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# Experimental Studies of Immobilized-Yeast, Packed-Bed Reactors with Reduced $CO_2$ Entrapment

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## ABSTRACT

A horizontal packed-bed reactor with baffles (HPBR) and a continuously stirred tank reactor with intermittent paddle agitation have been shown to considerably reduce the $CO_2$ entrapment when glucose is fermented with immobilized baker's yeast in calcium alginate beads. Using high cell contents in the gel resulted in internal mass transfer hindrance. The highest productivity was obtained with the HPBR giving 29 g EtOH/L·h at an ethanol yield of 90%. The substrate used was 100 g/L glucose. Fermentation of lactose and deproteinized whey by coimmobilized baker's yeast and β-galactosidase resulted in much lower productivity—about 5 g EtOH/L·h because of the slow fermentation of galactose.

**Index Entries:** Immobilization; coimmobilization; alginate; yeast; $CO_2$ entrapment; reactor.

## INTRODUCTION

During recent years considerable attention has been given to the continuous production of ethanol with immobilized cells. Frequently used support materials are different kinds of gels such as carragenan and calcium alginate (*1,2*) with the cells immobilized in small beads. The advantages quoted are numerous: high productivity, low capital costs due to reduced equipment size, and low separation costs as no cell separation after the fermentation step is necessary. However, there are as yet no large-scale applications. Some of the reasons for this are problems with contamination in continuous production and a lack of adequate methods

for the scale-up of bioreactors. The engineering principles of whole-cell immobilization are discussed in an excellent review by Karel et al. (*3*).

The design of the bioreactor requires an understanding of the mass transfer characteristics (*4*). A knowledge of the internal mass transfer is essential, especially with a high cell content in the calcium alginate carrier. Diffusion limitations will arise causing a reduction in reaction velocity (*5,6*). At the same time this can cause bead rupture. This is caused by supersaturation of carbon dioxide within the beads (*7*).

The external mass transfer can, of course, influence the reaction velocity to a certain extent, but this is often negligible (*8,9*). A much more important drawback in bioreactor performance is the so-called $CO_2$ gas effect. According to the reaction

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

equimolar amounts of ethanol and carbon dioxide are produced. The gas evolved at a very high rate causes dead spaces in the packed bed. This will make a large portion of the immobilized cells inactive. In a vertical packed-bed reactor the increased hydrostatic pressure will result in compaction of the bed, especially in the top of the reactor (*10,11*).

Many bioreactors of varying degrees of sophistication have been suggested for minimizing the gas effect. A multistage reactor was shown to give a better utilization of the reactor than a vertical tubular reactor (*11*). Other reactors reported are the pulsed-packed-bed reactor (*7*), the rhombus-shaped bioreactor (*12*), the rotating packed-drum reactor (*13*), and the stirred basket reactor (*14*).

Another possibility is to use film reactors with the cells immobilized in gel films or on sheets (*15–18*). These kinds of reactors avoid the gas effect but can give a lower reactor productivity because the carrier for the film will reduce the active reactor volume.

One of the largest semicommercial plants using cells immobilized in calcium alginate beads for ethanol production has a total reactor volume of 20 $m^3$. This is divided into two column reactors with a ht/diam ratio of about 6 (*19,20*). Fluid-bed reactors can reduce the gas effect considerably (*21*). $Al_2O_3$, $Fe_2O_3$, or some other agent is often immobilized in the beads to enhance the fluidization properties. This kind of reactor is especially suitable for aerobic fermentation.

Some interest has been shown in the use of horizontal reactors (*22,23,7,24,25*). The adverse effect of the $CO_2$ gas is minimized as a result of the shorter path length of the gas leaving the bed. A horizontal bed reactor can be improved by careful design to minimize backmixing, which is detrimental to the ethanol fermentation, especially at high ethanol concentration.

From a strictly kinetic point of view the best choice ought to be a continuous stirred tank reactor (CSTR) followed by a plug flow reactor (PFR) in series. With this arrangement the substrate inhibition is reduced by an immediate decrease in the substrate concentration. In the following, PFR

product inhibition is less severe because of the minimized backmixing. However, this arrangement is not used very often.

From an engineering point of view, it is of great advantage to use as simple a reactor as possible, thus facilitating operation.

The purpose of this study was to examine the performance of a horizontal reactor and compare it with that of a conventional tank reactor with intermittent stirring. Model systems used are anaerobic fermentation of glucose to ethanol with baker's yeast immobilized in calcium alginate gel and anaerobic fermentation of lactose and whey to ethanol with coimmobilized yeast and β-galactosidase (*26–28*).

## MATERIALS AND METHODS

### *Materials*

Sodium alginate was of the type Manugel GMB. *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethyl-amino-propyl-1)-carbodi-imid HCl (EDC) were purchased from Sigma Chemical Co. The β-galactosidase, from *Aspergillus oryzae,* was a generous gift from Miles Kali-Chemie GmbH&Co., KG, Hannover, Germany. The glucose used was dextrose monohydrate. Sweet spray-dried whey permeate powder was kindly supplied by the diary in Götene, Sweden. Other chemicals were of reagent grade and purchased from other commercial sources.

### *Microorganism*

Baker's yeast (Svenska Jästbolaget, Sweden) was used in the glucose fermentations. Dried baker's yeast (Danstar Ferment Ltd., Denmark) was used in the lactose and whey fermentations.

### *Medium*

#### *Glucose and Lactose Fermentation Medium*

Glucose 100 g/L or lactose 48.3 g/L, yeast extract (Difco) 2.5 g/L, $(NH_4)_2HPO_4$ 0.25 g/L, $MgCl_2 \cdot 6H_2O$ 0.025 g/L, KCl 0.5 g/L and $CaCl_2 \cdot 2H_2O$ 1.47 g/L were dissolved in an acetate buffer solution (0.1 M) to a pH of 4.5. $CaCl_2$ was added to stabilize the alginate gel used as the immobilization matrix.

#### *Whey Fermentation Medium*

Whey permeate powder, 55.6–109.6 g/L, was dissolved in acetate buffer to a pH of 4.5.

