ENGINEERING ASPECTS OF IMMOBILIZED BIOCATALYSTS

SHINTARO FURUSAKI
University of Tokyo, Tokyo 113

Key Words: Biochemical Engineering, Immobilized Enzymes, Immobilized Microorganisms, Immobilized Mammalian Cells, Immobilized Plant Cells, Reactors, Mass Transfer Effects, Bioreactors

Engineering problems in applying immobilized biocatalysts to the production of useful materials are discussed. The scope of the review covers immobilized enzymes, immobilized microorganisms, immobilized mammalian cells, immobilized plant cells and the use of membranes for immobilization. Recent developments in these subjects are reviewed especially as to reaction analyses, mass-transfer effects, bioreactions using an organic phase and problems concerning reactor design. The future scope of research in this field is briefly described in the final section.

1. Introduction

Utilization of biocatalysts, such as enzymes, microorganisms, organelles, mammalian cells and plant cells, arrived at a breakthrough point when the immobilized enzyme was industrialized successfully by Chibata *et al.* of Tanabe Seiyaku Co. in 1969. Many papers have since been published on various kinds of immobilized biocatalysts and industrial processes have been developed, as shown in **Table 1**. Immobilization is now one of the major considerations in the design of bioreactors. Many review papers[B8, C15, C16, H9, L14, R7, T5] have been published, especially regarding research on immobilization methods and applications of immobilized biocatalysts. Brief historical surveys were also included in those reviews.

In this review article, research on immobilized biocatalysts is presented from an engineering point of view. Immobilization methods are not emphasized, but behavior in reactors is discussed from an engineering perspective. Topics will cover this "art" of biotechnology, i.e. use of immobilized enzymes, microbial, mammalian and plant cells. Bioreactor designs using immobilized biocatalysts are also important in the field of biotechnology and are included in the discussion. Finally, future problems will be discussed briefly in the closing remarks.

2. Immobilized Enzymes

Methods of immobilization have been investigated quite extensively. Immobilization techniques and the application of immobilized enzymes have been reviewed in many reports.[C14, H9, L4, L10, P6, T5] Among these, the book edited by Chibata[C14] probably holds the widest coverage of references. It introduces each general method of immobilization and important applications of the immobilized enzymes and biocatalysts. Weetall and Pitcher[W10] describe important factors in scaling up immobilized enzyme systems.

Engineering topics in immobilized enzymes that appeared in recent papers are classified in **Table 2**. Clearly many studies have been published from various engineering perspectives. Some characteristic features are given below. Topics on membrane reactors will be described in the later sections.

2.1 Immobilization techniques

Immobilization techniques may be classified into

Table 1. Industrialized processes using immobilized biocatalysts

Biocatalyst	Application
a. Enzyme	
Aminoacylase	Production of L-amino acid (optical isomerization)
Glucose isomerase	Production of invert sugar
Lactase	Reforming of milk (decomposition of lactose)
Penicillin amidase	Production of 6-aminopenicillanic acid (6-APA)
GL-7-ACA amidase	Production of 7-aminocephalosporanic acid (7-ACA)
Aspartase (in *Escherichia coli*)	Production of L-aspartic acid
Fumarase (in *Brevibacterium ammomiagenes*)	Production of L-malic acid
Aspartase + L-aspartic acid 4-decarboxylase (in *Pseudomonas dacunhae*)	Production of L-alanine
α-Glucosyltransferase (in *Protaminobacter rubitum*)	Production of palatinose
b. Viable microorganism	
Saccharomyces sp.	Production of ethyl alcohol
Corinebacterium sp.	Production of acrylamide
c. Human cell (on microcarrier)	Production of interferron

Received December 5, 1987. Correspondence concerning this article should be addressed to S. Furusaki.

Table 2. Engineering problems of immobilized enzymes

Item	Detail	Literature
Immobilization	Specific immobilization	I5, N12
	Soluble supports	F3, W12
	Reaction accompanied with organic phase	J3, K20, L9, N7, O4, O5, P1, P4, T10, Z1
	Electric field effect	F11, K2, V1
	Two-phase systems	A7, B1, B5, B12, F6, H3, K13, L1, L2, L9, L21, M8, M9, M13, S1–S3, S16, S26
	Control of enzyme direction upon immobilization	W4, W5
	Inorganic supports	A5, C2, C21, H21, S17, S22, W8
	Fibrous supports	I1
	Photosynthetic supports	F5, I2, I3, M10
Co-immobilization	Multienzymes	E3, H8, K1, S18
	NAD recycling (using membranes)	L7, M15, M16
Reactor system	Industrial application	B4, H23, P6, S28
	Economic evaluation	D6, D7, L5
	Specific reactors	N6, S22
Engineering analysis of immobilized system	Distribution of enzymes	B7, C20, D2, D4, D5, H22, H24, M19
	Stability of enzymes, diffusion effect	C19, C5, D3, K19, K22, L6, P8, S12, T16
	Reaction analysis	C4, C17, I7, P3, R3, T17
	Effectiveness factor	B10, C7, C8, H10, H11, L3, L8, M3, Y2, Y3
	pH profile	C10, L18
	Oscillation, unsteadiness	K10
	Compaction	F18, N5, S6, S7, U1

the following categories:[C13,C14] covalent bonding, bridging, entrapping, ionic and/or physical adsorption. A large number of proposals on covalent bonding immobilization have been presented, but complex reaction steps are not economical and are considered undesirable with regard to enzyme stability. Entrapment and ionic adsoption are more practical although the former method may cause leakage of enzymes of low molecular weight. A chemical treatment using glutaraldehyde often gives better results in this case.

The photochemical entrapment approach of Ichimura and coworkers[I2,I3,M10] is said to be easy to handle and to be moderate for biocatalysts since visible light can initiate the entrapping polymerization. The drawback of photo-polymerization is that a cutting process is necessary to obtain the biocatalyst particles although some techniques are available to obtain globular particles. Photo-polymerization by using prepolymers and near-UV light[F5] is also used to immobilize enzymes and microbes. The super-fine fibrous ion exchanger[I1] can be used as a carrier of enzymes. This type of immobilization will lead to a large surface area and low pressure drop when used in fixed beds. Nozawa *et al.*[N12] proposed the application of the avidin-biotin affinity system, but economic problems remain unsolved.

Enzyme reactions with high-polymer substrates, such as cellulose hydrolysis, are theoretically difficult when applying immobilized enzymes. Fujimura *et al.*[F3] proposed an interesting immobilization technique. By using a polymer from methacrylic acid, methyl acrylate and methyl methacrylate, their enzyme can become soluble to a medium during reaction and then insoluble when it is to be recovered. Yield of recovery of enzymes by the gelation-precipitation should be high enough for this immobilization to be economically feasible. Kajiwara and Maeda[K1] proposed co-immobilization of malate dehydrogenase and formate dehydrogenase with NAD so that regeneration of the coenzyme proceeds inside the immobilized biocatalyst particles. The immobilization was accomplished by using a polyethyleneglycol diacrylate gel.

2.2 Enzyme reactions in the organic phase

Some enzyme reactions proceed efficiently using the oil phase as a reaction medium. Three types of reactions using this method have been widely studied: lipid conversion by lipase, peptide production, and transformation of steroids.

One approach to immobilized enzyme reactions in organic media is to adsorb enzymes onto hydrophilic carriers such as silica gel.[K20,L9,T10] A small amount of adsorbed water often enhances the extent of reaction compared with the abundant use of water in interesterification reactions. This is probably due to the fact that concentration of glycerol becomes higher in the small aqueous portion and it is rapidly consumed there. Thus, water content in the immobilized enzyme particles plays an important role in the reaction scheme. As a modification of this procedure Yokozeki *et al.*[Y7] used a polypropylene glycol-based prepolymer[O3] to entrap the celite-bound lipase. Reaction behavior of immobilized thermolysin[N7,O5] and chymotrypsin[J3,P4] was studied in relation to the influence of organic solvents.

As an alternative approach to reactions in the organic phase, solid enzyme powder can be suspended in the organic phase.[Z1] Harano *et al.*[H7, O4] studied the reaction behavior of solid thermolysin to produce aspartame precursor.

Two-phase systems can be applied to enzyme reactions in which an organic phase takes part. Use of reversed micelles has recently been investigated intensively.[B5, H3, L21, M7, M8, M13, S16, S26] This method may be considered a variation of immobilization, since enzymes are confined in the reversed micelles in the organic phase. Aerosol OT®, (AOT®), i.e. bis(2-ethylhexyl) sodium sulfosuccinate is the most popular surfactant used to immobilize positively charged enzymes (at a pH lower than the isoelectric point). Cetyltrimethylammonium bromide (CTAB) or trioctylmethylammonium chloride (TOMAC) are mostly used for the pH range of the opposite charge. Vesicles using phospholipids may also be used to immobilize hydrophobic membrane-bound enzymes.[S1, S2, S3]

Membrane bioreactors are also applied to enzyme reactions using organic phase. They will be discussed in a later section. Polyethylene glycol-modified enzymes were found to be soluble and active in organic phase.[T1, T1] They are not among the immobilized enzymes, and are outside the scope of this review.

