol concentration in the effluent. In these experiments the bed volume during steady state operation was standardised for both the polyacrylamide and calcium alginate gels. The result shown in Fig. 4 demonstrates that calcium alginate gel is superior to polyacrylamide gel with respect to its effect on ethanol productivity. This is probably because of the difference in specific area of the gels as well as their different nature. The polyacrylamide gel particles were cubic while the calcium alginate gel particles were spherical. Fig. 5 shows the dependence of various variables in the effluent on the space velocity. The cell concentrations were less than 1.0 g dry cell l^{-1}, so the proportion of glucose converted to cell mass was small (about 0.6% calculated on carbon conversion). The maximum productivity was 46 $gl^{-1}h^{-1}$ at 0.57 h^{-1} of space velocity. It should be noted, however, that at this space velocity the yield of ethanol from the supplied glucose was 80% with 29 gl^{-1} of the residual glucose wasted.

Profile of Various Variables at Different Sampling Sites

Various variables were analyzed at different sites along the calcium alginate gel-packed bed of the horizontal bioreactor once steady state had been reached (Fig. 6). The most

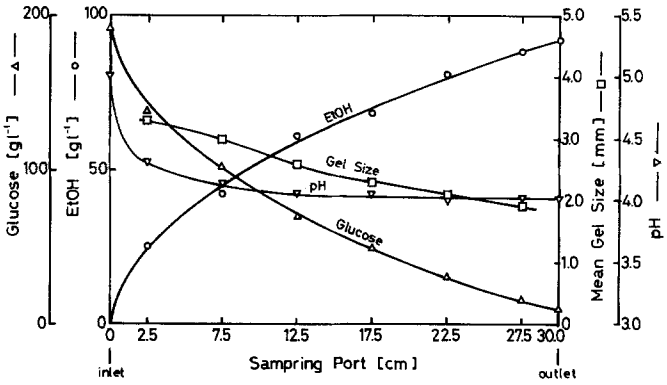

Fig. 6.
Profile of various variables at different sampling sites in the horizontal packed-bed bioreactor (using calcium alginate gel, space velocity; 0.39 h^{-1})

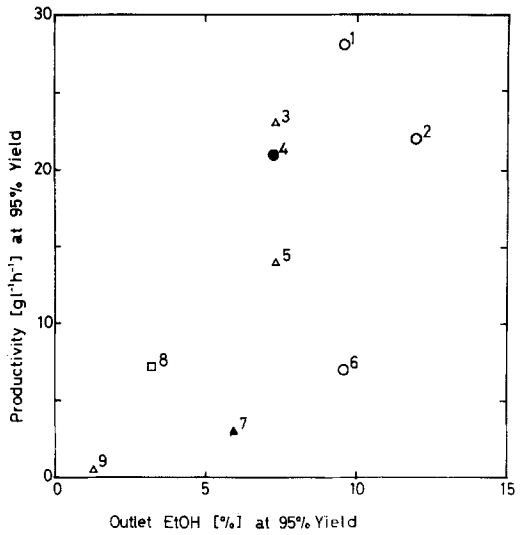

Fig. 7. Comparison of various processes for continuous ethanol production. 95% yield means that the actual amount of ethanol formed is 95% of the stoichiometric amount of ethanol calculated from the amount of glucose supplied. ○, Immobilized yeast in packed-bed [*1*, this work; *2*, assuming 50% fraction void (Wada et al. 1981) and *6*, Ghose and Bandyopadhyay 1980]. △, Suspended free yeast in chemostat [*3*, with cell recycle (Ghose and Tyagi 1979); *5*, without cell recycle (Ghose and Tyagi 1979) and *9*, without cell recycle (Sitton and Graddy 1980)]. ●, Immobilized *Zymomonas mobilis* in packed-bed (*4*, Grote et al. 1980). ▲, Suspended free *Zymomonas mobilis* in chemostat without cell recycle (*7*, Lee et al. 1979) □, Flocculent yeast in fluidized-bed (*8*, Greenshields and Smith 1971)

notable feature was that the mean gel size increased gradually towards upstream (from 1.9 mm to 3.3 mm). In the inlet region, the gel tended to swell due to osmotic pressure caused by the high glucose concentration whereas near the outlet region, gel "growth" was repressed due to product (ethanol) inhibition. Note that the initial gel size had been about 1.5 mm. The pH had dropped sharply in the inlet region but was almost unchanged downstream.

From the glucose and ethanol profiles the bioreactor appeared to be similar to a plug-flow reactor although there may have been some axial dispersion of liquid. Tracer experiments with a horizontal fixed-bed bioreactor (10 cm broad by 100 cm long by 10 cm high) packed with 3 mm glass beads revealed that the Peclet number was about unity which is slightly lower than in an ordinary packed-bed bioreactor (Yamané, T., unpublished data). In a horizontal packed-bed bioreactor, when the feed rate is raised part of the feed liquid flows over the bed causing a short-cut flow. The limiting space velocity above which this undesirable flow appeared was found to be around 4.0 h^{-1} for this particular bed packed with glass beads (Yamané, T., unpublished data). The operational space velocity in the horizontal packed-bed ethanol producer is probably less than 0.6 h^{-1} (Fig. 5) which is much lower than the limiting space velocity.

