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# Novel Hollow Fiber Immobilization Techniques for Whole Cells and Advanced Bioreactors

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## ABSTRACT

A novel microporous hollow fiber membrane-based immobilization technique for whole cells has been developed. Yeast cells (*Saccharomyces cerevisiae*) were grown on chopped hydrophobic microporous hollow fibers as well as on hydrophilic hollow fibers. This immobilization support was used to carry out fermentation in a tubular bioreactor. Air was passed from time to time to facilitate cell growth. The microbial culture reached a very high cell density level of around $10^{10}$/mL of fiber lumen volume. An ethanol concentration of 45 g/L and productivity of 41 g/L-h were obtained with an initial glucose concentration of 100 g/L. The present technique does not have the shortcomings of conventional immobilization methods.

**Index Entries:** Immobilization; hollow-fibers; microporous; fermentation; yeast.

## INTRODUCTION

Different techniques and supports used for immobilization of whole cells and enzymes have been reviewed extensively by Chibata (1978), Venkatasubramanian (1980), Klibanov (1983), and Karel et al. (1985). Among these, hollow fiber entrapment of whole cells is being increasingly investigated. Examples include yeast immobilization (Inloes et al., 1983), plant cell immobilization (Shuler, 1981; Prenosil and Pedersen, 1983) and animal cell immobilization (Chick et al., 1979).

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In such hollow fiber immobilization systems, the cells may be entrapped in the fiber lumen with substrate solution flowing on the shell side of the device. Alternately, the cells are entrapped on the shell side (the extra capillary space) and substrate solution flows generally through the fiber lumen. Higher cell densities, maintenance of cell viability for extended periods, continuous removal of product and inhibitory wastes, isolation of cells from the main substrate stream, and higher volumetric productivities are major advantages for such devices. Hollow fiber wall ruptures caused by uncontrolled cell growth, diffusional limitations, membrane leakage, gas supply, and removal problems create significant limitations of such methods of cell culture and fermentation processes.

The purpose of this work is to introduce an alternate hollow fiber immobilization technique and study it in the context of various bioreactors. In this technique, we used chopped single hollow microporous fibers or bundles of chopped hollow microporous fibers to grow cells in the fiber lumen as well as in the fiber wall surfaces (and in the interfiber space, in the case of bundles). Such chopped hollow fibers or bundles of hollow fibers with entrapped cells were then utilized in shaker culture flask to demonstrate the wide applicability of this technique of cell immobilization.

The proposed technique is quite advantageous compared to the traditional methods. Uncontrolled cell growth, leading to membrane damage or disruption (Inloes et al., 1983) is of no consequence; a corollary suggests that defective fibers can be used for immobilization. In hollow fiber bioreactors, continuous lengths of hydrophobic hollow fibers may be used for dispersion-free oxygen supply, carbon dioxide removal, and product extraction (Frank and Sirkar, 1985, 1986; Kang et al., 1988) uncoupled from the requirement (e.g., hydrophilicity or wetting of hydrophobic fibers) of cell immobilization. The chopped hollow fibers with immobilized cells are almost neutrally buoyant when wetted hydrophobic polypropylene fibers are used. Further, they do not impose any pH limitations encountered in gel entrapment processes. Additionally such a support, quite often biodegradable, does not pose waste disposal problems as diatomaceous earth particles do and if necessary can be recycled after sterilization.

We present here results of our studies using free and bundled chopped hollow microporous fibers of hydrophobic polypropylene as well as hydrophilic regenerated cellulose for the growth of *Saccharomyces cerevisiae* cells. Hydrophobic polypropylene microporous fibers were utilized generally after they were wetted. Limited studies were also made with chopped fibers whose outside was hydrophilic around a hydrophobic core. These studies were carried out in shaker culture flasks for ethanol production. Studies of ethanol fermentation in the tubular packed bed configuration with chopped hollow fibers have also been made and are reported here. Such studies have also been extended to bioreactors with continuous

Table 1
Physical Properties of the Hollow Fiber Membranes Used[a]

| Membrane | Material | Pore size, μm | O.D., μm | I.D., μm | Porosity |
|---|---|---|---|---|---|
| Celgard X-10 | Polypropylene Hydrophobic | 0.03 | 150 | 100 | 0.2 |
| Celgard X-20 | Polypropylene Hydrophobic | 0.03 | 290 | 240 | 0.4 |
| Cuprophan[b] | Regenerated Cellulose Hydrophilic | — | 200[c] | 140[c] | 0.55[c] |

[a]From manufacturer's catalog.
[b]Pore size not available in manufacturer's catalog.
[c]Measured in our laboratory.

lengths of hydrophobic microporous hollow fibers used for gas supply and product removal *in situ* using dispersion-free techniques (Kiani et al., 1984; Prasad and Sirkar, 1988). These results will be presented elsewhere.

## MATERIALS AND METHODS

### Microorganism

The yeast used was *Saccharomyces cerevisiae* (NRRL Y-132) supplied by Northern Regional Research Laboratories (Peoria, IL). The composition of the medium was the same as that used by Gencer and Mutharasan (1983). This medium was autoclaved and used for fermentation. Inoculum level was about 3% of total volume.

### Materials

Hydrophobic microporous hollow fibers (Celgard X-20, X-10) were obtained from Questar, Charlotte, NC. Cuprophan hollow fibers which are hydrophilic, were procured from Enka (Ashville, NC). The physical characteristics of these fibers are given in Table 1.

### Wetting Procedure

The procedure adopted for wetting the hydrophobic Celgard X-10 and X-20 hollow fibers is similar to that used earlier by Bhave and Sirkar (1987). Basically, this involved wetting the membrane first with a 60% alcohol solution and then replacing the alcohol slowly by sterile water in an exchange process.