2.3 Reaction analysis

The effectiveness factor of immobilized enzymes has been studied by many researchers. The fundamental approach was based upon Bischoff's analysis.[B9] His results can be applied to the calculation of the effectiveness factor for various reaction types, especially for the case of flat immobilized enzymes. For shapes other than flat board, Aris[A10] presented convenient general variables to reach good estimations.

Kobayashi and coworkers[K23, M18] extended the above approach to various types of enzyme kinetics and presented a simple approximation method[K24] to obtain the effectiveness factor for Michaelis-Menten type reactions. This relation has widely been used in the field of biochemical engineering. Gondo *et al.*,[G9] Yamane *et al.*[Y2, Y3] and Toda *et al.*[T12, T14] also gave useful relations including the film mass transfer effect. Do *et al.*[C20, D5, H22, H24] studied the distribution of enzymes inside the carrier when enzymes were diffused from outside the carrier. The distribution certainly affects the reaction behavior.

It is well known that immobilization increases the stability of enzymes. One of the reasons is a more stable structure due to physical and chemical interactions with the carrier structure. The intraparticle diffusion resistance sometimes enhances the stability in the case of possible deactivation by substrate, etc. Apparent rate of deactivation is lower than that of native enzymes because of the diffusional resistance.[O1] Effective use of inner-bound enzymes increases with the progress of deactivation. Klibanov[K19] presented a good review of this topic. Kobayashi,[K22] Lee and Reilly,[L6] and Chung and Chang[C19] studied this behavior further. Furusaki and coworkers[F14 - F16] studied the cascade operation of deactivating enzymes. This study is applicable not only to packed-bed or membrane bioreactors; the principle can also be extended to the circulatory use of a set of several reactors.[F9] An industrial application of this method has been realized.[I8] According to the model calculation three or four cascade stages will be sufficient.

When the temperature can be raised above the normal level, the optimal temperature policy[H1, S4] can be applied.

Organic supports of immobilized biocatalysts often suffer from bed compaction. This compaction causes reduction in surface area of the support carriers and thus decreases the effectiveness factor of the biocatalyst.[F18, S6, S7, U1] To avoid compaction, a baffled packed bed[F8] was proposed and resulted in success. Nakamura *et al.*[N5] proposed a rigid shell surrounding soft carriers. Inorganic supports such as porous glass are resistant to this phenomenon. Kusunoki and coworkers studied the use of ceramic honeycomb carriers.[S22]

Kerneves *et al.*[K10] studied multiple steady states and oscillation in immobilized enzyme systems. This is another important problem to be solved by chemical engineers.

3. Immobilized Microorganisms

Immobilized microorganisms can be classified into two categories, i.e. immobilized dead cells and immobilized living cells. The latter is subdivided into immobilized resting cells and immobilized growing cells. Immobilized dead cells use enzymes exuded from cell debris and retained inside carriers. They behave similarly to immobilized enzymes although cellular membranes may cause complications in transport phenomena. Immobilized resting cells are cells characterized by homogeneous distribution inside the particles. This will happen where cells do not grow after immobilization. If the cells grow, the cell-density distribution is not homogeneous. Usually the cell-density becomes large near the surface. This is the case for immobilized growing cells. The cell growth must be regulated in this case so that the grown cells do not leak into the solution.

General reviews[K9] on immobilized microorganisms have been published by many authors. Applications of immobilized microorganisms were given by Corcoran.[C23] Karel *et al.*[K3] presented an extensive

Table 3. Engineering problems of immobilized microorganisms

Item	Detail	Literature
Application	General	C23, K9, T6
	Antibiotics, peptides, proteins, amino acids, etc.	E1, H19, J2, K5, K12, K21, N2, W1
	Immobilization methods	A9, C4, E1, E2, F4, G1, J2, K6, K12, M6, M21, N2, O2, O3, T15, V5, W4, Y5
Apparatus	Compaction	F7, F8
	Fluidized bed	A4, F1, H12, M17, T9
	Spouted bed	W4
Transport phenomena	Diffusion	H6, H13, R1, R2, T8
	O_2 Transport	A1, A3, H14, T13, W11
	Effectiveness factor, modelling	B13, C11, F19, K17, K18, S13
	Dynamics, stability, oscillation	B2, D8, T3
	Measurement of immobilized systems	L19, W11
Genetic engineering	Immobilization of microorganism with recombinant DNA	C12, D1, G4, H5, M20
Ethanol production	General	B11, N3, N10, S8, Y1
	Immobilized yeast	C22, D9, G3, G6, G7, G8, H2, H15, H16, M2, R9, T18, V3
	Immobilized *Zymomonas*	K15, L20, M5, M12
	Flocculant yeast	N8, P9
	Extractive fermentation	C24, K25
	Apparatus	C18, F13, H4, H12, N1, S19

review including engineering problems such as mass transfer, kinetics and reactor design. Regarding the influence of diffusional resistance, Radovich[R1,R2] presented precise reviews including the determination of mass transfer resistances and a discussion of reactor design. Engineering problems concerning immobilized microorganisms are listed in **Table 3**.

3.1 Application of immobilized microorganisms

After the success in 1969 of Tanabe Seiyaku Co.'s immobilized enzyme process, the application of immobilized microorganisms was investigated by many institutions. Immobilized *Escherichia coli* for production of L-aspartic acid, immobilized *Brevibacterium ammoniagenes* for L-malic acid and immobilized *Pseudomonas docunhae* for L-alanine have been successfully applied industrially. These are immobilized dead cells using enzymes that leak from cell debris. Fukui and coworkers[F4,O2,O3,Y5] used prepolymers as a starting material of cell-entrapment and carried out research into steroid transformation by immobilized-cell enzymes. The reaction was carried out in organic solvents and proceeded similarly to that in aqueous phase. The choice of an organic solvent was important and interesting discussions[B12,L1,L2] were presented with regard to catalyzed reactions by immobilized-cell enzymes in organic phase. Recently, immobilized *Corynebacterium* was used for production of acrylamide from acrylonitrile by Nitto Chemical Industries Co.[N9] This is the first process to produce a commodity chemical using an immobilized biocatalyst. Immobilized yeast was studied for production of ethanol in a pilot plant,[N10] and the process is said to be commercially feasible.[N3,S8] These latter cases are examples of the use of immobilized living cells.

Generally, products of microbial fermentation, e.g. amino acids, organic acids, peptides, saccharides, steroids and antibiotics, can be obtained by using immobilized microbes. However, immobilized aerobes have not been applied although many studies have been carried out. This is probably due to the inhibiting effect of mass transfer resistance on the oxygen demand. Also, aerobe cells leaked from carriers contribute to a large extent, and the role of immobilized microorganisms is becoming smaller as time goes on. If the application of immobilized aerobes becomes possible, the use of immobilized microorganisms will become much greater. However, this seems difficult for the reasons mentioned above.

The application of immobilized microorganisms is competitive with the use of flocculant microorganisms and of membrane reactors. The latter two methods can provide a dense cell suspension in the fermentation medium, so the reaction rate based upon unit reactor volume can become as high as that using immobilized cells. Adaptation to the concentrating processes depends on the character of the microorganisms. It is thus necessary to evaluate carefully which process is most appropriate on a case-by-case basis.

Immobilized microorganisms have the following merits: (1) The reaction rate is high because of high cell density. Therefore, the reactor volume becomes smaller. (2) The separation of cells from fermentation media is easy. (3) Innoculation and batchwise cultivation of microorganisms are not necessary. However, the stabilization effect observed in the case of immobilized enzymes is not always possible.

Therefore, other methods having the merits described above could possibly take the place of immobilized microorganisms.

3.2 Developments in immobilization of microorganisms

Microorganisms are mostly immobilized by an entrapment method. Details of the method have been described in many reviews and will not be discussed here. Co-immobilization of two microorganisms is possible if the optimum temperature and pH are common. Tanaka *et al.*[T7] co-immobilized *Aspergillus awamori* and *Zymomonas mobilis* to produce ethanol from starch. With the laspe of fermentation *Z. mobilis* became rich near the center of the carriers and *A. awamori* became rich near the surface due to oxygen transfer resistance. *Chlorella* and *Gluconobacter* were co-immobilized similarly by Adlercreutz *et al.*[A2] Substrate and micoorganisms are co-immobilized when the solubility of the substrate in aqueous medium is very low. Kaul *et al.*[K6] co-immobilized steroids with *Arthrobacter simplex* in Ca-alginate gel. This method may be applied to bioconversion of materials which normally dissolve in an organic phase. Enzymes and microorganisms can also be co-immobilized. For example, β-galactosidase and *Saccharomyces cerevisiae* were coimmobilized to produce ethanol.[H2]

Fungi immobilization was also investigated using Ca-alginate and κ-carageenan gels.[B6,L15,L16] Immobilization of fungi gives different aspects from that of microbial cells. Gbewonyo and Wang,[G1] Jones *et al.*[J2] and Kim *et al.*[K12] used celite carrier to culture *Penicillium chrysogenum* in a liquid fluidized bed. Celite was used as a carrier of *Streptomyces* sp.[A9] as well. The small pore of celite was said to provide a good environment for fungi to grow in, and the immobilized fungus was easy to handle in three-phase fluidized beds. Endo *et al.*[E1,E2] used foamed polyurethane sponge as a carrier. By applying the carrier the fungus does not form a pellet or pulpy viscous solution. It forms a biofilm near the surface and mass transfer resistance decreases. The production rate of penicillin increased to a much higher level than that by natural cultivation. *Aspergillus niger* was immobilized by polyacrylamide[H19] or Ca-alginate[T15] gels, as well. Electrostatic effects on adhesion of microorganisms to carriers are discussed by Mozes *et al.*[M21]

3.3 Apparatus

Apparatus for immobilized microorganisms is generally the same as the chemical reactors used for liquid-phase catalytic reactions. Fixed and fluidized beds are common. Stirred tanks, trickle beds, bubble columns and rotary discs are often utilized. Reactors for ethanol production require CO_2 removal and plug flow behavior to avoid product inhibition. Three-stage conical fluidized beds,[H12] tapered packed beds,[H4] horizontal cross-flow reactors[C18,S19] and rotary column reactors[F12,F13] have been proposed for this purpose.

