APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY 9, 117–130 (1984)

# The Potential of Immobilized Biocatalysts for Production of Industrial Chemicals

G. B. BORGLUM* AND J. J. MARSHALL

*Biotechnology Research, Miles Laboratories, Inc., PO Box 932, Elkhart, IN 46515*

Received July 1, 1983; Accepted October 26, 1983

## Abstract

The successful translation from conception to practice of processes based on immobilized biocatalyst technology has been slower than anticipated. There are severe barriers, both technical and economic, limiting the introduction of immobilized biocatalyst technology to replace conventional processing procedures and processes for the production of chemicals by synthetic or fermentative routes. A small number of immobilized enzyme processes are in operation commercially, the most noteworthy being in food-related processes and in the pharmaceutical industry, where they are used for carbohydrate conversions and antibiotic transformations, respectively. There does not, as yet, appear to be any large-scale industrial application of immobilized cell technology. Examples from our laboratory—immobilized yeast for ethanol production and *Aspergillus niger* for citric acid synthesis—illustrate the problems that have to be overcome.

**Index Entries:** *Aspergillus niger,* entrapped in κ-carrageenan; citric acid, synthesis by immobilized *Aspergillus niger;* ethanol, immobilized yeast for production of; yeast, entrapped in κ-carrageenan.

## Introduction

Biocatalysts—i.e., enzymes and micoorganisms—are extensively used in the food industry (*1*). Their potential in supplementing or displacing chemical indus-

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


0273–2289/84/0200–0117$02.80

try processes will require an immobilized biocatalyst to achieve the economics of a continuous process. In the case of immobilized enzymes, the barriers to be overcome are quite formidable. Even in the case of simple enzyme reactions (e.g., hydrolytic), there are few examples of commercial applications. Glucose isomerase for producing fructose from dextrose and penicillin acylase for hydrolyzing penicillin to 6-aminopenicillanic acid are in greatest use (*2*). In fairness, it must be recognized, however, that this situation does not necessarily result from the lack of suitable technology, but often from the low cost of the enzymes and the existing plant capacity for carrying out batch reactions. Many immobilized enzyme processes have been described, but most are not of any commercial/industrial significance. When it is recognized that most of the enzymes likely to be useful for the production of commodity chemicals would probably require cofactors or an energy-rich cosubstrate, such as ATP, and that use of these compounds without an efficient regeneration process would render any such process impossible for economic reasons, the barriers to be overcome are even more apparent. An example of an ATP regeneration process is provided in the study of Murata and coworkers (*3*) in immobilizing *Escherichia coli* cells containing glutathione synthetase and acetate kinase in κ-carrageenan for the synthesis of glutathione. The ATP required in the synthesis reactions was regenerated from ADP and acetyl phosphate by the acetate kinase.

In the case of immobilized cell systems, cofactor regeneration and the synthesis and regeneration of high energy cosubstrates is taken care of automatically without the requirement for any special technology, other than to ensure that the cells used are immobilized without destroying cell viability and to ensure that the immobilized cells are supplied with at least the minimum level of nutrients required to maintain cell viability.

It therefore seems probable that among the first major applications of immobilized biocatalysts will be the use of immobilized cells for the production of fermentation products presently produced by conventional fermentation processes.

We will not try to review all the processes that have been developed for the immobilization of enzymes and cells or to provide a comprehensive list of potentially useful immobilized biocatalyst systems that have been developed. Rather, in the light of the above comments, we will restrict this discussion to consideration of processes for the production of immobilized viable microbial cells, provide examples of the use of such systems for the production of two common commodity chemicals (ethanol and citric acid), and consider the possibilities of using such systems for the production of other industrial chemicals.

We are a long way from seeing large quantities of commodity chemicals produced by immobilized enzyme or immobilized cell processes. In the case of commodity industrial chemicals, we can at this time only consider potential, rather than demonstrated, applications.