## Surface Treatment of Hydrophobic Hollow Fibers

The outer surface of the hydrophobic Celgard X-20 fiber was treated to make it hydrophilic. The procedure adopted is the same as that used by Patel (1987). This involved treating the outside surface of the membrane with a solution of potassium permanganate in concentrated sulfuric acid for four hours. The treated surfaces were washed with sulfuric acid and hydrogen peroxide solutions.

## Analytical Methods

Ethanol concentration was determined in a Hewlett Packard gas chromatograph (model 5890A) using Porapak Q (80/100) or Tenax GC (80/100) column and a flame ionization detector. The glucose concentration was analyzed by a YSI model 27 glucose analyzer. Cell growth in the broth was estimated by measurement of the optical density at 540 nm using a Bausch and Lomb spectrophotometer (model 1001). Cell growth in hollow fibers was measured by subtracting the weight of hollow fibers from the weight of hollow fibers containing cells (these were dried earlier in incubator overnight at 60°C). The growth in hollow fibers was observed by a SEM (model JEOL JSM-80) using samples appropriately prepared (*see next section*).

## Experimental Procedure

The hollow fibers Celgard X-10 and X-20 were chopped to required sizes and then wetted. Cuprophan fibers were also chopped to required sizes. Fibers of 0.25, 0.5, 1, 1.5, and 2 in. length were used. The total length of fibers used was always kept constant at 192 in. The hollow fibers were weighed before putting them in the medium.

One hundred mL of medium was transferred into each of three 250 mL flasks. These flasks were previously sterilized. The wetted hollow fibers Celgard X-10, X-20, and Cuprophan respectively were transferred into these flasks. These flasks were later kept in an incubator at 30°C for 120 h. After cell growth, fibers were withdrawn, washed, and dried overnight in the incubator at 60°C. The weight of hollow fibers was determined and the initial weight of fibers before fermentation was subtracted. The difference in weight of the fibers provided the amount of cells entrapped in the fibers. The cells were also taken out from chopped hollow fibers by stirring these fibers in ethanol overnight. The cells were resuspended and centrifuged, washed, and dried. The same procedure was repeated for every fiber length and type.

In another set of experiments, the outer surface of Celgard X-20 fibers was treated to make it hydrophilic and the above procedure was repeated to carry out cell growth. After growth, the fibers were taken out, washed,

and dried overnight at 60°C in incubator. All the dried fibers were potted in plastic vials with epoxy (Armstrong C-4D type, Beacon Chemicals Co., Mt. Vernon, NY). Cell growth in the pores, lumen and outside surface of fiber was studied using SEM.

The chopped hollow fibers (Celgard X-20) of 1/4 in. length were filled in a tubular fermentor (length 1.5 ft, I.D. 1 in.). Total volume of fermentor was 94.6 mL and void volume was 73 mL. The total weight of chopped hollow fiber in fermentor was 3.804 g. Ethanol (50% v/v), introduced in the fermentor to wet the fibers, was kept for 4 h. After that sterile water was passed continuously to replace ethanol. After wetting, the medium was introduced into the fermentor and the fermentor was then inoculated with previously grown cells. The cells were allowed to grow for 2 d. After growth, the broth was drained and regular medium was allowed to flow.

## RESULTS AND DISCUSSION

We first consider experiments designed to find out whether cells will grow on hydrophobic fibers that were not wetted. Previous extractive fermentation studies with nonwetted hydrophobic hollow fibers in a tubular reactor (Frank and Sirkar, 1985, 1986) had indicated indirectly that cells do not grow on the fibers if they are not wetted.

Yeast cells were grown on wetted and nonwetted microporous hydrophobic X-20 polypropylene fibers in one set of experiments to observe the growth of cells as a function of wetting. Fibers of one inch length were transferred into 100 mL medium in both cases. The total length of fibers was always 192 in. These flasks were inoculated with the yeast cells and incubated at 30°C. After 120 h of growth, fibers were taken out and washed. These fibers were potted with epoxy in plastic vials and growth was examined by a microscope, which was connected to a video screen. The slides for the microscope were prepared with the help of a sladge type microtome Model 1400 (E. Leitz Inc., Rockleigh, NJ). The photographs of growth in lumen were taken by a 35 mm camera from video screen of Zeiss universal microscope (Sylvax Scientific Inc., Morris Plains, NJ). These results are shown in Figs. 1, 2, and 3.

Figure 1 shows the photomicrograph of the end of a nonwetted fiber (X-20) after growth. Figures 2 and 3 show the photomicrographs of wetted fibers after growth at the ends and middle of fibers (X-20), respectively. It is clear that there was no growth in the nonwetted fibers in lumen as well as on surface while growth is evident in wetted fibers at the ends as well as in the middle. Since X-20 polypropylene fibers are naturally hydrophobic, medium can not enter inside the fiber pores and in the fiber lumen. In wetted fibers, water is immobilized in the pores and in the lumen; the nutrients in the medium, therefore, diffuse easily. It is also clear that cells