As an example of a different type of reactor, a spouted bed using compressed air was presented by Webb *et al.*[W4] for production of cellulase. Gas-phase fluidized beds are applied to some reactions.[A4,T9]

3.4 Mass transfer

Intraparticle diffusion effects on the reactivity of immobilized microorganisms have been investigated similarly to the case of immobilized enzymes. The principal difference is the existence of cells in the biocatalyst domaim. When the substrate reaches the surface of cells, bioconversion is initiated. Thus, the substrate has to migrate through the space between the cells in order to react. This situation resembles diffusion through solid catalyst particles. Klein *et al.*[K16,K17] presented the concept that the effective diffusivity, D_e, depends on the cell concentration in the gel, i.e. the diffusivity decreased with increasing cell concentration. Furusaki *et al.*[F19] formulated this property using the random pore theory of Wakao and Smith.[W2] Thus, D_e is related to the diffusivity in the gel, D_g:

$$\frac{D_e}{D_g}=\varepsilon_c^2 \tag{1}$$

where ε_c is the volume fraction of gel occupied by cells. If the specific volume of cells, v_c, and the cell concentration c_c is used, the following equation holds using a consistent unit system.

$$\frac{D_e}{D_g}=(1-v_c c_c)^2 \tag{2}$$

The value of D_g was independent of Ca-alginate gel concentration within the range of the experimental condition (Ca-alginate conc. $<5\%$). It is in general 60–80% of the diffusivity in water. Equation (2) was used in the analysis of immobilized resting *S. cerevisiae*[F19] and *Z. mobilis*[S5] and immobilized growing *S. cerevisiae*.[S13]

4. Immobilized Mammalian Cells

Mammalian cells are used to produce interferons, interleukins, vaccines, antibodies and a tissue-type plasminogen activator (TPA). There are many prospective possibilities of producing hormones, e.g. human growth hormone and kallikrein, and blood ingredients, e.g. factors VIII, IX, and protein C. Arathoon and Birch[A8] clarified problems related to the industrialization of large-cell culture systems.

Immobilization of mammalian cells is useful when they require solid surface to grow on and/or to protect them from shear stress. For the former requirement, microcarriers are used. Microcarrier for

cell cultivation was proposed also to increase the surface area on which cells grow.[V2] By using a microcarrier, e.g. DEAE-dextran, cells grow on the surface of the carrier in a monolayer. Operation is normally batchwise because fine carriers are used. Research has been carried out on this process,[R4, S9] and it was industrialized successfully by Toray Industries, Ltd.[I4, I10, Y6] Ceramic support[M4] for hybridoma cells was also tried for production of monoclonal antibodies. Matrix perfusion in microcarrier beds was another approach.[S27]

Gel entrapment was tried as a means of overcoming shear stress. Although normal mammalian cells can grow only on a solid surface, hybridoma cells can grow in culture medium in suspension. In this case, entrapment by alginate gels can be applied to obtain immobilized mammalian cells.[S21] Normal adhesive cells are found to be entrapped by alginate gels[S20] as well. When a large-scale fermentor is used for cell culture, the problem of shear stress becomes important since the turbulence effect becomes larger upon reactor scale-up. Turbulent viscosity, which is the proportional factor of the Reynolds stress against the mean velocity gradient, is proportional to 1.5–1.7 power of the column diameter for bubble columns.[M14, U2]

The effect of shear stress on the cells was studied in relation to the laminar velocity gradient,[S25] measuring the extent of lactate dehydrogenase leakage from the cells. Cells suffer normally from shear stress in the turbulent region, so this result is not directly related to the real case. Nevertheless, it gives useful information as to fundamental results of the physical strength of cells. To prevent damage to microcarrier-anchored cells due to shear stress, Kariya and Tozaki[K4] proposed a novel rotary reactor.

Membrane reactors to entrap mammalian cells have also been studied.[A6, K14] Microcapsules were used to entrap the cells, as well.[G5, J1, L11]

The problem associated with the entrapment of mammalian cells is transfer of substrates, products and oxygen. Sensitivity to the mass transfer limitation differs according to species. Thus, once again the applicability must be judged on a case-by-case basis.

5. Immobilized Plant Cells

Plant cells produce biologically active products that microorganisms cannot produce. This is why plant cells play an important role in biotechnology. Some of the useful products of plant cells are given in **Table 4**. Immobilization of plant cells was first investigated by Brodelius *et al.*[B16] Many reviews of research on immobilized plant cells have been published.[B14, B15, F21, P7, R5, R8, S14, Y8] Most are concerned with application of plant cells; the chemical engineering approach has not yet been sufficiently considered.

Table 4. Examples of products of plant cells anticipated in biotechnology

Plant	Product
Catharanthus roseus	Ajmalicin, vinblastine, serpentine
Papaver somniferum	Codeine
Nicotiana rustica	Nicotine
Digitalis lanata	Digoxin, digitoxin
Daucus carota	Anthocyanin, periprogenin, 5-hydroxydigitoxigenin
Morinda citrifolia	Anthraquinone
Nicotiana tabacum	Ubiquinone, nicotine
Coptis japonica	Berberin
Lavendula vera	Biotin, L-cysteine
Dioscorea deltoides	Diosginin
Beta vulgaris	Betacyanin
Coffea arabica	Caffeine
Panax ginseng	Saponins
Lithospermum erythrorhizon	Shikonin

Immobilization of plant cells has advantages similar to immobilization of microorganisms, such as ease of continuous operation, high resistance to contamination and to shear stress, and ease of separation of biocatalysts. In addition to these advantages, immobilized plant cells often produce more secondary metabolic products than do native cells. This may be caused by physical stresses[F2] due to immobilization exerted on the cells.[A11, F20] The increase in productivity should be weighed against the demerits such as mass transfer limitation.

Studies of mass transfer concerning immobilized plant cells are few. Furuya *et al.*[F22] presented the effect of aeration on immobilized *Papaver somniferum*. The cell required vigorous aeration in order to maintain its activity. Later intraparticle oxygen transfer was found to limit viability of immobilized *P. somniferum*, from a reaction engineering analysis.[I9] That is, oxygen concentration becomes low in the central region of the particles.[M11] The influence of nutrient limitation of immoblilized *Capsicum frutescens* was physiologically studied.[L12] The diffusivity of glucose in callus tissue was measured by Mavituna *et al.*[M11]

The immobilization of photosynthetic algae was studied to learn the immobilization effect on photosynthetic activity.[B3, K8, T4, W9] This approach, considering its stability, is more practical than immobilizing chloroplasts. Immobilization of protoplasts[C1, L17, S15] is a prospective way to remove mass transfer resistance by cell walls. Co-immobilization of plant cells and microorganisms was studied by Adlercreutz *et al.*[A2] By their system oxygen was supplied by *Chlorella pyrenoidosa* and consumed by *Gluconobacter oxydns*. Balance of oxygen supply is subtly achieved. Light supply is an important

problem of reactors with immobilized biocatalysts demanding light intensity. A novel method of immobilization using polyurethane foam particles was proposed.[L13,R6]

Reactors using immobilized plant cells have not been investigated sufficiently and will be a future topic of research. Most of the reaction studies were carried out in mixing vessels or bubble columns. An airlift loop reactor was proposed by Verlaan *et al.*[V4] Membrance reactors for plant cells have been investigated by Shuler *et al.*[S23]

In summary, plant cell immobilization is in the earlier stage of research and further work is necessary on stability, mass transfer, and the change of productivity of secondary metabolites due to immobilization.

6. Membrane Immobilization

Immobilization using membranes, by means of membrane bioreactors, has been investigated extensively for enzymes, microorganisms, mammalian and plant cells. Various methods of membrane application are possible, such as the hollow-fiber reactor[C6,K7,W7] and use of membrane modules attached to fermentors. The latter is used for handling viable cells because aeration or gas removal is possible. Methods of application of solid membranes can be classified as follows[F10] (**Fig. 1**): (a) filtering bioproducts by membrane out of reactors, (b) permeating substrates and products to and from a biocatalyst-containing compartment separated by membrane, (c) use of biocatalysts immobilized in the form of membranes, (d) use of biocatalyst-containing membranes simultaneously permeating reactants and products, and (e) use of multilayer membranes composed of a biocatalyst layer and a separation layer to carry out simultaneous reaction and permeation. There are many reviews of membrane bioreactors.[C6,G2,T11] Recent topics are discussed extensively by Chang.[C9]

A characteristic feature using membranes is the ease of immobilizing biocatalysts. Due to this merit membrane reactors are used for coenzyme-dependent systems[M15,M16,S11] and multienzyme systems.[H20,S24,W3] Dynamic membrane bioreactors, where enzyme gel accumulates on the surface of UF membranes, are a recent subject in this field.[G10,N4,P10] This method can be applied to separation of optical isomers.[I6]

Membranes can also be used to retain immobilized microorganisms, and mammalian and plant cells. Fermentation by high cell density is possible by this method. In that case, however, adhesion of cells to the surface of membranes can be a crucial problem. The pressure-swing reactor[F14,F17,K11,P2] is effective in preventing fouling of membranes caused by cell adhesion.

