Microbial fermentations with immobilized cells

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Immobilization of microbial cells in biological processes can occur either as a natural phenomenon or through artificial process. While the attached cells in natural habitat exhibit significant growth, the artificially immobilized cells are allowed restricted growth. Since the time first reports of successful application of immobilized cells in industrial applications, several research groups worldover have attempted whole-cell immobilization as a viable alternative to conventional microbial fermentations. Various immobilization protocols and numerous carrier materials were tried. The cell immobilization process has also triggered our interest in bioreactor design. Using immobilized cells, different bioreactor configurations were reported with variable success. The study on the physiology of immobilized cells and development of noninvasive measuring techniques have remarkably improved our understanding on microbial metabolism under immobilized state.

THE industrial biotechnology processes using microorganisms are generally based on the exploitation of the cells in the fermentation medium during the process. The classical fermentations suffer from various constrains such as low cell density, nutritional limitations, and batch-mode operations with high down times. It has been well recognized that the microbial cell density is of prime importance to attain higher volumetric productivities. The continuous fermentations with free-cells and cell recycle options aim to enhance the cell population inside the fermenter. However, the free-cell systems cannot operate under chemostatic mode that decouples specific growth rate and dilution rates. During the last 20–25 years, the cell immobilization technology, with its origins in enzyme immobilization, has attracted the attention of several research groups. This novel process eliminates most of the constrains faced with the free-cell systems. The remarkable advantage of this new system is the freedom it has to determine the cell density prior to fermentation. It also facilitates operation of microbial fermentation on continuous mode without cell washout. The whole-cell immobilization process decouples microbial growth from cellular synthesis of favoured compounds. Since the early 70s, when Chibata's group announced successful operation of continuous fermentation of L-aspartic acid, numerous research groups have attempted various microbial fermentations with immobilized cells. During these years, over 2500 research papers on various aspects of whole-cell immobilization have been published. Several comprehensive reviews[1] as well as specialized reviews[2–7] have been published on some important aspects of this field. There are also specialized monograms[8], and conference proceedings[9] pertaining to cell immobilization technology, which have excited microbiologists and bioengineers.

We have presented an overview of this field. It is extremely difficult to include all the aspects of cell immobilization technology and cover every report. Though physiology of immobilized cells is an important branch of this technology, it is omitted in the present article due to paucity of space.

Rationale for whole-cell immobilization

Many processes have been practised traditionally, embodying the basic principle of microbial conversions offered by cells bound to surfaces. Waste treatment in trickling filters and ethanol oxidation to produce vinegar are but a few examples of such processes. Immobilization of cells is the attachment of cells or their inclusion in distinct solid phase that permits exchange of substrates, products, inhibitors, etc., but at the same time separates the catalytic cell biomass from the bulk phase containing substrates and products. Therefore it is expected that the microenvironment surrounding the immobilized cells is not necessarily the same experienced by their free-cell counterparts.

Immobilization commonly is accomplished using a high molecular hydrophilic polymeric gel such as alginate, carrageenan, agarose, etc. In these cases, the cells are immobilized by entrapment in the pertinent gel by a drop-forming procedure. When traditional fermentations are compared with the microbial conversions using immobilized cells, the productivity obtained in the latter is considerably higher, obviously partly due to high cell density and immobilization-induced cellular or genetic modifications. Nevertheless, a few critical parameters such as the cost of immobilization, mass transport limitations, applicability to a specific end-product, etc. are to be carefully examined before choosing any particular methodology.

The use of immobilized whole microbial cells and/or organelles eliminates the often tedious, time consuming, and expensive steps involved in isolation and purification

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of intracellular enzymes. It also tends to enhance the stability of the enzyme by retaining its natural catalytic surroundings during immobilization and subsequent continuous operation. The ease of conversion of batch processes into a continuous mode and maintenance of high cell density without washout conditions even at very high dilution rates, are few of the many advantages of immobilized cell systems. The metabolically active cell immobilization is particularly preferred where co-factors are necessary for the catalytic reactions. Since co-factor regeneration machinery is an integral function of the cell, its external supply is uneconomical. There is considerable evidence to indicate that the bound-cell systems are far more tolerant to perturbations in the reaction environment and similarly less susceptible to toxic substances present in the liquid medium. The recent reports on higher retention of plasmid-bearing cells have further extended the scope of whole-cell immobilization to recombinant product formation. Another important advantage of immobilization, particularly in the case of plant cells, is the stimulation of secondary metabolite formation and elevated excretion of intracellular metabolites.

Immobilization methods

Many methods namely adsorption, covalent bonding, crosslinking, entrapment, and encapsulation are widely used for immobilization. These categories are commonly used in immobilized enzyme technology. However, due to the completely different size and environmental parameters of the cells, the relative importance of these methods is considerably different. The criteria imposed for cell immobilization technique usually determine the nature of the application.