I would like to briefly consider the elegant applied biochemistry of the Cetus Corporation process for the enzymatic epoxidation of an alkene coupled with the enzymatic oxidation of a substrate to coproduct (*4*). Several alkenes and sub-

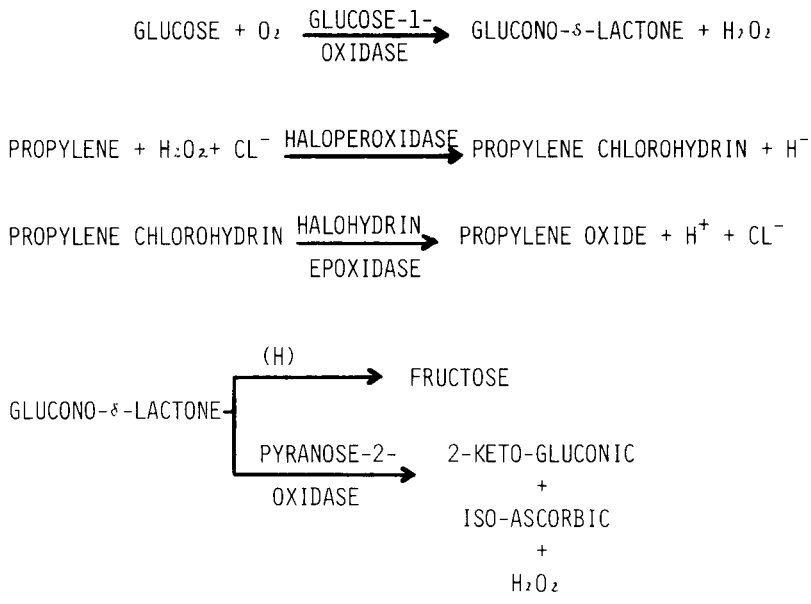


Fig. 1. Enzyme reactions of the Cetus process (*4*).

strates can be employed; in the following illustration, propylene and glucose will be used. The process involves the concerted action of three enzymes (Fig. 1).

Glucose is oxidized to glucono-delta-lactone and hydrogen peroxide by glucose-l-oxidase. Chloride ion and the hydrogen peroxide react with propylene through the action of haloperoxidase to form propylene chlorohydrin and hydroxide ion. The third enzyme, halohydrin epoxidase, catalyzes the formation of propylene oxide, hydrogen ion, and chloride ion. The chloride ion is reused in the haloperoxidase reaction, eliminating the problem of coproduct disposal. The process coproduct from the hydrogen peroxide generation reaction, glucono-delta-lactone, can be hydrogenated to fructose or further oxidized by a fourth enzyme, pyranose-2-oxidase, to 2-keto-gluconic acid and iso-ascorbic acid. These two products can then be converted chemically to furfural. The second enzymatic oxidation also yields a mole of hydrogen peroxide per mole of substrate for use in the chlorohydrin reaction.

The process is based on several objectives. The epoxidation reaction sequence requires an inexpensive source of hydrogen peroxide for an economic process. The hydrogen peroxide generation reaction yields a coproduct that can be marketed to recover the substrate cost and thus provide an economic source of hydrogen peroxide. The process is not limited to propylene and glucose, but can utilize several alkenes, and other substrates, such as methanol oxidation to formaldehyde with alcohol oxidase, can be used for generating hydrogen peroxide. In principle, the process offers the economic advantage of converting, as an example, propylene and glucose to the coproducts propylene oxide and furfural, having approximately double the value of the starting materials. The, so far, unsuccessful com-

mercialization of the process indicates insurmountable obstacles were encountered in translating the coupled reactions to an industrial process.

## Processes for Immobilization of Microbial Cells

The numerous methods that have been developed for immobilizing enzymes can be classified under three headings: adsorption on a support, entrapment in a hydrophilic matrix, and covalent bonding to a support. The published techniques do not all neatly fit these three categories. For example, glucose isomerase from *Streptomyces* can be entrapped within the microbial cells, which in turn are crosslinked to form the support matrix (*5,6*). In principle, microbial cells can be immobilized by the techniques developed for enzymes (Table 1). The requirement for maintaining cell viability has resulted in the choice of adsorption on a support of entrapment in a matrix for the majority of published studies of microbial cell immobilization.

### *Adsorption*

Immobilization by adsorption is the most facile technique and potentially offers the lowest cost process. Adsorbed microorganisms have been used for several years in vinegar production via a packed tower fermentation and the purification of wastes by trickling filters (*7*). The trickling filters are colonized by microorganisms during use. Many and perhaps most micoorganisms have the ability to adhere to surfaces. Gerson and Zajic (*8*) have described the mechanisms of cell attachment to involve (a) microexudates the cells excrete that act as an adhesive to affix the cell to the support, (b) electrostatic attraction between cell wall and the inert surface, and (c) cell projections that cling to the support surface. Kolot (*9, 10*) has recently reviewed the literature dealing with supports for microbial cells. A variety of materials have been used, for example, crushed brick, plastics, wood chips, cordierite, ion-exchange resins, and inert supports coated with crosslinked gelatin.