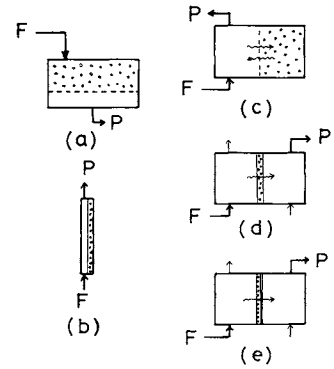

Fig. 1. Typical membrane reactors
F = feed; P = product. Points denote biocatalysts. (a) filtration type; (b) solid membrane biocatalyst; (c) pressure swing type; (d) simultaneous reaction and permeation; (e) use of multilayer membrane.

Several bioreactions are preferable in the organic phase, as stated in the previous section. Membrane reactors can contribute to this field as well. Yamane *et al.* studied reactions of lipids using lipase by membrane reactors.[H17,H18,Y4]

Liquid membranes, especially W/O/W surfactant membranes, are also used to immobilize enzymes.[K26] In this case immobilization of biocatalysts and selective migration of substances are realized simultaneously. Schepper *et al.*[S9] investigated this process using the hydrolysis of L-phenylalanine methyl ester by α-chymotrypsin. Separation of DL-isomers was possible by using this reaction. A liquid anion exchanger was used to migrate the L-phenylalanine produced and to control pH in the inner aqueous phase. Immobilization of coenzymes is also possible by liquid membrane since the solubility of coenzymes in the organic phase is very small. Coenzyme uridine diphosphoglucoronate (UDPGA) was immobilized together with UDP-glucoronyltransferase to remove phenol from serum.[P5,V6] As another example of a coenzyme, a liquid membrane system using leucine dehydrogenase and formate dehydrogenase (FDH) together with NAD was investigated.[M1]

Immobilization of enzymes by reversed micelles or by vesicles may be considered as a form of immobilization by liquid membranes. This was discussed above in the section of immobilized enzymes.

Conclusion/Future View

Immobilized biocatalysts have the following advantages: (a) ease of developing continuous processes, (b) high stability and resistance against shear stress and contamination, (c) fast reaction rate due to high catalyst concentration, (d) easy separation of biocatalysts from fermentation medium, and (e) repetitive use of biocatalysts. Of course, there are

demerits such as the existence of mass transfer resistances, the necessity of immobilization processes and the additional cost of immobilizing reagents. Selectivity may be varied by the mass-transfer effect. Because of the above merits, however, investigation of immobilized biocatalysts has been carried out extensively in various fields. Although practical industrialization is limited at the moment compared with the extent of research, steady growth of use of this method seems likely.

Recent development of DNA recombination might induce the next important topics in this field. A number of recombinant cells have been developed, and they will come to be involved in the object of immobilization. As a result, such problems as those concerning stability of recombinant genes or optimization during the course of gene amplification will be investigated with respect to physiological shock caused by immobilization.

Another future aspect is the use of biomimetic catalysts, e.g. artificial enzymes. They are regarded as being stable and highly selective. The properties of immobilized biomimetic catalysts will become one of the main topics in the field of bioprocess engineering in the near future.

Literature Cited

A1) Adlercreutz, P.: *Biotechnol. Bioeng.*, **28**, 223 (1986).
A2) Adlercreutz, P., O. Holst and B. Mattiasson: *Enzyme Microb. Technol.*, **4**, 395 (1982).
A3) Adlercreutz, P. and B. Mattiasson: *Appl. Microbiol. Biotechnol.*, **20**, 296 (1984).
A4) Akao, T. and Y. Okamoto: *Kagaku Kōgaku*, **49**, 349 (1985).
A5) Allen, B. R. and D. J. Fink: "Enzyme Engineering," Vol. 5, p. 443, Plenum Press, New York (1980).
A6) Altshuler, G. L., D. M. Dziewulski, J. A. Sowek and G. Belfort: *Biotechnol. Bioeng.*, **28**, 646 (1986).
A7) Antonini, E., G. Carrea and P. Cremones: *Enzyme Microb. Technol.*, **3**, 271 (1981).
A8) Arathoon, W. R. and J. R. Birch: *Science*, **232**, 1390 (1986).
A9) Arcuri, E. J., J. R. Nichols, T. S. Brix, V. G. Santamarina, B. C. Buckland and S. W. Drew: *Biotechnol. Bioeng.*, **25**, 2399 (1983).
A10) Aris, R.: *Chem. Eng. Sci.*, **6**, 262 (1957).
A11) Ayabe, S., K. Iida and T. Furuya: *Plant Cell Rep.*, **3**, 186 (1986).
B1) Baerbel, H. H., E. Anderson, M. Larsson and B. Matiasson: "Biochem. Eng. III," Ann. N. Y. Acad. Sci., Vol. 413, p. 542 (1983).
B2) Bailey, K. M., K. Venkatasubramanian and S. B. Karkare: *Biotechnol. Bioeng.*, **27**, 1208 (1985).
B3) Bailliez, C., C. Largeau, C. Berkaloff and E. Casadevall: *Appl. Microbiol. Biotechnol.*, **23**, 361 (1986).
B4) Beck, M., T. Kiesser, M. Perrier and W. Bauer: *Can. J. Chem. Eng.*, **64**, 553 (1986).
B5) Berezin, I. V., Y. L. Khmelnitsky, N. L. Klyachko, A. V. Lavashov and K. Martinek: Proc. 3rd Eur. Cong. Biotechnol., Vol. 3, p. 449 (1984).
B6) Berk, D. L., A. Behie, A. Jones, B. H. Lesser and G. M. Gaucher: *Can. J. Chem. Eng.*, **62**, 112 (1984).
B7) Bernstein, H., Y. C. Yang and R. Langer: *Biotechnol. Bioeng.*, **30**, 196 (1987).
B8) Birnbaum, S., P. O. Larsson and K. Mosbach: *Chem. Anal.*, **66**, 679 (1983).
B9) Bischoff, K. B.: *AIChE J.*, **11**, 351 (1965).
B10) Borchert, A. and K. Buchholz: *Biotechnol. Bioeng.*, **26**, 727 (1984).
B11) Borglum, G. B. and J. J. Marshall: *Appl. Biochem. Biotechnol.*, **9**, 117 (1984).
B12) Brink, L. E. S. and J. Tramper: *Biotechnol. Bioeng.*, **27**, 1258 (1985).
B13) Brink, L. E. S. and J. Tramper: *Enzyme Microb. Technol.*, **8**, 334 (1986).
B14) Brodelius, P.: "Enzymes Immobilized Cells in Biotechnol.," Biotechnol. Ser., Vol. 5, p. 109 (1985).
B15) Brodelius, P.: *Trends Biotechnol.*, **3**, 280 (1985).
B16) Brodelius, P., B. Deus, K. Mosbach and M. H. Zenk: *FEBS Lett.*, **103**, 93 (1979).
C1) Cabral, J. M. S., P. Fevereiro, J. M. Novais and M. S. S. Pais: "Enzyme Eng. 7," Ann. N. Y. Accd. Sci., Vol. 434, p. 501 (1984).
C2) Cabral, J. M. S., J. M. Novais and J. P. Cardoso: *Biotechnol. Bioeng.*, **23**, 2083 (1981).
C3) Cabral, J. M. S., J. M. Novais, J. P. Cardoso and J. F. Kennedy: *J. Chem. Technol. Biotechnol.*, **36**, 24 (1986).
C4) Cabral, J. M. S., J. M. Novais and J. F. Kennedy: *Appl. Microbiol. Biotechnol.*, **23**, 157 (1986).
C5) Carter, R. S., J. E. Prenosil and J. R. Bourne: "Enzyme Eng.," Vol. 5, p. 321, Plenum Press (1980).
C6) Chambers, R. P., W. Cohen and W. H. Barricos: "Methods Enzymology," Vol. 44, p. 291, Academic Press, New York (1976).
C7) Chang, H. N.: *AIChE J.*, **28**, 1030 (1982).
C8) Chang, H. N.: *Korean J. Chem. Eng.*, **1**, 43 (1984).
C9) Chang, H. N.: *Biotechnol. Adv.*, **5**, 129 (1987).
C10) Chang, H. N. and I. S. Joo: *Chem. Eng. Commun.*, **34**, 15 (1985).
C11) Chang, H. N. and T. H. Park: *J. Theor. Biol.*, **116**, 9 (1985).
C12) Chang, Y. K. and H. C. Lin: *Biotechnol. Bioeng.*, **29**, 950 (1987).
C13) Chibata, I., "Immobilized Enzymes," Kodansha, Tokyo (1978).
C14) Chibata, I., "Koteika Seitaishokubai" (in Japanese), Kodansha Scientific, Tokyo (1986).
C15) Chibata, I., T. Tosa and T. Sato: "Enzymes and Immobilized Cells in Biotechnology," p. 37, Benjamin/Cummings, Menlo Park (1985).
C16) Chibata, I., T. Tosa and T. Sato: *J. Mol. Catal.*, **37**, 1 (1986).
C17) Ching, C. B. and Y. Y. Ho: *Appl. Microbiol. Biotechnol.*, **20**, 303 (1984).
C18) Chotani, G. K. and A. Constantinides: *Biotechnol. Bioeng.*, **26**, 217 (1984).
C19) Chung, B. H. and H. N. Chang: *Korean J. Chem. Eng.*, **3**, 39 (1986).
C20) Clark, D. S., J. E. Bailey and D. D. Do: *Biotechnol. Bioeng.*, **27**, 208 (1985).
C21) Clifford, C. and D. B. Johnson: *Biotechnol. Bioeng.*, **22**, 2441 (1980).
C22) Constantinides, A. and G. K. Chotani: "Enzyme Eng. 7," Ann. N. Y. Acad. Sci., Vol. 434, p. 347 (1984).
C23) Corcoran, E.: *Topics Enzyme Ferment. Biotechnol.*, **10**, 12 (1986).
C24) Crabbe, P. G., C. W. Tse and P. A. Munro: *Biotechnol. Bioeng.*, **28**, 939 (1986).
D1) Dhulster, P., J. N. Barbotin and D. Thomas: *Appl. Microb. Biotechnol.*, **20**, 87 (1984).