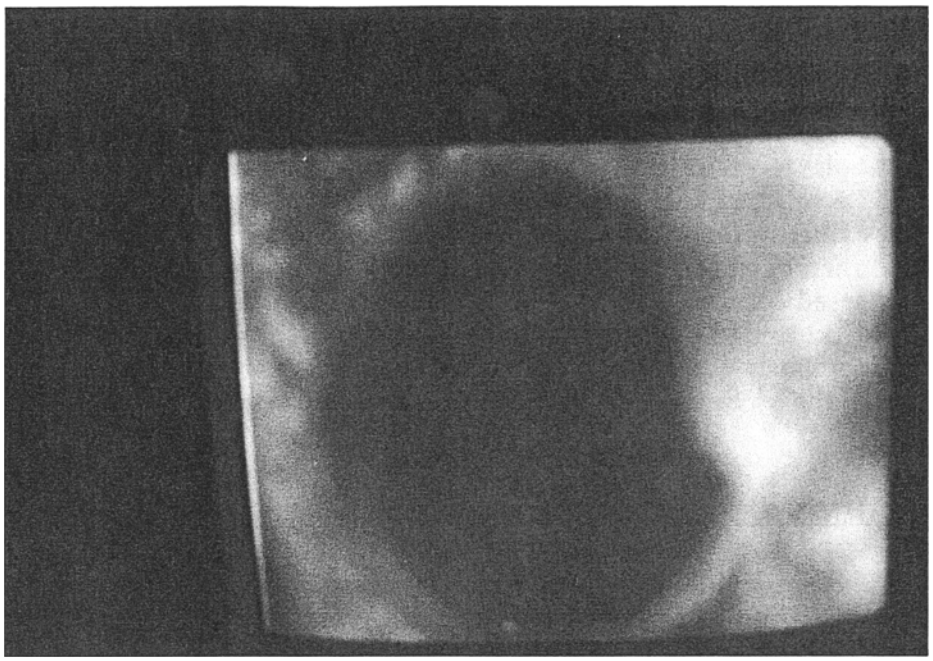

Fig. 1. Microphotograph of nonwetted Celgard X-20 fiber after growth at the end of fiber lumen.

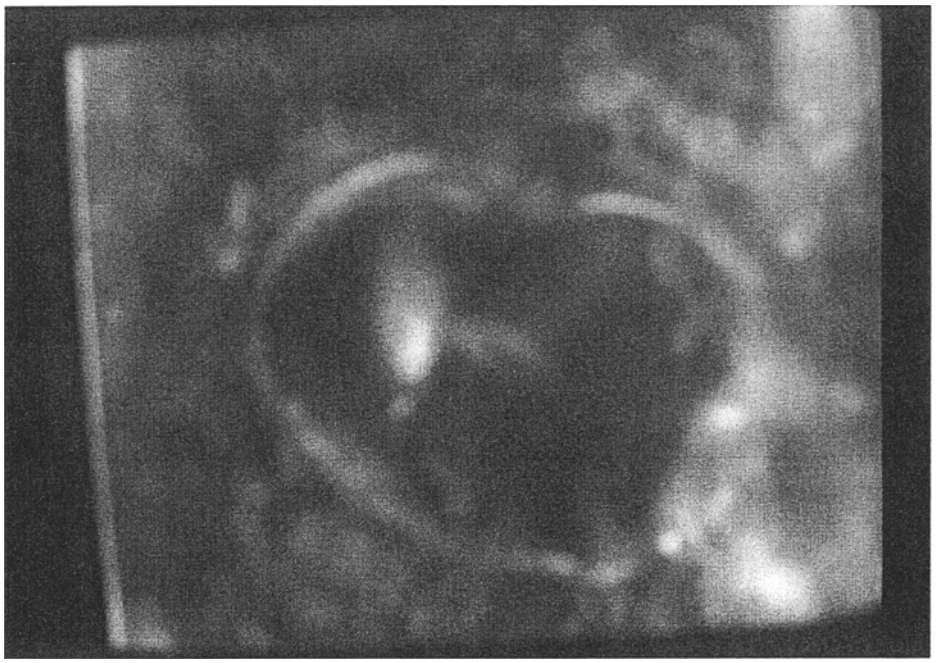

Fig. 2. Microphotograph of wetted Celgard X-20 fiber after growth at the end of fiber lumen.

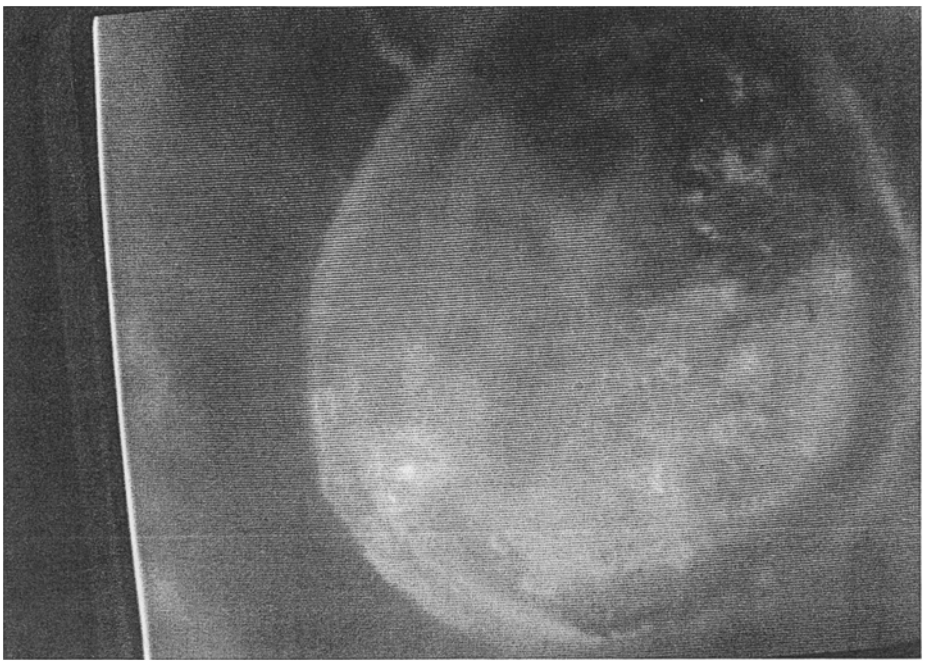

Fig. 3. Microphotograph of wetted Celgard X-20 fiber after growth at the middle of fiber lumen.

grow along the length of the wetted fibers in the lumen as well as on outside surface.