### *Immobilization of Yeast Cells*

A 2.1% (w/v) sodium alginate solution was prepared using distilled water. This was mixed with a yeast suspension resulting in a yeast cell

concentration of 17.1 or 29.2% (w/v, wet basis), corresponding to a yeast cell concentration of 7.0 and 11.0% (dw) in the gel after shrinkage. The alginate–yeast suspension was injected into a calcium chloride solution (0.1*M*) through a nozzle. The nozzle consisted of 19 needles, each with an inner diameter of 0.55 mm. The flow of the suspension through the nozzle was regulated by applying an air pressure on the liquid surface.

After curing in a 0.01 M $CaCl_2$ solution and activation in a glucose medium (50 g/L) the average size of the beads obtained was 2–2.5 mm throughout all the experiments.

### *Coimmobilization of Yeast Cells and β-Galactosidase*

Baker's yeast cells were coimmobilized with β-galactosidase as previously described (*26–28*). The enzyme (75 g) was bound to 15 g sodium alginate using 9 g NHS and 15 g EDC in 1200 mL of water. The enzyme coupling reaction was carried out at room temperature and was completed after 15 h. The alginate β-galactosidase complex was then coentrapped with 135.7 g (dw) yeast cells in another 15 g sodium alginate solution (1200 mL water) in the form of beads as described earlier.

Curing in 0.01 M $CaCl_2$ and activation in a lactose medium (50 g/L) resulted in a shrinkage of the beads to 55% of the original volume. Therefore this process resulted in 1475 g beads (wet weight).

### *Analytical Methods*

The effluent samples were filtered through a 0.45-μm filter before analysis. The samples from the lactose and whey fermentations were also heat-treated in a microwave oven for 10 s to prevent any hydrolysis reaction in the samples if enzyme was lost from the carrier.

Glucose, galactose, lactose, and ethanol were analyzed using high-performance liquid chromatography. A Shimadzu chromatograph was used, with a column of 0.78 cm inner diameter and length of 30 cm (Biorad Aminex HPX-87H) placed in an oven at 60°C. Diluted $H_2SO_4$ (5 m*M*) was used as the diluent at a flow rate of 0.5 mL/min. A refractive index detector, ERMA ERC-7510, was used.

The optical density of the effluent was measured with a colorimeter (Beckman-C) at 520 nm, and the cell concentration was determined from the calibration curve.

## BIOREACTORS

### *Small-scale Packed-bed Reactors*

Two identical reactors with a total volume of 56.5 mL (diam 3 cm, ht 8 cm), respectively, were packed with different amounts of gel beads (9.4 mL and 28.3 mL). The reactors were continuously operated by feeding the substrate into the bottom of the reactors.

*Reactor with Alginate Gel Films (Fig. 1a)*

Six rectangular gel films (7 cm × 50 cm) were mounted in parallel in a stainless steel holder. The films were placed in a plexiglass reactor that could be run vertically or horizontally. The total reactor volume was 1730 mL; length 500 mm, height 70 mm, and width 40 mm.

To make a stable gel film a nylon net (opening 1.0 mm, nylon line diameter 0.4 mm) was used as reinforcement. This was fixed onto a stainless steel frame. The gel film was prepared by first submerging the nylon net in a yeast-alginate suspension. Immediately afterwards, the net was placed in a liquid bath of 0.1 M $CaCl_2$ solution. After about an hour the gel sheet was stable enough to be removed and placed in the reactor which was filled with a 0.01 M $CaCl_2$ solution. The gelled preparation was cured for about 1 d.

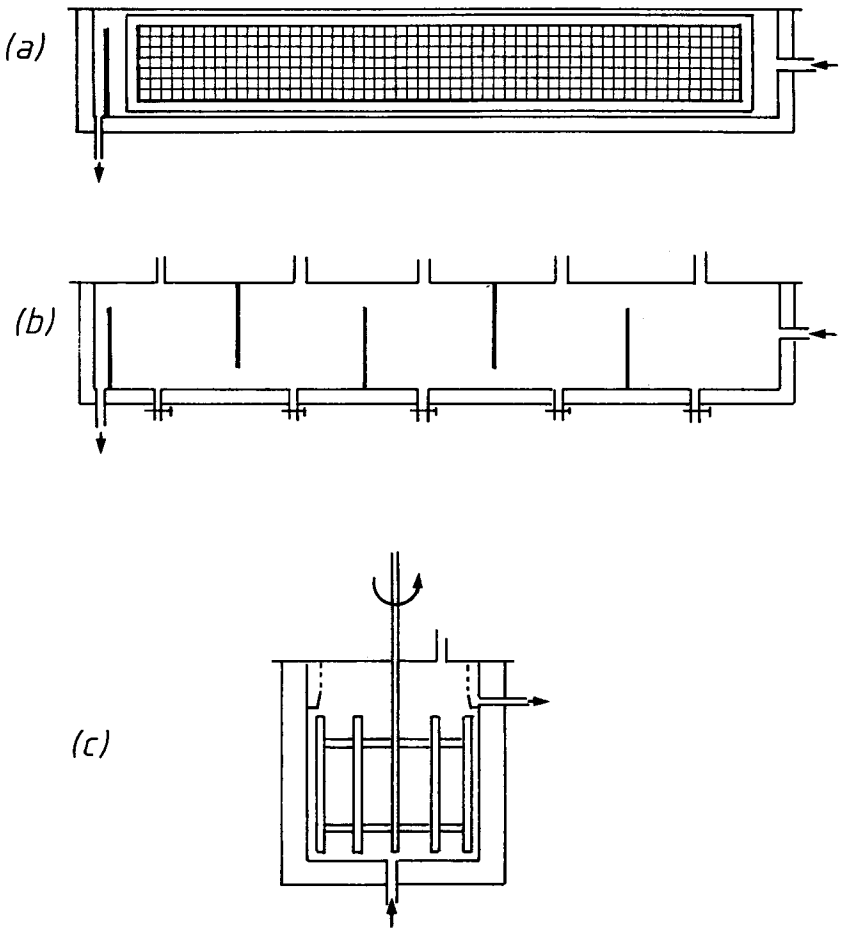


Fig. 1. (a) Reactor with yeast immobilized in alginate sheets; (b) horizontal packed bed reactor with baffles (HPBR); (c) intermittently stirred tank reactor (ISTR).