D2) Do, D. D.: *Biotechnol. Bioeng.*, **26**, 1032 (1984).
D3) Do, D. D.: *Chem. Eng. J.*, **28**, B51 (1984).
D4) Do, D. D. and M. M. Hossain: *Biotechnol. Bioeng.*, **28**, 486 (1986).
D5) Do, D. D. and M. M. Hossain: *Biotechnol. Bioeng.*, **29**, 545 (1987).
D6) Domach, M. M.: *Chem. Eng. J.*, **29**, B1 (1984).
D7) Domach, M. M.: *World Biotechnol. Rep.*, **2**, A31 (1984).
D8) Doran, P. M. and J. E. Bailey: *Biotechnol. Bioeng.*, **29**, 892 (1987).
D9) D'Souza, S. F., J. S. Melo, A. Deshpande and G. B. Nadkarni: *Biotechnol. Lett.*, **8**, 643 (1986).
E1) Endo, I., T. Nagamune and I. Inoue: *Kagaku Kōgaku*, **49**, 293 (1985).
E2) Endo, I., T. Nagamune, N. Kato, M. Nishimura and T. Kobayashi: *Bioprocess Eng.*, **3**, to be published (1988).
E3) Erekin, N. and M. E. Friedman: *J. Solid-Phase Biochem.*, **4**, 123 (1979).
F1) Fan, L. S., K. Fujie, T. R. Long and W. T. Tang: *Biotechnol. Bioeng.*, **30**, 498 (1987).
F2) Frischknecht, P. M. and T. W. Baumann: *Phytochemistry*, **24**, 2255 (1985).
F3) Fujimura, M., T. Mori and T. Tosa: *Biotechnol. Bioeng.*, **29**, 747 (1987).
F4) Fukui, S., S. A. Ahmed, T. Omata and A. Tanaka: *Eur. J. Appl. Microbiol. Biotechnol.*, **10**, 289 (1980).
F5) Fukui, S. and A. Tanaka: *Hakko to Kogyo*, **41**, 552 (1983).
F6) Fukui, S. and A. Tanaka: *Endeaver*, **9**, 10 (1985).
F7) Furui, M.: *Kemikaru Enjiniaringu*, **30**(2), 107 (1985).
F8) Furui, M. and K. Yamashita: *J. Ferment. Technol.*, **63**, 73 (1985).
F9) Furusaki, S.: "Kosokogaku," p. 330, Tokyo Kagaku Dojin, Tokyo (1981).
F10) Furusaki, S.: *Membrane*, **9**, 93 (1984).
F11) Furusaki, S. and N. Asai: *Biotechnol. Bioeng.*, **25**, 2209 (1983).
F12) Furusaki, S. and T. Kikuchi: *Kemikaru Enginiaringu*, **30**(6), 53 (1985).
F13) Furusaki, S., T. Kikuchi and K. Kijima: *J. Fac. Eng., Univ. Tokyo*, **B38**, 1 (1985).
F14) Furusaki, S., T. Kojima and T. Miyauchi: *J. Chem. Eng. Japan*, **10**, 233 (1977).
F15) Furusaki, S., I. Matsuura and T. Miyauchi: *J. Chem. Eng. Japan*, **13**, 304 (1980).
F16) Furusaki, S. and T. Miyauchi: *J. Chem. Eng. Japan*, **10**, 247 (1977).
F17) Furusaki, S. and T. Miyauchi: *J. Chem. Eng. Japan*, **14**, 479 (1981).
F18) Furusaki, S., Y. Okamura and T. Miyauchi: *J. Chem. Eng. Japan*, **15**, 148 (1982).
F19) Furusaki, S. and M. Seki: *J. Chem. Eng. Japan*, **18**, 389 (1985).
F20) Furuya, T.: *Plant Cell Rep.*, **3**, 186 (1986).
F21) Furuya, T.: *Yakugaku Zasshi*, **106**, 856 (1986).
F22) Furuya, T., T. Yoshikawa and M. Taira: *Phytochemistry*, **23**, 999 (1984).
G1) Gbewonyo, K. and D. I. C. Wang: *Biotechnol. Bioeng.*, **25**, 967 (1983).
G2) Gekas, V. C.: *Enzyme Microb. Technol.*, **8**, 450 (1986).
G3) Gencer, M. A. and R. Mutharasan: *Biotechnol. Bioeng.*, **25**, 2243 (1983).
G4) Georgiou, G., J. J. Chalmers, M. L. Shuler and D. B. Wilson: *Biotechnol. Progr.*, **1**, 75 (1985).
G5) Gharapetian, H., N. A. Davis and A. M. Sun: *Biotechnol. Bioeng.*, **28**, 1595 (1986).
G6) Ghose, T. K. and K. K. Bandyopadhyay: *Biotechnol. Bioeng.*, **22**, 1489 (1980).
G7) Ghose, T. K. and K. K. Bandyopadhyay: *Biotechnol. Bioeng.*, **24**, 797 (1982).
G8) Gianetto, A., V. Specchia and G. Genon: *Chem. Eng. Commun.*, **23**, 251 (1983).
G9) Gondo, S., S. Isayama and K. Kusunoki: *J. Chem. Eng. Japan*, **7**, 64 (1974).
G10) Greco, Jr. G., D. Albanesi, M. Cantarella and V. Scardi: *Biotechnol. Bioeng.*, **22**, 215 (1980).
H1) Haas, W. R., L. L. Tavlarides and W. J. Wnek: *AIChE J.*, **20**, 707 (1974).
H2) Hahn-Hagerdal, B.: *Appl. Biochem. Biotechnol.*, **7**, 43 (1982).
H3) Han, D., J. S. Rhee and S. B. Lee: *Biotechnol. Bioeng.*, **30**, 381 (1987).
H4) Hamamci, H. and D. D. Y. Ryu: *Biotechnol. Bioeng.*, **29**, 994 (1987).
H5) Hamstra, R. S., M. R. Murris and J. Tramper: *Biotechnol. Bioeng.*, **29**, 884 (1987).
H6) Hannoun, B. J. M. and G. Stephanopoulos: *Biotechnol. Bioeng.*, **28**, 829 (1986).
H7) Harano, Y., H. Mori and H. Ooshima: Proc. World Cong. III. Chem. Eng., Vol. 1, p. 984, Tokyo (1986).
H8) Harder, A. and J. Wilms: Proc. 3rd Eur. Congr. Biotechnol., Vol. 4, p. 345 (1985).
H9) Hartmeier, W.: *Trends Biotechnol.*, **3**, 149 (1985).
H10) Hashimoto, K., S. Adachi and M. Yokoyama: *Mem. Fac. Eng. Kyoto Univ.*, **47**, 269 (1985).
H11) Hashimoto, K., S. Adachi, M. Yokoyama, H. Imakoma, G. Gelif and T. Ikeda: *Mem. Fac. Eng., Kyoto Univ.*, **47**, 276 (1985).
H12) Hatakeyama, H. and H. Noda: *Kagaku Kōgaku*, **48**, 603 (1984).
H13) Hiemstra, H., L. Dijkhuizen and W. Harder: *Eur. J. Appl. Microbiol. Biotechnol.*, **18**, 189 (1983).
H14) Ho, C. S., E. Livio and L. K. Ju: Proc. World. Congr. III Chem. Eng., Vol. 1, p. 855, Tokyo (1986).
H15) Holcberg, I. B. and P. Margalith: *Eur. J. Appl. Microb. Biotechnol.*, **13**, 133 (1981).
H16) Honda, H., M. Taya and T. Kobayashi: *J. Chem. Eng. Japan*, **19**, 268 (1986).
H17) Hoq, M. M., T. Yamane, S. Shimizu, T. Funada and S. Ishida: *J. Am. Oil Chem. Soc.*, **61**, 776 (1984).
H18) Hoq, M. M., T. Yamane, S. Shimizu, T. Funada and S. Ishida: *J. Am. Oil Chem. Soc.*, **62**, 1016 (1985).
H19) Horitsu, H., S. Adachi, Y. Takahashi, K. Kawai and Y. Kawano: *Appl. Microbiol. Biotechnol.*, **22**, 8 (1985).
H20) Horvath, C.: "Prog. Clin. Enzymol. III," p. 219, ed. by D. M. Goldberg and M. Werner, Masson Publ. (1983).
H21) Hossain, M. M. and D. D. Do: *Biotechnol. Bioeng.*, **27**, 842 (1985).
H22) Hossain, M. M. and D. D. Do: *Biotechnol. Bioeng.*, **27**, 1126 (1985).
H23) Hossain, M. M. and D. D. Do: *Chem. Eng. Sci.*, **42**, 255 (1987).
H24) Hossain, M. M., D. D. Do and J. E. Bailey: *AIChE J.*, **32**, 1088 (1986).
I1) Ichijo, H., T. Suehiro, J. Nagasawa, A. Yamauchi and M. Sagesaka: *Sen-I Gakkaishi*, **41**, T-303 (1985).
I2) Ichimura, K.: *J. Polym. Sci., Polym. Chem. Ed.*, **20**, 1411 (1982).
I3) Ichimura, K. and S. Watanabe: *J. Polym. Sci., Polym. Chem. Ed.*, **20**, 1419 (1982).
I4) Iizuka, M., E. Sano, H. Kubota and S. Kobayashi: Proc. 3rd Conf. Recovery Bioprod., p. 28, Uppsala (1986).
I5) Ikura, K., K. Okumura, M. Yoshikawa, R. Sakaki and H.