Another set of experiments was conducted to confirm that no epoxy came inside the fiber lumen during potting for sample preparation. The wetted X-20 fibers without cell growth were potted in plastic vials and then photographed. This is shown in Fig. 4, and it is quite clear that epoxy does not come inside the fiber lumen. This also confirmed growth in wetted fibers.

The nutrients and glucose diffuse inside the chopped fibers through the lumen as well as the pores. Therefore, cell growth is likely to be dependent on the length of fibers. To explore this hypothesis, a series of experiments were conducted with three different types of fibers, Celgard X-10, X-20, and Cuprophan. Celgard fibers were hydrophobic, whereas Cuprophan fibers were hydrophilic. The Celgard fibers were chopped into required sizes, wetted, and then used. The fibers were chopped into 0.25, 0.50, 1, 1.5, and 2 in. sizes but total length was always 192 in. Each set of fibers was transferred to a 250 mL flask containing 100 mL medium. These flasks were inoculated and incubated at 30°C. The fibers were taken out after 120 h of growth and washed.

Sample of these fibers were potted in plastic vials and growth was examined by SEM. The results are shown in Figs. 5–9. It is clear from Fig. 5

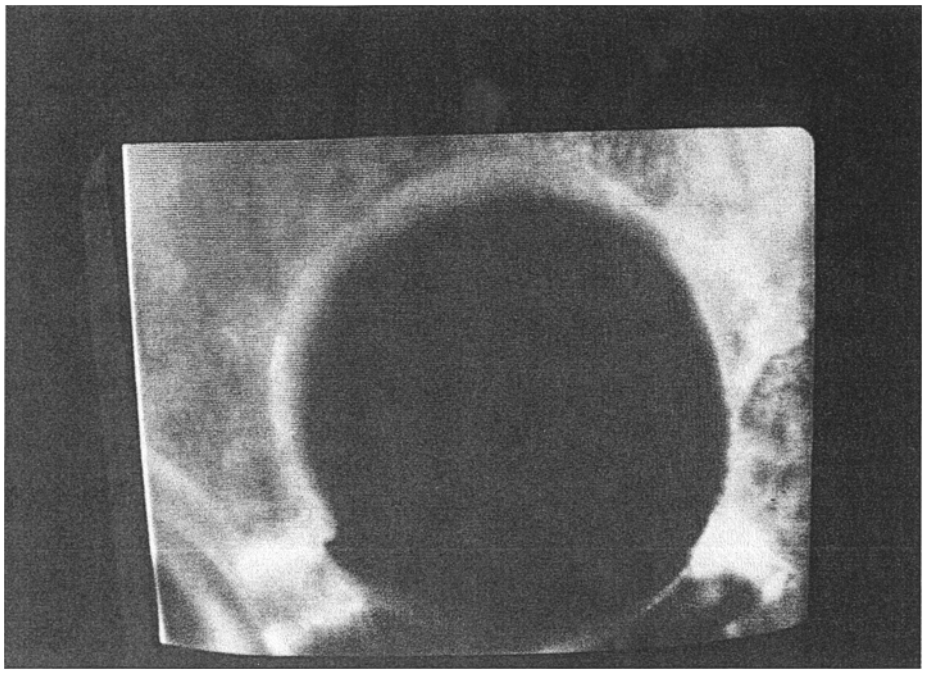

Fig. 4. Microphotograph of wetted Celgard X-20 fiber potted with epoxy in plastic vial without growth at the end of fiber lumen.

that cells do not grow in the nonwetted fibers (X-20), although they grow in wetted fibers such as X-20 and X-10. This can be observed in Figs. 6 and 7, respectively. The same is evident in Cuprophan fibers (Fig. 8). The SEM photographs of the outside surface are shown in Fig. 9. It is clear that cells grow on surface.

The chopped hollow fibers after 120 h growth were taken out, washed, dried, and weighed. After subtracting the weight of hollow fibers, the dry weight of cells was calculated. It is clear from the data shown in Table 2 that cell growth depends on the length of fibers. The cells are growing from the ends to the interior of fiber lumen as well as on the surface. The substrate and nutrients may not be able to diffuse effectively to the interior cells. This explains the dependency of cell growth on the length of fibers. We further note that, the cell growth is less in Cuprophan fibers. Cuprophan fibers have a density much higher than that of the medium unlike the Celgard fibers. Because they settle to the bottom more often, part of the surface as well as ends may not be available for cell growth. In addition, the pore size in Cuprophan is much smaller indicating higher resistance to nutrient and substrate transport through the wall pores.

Bundled fibers of Celgard X-20 were also used for cell growth. Each bundle contained 100 fibers at 1 in. length and was tied at the middle.