TABLE 1
Microbial Cell Immobilization

| | | |
|---|---|---|
| | *Adsorbents* | |
| Crushed brick | Plastics | Wood chips |
| Cordierite | Ion-exchange resins | Coated support |
| | *Gel Matrices* | |
| Polyacrylamide | | Agar |
| Alginate | | κ-Carrageenan |
| | *Bifunctional Agents for Covalent Attachment* | |
| Glutaraldehyde | | Cyanuric chloride |
| Diisocyanates | | |

The retention of the microorganism on the support is affected by the physiological state of the organism. The pH can alter the charge of the support causing desorption.

*Entrapment*

A number of hydrophilic gels have been used as entrapment matrix. Polyacrylamide has been used for immobilizing bacteria, fungi, and yeast (*9,10*). A disadvantage of this gel is the toxicity exhibited by the monomer, acrylamide, toward microorganisms. Techniques reported have been to polymerize the monomer mixed with the microorganism as a slab and then to fabricate smaller particles by cutting the slab, mincing the slab in a blender, or forcing the gel through wire mesh. Techniques to produce gel beads are to inject the polymerization mixture into a water-immiscible liquid such as mineral oil (*11*) or to polymerize the monomer after emulsifying the aqueous mixture in an organic liquid. The reaction is a free radical polymerization, so care must be taken to scavenge the oxygen dissolved in the aqueous and organic phases.

Several workers have reported the successful immobilization of microorganisms in algal gums: agar, alginate, and κ-carrageenan (*9,10*). All three gums are used as food ingredients and exhibit no toxicity to microorganisms. We used agar and κ-carrageenan in our studies. Agar has the unique characteristic of melting near the boiling point of water and gelling at about 38°C. To produce a spherical bead, we used the apparatus shown schematically in Fig. 2. Molten agar cooled to 40°C was mixed with the microorganism and the mixture transferred to a glass coil, used as a reservoir, submerged in the water bath. The agar mixture was injected into a flowing mineral oil stream at 40°C by displacing the mixture from the coil with water. The molten agar drops were gelled by cooling the oil stream in an ice bath. The beads were collected in a reservoir and the oil was recirculated.

Sodium alginate dissolves in water without need for heating. After suspending the microorganism in the gum solution, gel beads are prepared by pumping the mixture to form free-falling drops into a calcium ion solution. Beads of calcium

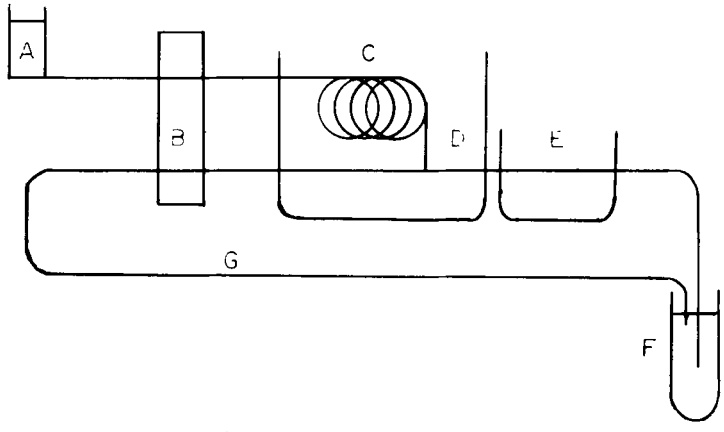


Fig. 2. Apparatus for preparing agar gel beads: A, water for displacing agar solution; B, pump; C, mycelium–gum agar reservoir; D, bath, 40°C; E, ice bath; F, bead collection reservoir; G, mineral oil line.

alginate gel are formed. The gel dissolves upon contact with phosphate and citrate however Birnbaum and coworkers (*12*) have reported successfully stabilizing the gel by treating the beads subsequent to gelation with polyethylenimine and cross-linking with glutaraldehyde.

Chibata and coworkers (*13*) have extensively studied the gelation of κ-carrageenan. Their preferred gelling agent is potassium ion, which produces a sturdy gel. Bead-shaped gel is prepared in the same manner as for calcium alginate beads. Drops of gum containing suspended microorganisms are allowed to fall into a solution of gelling agent. Most κ-carrageenan products contain a small amount of potassium and calcium ions and require heat for solution. Ion exchange treatment of the κ-carrageenan to the sodium salt allows dissolving the gum at room temperature or at slightly elevated temperature. For gum stability, the fermentation medium must contain potassium ion. In our work, we have prepared insoluble gel beads using polyethylenimine or a commercial floccing agent, Betz 1180, as gelling agent (*14*).