Chiba: *J. Appl. Biochem.*, **6**, 222 (1984).
I6) Ishikawa, H., H. Nishida and H. Hikita: *J. Chem. Eng. Japan*, **19**, 149 (1986).
I7) Ishikawa, H., T. Tanaka, K. Kurose and H. Hikita: *Biotechnol. Bioeng.*, **29**, 924 (1987).
I8) Ishimura, F.: "Kagaku Kikai Gijutsu," No. 35, p. 79, Soc. Chem. Eng. Japan (1983).
I9) Isohara, T., T. Nozawa, S. Furusaki and T. Furuya: Preprint 52th Ann. Meeting, Soc. Chem. Eng. Japan, Nagoya, I302, p. 379 (1987).
I10) Iwamura, T.: Proc. 4th Korea-Japan Techn. Exchange Seminar, p. 57 Seoul (1987), also Sep. Process Eng., **17**, 283 (1987).
J1) Jarvis, A. P. and T. A. Gardia: *Biotechnol.*, **1**, 17 (1983).
J2) Jones, A., D. N. Wood, T. Raznieswa, G. M. Gaucher and L. A. Behie: *Can. J. Chem. Eng.*, **64**, 547 (1986).
J3) Jones, J. B. and D. H. Pliura: *Can. J. Chem.*, **59**, 2921 (1981).
K1) Kajiwara, S. and H. Maeda: *Biotechnol. Bioeng.*, **28**, 1794 (1986).
K2) Kalthod, D. G. and E. Ruckenstein: *Biotechnol. Bioeng.*, **24**, 2189 (1982).
K3) Karel, S. F., S. B. Libicki and C. R. Robertson: *Chem. Eng. Sci.*, **40**, 1321 (1985).
K4) Kariya, M. and C. Tozaki: *Hakko to Kogyo*, **45**, 206 (1987).
K5) Karube, I., S. Suzuki and E. J. Vandamme: *Drugs Pharm. Sci.*, **22**, 761 (1984).
K6) Kaul, R., P. Adlercreutz and B. Mattiasson: *Biotechnol. Bioeng.*, **28**, 1432 (1986).
K7) Kawakami, K., T. Hamada and K. Kusunoki: *Enzyme Microb. Technol.*, **2**, 295 (1980).
K8) Kayano, H., T. Matsunaga, I. Karube and S. Suzuki: *Biochem. Biophys. Acta*, **638**, 80 (1981).
K9) Kennedy, J. F. and J. M. S. Cabral: *Appl. Biochem. Bioeng.*, **4**, 189 (1983).
K10) Kernevez, J. P., E. J. Doedel and D. Thomas: *Biomed. Biochim. Acta*, **44**, 993 (1985).
K11) Kim, I. H. and H. N. Chang: *AIChE J.*, **29**, 910 (1983).
K12) Kim, J. H., D. K. Oh, S. K. Park, Y. H. Park and D. A. Wallis: *Biotechnol. Bioeng.*, **28**, 1838 (1986).
K13) Kiyachko, N. L., A. V. Lavashov and K. Martinek: *Mol. Biol.* (Moscow), **18**, 1019 (1984); refer to *Chem. Abstr.*, **101**, 146792.
K14) Klausner, A.: *Biotechnol.*, **1**, 736 (1983).
K15) Klein, J. and B. Kressdorf: *Biotechnol. Lett.*, **8**, 739 (1986).
K16) Klein, J. and G. Manecke: "Enzyme Engineering," Vol. 6, Chibata, I., S. Fukui and L. B. Wingard ed., p. 181, Plenum Press, New York (1982).
K17) Klein, J. and P. Schara: *Appl. Biochem. Biotechnol.*, **6**, 91 (1981).
K18) Klein, J., K. D. Vorlop and F. Wagner: "Enzyme Eng. 7," Ann. N. Y. Acad. Sci., Vol. 434, p. 437 (1984).
K19) Klibanov, A. M.: *Anal. Biochem.*, **93**, 1 (1979).
K20) Klibanov, A. M., G. P. Samokhin, K. Martinek and I. V. Benezin: *Biotechnol. Bioeng.*, **19**, 1351 (1977).
K21) Kloosterman, J. and M. D. Lilly: *Biotechnol. Lett.*, **7**, 25 (1985).
K22) Kobayashi, T., K. Katagiri, K. Ohmiya and S. Shimizu: *J. Ferment. Technol.*, **58**, 23 (1980).
K23) Kobayashi, T. and K. J. Laidler: *Biochim. Biophys. Acta*, **302**, 1 (1973).
K24) Kobayashi, T., K. Ohmiya and S. Shimizu: *J. Ferment. Technol.*, **54**, 260 (1976).
K25) Kollerup, F. and A. J. Danglis: *Can. J. Chem. Eng.*, **64**, 588 (1986).
K26) Kusunoki, K.: *Kagaku Kōgaku*, **45**, 523 (1981).
L1) Laane, C., S. Boeren and K. Vos: *Trends Biotechnol.*, **3**, 251 (1985).
L2) Laane, C., S. Boeren, K. Vos and C. Vaeger: *Biotechnol. Bioeng.*, **30**, 81 (1987).
L3) Lakshmann, C.: *AIChE J.*, **29**, 876 (1983).
L4) Laskin, A. I.: "Enzymes and Immobilized Cells in Biotechnol.," Benjamin/Cummings, Menlo Park, CA (1985).
L5) Laszlo, E. and J. Hollo: *Starch*, **38**, 168 (1986).
L6) Lee, G. K. and P. J. Reilly: *Chem. Eng. Sci.*, **36**, 1967 (1981).
L7) Legoy, M. D., J. M. LeMoullec and D. Thoma: *FEBS Lett.*, **94**, 335 (1978).
L8) Lencki, R. W. and R. J. Neufeld: Proc. World Congr. III Chem. Eng., p. 949 (1986).
L9) Lilly, M. D.: *J. Chem. Technol. Biotechnol.*, **32**, 162 (1982).
L10) Lilly, M. D. and P. Dunnill: *Process Biochem.*, **6**, 29 (1971).
L11) Lim, F. and A. M. Sun: *Science*, **210**, 908 (1980).
L12) Lindsey, K.: *Planta*, **165**, 126 (1985).
L13) Lindsey, K., M. M. Yeoman, G. M. Black and F. Mavituna: *FEBS Lett.*, **155**, 143 (1983).
L14) Linko, M.: Proc. 20th Eur. Brew. Conv., p. 39 (1985).
L15) Linko, Y. Y. and G. X. Li: "Enzyme Eng. 8," Ann. N. Y. Acad. Sci., Vol. 501, p. 377 (1987).
L16) Linko, Y. Y., G. X. Li and P. Linko: "Enzyme Eng. 8," Ann. N. Y. Acad. Sci., Vol. 501, p. 381 (1987).
L17) Linse, L. and P. Brodelius: "Enzyme Eng. 7," Ann. N. Y. Acad. Sci., Vol. 434, p. 487 (1984).
L18) Liou, J. K. and I. Rousseau: *Biotechnol. Bioeng.*, **28**, 1582 (1986).
L19) Lovitt, R. W., R. P. Walter, J. G. Morris and D. B. Kell: *Appl. Microbiol. Biotechnol.*, **23**, 168 (1986).
L20) Luong, J. H. T. and M. C. Tseng: *Appl. Microbiol. Biotechnol.*, **19**, 207 (1984).
L21) Luthi, P. and P. L. Luisi: *J. Am. Chem. Soc.*, **106**, 7285 (1984).
M1) Makryaleas, K., T. Schepper, K. Schugerl and H. R. Kula: *Ger. Chem. Eng.*, **8**, 345 (1985).
M2) Mandenius, C. F., B. Mattiasson, J. P. Axelsson and P. Hagander: *Biotechnol. Bioeng.*, **29**, 941 (1987).
M3) Manjon, A., J. L. Iborra, J. L. Gomez, E. Gomez, J. Batida and A. Bodalz: *Biotechnol. Bioeng.*, **30**, 491 (1987).
M4) Marcipor, A., P. Henno, E. Lentwojt, A. Rosets and G. Broun: "Biochem. Eng. III," Ann. N. Y. Acad. Sci., Vol. 413, p. 416 (1983).
M5) Margaritis, A. and J. B. Wallace: *Biotechnol. Bioeng. Symp.*, **12**, 147 (1982).
M6) Markt, M., J. Kas, O. Valentova, K. Demnerova and Z. Vodrazka: *Biotechnol. Lett.*, **8**, 721 (1986).
M7) Martinek, K., A. V. Lavashov, N. L. Klyachko and I. V. Berezin: *Dokl. Akad. Nauk SSSR*, **236**, 951 (1978).
M8) Martinek, K., A. V. Lavashov, N. L. Klyachko, Y. L. Khmelnitskii and I. V. Berezin: *Biol. Membrane*, **2**, 669 (1985); refer to *Chem. Abstr.*, **103**, 103381.
M9) Matiasson, B. and M. Larsson: Proc. 3rd Eur. Congr. Biotechnol., Vol. 4, p. 375 (1985).
M10) Matsumoto, K., H. Mizukuchi and K. Ichimura: *Kobunshi Ronbunshu*, **41**, 221 (1984).
M11) Mavituna, F., J. M. Park and D. Gardner: *Chem. Eng. J.*, **34**, B1 (1987).
M12) Melick, M. R., M. N. Karim, J. C. Linden, B. E. Dale and P. Mihaltz: *Biotechnol. Bioeng.*, **29**, 370 (1987).
M13) Menger, F. M. and K. Yamada: *J. Am. Chem. Soc.*, **101**, 6731 (1979).
M14) Miyauchi, T., S. Furusaki, S. Morooka and Y. Ikeda: "Adv. Chem. Eng.," Vol. 11, p. 275, Academic Press, New York (1981).