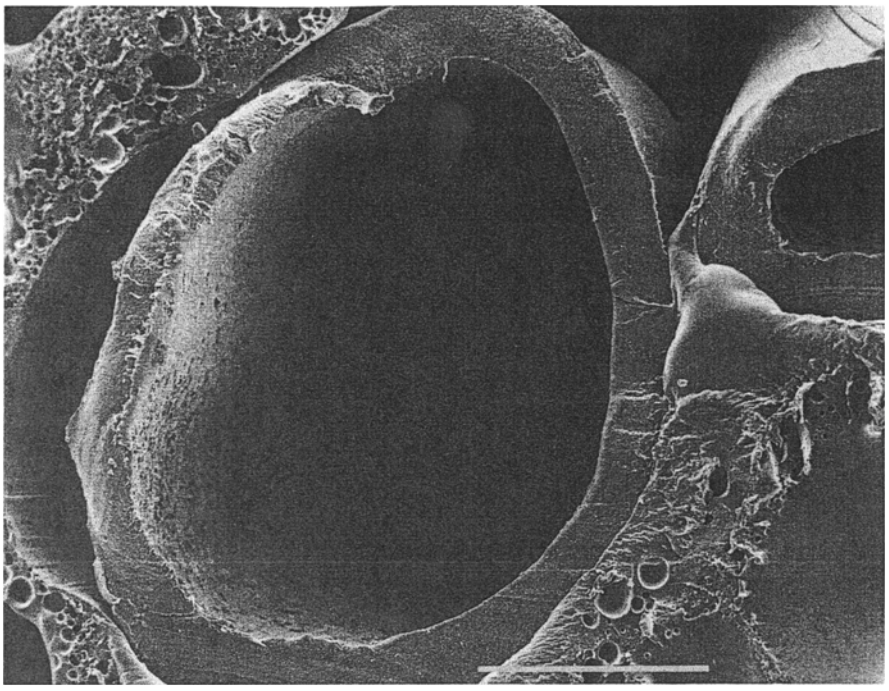

Fig. 5. SEM photograph of nonwetted Celgard X-20 fiber after growth at the end of fiber lumen.

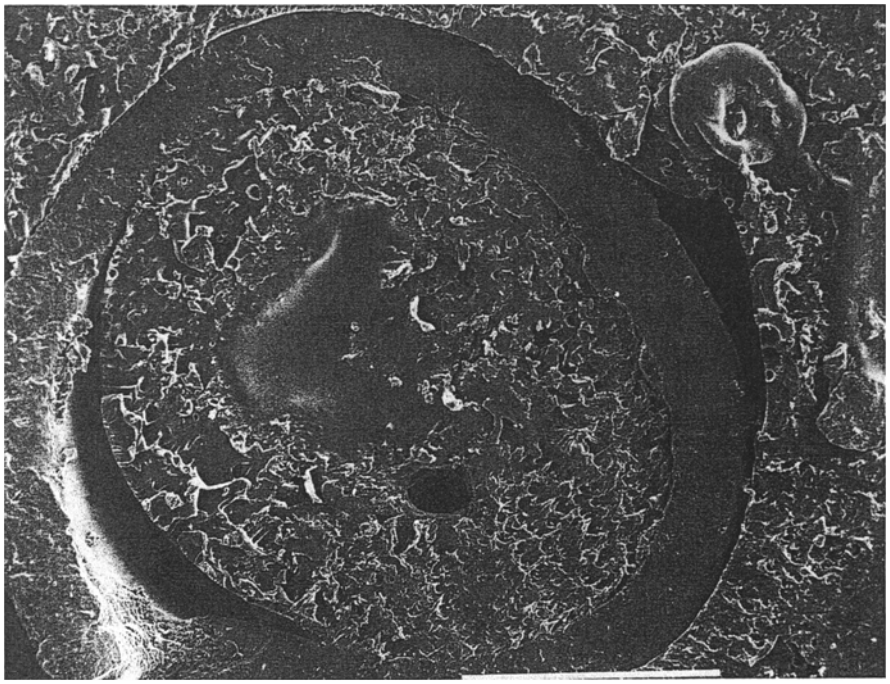

Fig. 6. SEM photograph of wetted Celgard X-20 fiber after growth at the end of fiber lumen.

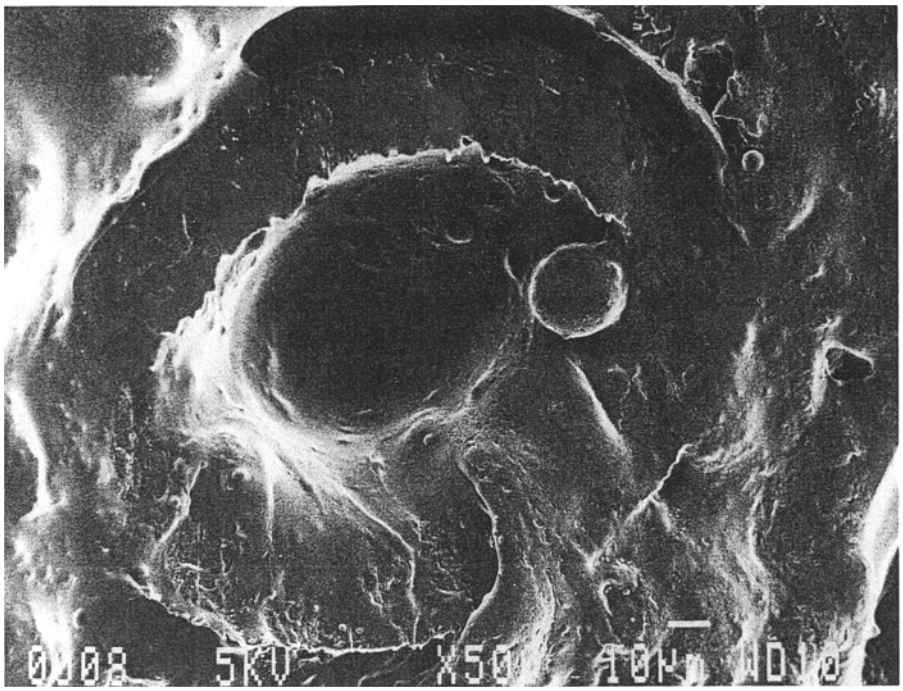

Fig. 7. SEM photograph of wetted Celgard X-10 fiber after growth at the end of fiber lumen.