### *Covalent Bonding to Support*

The microbial cell wall has attached groups suitable for forming bonds with activated supports, for example, amino, carboxyl, and hydroxyl groups. The immobilized enzyme techniques of activating a support with a bifunctional compound followed by reaction of the activated support with living cells can be used. Glutaraldehyde, cyanuric chloride, and disocyanates are well-known compounds for bonding amino groups on support and cell wall.

## Application of Immobilized Microbial Cells

### *Production of Ethanol*

The oil supply crisis a few years ago spurred a great research effort to investigate alternate energy sources and especially other sources of liquid fuel for our automobiles. The fuel shortages and resulting high fuel cost also increased consumer efforts in conserving fuel use and in choosing more fuel-efficient autobmobiles so we no longer have fuel shortages though we still have the high fuel prices. The world's oil reserves are not unlimited, however, and alternate sources for energy and for supplying the chemicals derived from oil will have to be developed.

One of the first proposals to reduce the nation's dependence on oil was to mix ethanol with gasoline to produce gasohol. As a result, an increasing portion of the scientific literature has reported studies of greater fermentation production through improved fermentation techniques and ethanol recovery, the development of fermentation processes making use of agricultural biomass or waste products (*15–17*), and the immobilization of yeast for a continuous fermentation process (*18*).

Yeast have been immobilized by all three types of immobilization process. Examples of yeast entrapment in hydrophilic gels are the most numerous. There are fewer examples of yeast adsorption, possibly because of the need to select a yeast

strain that readily adsorbs to a surface. Immobilization by covalent bond between yeast cell wall and support has been reported in few publications.

Moo-Young and coworkers (*19*) have immobilized yeast by adsorption on beechwood chips. The support is inexpensive and the column was active for 3 months. In our studies we were unable to adsorb yeast on activated alumina or on active carbon granules. The active carbon appeared to be deleterious to the yeast.

Chibata and coworkers (*20*) have extensively investigated the entrapment of bacteria and yeast in κ-carrageenan, bacteria for amino acid synthesis, and yeast for alcohol production. They were the first to use κ-carrageenan as a support matrix, and through yeast strain selection have achieved tolerance to high glucose and alcohol concentrations. They have recently reported producing 11.4% w/v ethanol from 25% w/v glucose medium for a period of 62 d. Their data showed no decline in productivity at the end of the period (*21*).

In our studies, we have investigated coimmobilizing yeast and glucoamylase for the fermentation of a liquefied starch substrate. Entrapping the enzyme in κ-carrageenan gelled with Betz 1180 polymer was not successful; the enzyme readily diffused out of the gel. To increase the size of the enzyme the glucoamylase was bound to glutaraldehyde—activated alkylamine porous glass beads (*22*). The enzyme–glass beads and yeast were then mixed with κ-carrageenan solution and successfully coimmobilized as gel beads.

The beads were packed in a column and supplied a 14% w/v glucose medium for the initial yeast growth period and for a short anaerobic fermentation period. A clarified liquefied starch medium, 15–22% w/v dry solids, prepared from corn flour, was then fed to the column for an 81-d period. The average effluent ethanol concentration was 4% w/v and fermentation efficiency averaged 90%. We experienced difficulty with yeast deposits plugging the column; the yeast strain may have been a flocculating strain. We observed complete consumption of the carbohydrates: glucose to glucohexaose. Heptaose and larger oligomers were present in the column effluent and presumably diffused into the gel too slowly for complete hydrolysis.

Navarro and Durand (*23*) grafted aminopropyl groups to porous silica beads and investigated the immobilization of yeast on the untreated and glutaraldehyde-activated amino silica beads. They reported the greatest number of yeast cells were immobilized on the highest porosity glutaraldehyde-activated beads. The same support without glutaraldehyde activation retained essentially no yeast cells. They concluded the yeast cells were immobilized by imine bonds between carbonyl groups of the activated support and amino groups on the cell wall. They observed increased cell metabolism and ethanol production due to immobilization.

### *Synthesis of Citric Acid*

Citric acid is widely used as an acidulant in the food industry. Its ability to form chelates with divalent and trivalent cations has led to several nonfood uses for citric acid or its salts. Trisodium citrate is useful as a builder in detergents. Scale and metal corrosion removal can be effected by citric acid, a biodegradable compound.

Essentially all citric acid is manufactured by fermentation of sucrose or dextrose by selected strains of *Aspergillus niger* (*24*). The production of citric acid by yeast has been reported; however, the yeast fermentation also produced isocitric acid, whereas the fungal fermentation produces no detectable isocitrate.