M15) Miyawaki, O., K. Nakamura and T. Yano: *Agric. Biol. Chem.*, **46**, 2725 (1982).
M16) Miyawaki, O., K. Nakamura and T. Yano: *J. Chem. Eng. Japan*, **15**, 224 (1982).
M17) Moebus, O. and M. Tenber: *Forum Microbiol.*, **9**, 187 (1986).
M18) Moo-Young, M. and T. Kobayashi: *Can. J. Chem. Eng.*, **50**, 167 (1972).
M19) Morbidelli, M., A. Servida and A. Varma: *Biotechnol. Bioeng.*, **26**, 1508 (1984).
M20) Mosbach, K.: *Nature*, **302**, 543 (1983).
M21) Mozes, N., F. Marchal, M. P. Hermesse, J. L. Van Haecht, L. Reuliaux, A. J. Leonard and P. G. Rouxhet: *Biotechnol. Bioeng.*, **30**, 439 (1987).
N1) Nagamune, T. and I. Endo: *Rikagaku Kenkyusho Hokoku*, **62**, 135 (1986).
N2) Nagamune, T., I. Endo, N. Kato, M. Nishimura and T. Kobayashi: *Bioprocess Eng.*, **3**, to be published (1988).
N3) Nagashima, M., M. Azuma, S. Noguchi, K. Inuzuka and H. Samejima: *Biotechnol. Bioeng.*, **26**, 992 (1984).
N4) Nakajima, M., N. Jimbo, K. Nishizawa, H. Nabetani, A. Watanabe and S. Nakao: Proc. Intern. Cong. Membrane, 1987, p. 453 (1987).
N5) Nakamura, K., K. Hibino and T. Yano: Proc. PACHEC 83, Vol. 4, p. 197, Seoul (1983).
N6) Nakamura, K., H. Kumagai and T. Yano: *Agric. Biol. Chem.*, **48**, 1131 (1984).
N7) Nakanishi, K., T. Kamikubo and R. Matsuno: *Bio/Technol.*, **3**, 459 (1985).
N8) Netto, C. B. and G. Goma: *Biotechnol. Bioeng.*, **30**, 320 (1987).
N9) Nishikawa, S., Y. Ashina and I. Watanabe: *Kagaku Kōgaku*, **51**, 423 (1987).
N10) Noguchi, S., M. Nagashima and M. Azuma: Proc. Pan-Pacific Synfuels Confs., Vol. 2, p. 464 (1982).
N11) Nozawa, T. and S. Furusaki: Preprint 20th Autumn Meeting, Soc. Chem. Eng. Japan, Himeji, SG203, p. 348 (1987).
N12) Nozawa, T., H. Kuzui and S. Furusaki: Proc. Chemeca 87, Vol. 2, p. 99.1, Melbourne (1987).
O1) Ollis, D. F.: *Biotechnol. Bioeng.*, **14**, 871 (1972).
O2) Omata, T., T. Iida, A. Tanaka and S. Fukui: *Eur. J. Appl. Microbiol. Biotechnol.*, **8**, 143 (1979).
O3) Omata, T., A. Tanaka and S. Fukui: *J. Ferment. Technol.*, **58**, 339 (1980).
O4) Ooshima, H., H. Mori and Y. Harano: *Biotechnol. Lett.*, **7**, 789 (1985).
O5) Oyama, K., S. Nishimura, Y. Nonaka, K. Kihara and T. Hashimoto: *J. Org. Chem.*, **46**, 5242 (1981).
P1) Park, J. M. and Y. C. Lee: *Hanguk Sikpum Kwahakhoechi*, **17**, 75 (1985).
P2) Park, T. H., J. H. Kim and H. N. Chang: *Biotechnol. Bioeng.*, **27**, 1185 (1985).
P3) Patwardhan, V. S. and N. G. Karanth: *Biotechnol. Bioeng.*, **24**, 763 (1982).
P4) Pliura, D. H. and J. B. Jones: *Can. J. Chem.*, **58**, 2633 (1980).
P5) Poppe, W., W. Halwachs and K. Schugerl: *Chem. Ing. Techn.*, **56**, 70 (1984).
P6) Poulsen, P. B.: Proc. 3rd Eur. Congr. Biotechnol., Vol. 4, p. 339 (1985).
P7) Prenosil, J. E. and H. Pedersen: *Enzyme Microb. Technol.*, **5**, 323 (1983).
P8) Prenosil, J. E., J. Peter and J. R. Bourne: "Enzyme Engineering," Vol. 5, p. 317, Plenum Press, New York (1980).
P9) Prince, I. G. and J. P. Barford: *Biotechnol. Lett.*, **4**, 469 (1982).
P10) Pronk, W., A. Knol and K. van't Riet: Proc. Intern. Cong. Memb. 1987, p. 637 (1987).
R1) Radovich, J. M.: *Biotechnol. Adv.*, **3**, 1 (1985).
R2) Radovich, J. M.: *Enzyme Microb. Technol.*, **7**, 2 (1985).
R3) Rees, D. C.: *Bull. Math. Biol.*, **46**, 229 (1984).
R4) Reuveny, S., A. Mizrahi, M. Kotler and A. Freeman: *Biotechnol. Bioeng.*, **25**, 469 (1983).
R5) Rhodes, M. J. C.: *Topics Enzyme Ferment. Biotechnol.*, **10**, 51 (1985).
R6) Rhodes, M. J. C., J. I. Smith and R. J. Robins: *Appl. Microbiol. Biotechnol.*, **26**, 28 (1987).
R7) Rosen, C. G.: *Chem. Economy Eng. Rev.*, **17**(7/8), (No. 190), 5 (1985).
R8) Rosevear, A. and C. A. Lambe: *Adv. Biochem. Eng. Biotechnol.*, **31**, 37 (1985).
R9) Ryu, D. D. Y., H. S. Kim and H. Taguchi: *J. Ferment. Technol.*, **62**, 255 (1984).
S1) Sada, E., S. Katoh and M. Terashima: *Biotechnol. Bioeng.*, **25**, 317 (1983).
S2) Sada, E., S. Katoh, M. Terashima and Y. Kikuchi: *Biotechnol. Bioeng.*, **30**, 117 (1987).
S3) Sada, E., S. Katoh, M. Terashima and Y. Takada: *AIChE J.*, **31**, 311 (1985).
S4) Sadana, A.: *Chem. Eng. Sci.*, **37**, 492 (1982).
S5) Sakaki, K., T. Nozawa and S. Furusaki: *Biotechnol. Bioeng.*, **31**, 603 (1988).
S6) Sakaki, K., K. Ueyama and S. Furusaki: *Kagaku Kogaku Ronbunshu*, **12**, 90 (1986).
S7) Sakata, M. and S. Furusaki: *Kagaku Kogaku Ronbunshu*, **11**, 63 (1985); *Intern. Chem. Eng.*, **26**, 680 (1986).
S8) Samejima, H., M. Nagashima, M. Azuma, S. Noguchi and K. Inuzuka: "Enzyme Eng. 7," Ann. N. Y. Acad. Sci., Vol. 434, p. 394 (1984).
S9) Sato, S., I. Kawamura and N. Fujiyoshi: *Soshiki Baiyo*, **9**, 286 (1983).
S10) Schepper, T., W. Halwachs and K. Schugerl: *Chem. Eng. J.*, **29**, B31 (1984).
S11) Schmidt, E., B. Bossow, R. Wichmann and C. Wandrey: *Kem. Ind.*, **35**, 71 (1986).
S12) Scott, T. C. and C. G. Hill, Jr.: *Biotechnol. Bioeng. Symp.*, **15**, 431 (1986).
S13) Seki, M. and S. Furusaki: *J. Chem. Eng. Japan*, **18**, 461 (1985).
S14) Shargool, P. D.: *Biotechnol. Adv.*, **3**, 29 (1985).
S15) Sheurich, P., H. Schnabl, U. Zimmermann and J. Klein: *Biochim. Biophys. Acta*, **598**, 645 (1980).
S16) Shield, J. W., H. D. Fergason, A. S. Bommarius and T. A. Hatton: *Ind. Eng. Chem. Fundam.*, **25**, 603 (1986).
S17) Shimizu, K. and M. Ishihara: *Biotechnol. Bioeng.*, **29**, 236 (1987).
S18) Shimizu, S. Y. and H. M. Lenhoff: *J. Solid-Phase Biochem.*, **4**, 109 (1979).
S19) Shiotani, T. and T. Yamane: *Eur. J. Appl. Microbiol. Biotechnol.*, **13**, 96 (1981).
S20) Shirai, Y., K. Hashimoto, H. Kawahara, R. Sasaki and H. Chiba: Preprint 20th Autumn meeting, Himeji, p. 347 (SG202), Soc. Chem. Eng. Japan (1987), also *Appl. Microb. Biotechnol*, to be published (1988).
S21) Shirai, Y., K. Hashimoto, H. Yamachi and M. Tokashiki: Preprint Ann. Meeting, Soc. Chem. Eng. Japan, Osaka, I314 (1986).
S22) Shiraishi, F., K. Kawakami, K. Kato and K. Kusunoki: *Kagaku Kogaku Ronbunshu*, **8**, 316 (1983).
S23) Shuler, M. L., O. P. Sahai and G. A. Hallsby: "Biochem.