Comparison of Various Processes for Continuous Ethanol Production

Two factors will be important for the economic production of ethanol; to increase the reactor productivity and to increase the ethanol concentration in the effluent. The latter is important for reducing the cost of seperating ethanol from the effluent (Hartline 1979). All previous papers have only compared the maximum productivities at residual sugar concentrations which were too high to be ignored. In order not to waste residual sugar in the effluent a setup for the economical production of ethanol would probably be operated at a much smaller dilution rate or space velocity than that which gives maximum productivity. To take account of the two factors mentioned above, the practical performance of the bioreactor can be assesed graphically at the dilution rate or space velocity where the yield of ethanol from glucose is 95% (Fig. 7). The productivity at 95% yield is plotted as the y-coordinate against the ethanol concentration of the effluent at 95% yield, plotted as the x-coordinate. The term, *yield* is the proportion of the actual amount of ethanol formed to the stoichiometric amount of ethanol which could be

produced from the glucose supplied, i.e., the actual conversion of glucose to ethanol relative to the theoretical one. This definition of yield is commonly used in chemical reaction engineering. From the definition the outlet ethanol concentration for 95% yield is calculated by

$$\text{Outlet ethanol concentration } (gl^{-1}) = 0.486 \times \text{Inlet glucose concentration } (gl^{-1})$$

It should be noted that a 95% yield would be the upper limit attainable with a chemostat (Ghose and Tyagi 1979) as well as with a packed-bed bioreactor using immobilized growing cells (Wada et al. 1981). Figure 7 includes most of the recently published data for continuous alcohol production and gives performance comparisons on a more realistic basis than previously. Clearly, the larger the values of x and y, the better is the process. The data for the horizontal packed-bed bioreactor (x, 96 gl^{-1} and y, 28 $gl^{-1}h^{-1}$) places it amongst the processes which have the highest performances. This bioreactor is hence promising for use in the economic production of ethanol.

Acknowledgements. The calcium alginate as a excellent gel for yeast immobilization was suggested to us first by Prof. Susumu Fukushima, Department of Chemical Engineering, Faculty of Engineering, Kansai University, to whom the authors are very grateful. The authors would like to thank Messrs. Banba, K. and Hirano, T. for technical assistance.

References

Arcuri JE, Worden RM, Schumate II SE (1980) Ethanol production by immobilized cells of *Zymomonas mobilis*. Biotechnol Lett 2:499–504

Chibata I, Tosa T, Sato T (1974) Immobilized aspartase-containing microbial cells: preparation and enzymic properties. Appl Microbiol 27:878–885

Ghose TK, Tyagi RD (1979) Rapid ethanol fermentation of cellulose hydrolysate I. Batch versus continuous systems. Biotechnol Bioeng 21:1387–1400

Ghose TK, Bandyopadhyay KK (1980) Rapid ethanol fermentation in immobilized yeast cell reactor. Biotechnol Bioeng 22: 1489–1496

Greenshields RN, Smith EL (1971) Tower-fermentation systems and their applications. The Chem Engr May 1971:182–190

Grote W, Lee KJ, Rogers PL (1980) Continuous ethanol production by immobilized cells of *Zymomonas mobilis*. Biotechnol Lett 2:481–486

Hartline FF (1979) Lowering the cost of alcohol. Science 206, October:41–42

Kierstan M, Bucke C (1977) The immobilization of microbial cells, subcellar organelles and enzymes in calcium alginate gels. Biotechnol Bioeng. 19:487–497

Lee KJ, Tribe DE, Rogers, PL (1979) Ethanol production by *Zymomonas mobilis* in continuous culture at high glucose concontrations. Biotechnol Lett 1:421–426

Moo-Young M, Lamptey J, Robinson CW (1980) Immobilization of yeast cells on various supports for ethanol production. Biotechnol Lett 2:541–548

Navarro JM, Durand G (1977) Modification of yeast metabolism by immobilization onto porous glass. Eur J Appl Microbiol 4:243–254

Siess MH, Divies C (1981) Behaviour of *Saccharomyces cerevisiae* cells entrapped in a polyacrylamide gel and performing alcoholic fermentation. Eur J Appl Microbiol Biotechnol 12:10–15

Sitton OC, Gaddy JL (1980) Ethanol production in an immobilized cell reactor. Biotechnol Bioeng 22:1735–1748

Wada M, Kato J, Chibata I (1980) Continuous production of ethanol using immobilized growing yeast cells. Eur J Appl Microbiol Biotechnol 10:275–287

Wada M, Kato J, Chibata I (1981) Continuous production of ethanol in high concentration using immobilized growing yeast cells. Eur J Appl Microbiol Biotechnol 11:67–71

Yamané T, Nakatani H, Sada E, Omata T, Tanaka A, Fukui S (1979) Steroid bioconversion in water-insoluble organic solvents: Δ^1-dehydrogenation by free microbial cells and by cells entrapped in hydrophilic or lipophilic gels. Biotechnol Bioeng 21:2133–2145

Yamané T, Shiotani T (1981) Interrelationship among specific rates of cell growth, substrate consumption and metabolite formation in some simple microbial reactions producing primary metabolites. Biotechnol Bioeng 23:1373–1387

Received June 12, 1981