Citric acid is an intermediate compound of the Krebs cycle and is normally further metabolyzed rather than excreted. High conversion of carbohydrate to citric acid requires supplying phosphate ion and cations to enhance citric acid synthesis and to limit, but not prevent growth of the fungus. The concentrations of the ions are determined empirically for the selected strain of *Aspergillus niger*. For growth, fungi require phosphorus supplied as phosphate, potassium, sulfur supplied as sulfate and magnesium. They also require iron, zinc, and copper as micronutrients (*25*). Several studies investigating the role of these ions in citric acid production have been published. Röhr and Kubicek (*24*) have postulated the ion limitation in a citric acid fermentation medium results in a high intracellular ammonium ion pool that counteracts the feedback inhibition of phosphofructokinase by citrate and ATP.

The citrate synthesis is thereby unregulated. The high intracellular ammonium ion and glucose concentrations repress the synthesis of 2-keto-glutarate dehydrogenase, inhibiting citric acid metabolism in the Krebs cycle and resulting in excretion of the excess citric acid.

In a typical submerged fermentation, sterile medium is innoculated with *Aspergillus niger* spores and aerated. The fungus grows in a spherical pellet form rather than as filamentous mycelium because of strain selection and the limiting nutrient concentration. The typical conversion yield is 0.7 mol citric acid/mol monosaccharide consumed.

Anderson and coworkers (*26*) have reported immobilizing *Aspergillus niger* by adsorption in a film fermenter. The fungus grew in the filamentous form on a series of vertical polypropylene discs that rotated half-submerged in fermentation medium. They conducted a continuous fermentation for 27 d and achieved a maximum synthesis rate of 0.07 mol/L-d.

Vieth and Venkatsubramanian (*27*) immobilized *A. niger* cells in collagen membrane crosslinked with glutaraldehyde. The immobilized cells exhibited citric acid synthesis activity that was 50% of the activity of free cells. Half-life of the immobilized cells was 138 h.

Vaija et al. (*28*) reported obtaining a maximum citric acid yield of 12 g/L in a continuous fermentation using *A. niger* immobilized in calcium alginate beads. They reported an overall fermentation efficiency of 40% and a half-life of about 1 month for the immobilized mycelial pellets.

Entrapping mycelium in hydrophilic gels was chosen as immobilization method for our studies. Mycelial pellets were immobilized after a 5-d fermentation, the stage of peak synthesis capability. *Aspergillus niger* mycelium was sensitive to acrylamide, but appeared able to recover from exposure to the monomer. The mycelium was immobilized in polyacrylamide by emulsion polymerization. Upon subsequent incubation of the immobilized mycelium in substrate, no growth or citric acid production was observed.

Algal gums have been extensively used for immobilizing living microorganisms. The gums have several uses in the food industry; thus, the use of these food-approved materials for immobilizing *Aspergillus niger* would be desirable since citric acid is marketed as a food ingredient.

Immobilization of mycelium in agar was studied using the apparatus previously described. Beads entrapping mycelium were prepared with 2.5, 3, and 4% agar and evaluated in shaken fermentation (Table 2). After 2 and 5 d, the beads were transferred to a fresh fermentation medium. Also at these time periods unimmobilized mycelium used as control fermentations were also transferred.

The rate of citric acid formation by immobilized mycelium was about 25% of the rate of the control preparations for a 9-d period. Small amounts of free mycelium accumulated during the second and third fermentation periods using the immobilized preparations. The amounts of free mycelium were too small to contribute significantly to the synthesis rates observed.

*Aspergillus niger* mycelium was immobilized in κ-carrageenan using as gelling agents: 1,6-diaminohexane and a high molecular weight polyamine flocculating agent, Betz Polymer 1180 manufactured by Betz Laboratories, Inc. Beads were prepared with gelling agent alone and also with gelling agent followed by glutaraldehyde crosslinking.

The immobilized preparations were evaluated by shaken batch fermentation; the substrate was replaced at 2 or 3-d intervals. The results are presented in Table 3. The beads were reused until they dissolved, citric acid synthesis halted, or a great deal of free mycelium accumulated. The data indicated that beads prepared with 1,6-diaminohexane must be crosslinked to render the beads insoluble. Beads gelled with the high molecular weight polyamine were insoluble without glutaraldehyde crosslinking. Glutaraldehyde was toxic to *Aspergillus niger*.