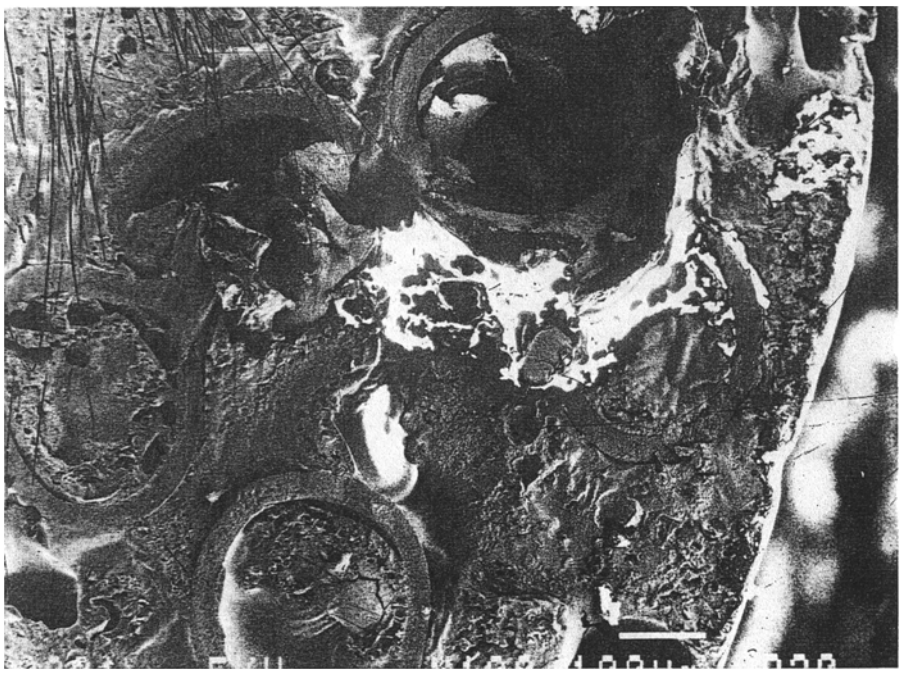

Fig. 8. SEM photograph of Cuprophan fiber after growth at the end of fiber lumen.

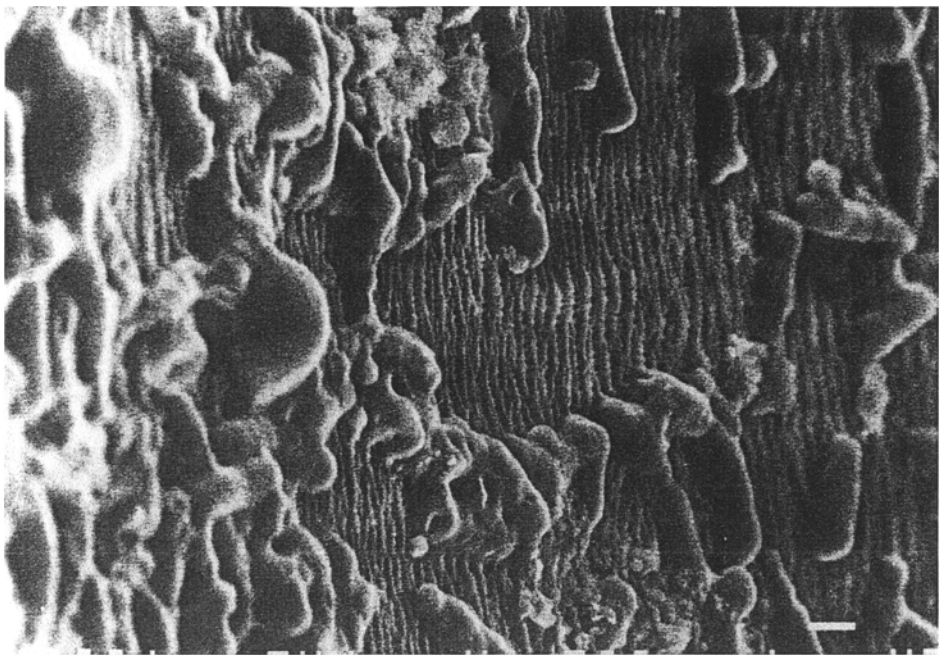

Fig. 9. SEM photograph of outer surface of wetted Celgard X-20 fiber after growth.

Table 2
Cell Growth in Fibers[a]

| Length of fibers | Type of fibers | | |
|---|---|---|---|
| | Celgard X-10 | Celgard X-20 | Cuprophan |
| 2 in. | 23.00 | 19.00 | 10.71 |
| 1.5 in. | 25.00 | 23.53 | 11.63 |
| 1.0 in. | 28.00 | 25.95 | 13.02 |
| 0.5 in. | 31.25 | 31.39 | 14.72 |
| 0.25 in. | 63.30 | 57.10 | 20.00 |

[a]Cell growth is calculated as percentage of dry weight of cells in the fibers to total weight of cells and fibers. These fibers were all wetted; there was no growth on non-wetted fibers.

These were used for cell growth. It was found that dried cell amount is 26.34% of the total fiber and cell weight, which is quite close to that for loose fibers (Table 2). This indicates that growth is mainly from the ends of fibers to the interior of fiber lumen. The bundled fibers after cell growth were washed and used for fermentation as an immobilized cell source. It can be noted from the data shown in Table 3 that fermentation was going well and after 90 h, a yield of 0.425 was obtained. Bundled fibers were

Table 3
Ethanol Fermentation in Presence of Bundled Fibers

| Time H | Ethanol weight, g/L | Ethanol yield, g/g | Glucose consumed, g/L |
|---|---|---|---|
| 18 | 25.0 | 0.25 | 50.02 |
| 27 | 27.5 | 0.275 | 55.26 |
| 42 | 31.5 | 0.315 | 62.89 |
| 50 | 37.6 | 0.376 | 78.21 |
| 66 | 39.6 | 0.396 | 81.25 |
| 74 | 41.8 | 0.418 | 85.89 |
| 90 | 42.5 | 0.425 | 93.87 |