*Horizontal Packed-bed Reactor (HPBR) (Fig. 1b)*

The final design of the HPBR is shown in Fig. 1b. The total reactor volume was 1680 mL with a length of 500 mm, height of 70 mm, and width of 40 mm. It was constructed in plexiglass with a thermostat jacket covering the sides and the bottom. The reactor was equipped with four baffles dividing the reactor into five compartments of equal size. Besides the baffles, each compartment was divided from the others by a nylon net to avoid movement of the gel beads between the compartments.

*Intermittently Stirred Tank Reactor (ISTR) (Fig. 1c)*

Two identical reactors of this kind were constructed in plexiglass with thermostat jackets, as can be seen in Fig. 1c. The total reactor volume was 1900 mL with an inner diameter of 140 mm and a liquid height of 140 mm. The substrate flow was fed into the bottom of the reactor and the effluent left the reactor via an overflow arrangement. The agitator used was a paddle or gate impeller. The agitator was equipped with an agitator speed controller and a timer with which the agitator could be driven intermittently.

## EXPERIMENTAL SET-UP

A schematic layout of the experimental setup is shown in Fig. 2. The substrate was stored in a refrigerator at 8°C and pumped continuously to the reactor with a peristaltic pump. The temperature in the reactor was kept at 30°C. The flow of substrate was measured by sampling the outflow during a given period and was double-checked against the decrease in the substrate tank. The flow from the ISTR was found to be very stable. The flow from the HPBR sometimes varied by up to ±15%, especially at high space velocities. This was caused by variation in the gas holdup in the reactor. The space velocities calculated were therefore mean values. The rate of $CO_2$ gas evolution was measured by collecting

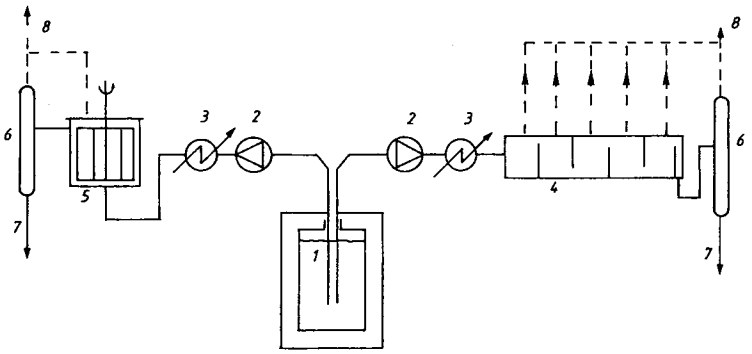


Fig. 2. Experimental setup. 1. Substrate tank; 2. peristaltic pump; 3. heat exchanger; 4. HPBR; 5. ISTR; 6. gas–liquid separator; 7. product outlet; 8. $CO_2$ vent.

800 mL of gas and noting the time required. This was used as an indication of reactor performance. When the gas production was constant, steady state was assumed to have been attained and samples were then taken from the effluent. The cell concentration was not monitored regularly. Samples were taken occasionally to determine maximum cell concentration in the effluent.

After the beads had been cured for about 24 h they were put into the reactors. In most cases the activation was performed with the fermentor medium. In the glucose fermentations up to 50 h were required to attain steady state. In the lactose fermentation this time was considerably longer (up to 80–90 h). The experimental runs lasted for up to 14 d. During this time the space velocity was varied. At least five reactor volumes of the fermentor medium had to pass through the apparatus before a new steady-state condition was obtained. To check the reproducibility the experimental run was concluded by reproducing the startup conditions. If these were not consistent the run was rejected.

As the microorganism used in these investigations was commercial baker's yeast, it was to be expected that the activity and the kinetic behavior would vary for different experiments. Therefore, the reactors were run in parallel with beads from the same batch, to facilitate a true comparison. Up to three reactors could be run simultaneously.

## RESULTS AND DISCUSSION

For all the experiments, the results were evaluated as the yield of ethanol expressed as percent of theoretical maximum. This means that 100% ethanol yield would only be obtained if all the glucose or lactose was fermented to ethanol. Of course, a portion of the substrate is utilized for cell mass, maintenance, and secondary product formation. The yield of ethanol based on fermentable sugar varied between 90–95%. This is somewhat higher than that expected from an ATP balance. Space velocity (SV) is defined as flow/gel volume (l/l gel·h) and dilution rate (DR) as flow/reactor volume (l/l·h). Finally, productivity is expressed as mass flow of ethanol/reactor volume (g EtOH/l·h).

### *Small-scale Reactor Experiments*

The influence of the external mass transfer has been studied by several researchers (*8,9,29*) and found to be negligible. This means that the ethanol yield must be constant if the space velocity is kept constant while the gel volume or cell concentration in the reactor is varied. Two different gel volumes were used, 9.4 and 28.3 mL in two small packed-bed reactors run in parallel with a total reactor volume of 56.5 mL. The space velocity was kept constant by increasing the flow velocity from 14 to 42 mL/h. From Fig. 3 it can be concluded that the ethanol yield is about the same for the two reactors, at least for concentrations greater than 100 g/L. To

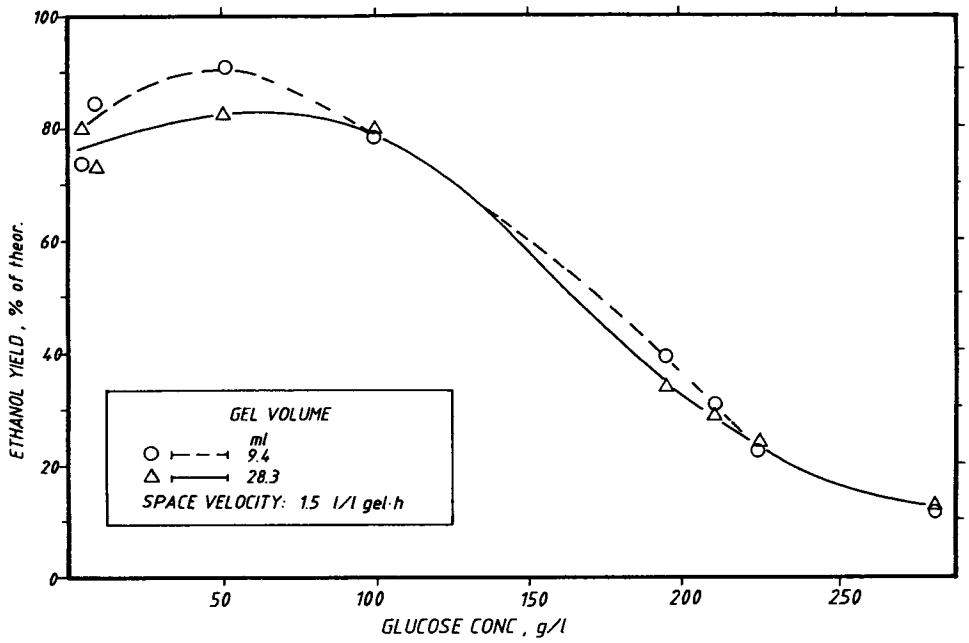


Fig. 3. Influence of glucose concentration on ethanol yield at constant space velocity (sv).