Mycelium was immobilized in sodium κ-carrageenan using the Betz polymer or polyethylenimine as gelling agents. The citric acid synthesis by the immobilized mycelium was evaluated in shake flask fermentation. The substrate was replaced at 2- or 3-d intervals because of the accumulation of free mycelium. The citric acid synthesis rate attained a maximum level of 0.07 mol/L-d (Table 4). The immobilized mycelium was reused for 31 d.

TABLE 2
Production of Citric Acid by *A. niger* Immobilized in Agar Gel Beads

| | Citric acid synthesis rate[a] | | |
|---|---|---|---|
| Agar concentration | Day 2 | Day 5 | Day 9 |
| 2.5%, w/v | 20 | 30 | 20 |
| 3.0%, w/v | 50 | 30 | 30 |
| 4.0%, w/v | 20 | 25 | 10 |

[a]Synthesis rate is expressed as percent of rate observed for unimmobilized mycelium.

TABLE 3
Production of Citric Acid by *A. niger* Immobilized in κ-Carrageenan Beads

| Gelling agent | Days used | Total citric acid, mmol |
|---|---|---|
| 1,6-Diaminohexane | 4 | 1.1 |
| 1,6-Diaminohexane glutaraldehyde[a] | 7 | 0.7 |
| Polymer 1180 | 18 | 5.2 |
| Polymer 1180 glutaraldehyde[a] | 9 | 0.7 |

[a]Gelling agent was crosslinked after bead gellation.

The use of immobilized *Aspergillus niger* in an aerated column was unsuccessful. The citric acid fermentation required a high aeration rate, which caused problems of foaming and liquid evaporation. Foaming could be controlled by antifoam addition, but the quantity of antifoam required was inhibitory to the fermentation.

A citric acid fermentation using immobilized *Aspergillus niger* can displace the traditional batch fermentation using unimmobilized *Aspergillus niger* if it offers the advantages of a continuous high rate of citric acid production and efficient and total use of glucose. These advantages could not be demonstrated in this study. Mycelial growth cannot be prevented without cessation of citric acid production; thus, in a continuous reactor of immobilized *Aspergillus niger*, the free mycelium formed must be continuously removed from the fermenter. The diffusion resistance of the gel to substrate and oxygen transfer to the immobilized mycelium limits the synthesis rate attainable. The rates observed in this study may be the maximum rates attainable by *Aspergillus niger* in a gel matrix. Complete sugar consumption was not investigated in this study because of the accumulation of free mycelium.

### *Production of Other Chemicals*

The fermentation production of ethanol and citric acid have been established for many years. The production of other industrially significant chemicals by fermentation has not developed because of competition from more economic processes

TABLE 4
Fermentation Rate of *A. niger* Immobilized in κ-Carrageenan Beads

| Day | Polymer 1180[a] | Polyethylenimine[a] |
|---|---|---|
| 3–13 | 0.03–0.05[b] | 0.03–0.05[b] |
| 14–30 | 0.05–0.07 | 0.05–0.07 |
| 31 | 0.05 | 0.05 |

[a]Gelling agent for κ-carrageenan.
[b]Fermentation rate is expressed in moles of citric acid synthesized per liter-day.

based on petroleum. The economic advantage of petroleum will diminish as petroleum supplies become limited and thus more costly.

Krouwel and coworkers (*29*) immobilized *Clostridium butylicum* in calcium alginate beads. This is an anaerobic microorganism that produces *n*-butanol and isopropanol from glucose. The beads were packed in a conical column to reduce slug formation caused by the gases produced during fermentation. They reported a yield of about 30% for the two alcohols in a fermentation continued for 9 d.

Häggström and Enfors (*30*) immobilized spores of *Clostridium acetobutylicum* in calcium alginate beads. The immobilized spores were heat-activated. After a 3-d vegetative growth period the steady-state productivity was 67 g butanol/L reactor volume/d. The authors state that the productivity of the immobilized cells was about eight times the productivity of the batch process. Butanol and acetone were coproducts in weight ratio of 5.5:1.

The production of α-keto acids from amino acids by yeast immobilization in calcium alginate has been described by Brodelius et al. (*31*). The yeast, *Trigonopsis variabilis*, contains D-amino acid oxidase, thus a substrate of racemic amino acid yields the alpha-keto acid and the L-amino acid. The alpha-keto acids are useful in treating acute uremia.

## Perspective and Prospect

The potential advantages of immobilized biocatalysts are well-known (Table 5). Enzyme reuse permits the economical utilization of an otherwise cost-prohibitive enzyme. One of the factors in the industrial acceptance of glucose isomerase was its immobilization. The production cost of the enzyme was too great for a single use.