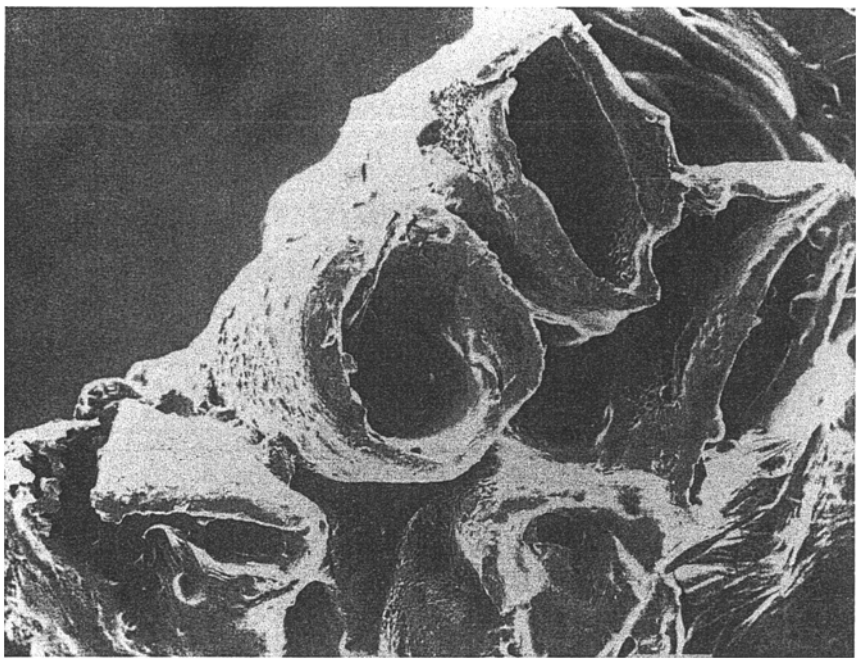

Fig. 10. SEM photograph of hydrophilic/hydrophobic fiber after growth at the end of fiber lumen.

also reused after sterilization and showed considerable cell attachment and growth.

Cells were grown in the same way on hydrophilic-hydrophobic fibers and photographed by SEM. The result of growth in the hydrophobic core is shown in Fig. 10. It is clear that there is no growth on the inside surface of fiber.

The dependence of cell growth on length of chopped fiber suggests considerable resistance to substrate diffusion to inside of fiber lumen. Obviously, 0.25 in. fiber length appears to be highly useful. The cells grow

from the ends of fiber lumen to the fiber lumen interior. As the growth proceeds, the ends are blocked by the cells and the substrate may not be able to diffuse far down the fiber lumen. We have modeled this observed behavior, and the results are communicated elsewhere.

We have also carried out ethanol fermentation in a tubular bioreactor with wetted chopped Celgard X-20 hollow fibers (0.25 in. long) for ethanol production using *Saccharomyces cerevisiae*. Samples of the chopped hollow fibers were taken out from time to time from tubular fermentor. Cells were taken out, washed, and dried as described earlier. It is to be noted that cell concentration is calculated as weight of cells/U fiber lumen volume. The maximum number of cells obtained is $9.3 \times 10^9$/mL volume of fiber lumen. This compares quite well with the highest estimates of yeast cell concentration obtained in traditional hollow fiber entrapment technique (Inloes et al., 1982). The cell rupture, the diffusional limitations, the pressure drop or gas bubble problem are of no concern to us in this immobilization technique. Such an immobilization technique can also be used in an integrated fermentor-extractor (Frank and Sirkar, 1985, 1986).

The glucose concentration remaining in the waste substrate exiting the reactor has been plotted against time at different flowrates as well as at two values of initial glucose concentrations in Fig. 11. We can see that the total amount of glucose is consumed quickly when initial glucose concentration was 100 g/L, whereas it is not true with an initial concentration of 200 g/L. In the latter case, the glucose consumption stops when residual concentration reaches about 34 g/L. It indicates that ethanol concentration has reached the toxicity level. The ethanol concentration and ethanol productivity are shown in Fig. 12. An ethanol concentration of 45 g/L and an ethanol productivity of 41 g/L-h are obtained at an initial glucose concentration of 100 g/L. The ethanol productivity can be increased further without much difficulty.

## CONCLUDING REMARKS

It can be concluded that yeast cells grow on wetted microporous hydrophobic as well as in microporous hydrophilic hollow fibers. The growth in Celgard hydrophobic polypropylene hollow fibers is found to be higher; further this growth depends strongly on fiber length. Cell density per unit length increases as the length of fibers decreases. Cells could not occupy the pores owing to their large size *vis-a-vis* the small pore size. A high cell density of $9.3 \times 10^9$/mL volume of fiber lumen is obtained inside a tubular fermentor using chopped hollow fibers as immobilization support.