Adsorption

This was apparently the first example of cell immobilization. Hattori and Furusaka[10,11] reported the binding of *Escherichia coli* cells on to an ion exchange resin. Subsequently, a variety of microbial cells were immobilized by adsorption on different supports like kieselguhr, wood, glass ceramic, plastic materials, etc. Klein and Ziehr[5] have reviewed the immobilization of microbial cells by adsorption. Since the adsorption phenomenon is based on electrostatic interactions (van der Walls forces) between the charged support and microbial cell, the actual zeta potential on both of them plays a significant role in cell-support interactions. Unfortunately, the actual charge on support surfaces is still unknown and this limits the proper choice for microbial attachment. Along with charge on the cell surface, the composition of cell wall carrier composition will also play a predominant role[12]. Cells of *Saccharomyces cerevisiae* and *Candida utilis* contain α-mannans in the cell wall. The cells of latter have a strong affinity to Cancanavalin-A-activated carrier[13]. Carrier properties, other than zeta potential, will also greatly influence cell-support interaction. All glasses or ceramic supports are comprised of varying proportions of oxides of alumina, silica, magnesium, zirconium, etc. which result in bond formation between the cell and the support. Several procedures of cell adsorption based on pH dependence are reported in the literature[5].

Covalent bonding

The mechanism involved in this method is based on covalent bond formation between activated inorganic support and cell in the presence of a binding (cross-linking) agent. For covalent linking, chemical modification of the surface is necessary. Cells of *S. cerevisiae* were immobilized by coupling silinized silica beads[14]. The reaction requires introduction of reactive organic group on inorganic silica surface for the reaction between the activated support material and yeast cells. α-amino propyl triethoxy silane is generally used as the coupling agent. This inorganic functional group condenses with hydroxyl group on silica surface. As a result, the organic group is available for covalent bond formation on the surface of silica. Covalent bonding can also be achieved by treating the silica surface with glutaraldehyde and isocyanate. A system of more general interest has been developed by Kennedy and Cabral[15], using inorganic carrier system. The addition of Ti^{4+} or Zr^{4+} chloride salts to water results in pH-dependent formation of gelatinous polymeric metal hydroxide precipitates wherein the metals are bridged by hydroxyl or oxide groups. By conducting such a precipitation in a suspension of microbial cells, the cells have been entrapped in the gel-like precipitate formed. In continuous operation, titanium hydroxide-immobilized cells of *Acetobacter* were employed to convert alcohol to acetic acid.

Cross linking

Microbial cells can be immobilized by cross-linking each other with bi- or multifunctional reagents such as glutaraldehyde[16], toluenediisocyanate[17]. The toxicity of the chemicals used for cross-linking obviously imposes limitations for the general applicability of these procedures. Apart from chemical cross-linking, procedures employing physical processes, such as flocculation[18] and pelletization[19], also benefit the immobilization techniques because of strong mutual adherence forces of some microbial cell cultures.

Entrapment

The most extensively studied method in cell immobilization is the entrapment of microbial cells in polymer

matrices. The matrices used are agar, alginate, carrageenan, cellulose and its derivatives, collagen, gelatin, epoxy resin, photo cross-linkable resins, polyacrylamide, polyester, polystyrene and polyurethane. Among the above matrices, polyacrylamide has been widely used by several workers. This gel was first used for immobilization of enzymes[20]. Later this technique was successfully applied to immobilization of lichen cells[21]. As a rule, the entrapment methods are based on the inclusion of cells within a rigid network to prevent the cells from diffusing into surrounding medium while still allowing penetration of substrate. The precise mode of entrapment of cells in polyacrylamide is critical for satisfactory retention of activity. The factors affecting the gel preparation are the content of acrylamide, the ratio of cells to acrylamide, and the size of the gel particles. While the former two influence the hardness of the resulting gel and the pore size of the microlattice in which the cells are entrapped, the third factor determines the activity, stability, and the pressure drop when packed in a reactor. The other procedures for network formation for cell entrapment are precipitation, ion exchange gelation, and polymerization. The precipitation techniques are exemplified by collagen[22], cellulose and carrageenan[23]. By changing a parameter such as temperature, salinity, pH, etc. polymer precipitate can be prepared from a homogeneous solution of linear chain polymers. The networks are primarily formed by precipitation with salt solutions, ones that are constituted by secondary valency forces ranging from dispersion to hydrogen bonding. Network formation procedures where the cross-linking is established by ionic bonds between linear polyelectrolytes and multivalent cations have been extensively tried. Entrapment of cells in alginate gel is popular because of the requirement for mild conditions and the simplicity of the used procedure. Several reports are available employing alginate gel[24].