Immobilization of enzymes and microorganisms allows their use in a continuous process to achieve more efficient use of the equipment and improved process control. Using a packed bed reactor of immobilized enzyme for illustration, the ratio of enzyme to substrate is considerably greater than the ratio used for a soluble enzyme process resulting in a short reaction time. Soluble glucoamylase used under recommended conditions (*32*) hydrolyzes dextrins to glucose in 72 h. Lee and coworkers (*33*) reported use of a 9-min residence time in a packed bed of glucoamylase immobilized on porous silica to hydrolyze a dextrin solution to glucose. The extent of reaction is readily controlled by changing the feed pumping

TABLE 5
Advantages of Immobilized Biocatalysts

| |
|---|
| Extended use |
| Continuous process |
| Improved process control |
| High biocatalyst-to-substrate ratio |
| Shortened reaction time |
| Limited nutrient needed for biomass production |
| Savings in capital costs |

rate to vary residence time. The ratio of reaction periods, immobilized to soluble enzyme, in this example, is 1 to 480; thus, for the same production capacity, a considerably smaller reactor volume is needed.

The use of biocatalysts instead of chemical synthesis processes offers potential advantages (Table 6). The mild reaction conditions required by biocatalysts result in less stringent specifications for equipment, and hence lower capital requirement. Enzymes and microorganisms require a lower temperature than chemical catalysts, which reduces the energy needs. The feedstocks for a biocatalyst process are biorenewable raw materials, an advantage that will steadily increase in importance. There is a potential advantage that a multistep chemical synthesis can be simplified to one biosynthetic step.

On the other hand, there are several factors that limit the replacement of chemical processes with immobilized biocatalyst processes (Table 7). A microorganism must be found that will have the desired synthesis capability or which produces an enzyme that catalyzes the desired transformation of a compound. A microorganism having enhanced ability to produce a desired compound or that overproduces an enzyme has an ecologic disadvantage and cannot survive in competition with other microorganisms. A microorganism isolated from nature will be uneconomic for industrial use and genetic manipulation will be required to enhance the desired activity.

The cost of the immobilization process and immobilization support affect the economic success of a biocatalyst. An extended biocatalyst useful life and a support that can be reused would reduce immobilization costs.

For an acceptable immobilized biocatalyst the substrate to product conversion efficiency must be high enough to be economical; byproduct formation must be minimal. Conversion efficiency is usually not a problem in using immobilized

TABLE 6
Biocatalysts vs. Chemical Catalysts

| |
|---|
| Mild reaction conditions |
| Biorenewable feedstock |
| Simplified process |
| Selective reaction |
| Minimum byproduct formation |

TABLE 7
Limiting Factor for Biocatalyst Use

| |
|---|
| Microorganism selection and manipulation |
| Immobilization cost |
| Efficient substrate to product conversion |
| Rate of product formation |
| Product concentration |

enzymes. Byproduct formation can be controlled by feed rate and reaction conditions. With immobilized living microorganisms biomass production and normal cell metabolism compete with the desired synthesis pathway in use of substrate. Uncontrolled cell metabolism and cell growth lower production efficiency, but measures to control these factors must maintain cell viability.

The rate of product formation and the concentration of product in the process eluate stream affect the successful industrial use of immobilized biocatalysts. The desired product may be toxic or inhibitory to the immobilized microorganism as, for example, in ethanol or butanol production. A low product concentration increases product recovery cost.