We would like to note further that chopped fibers that are charged may be of special utility in immobilizing whole cells having opposite charges. Ion exchange hollow fibers are particular examples of charged

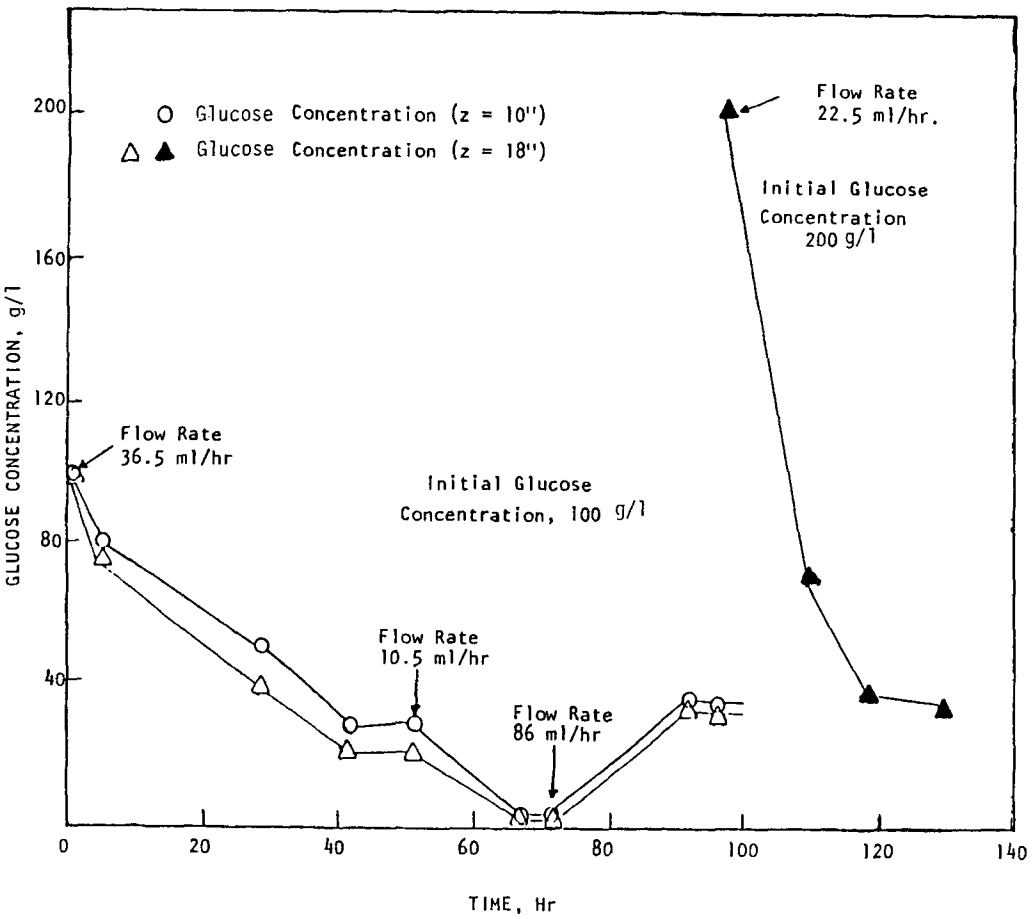


Fig. 11. Exit glucose concentration profiles in tubular fermentor (z is the distance of sampling port from the entrance of tubular fermentor).

surface in this connection. Correspondingly, enzyme immobilization by coupling chemistries on the chopped hollow fibers are straightforward extensions. Additionally, the wetting steps with hydrophobic fibers may be conveniently avoided by using surfactant-treated hydrophobic hollow fibers.

## ACKNOWLEDGMENT

We would like to acknowledge the generous supply of Celgard hollow fibers to our research by Robert W. Callahan of Separations Products Division of Hoechst Celanese Corporation, Charlotte, NC. The end residues from many modules, provided the source for chopped hollow fibers of this study.

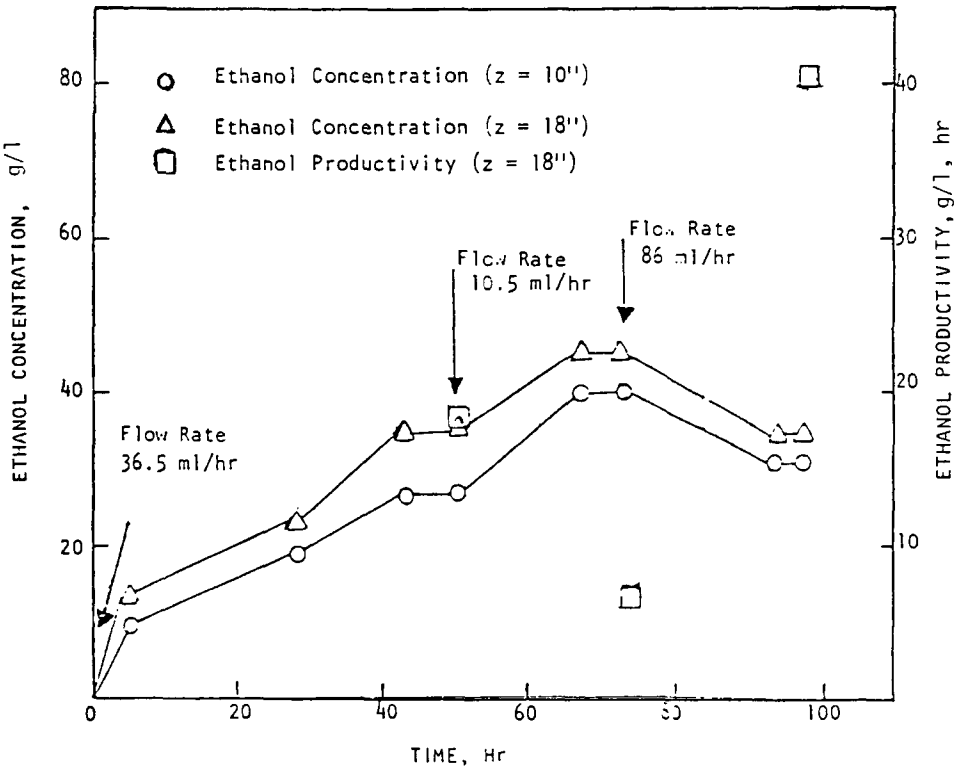


Fig. 12. Ethanol concentration and productivity profiles in tubular fermentor (z is the distance of sampling port from the entrance of tubular fermentor).

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