| | | |
|---|---|---|
| Gel volume (mL) | 9.4 | 28.3 |
| Liquid volume (mL) | 47.1 | 28.2 |
| SV. (1/1 gel·h) | 1.5 | 1.5 |
| DR. (1/1·h) | 0.25 | 0.74 |
| Flow (mL/h) | 14 | 42 |
| Cell conc. [% (dw) of gel] | 7.8 | 7.8 |

ensure a true comparison the experiment was started with a glucose feed concentration of 224 g/L and kept at this level until steady state was obtained (after 21 h). After changing to a new glucose level steady-state conditions were obtained after about five reactor volumes of substrate flow. To ensure full reproducibility the whole experimental run was concluded with a glucose feed concentration of 210 g/L, which gave an ethanol yield consistent with the startup conditions.

The difference at 50 g/L glucose concentration is probably caused by loss of ethanol in the vented $CO_2$ from the reactor with 28.3 mL gel vol. The $CO_2$ measurement indicated an 86% yield for this reactor on this occasion. The decrease in ethanol yield at lower concentrations is probably caused by maintenance. If a maintenance coefficient of 0.036 g glucose/g cells (dw)·h (*30*) is assumed this corresponds to a 20% consumption of glucose at a glucose concentration of 10 g/L. For lower concentrations the ethanol yield is expected to be even lower.

The most important finding from these small-scale experiments is that the ethanol yield should be interpreted as a function of space velocity expressed as l/l gel·h. The dilution rate, whether based on liquid

volume in the reactor or on total reactor volume, is not suitable for adequate comparisons between different reactors. The dilution rate based on total reactor volume, in this case 0.25 and 0.74 l/l·h, respectively, is instead important when reactor productivity is discussed.

### *Reactor with Gel Films*

An interesting alternative for minimizing the gas effect is to immobilize yeast cells on sheets or in films (*15–18*). Therefore a reactor with six parallel gel films was constructed. The reactor was continuously fed with 100 g/L glucose. The cell concentration in the gel was 11.0% (dw). During a period of 350 h the ethanol yield was monitored at three different space velocities. Experiments were performed with the reactor mounted horizontally and vertically. The results are presented in Table 1. There is obviously no difference between the vertical and horizontal reactor orientations. The main disadvantage is the low productivity caused by the limited gel volume in the reactor (19% of reactor volume). For practical reasons it was not possible to use more than six gel films in the reactor. The frames required as holders for the nylon nets took up too much space. Stainless steel nets could of course be used without frames, but the gel tended to slip off the steel wires and the film was destroyed. From an engineering point of view the problems involved in making this kind of gel film on a larger scale are obvious. The glucose utilization was higher than that indicated by the ethanol yield. A yield factor varying between 84–87% was obtained, i.e., much lower than in the other reactor experiments. The cell concentration in the outlet was not measured, but there was considerable cell growth on the gel surfaces. After the experiment was terminated a considerable amount of sediment was found in the reactor indicating excess cell growth. Although the ethanol yield was comparable to that of the other reactors used (Fig. 5) it was decided not to develop this reactor further.

### *Horizontal Packed Bed-reactor*

In order to increase the cell concentration the reactor was filled with alginate gel beads. The activation procedure was performed with and

Table 1
Reactor with Alginate Gel Films[a]

| SV, | DR, | Ethanol yield, % | | Productivity, g EtOH/1·h | |
|---|---|---|---|---|---|
| 1/1 gel·h | 1/1·h | Horizontal | Vertical | Horizontal | Vertical |
| 1.1 | 0.2 | 69 | 69 | 7.0 | 7.1 |
| 1.8 | 0.3 | 65 | 64 | 9.9 | 9.8 |
| 2.4 | 0.4 | 55 | 53 | 11.3 | 10.8 |

[a]Reactor volume: 1730 mL; gel volume: 328 mL; substrate: 100 g/L glucose; cell conc.: 11% (DW) of gel; gel film thickness: 1.5 mm.

without aeration with the same result. A difference is just noticeable at very low cell concentrations.

Since high productivity is valuable only if there is at least 90% utilization of the substrate, the space velocity was lowered until a 90% ethanol yield was obtained. About 61% of the total reactor volume was then filled by alginate beads (cell concentration 10.9% (dw) of the gel). The results are presented in Table 2 and Fig. 4. Extremely low space velocities had to be used to obtain sufficient glucose conversion. Large gas pockets could be seen in the packed bed. By coloring the substrate flow it was noticed that much of the liquid bypassed the packed bed by flowing over the bed. From this experiment it could be concluded that too great a gel volume can cause a detrimental effect on reactor performance. Gas pockets, making a large proportion of the bed inactive, in combination with a bypass flow, lowered the conversion considerably.

To minimize these effects the gel volume was reduced to 825 mL (47% of the total reactor volume) and four baffles were introduced into the reactor dividing it into five equal compartments containing equal amounts of gel beads. The cell concentration in the gel was kept approximately constant. The reactor performance was improved, as can be seen from Table 2 and Fig. 4. Bypass flow could still arise, as was seen by coloring the substrate flow.

Finally, the baffle arrangement was reconstructed, still dividing the reactor into five compartments, but ensuring that no bypassing was possible. The final reactor construction can be seen in Fig. 1b. When using the same gel volume and the same cell concentration the ethanol yield and productivity were considerably enhanced, as can be seen from Table 2 and Fig. 4.

The reactor with this final baffle arrangement was used hereafter and will be referred to as HPBR (horizontal packed-bed reactor with baffles).