Eng. III," Ann. N. Y. Acad. Sci., Vol. 413, p. 373 (1983).
S24) Silman, R. W., L. T. Black, J. E. McGhee and E. B. Bagley: *Biotechnol. Bioeng.*, **22**, 533 (1980).
S25) Smith, C. G., P. F. Greenfield and D. H. Randerson: Proc. Chemeca 87, Vol. 2, p. 97.1, Melbourne (1987).
S26) Srivastava, R. C., D. B. Madamwar and V. V. Vyas: *Biotechnol. Bioeng.*, **30**, 901 (1987).
S27) Strand, J. M., J. M. Quarles and S. McConnell: *Biotechnol. Bioeng.*, **26**, 508 (1984).
S28) Swaisgood, H. E.: "Enzymes and Immobilized Cells in Biotechnology," p. 1, Benjamin/Cummings Publ., Menlo Park (1985).
T1) Takahashi, K., Y. Kodera, T. Yoshimoto, A. Ajima, A. Matsushima and Y. Inada: *Biochem. Biophys. Res. Commun.*, **131**, 532 (1985).
T2) Takahashi, K., H. Nishimura, T. Yoshimoto, Y. Saito and Y. Inada: *Biochem. Biophys. Res. Commun.*, **121**, 261 (1984).
T3) Takamatsu, S., M. Ueba and K. Yamashita: *J. Chem. Eng. Japan*, **17**, 647 (1984).
T4) Tamponnet, C., F. Costantino, J. N. Barbotin and R. Calvayrac: *Physiol. Plant.*, **63**, 277 (1985).
T5) Tanaka, A.: *Kagaku no Seibutsu*, **23**, 112 (1985).
T6) Tanaka, A., K. Sonomoto and S. Fukui: "Enzyme Eng. 7," Ann. N. Y. Acad. Sci., Vol. 434, p. 479 (1984).
T7) Tanaka, H., H. Kurosawa and H. Murakami: *Biotechnol. Bioeng.*, **28**, 1761 (1986).
T8) Tanaka, H., M. Matsumura and A. Valiky: *Biotechnol. Bioeng.*, **26**, 53 (1984).
T9) Tanaka, M., A. Kawaide and R. Matsuno: *Biotechnol. Bioeng.*, **28**, 1294 (1986).
T10) Tanaka, T., E. Ono, M. Ishihara, S. Yamanaka and K. Takinami: *Agric. Biol. Chem.*, **45**, 2387 (1981).
T11) Thomas, D. and G. B. Brown: "Methods Enzymology," Vol. 44, p. 901, Academic Press, New York (1976).
T12) Toda, K.: *Biotechnol. Bioeng.*, **17**, 1729 (1975).
T13) Toda, K. and K. Sato: *J. Ferment. Technol.*, **63**, 251 (1985).
T14) Toda, K. and M. Shoda: *Biotechnol. Bioeng.*, **17**, 481 (1975).
T15) Tsay, S. S. and K. Y. To: *Biotechnol. Bioeng.*, **29**, 297 (1987).
T16) Tse, P. H. S. and D. A. Gough: *Biotechnol. Bioeng.*, **29**, 705 (1987).
T17) Tse, P. H. S., J. K. Leypoldt and D. A. Gough: *Biotechnol. Bioeng.*, **29**, 696 (1987).
T18) Tyagi, R. D. and T. K. Ghose: *Biotechnol. Bioeng.*, **24**, 781 (1982).
U1) Ueyama, K. and S. Furusaki: *Chem. Eng. Commun.*, **36**, 299 (1985).
U2) Ueyama, K. and T. Miyauchi: *AIChE J.*, **25**, 258 (1979).
V1) Vallin, D. and T. M. Cauh: *Biochim. Biophys. Acta*, **571**, 321 (1979).
V2) Van Wezel, A. L.: *Nature*, **216**, 64 (1967).
V3) Vega, J. L., E. C. Clausen and J. L. Gaddy: *Biotechnol. Bioeng.*, **29**, 429 (1987).
V4) Verlaan, P., A. C. Hulst, J. Tramper, K. van't Riet and K. C. A. M. Luyben: Proc. 3rd Eur. Conger. Biotechnol., Vol. 1, p. 151, Verlag Chemie, Berlin (1984).
V5) Vladimir, J.: *Biotechnol. Lett.*, **8**, 639 (1986).
V6) Volkel, W., W. Poppe, W. Halwachs and K. Schugerl: *J. Memb. Sci.*, **11**, 333 (1982).
W1) Wada, M., T. Uchida, J. Kato and I. Chibata: *Biotechnol. Bioeng.*, **22**, 1175 (1980).
W2) Wakao, N. and J. M. Smith: *Chem. Eng. Sci.*, **17**, 825 (1962).
W3) Wandrey, C., R. Wichmann, A. F. Bueckmann and M. R. Kula: "Enzyme Engineering 5," p. 453, Plenum Press (1980).
W4) Webb, C., H. Fukuda and B. Atkinson: *Biotechnol. Bioeng.*, **28**, 41 (1986).
W5) Webster, I. A.: *Biotechnol. Lett.*, **5**, 207 (1983).
W6) Webster, I. A., C. E. Schwier and F. S. Bates: *Enzyme Microb. Technol.*, **7**, 266 (1985).
W7) Webster, I. A. and M. L. Shuler: *Chem. Eng. Sci.*, **34**, 1273 (1979).
W8) Weetall, H. H.: *Trends Biotechnol.*, **3**, 276 (1985).
W9) Weetall, H. H. and L. O. Krampitz: *J. Solid-Phase Biochem.*, **5**, 115 (1980).
W10) Weetall, H. H. and W. H. Pitcher, Jr., *Science*, **232**, 1396 (1986).
W11) Wittler, R., H. Baumgartl, D. W. Lubbers and K. Schugerl: *Biotechnol. Bioeng.*, **28**, 1024 (1986).
W12) Wonkhalaung, C., Y. Kashiwagi, Y. Magae, T. Ohta and T. Sasaki: *Appl. Microbiol. Biotechnol.*, **21**, 37 (1985).
Y1) Yagi, Y.: *Hakko to Kogyo*, **41**, 728 (1983).
Y2) Yamane, T.: *J. Ferment. Technol.*, **59**, 375 (1981).
Y3) Yamane, T., S. Araki and E. Sada: *J. Ferment. Technol.*, **59**, 367 (1981).
Y4) Yamane, T., M. M. Hoq and S. Schimizu: *Yukagaku*, **35**, 10 (1986).
Y5) Yamane, T., H. Nakatani, E. Sada, T. Omata, A. Tanaka and S. Fukui: *Biotechnol. Bioeng.*, **21**, 2133 (1979).
Y6) Yamazaki, T. and M. Shiota: *Kagaku Kōgaku*, **50**, 388 (1986).
Y7) Yokozeki, K., S. Yamanaka, K. Takinami, Y. Hirose, A. Tanaka, K. Sonomoto and S. Fukui: *Eur. J. Appl. Microbiol. Biotechnol.*, **14**, 1 (1982).
Y8) Yoshikawa, T.: *Soshiki Baiyo*, **10**, 157 (1984).
Z1) Zaks, A. and A. M. Klibanov: *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 3192 (1985).