## References


1. Reed, G., ed. (1975), *Enzymes in Food Processing*, Academic Press, New York.
2. Brodelius, P. (1978), in *Adv. Biochem. Eng.* (Ghose, T. K., Fiechter, A., and Blakebrough, N., eds.), Vol. 10, pp. 75–129, Springer-Verlag, New York.
3. Murata, K., Tani, K., Kato, J., and Chibata, I. (1980), *Eur. J. Appl. Microbiol. Biotechnol.* **10,** 11.
4. Neidleman, S. L., Amon, W. F., Jr. and Geigert, J. (1981), US Patent 4,247,641, assigned to Cetus Corporation. Niedleman, S. L., Amon, W. F., Jr., and Geigert, J. (1981), US Patent 4,284,723, assigned to Cetus Corporation.
5. Borglum, G. B. (1980), US Patent 4,212,943, assigned to Miles Laboratories, Inc.
6. Zienty, M. F. (1973), US Patent 3,779,869, assigned to Miles Laboratories, Inc.
7. Halls, N. A., and Board, R. G. (1973), *J. Appl. Bact.* **36,** 465.
8. Gerson, D. F., and Zajic, J. E. (1979), in *Immobilized Microbial Cells* (Venkatsubramanian, K., ed.), *ACS Symposium Series* **106,** Amer. Chem. Soc., Washington, DC, pp. 29–57.
9. Kolot, F. B. (1981), *Proc. Biochem.* **16**(5), 2.
10. Kolot, F. B. (1981), *Proc. Biochem.* **16**(6), 30, 46.
11. Köstner, A., and Mandel, M. (1976), in *Methods in Enzymology* (Mosbach, K., ed.), Vol. 44, Academic Press, New York., pp. 191–195.
12. Birnbaum, S., Pendleton, R., Larsson, P., and Mosbach, K. (1982), *Biotechnol. Lett.* **3,** 393.
13. Takata, I., Tosa, T. and Chibata, I. (1977), *J. Solid-Phase Biochem.* **2,** 225.
14. Borglum, G. B. (1982), US Patent 4,347,320, assigned to Miles Laboratories, Inc.
15. Friend, B. A., and Shahani, K. M. (1981), in *Fuels from Biomass and Wastes* (Klass, D. L., and Emert, G. H., eds.), Ann Arbor Science Publishers, Ann Arbor, pp. 343–355.
16. Becker, D. K., Blotkamp, P. J., and Emert, G. H. (1981), in *Fuels from Biomass and Wastes* (Klass, D. L. and Emert, G. H., eds.) Ann Arbor Publishers, Ann Arbor, MI, pp. 375–391.
17. Katzen, R., Ackley, W. R., Moon, C. D., Jr., Messick, J. R., Brush, B. F., and Kaupisch, K. F. (1981), (Klass, D. L. and Emert, G. H., eds.) Ann Arbor Publishers, Ann Arbor, MI, pp. 393–402.
18. Chibata, I., and Tosa, T. (1981), in *Ann. Rev. Biophys. Bioeng.* (Mullins, L. J., ed.), Vol. 10, Annual Reviews, Palo Alto, pp. 197–216.
19. Moo-Young, M., Lamptey, J., and Robinson, C. W. (1980), *Biotechnol. Lett.* **2,** 541.

20. Chibata, I. (1979), in *Immobilized Microbial Cells* (Venkatsubramanian, K., ed.), *ACS Symposium Series* **106,** Amer. Chem. Soc. Washington, DC, pp. 187–202.
21. Wada, M., Kato, J., and Chibata, I. (1981), *Eur. J. Appl. Microbiol. Biotechnol.* **11,** 67.
22. Robinson, P. J., Dunnill, P., and Lilly, M. D. (1971), *Biochem. Biophys. Acta* **242,** 659.
23. Navarro, J. M., and Durand, G. (1977), *Eur. J. Appl. Microbiol.* **4,** 243.
24. Röhr, M., and Kubicek, C. P. (1981), *Process Biochem.* **16** (4), 34, 44.
25. Berry, D. R., Chmiel, A., and Alobaich, Z. (1977), in *Genetics and Physiology of Aspergillus* (Smith, J. E., and Patemann, J. A., eds.), Academic Press, New York, pp. 403–426.
26. Anderson, J. G., Blain, J. A., Divers, M., and Todd, J. R. (1980), *Biotechnol. Lett.* **2,** 99.
27. Vieth, W. R., and Venkatsubramanian, K. (1979), in *Immobilized Microbiol. Cells* (Venkatsubramanian, K., ed.), *ACS Symposium Series* **106,** Amer. Chem. Soc., Washington, DC, pp. 1–11.
28. Vaija, J., Linko, Y.-Y., and Linko, P. (1982), *Appl. Biochem. Biotechnol.* **7,** 51.
29. Krouwel, P. G., van der Laan, W. F. M., and Kossen, N. W. F. (1980), *Biotechnol. Lett.* **2,** 253.
30. Häggström, L., and Enfors, S.-O. (1982), *Appl. Biochem. Biotechnol.* **7,** 35.
31. Brodelius, P., Nilsson, K., and Mosbach, K. (1980), in *Advances in Biotechnology* (Moo-Young, M., ed.), Vol. 3, Pergaman, New York, pp. 373–376.
32. *Diazyme® L-100 for Dextrose Production,* Technical Bulletin, Enzyme Products, Miles Laboratories, Inc.
33. Lee, D. D., Lee, Y. Y., and Tsao, G. T. (1975), *Die Stärke* **27,** 384.