It is important to notice that a horizontal bed must be carefully designed to guarantee that the liquid will not find any "short-cuts." This bypass flow can be detrimental for the reactor performance. The main disadvantage is that the gel volume in the first compartment increased to some extent, as a result of cell growth, until a steady state was attained.

Table 2
Improving the Horizontal Reactor

| Reactor | Ethanol yield, % | SV, 1/1 gel·h | Reactor volume, mL | Gel volume | | Productivity, g EtOH/1·h |
|---|---|---|---|---|---|---|
| | | | | mL | % of reactor | |
| Without baffles | 90 | 0.08 | 1680 | 1020 | 61 | 2.3 |
| Baffles partly bypassed | 91 | 0.12 | 1770 | 825 | 47 | 2.8 |
| With baffles (HPBR) | 89 | 1.31 | 1680 | 830 | 49 | 29.4 |

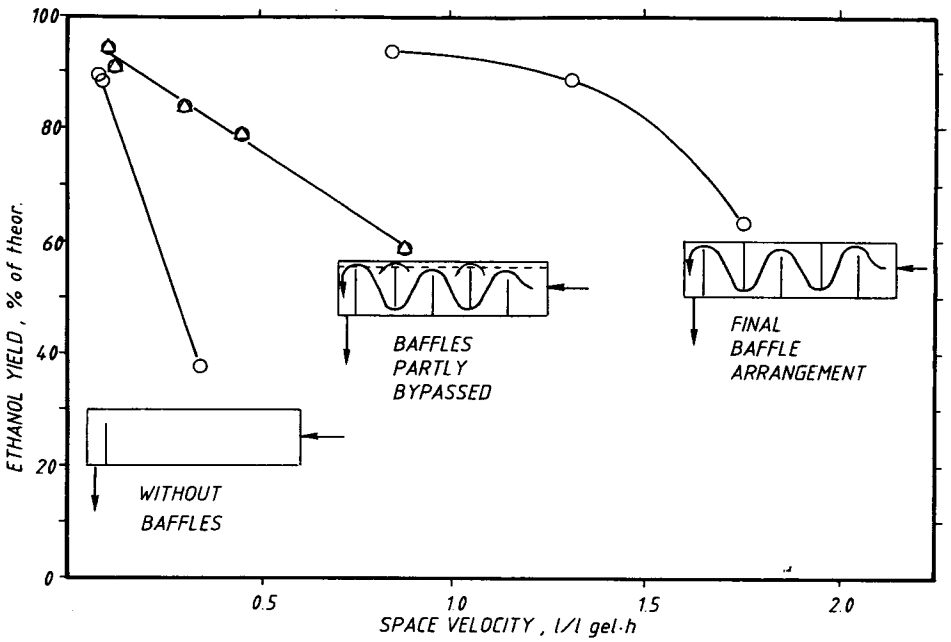


Fig. 4. Improving the design of the horizontal packed-bed reactor (data from Table 2).

As a consequence of this, bead rupture was much more frequent in this compartment.

Some of the gel beads were exposed to the gas phase above the liquid surfaces in all the compartments, which lowered the conversion efficiency.

### *Intermittently Stirred Tank Reactor (ISTR)*

As pointed out earlier a stirred tank reactor can be an alternative to the plug-flow reactor in certain cases, especially when the reaction velocity is reduced by substrate inhibition. The problem with stirred tank reactors using alginate beads is severe degradation of the beads by the high shear forces produced by the agitator. Therefore a paddle agitator was used to minimize the shear forces giving almost no bead degradation at all. As the external mass transfer is not rate-limiting the agitation was intermittent. With this arrangement there were no $CO_2$ gas pockets in the bed. The number of revolutions per minute varied with the flow of substrate but was kept at around 1–2 rpm with a paddle tip velocity of 1.5 cm/s. The results can be seen in Fig. 5. Two different gel volumes were used, 43% and 58% of the total reactor volume.

### *Comparison of ISTR and HPBR*

In Fig. 5 it can be seen that the ethanol yield was substantially lower in the ISTR than in the HPBR. It should be noted that the ISTR experi-

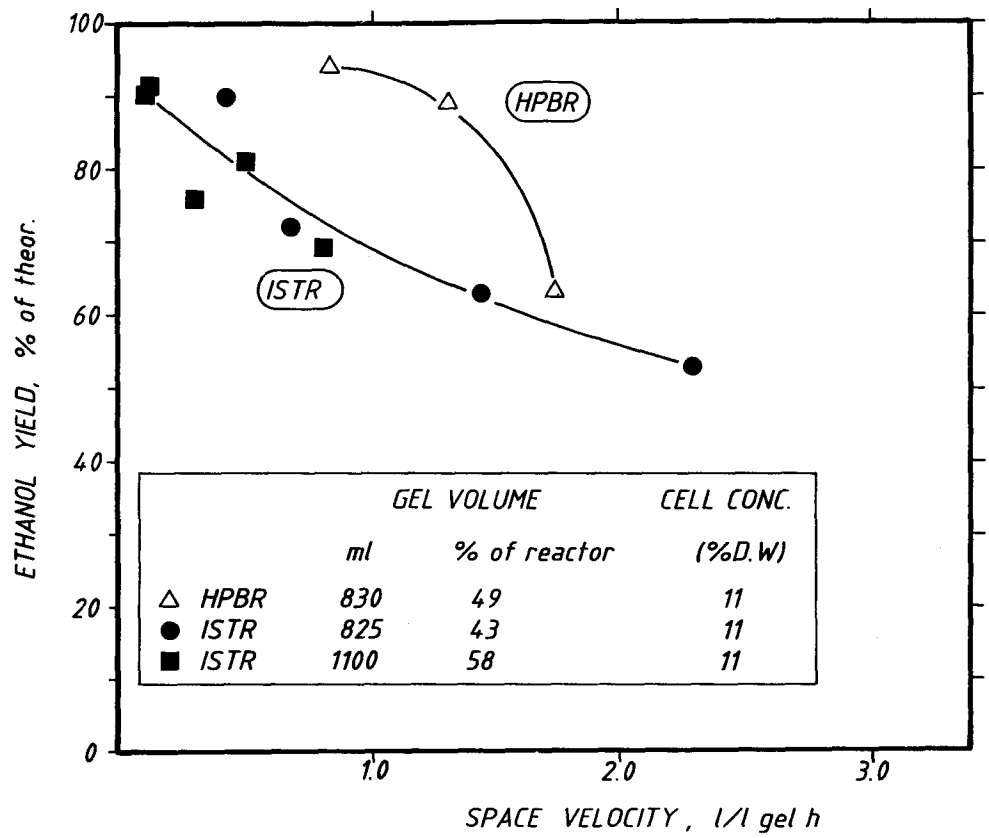


Fig. 5. Ethanol yield as a function of SV for the HPBR and the ISTR at high cell concentration.

| | HPBR (Δ) | ISTR (●, ■) |
|---|---|---|
| Reactor volume (mL) | 1680 | 1900 |
| Productivity at ~ 90% ethanol yield | 29.4 | 2.3 |
| SV. at ~ 90% ethanol yield | 1.31 | 0.11 |

ment with 1100 mL gel vol and the HPBR-experiment with 830 mL gel vol were run simultaneously with beads made from the same batch. It can be concluded that the plug-flow reactor is to be preferred at this glucose concentration.

Many authors have pointed out that the internal mass transfer can reduce the reaction velocity considerably. To explore the reactor performance at a lower cell concentration beads were made with a cell content of 7% (dw). The gel volumes used were 660 mL gel in the ISTR and 470 mL gel in the HPBR.

These experiments were run simultaneously with beads made from the same batch. The results are presented in Fig. 6. The difference between the two reactors is much smaller than when a higher cell concen-

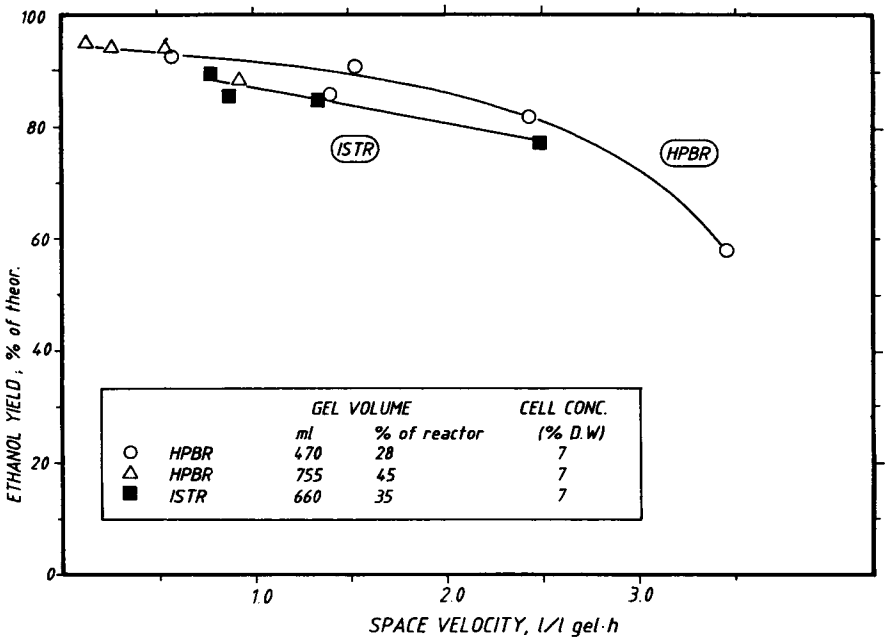


Fig. 6. Ethanol yield as a function of SV for the HPBR and the ISTR at low cell concentration.

| | HPBR (o) | HPBR (Δ) | ISTR (■) |
|---|---|---|---|
| Reactor volume (mL) | 1680 | 1680 | 1900 |
| Productivity at ~90% ethanol yield | 12.3 | 16.1 | 11.9 |
| SV. at ~90% ethanol yield | 0.98 | 0.79 | 0.76 |

tration was used. The most striking effect is that a higher ethanol yield is obtained for both reactors which indicates a severe internal diffusion hindrance by the high cell content in the gel matrix.

In Fig. 7 and 8 the experiments illustrated in Fig. 5 and 6 are compared for each reactor. The comparison is made on the basis of space velocity expressed as l/l gel·h which in this case is not quite adequate. If the space velocity is instead expressed as l/g cells·h, the difference would be even larger. This means that the conclusion of internal mass transfer hindrance still holds and is further stressed.

A final glucose experiment with the HPBR was performed with an increased gel volume (755 mL) and a cell content of 7% (dw). If the productivity values obtained are to be used for economical evaluation of the fermentation process, the ethanol yield must be high. Therefore this experiment was carried out with low space velocities to ensure sufficient glucose conversion. The results are presented in Fig. 6. This experiment extends the HPBR curve to lower space velocity values. It also confirms the earlier results that different gel volumes do not influence the ethanol yield when the space velocity is expressed as ℓ/ℓ/gel·h.

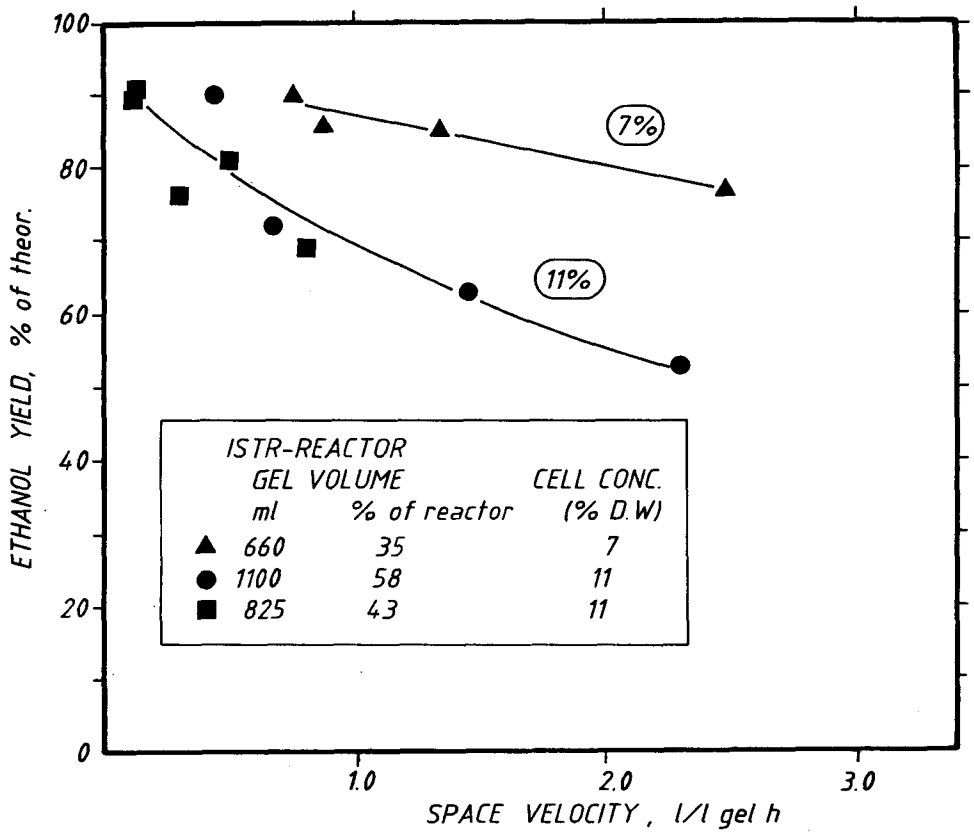


Fig. 7. Ethanol yield as a function of SV for the ISTR at different cell concentrations.

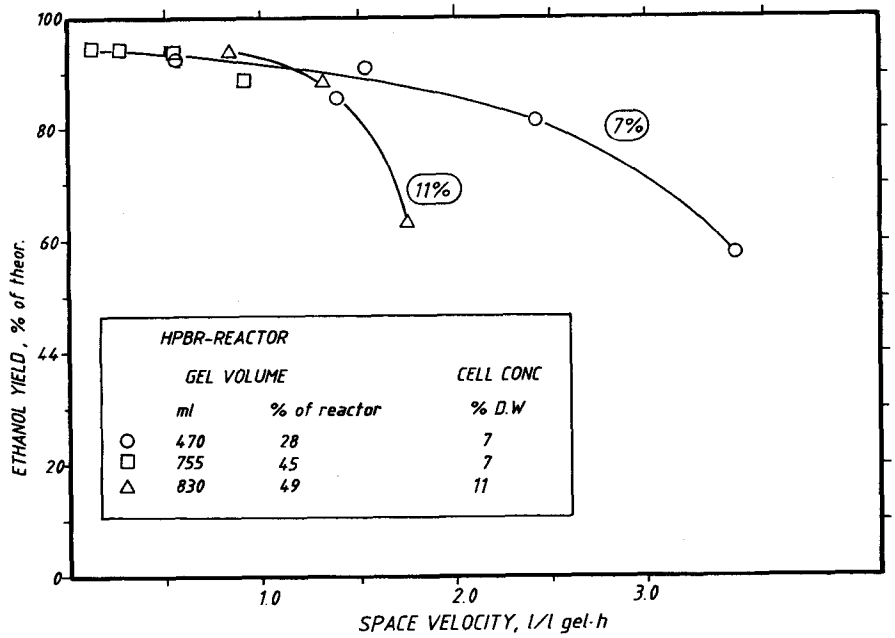


Fig. 8. Ethanol yield as a function of SV for the HPBR at different cell concentrations.

Throughout all the experiments there was no significant difference in outlet cell concentration which ranged from 0.1 to 0.2 g cell (dw)/L.

### *Reactor Experiments with Coimmobilized Yeast and β-Galactosidase*

The reaction sequence

$$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6(glu) + C_6H_{12}O_6(gal)$$
$$C_6H_{12}O_6(glu) \rightarrow 2C_2H_5OH + 2CO_2$$
$$C_6H_{12}O_6(gal) \rightarrow 2C_2H_5OH + 2CO_2$$

is more complicated than the glucose fermentation.

The hydrolysis is inhibited by galactose while the glucose and galactose fermentations are inhibited by the substrate and the product. This, coupled with the internal mass transfer behavior, requires mathematical modeling to make the right choice of the reactor. This work is under way and will be published (*31*).

Two initial experiments with the ISTR and HPBR were run in parallel to reveal possible critical factors. The experiment was started with lactose as substrate. After steady-state conditions had been attained the substrate was changed to whey. The results are presented in Table 3. In Fig. 9 it can be seen that steady-state is attained after about 80–90 h, which is considerably longer than in the glucose experiments. Sufficient time is required for the adaptation of the cells to ferment galactose.

From Fig. 9 it can be concluded that galactose is much more slowly fermented than glucose, which is to be expected. A surprising result is that the galactose is fermented more rapidly in the ISTR than in the HPBR, when rather the opposite is to be expected. In general, it is believed that glucose efficiently suppresses the galactose utilization until the glucose is exhausted. No simple explanation can be given for the

Table 3
Anaerobic Fermentation of Lactose and Whey in ISTR and HPBR

| | ISTR | | | HPBR | | |
|---|---|---|---|---|---|---|
| | Lactose | Whey | Whey | Lactose | Whey | Whey |
| Lactose, inlet (g/L) | 48.3 | 55.6 | 109.6 | 48.3 | 55.6 | 105.5 |
| Lactose, outlet (g/L) | 0.7 | 0.7 | 7.7 | 0.5 | 0.6 | 2.9 |
| Galactose, outlet (g/L) | 2.5 | 4.8 | 23.3 | 8.5 | 11.3 | 35.6 |
| Glucose, outlet (g/L) | 0.2 | 0.3 | 1.9 | 0.2 | 0.3 | 3.2 |
| Ethanol, outlet (g/L) | 23.7 | 24.0 | 34.6 | 20.7 | 20.6 | 35.2 |
| SV (1/1 gel·h) | 0.55 | 0.55 | 0.28 | 0.59 | 0.59 | 0.32 |
| Gel volume (mL) | 690 | 690 | 690 | 690 | 690 | 690 |
| Cell conc. (% dw) | 8.1 | 8.1 | 8.1 | 8.1 | 8.1 | 8.1 |
| DR (1/1·h) | 0.20 | 0.20 | 0.10 | 0.26 | 0.26 | 0.14 |
| Productivity (g EtOH/1·h) | 4.7 | 4.8 | 3.5 | 5.4 | 5.4 | 4.9 |

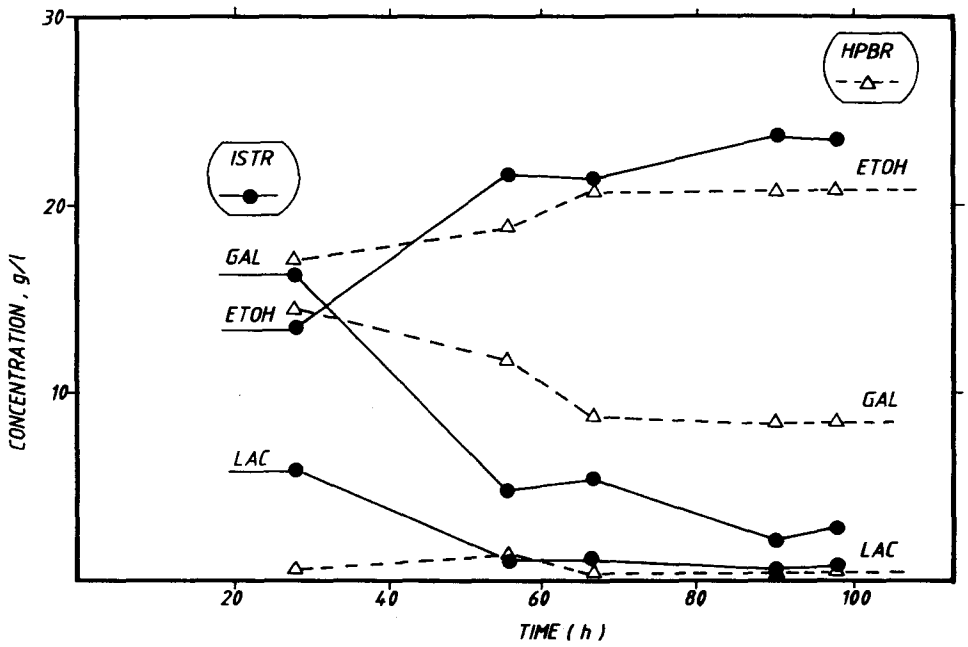


Fig. 9. Fermentation of lactose with coimmobilized baker's yeast and β-galactosidase in gel beads in the ISTR (solid lines) and the HPBR (dashed lines).

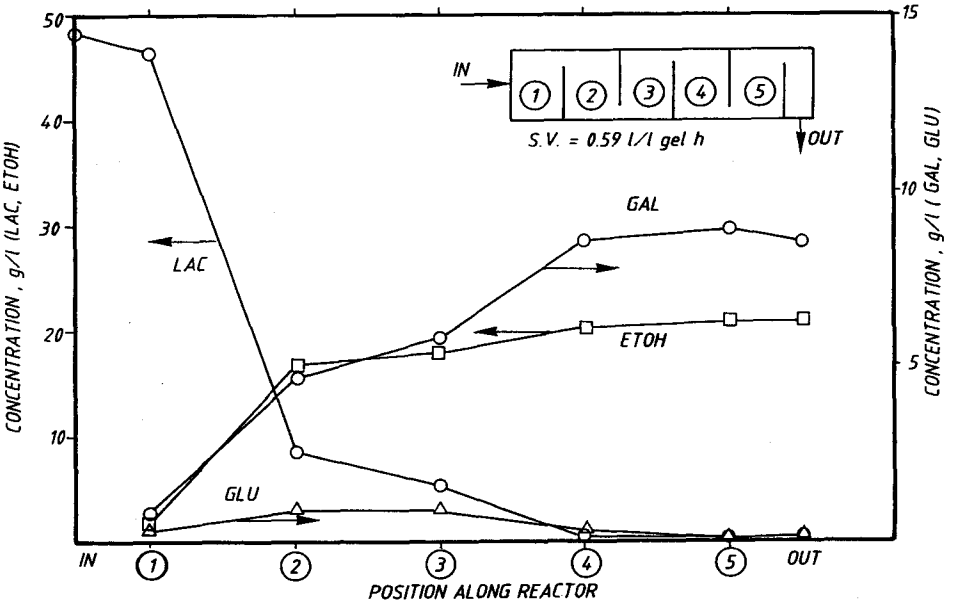


Fig. 10. Concentration profile for the HPBR. 48.3 g/L lactose fermented with coimmobilized baker's yeast and β-galactosidase.

results obtained at this point. The HPBR was reconstructed to allow samples to be taken from each compartment. It is, however, difficult to take a sample that is representative for the whole compartment as there is definitely a concentration gradient due to insufficient mixing. The concentration profile in Fig. 10 shows the approximate behavior of the HPBR. When the substrate was changed to whey, the ethanol yield decreased in both reactors by about 10%. This must be caused by some inhibiting component in the whey. The main bottleneck in the coimmobilization system is the slow fermentation of galactose resulting in much lower productivities (about 5.9 g EtOH/L·h) than in the glucose fermentation (29 g EtOH/L·h).

## CONCLUSIONS

As the external mass transfer not is rate-limiting in glucose fermentation it has been possible to show through small-scale reactor experiments, that different reactors must be compared on the basis of ethanol yield as a function of space velocity expressed as l/l gel·h as long as the cell content in the gel is constant. Using this approach a horizontal packed bed with baffles (HPBR) has been compared with an intermittently stirred tank reactor (ISTR). The experiments have shown that a HPBR must be carefully designed to prevent bypass flow in the reactor that can be detrimental to reactor performance. Continuously stirred reactors are often said to be unsuitable in combination with alginate beads because of bead degradation. With intermittent agitation using a paddle the packed bed is efficiently degassed from $CO_2$, even with a gel volume as high as 58% of the reactor volume.

Experiments with different cell contents in the gel point to a problem of internal mass transfer hindrance as a result of too high a cell content in the gel. This has been investigated further by measurements of effective diffusivities in alginate gels with different amounts of cells that confirm the results of the experiments (*6*).

For the glucose fermentation the highest productivity, 29 g EtOH/L·h at 90% ethanol yield, is obtained with the HPBR.

Fermentation of lactose or whey by coimmobilized yeast and β-galactosidase results in a much lower productivity—about 5 g EtOH/L·h. The main bottleneck in this case is the slow fermentation of galactose.

Further work is still needed to construct adequate mathematical models for predicting reactor performance and facilitating the choice of reactor (*31*).

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