κ-carrageenan is one of the earliest gel materials used for cell immobilization for continuous production of L-lactic acid by *Escherichia coli*[25]. The immobilization procedure is similar to alginate, and several other groups have used this polysaccharide as a preferred gel matrix either alone or in combination with other gums because of the mild conditions required and good gel stability. Using κ-carrageenan, Takata *et al.*[26] reported that the immobilized *Brevibacterium flavum* attained high stability against several denaturing chemicals. The rate of cell leakage could be lowered by hardening the gel with potassium cations. Similarly several other natural polymers such as agar, agarose, pectin and gelatin were also employed for cell immobilization. Casteillo *et al.*[27] employed gel as a carrier material for the immobilization of *Kluyveromyces fragilis* for β-galactadase activity and *E. coli* for penicillin acylase. The authors produced fibres instead of beads by direct extrusion in cold water. The fibres were further strengthened by treatment with 1.25% glutaraldehyde solution for 45 min.

The reversible network formed was affected by certain calcium-chelating agents like phosphates, Mg^{2+}, K^+ and EDTA and the gel integrity was poor. In ethanol fermentation, where large quantities of CO_2 are produced, the gel spheres disintegrate due to CO_2 pressure in the bed. Many variations in the immobilization procedure were tried. Chotani *et al.*[28] reported that incorporation of micron-sized silica in the bead improved the mechanical strength and internal space adhesion.

Available reports on entrapment by polycondensation reactions, indicate that condensation of epoxide with polyfunctional amines can well be adopted to prevailing physiological conditions[29]. Gel particles of high strength, elasticity, porosity, and high cell content can be prepared by the above procedure. The network is irreversibly formed and inert. Lorenz *et al.*[30] tried a new immobilization procedure using polyurethane ionomers. By coagulation with a salt solution, the mixture of cells and dispersion of amphiphillic polyurethane ionomers from hydrogel under the entrapment of the biocatalyst.

Some research groups in Japan have developed a novel immobilization procedure involving photo cross linkage resins[31,32]. Nojima[32] has reported glycolic cross linking with polyethylene. A prominent feature of such resins is that not only cells but also enzymes can readily be immobilized in their three-dimensional matrix, the size of which can be changed freely by adjusting the degree of polymerization of the polyethylene glycol molecules located between the two isophorone isocyanate molecules. Immobilized cells are produced by irradiating a mixture of such cells and the resin, using 300–400 nm wavelength from a lowpressure mercury lamp. By this method immobilized cell sheets of 50 cm width are prepared under aseptic conditions.

Modified methods of cell immobilization

Entrapment of microbial cells within the polymeric matrices is preferred by many researchers. Among the various methods, alginate gels have received maximum attention. There are several studies on the composition of alginate and their suitability for cell immobilization[33–35]. In the recent years, the diffusional characteristics of the immobilized system are being studied to enhance our understanding on the microenvironment prevailing near the immobilized cells[36–42], to optimize the immobilization protocols[43,44], and to improve the stability of the gel beads by modifying the protocols. According to Vorlop *et al.*[45], drying of immobilized cell sphere greatly increases the compression stress. In certain cases the beads are hardened by glutaraldehyde treatment[46,47]. Coating of immobilized biocatalyst gel particle with catalyst-free polymer is expected to be effective to enhance the stability. This could either be carried out by single-step gelation of the double-layered droplet using twin-nozzle, or by two-step procedure. Yamagiwa *et al.*[48] tried the two-

step preparation procedure by recoating the calcium alginate cell beads by plain alginate as a double layer to *enhance the gel stability*[49]. *Ruggeri et al.*[50] have attempted polyacrylamide coating over the alginate cell beads to enhance the structural stability. It was observed that Eudragit RL 100 (a copolymer of acrylic resin) coating of cell entrapped in alginate beads resulted in 15% higher diffusion of substrate, and the leakage of cells was considerably reduced. Recently, it has been reported that Ca-pectate gel could be a better alternative to Ca-alginate. Gameiner *et al.*[51,52] carried out a comparative evaluation of both the gels and observed that pectate gel is less sensitive to chelating anions and chemical agents, and hence the mechanical stability improved considerably[53]. The use of polyvinylalcohol (PVA) as a gel matrix has received attention in the recent past. Reports are available on the immobilization of various microbial cells in PVA either by freezing and thawing technique or gelling in saturated boric acid[54].

Incorporation of additional component into the gel matrix to improve the mechanical strength has been tried. Several components such as silica[55], sand, alumina, and various gums are generally used. In addition, the gel particles are further strengthened by treating with various cross-linking agents, such as glutaraldehyde. Recently, Chu *et al.*[56] reported the polyelectrolyte complex gel prepared from xanthan and chitosan for immobilization of *Corynebacterium glutamicum* having fumarase activity. By mixing two opposite-charged electrolytes, a complex resulted due to electrostatic interactions. Generally, these complexes were obtained as precipitates, but Sakiyama *et al.*[57] and Chu *et al.*[58] obtained moldable chitosan/κ-carrageenan and xanthan/chitosan complex gels in the presence of NaCl. It has been observed that the cells immobilized in these complexes were very stable and exhibited 5-fold higher activity compared to free-cells. The pore size was found to be similar to that of polysaccharide gel. In a similar study, Pandya and Knorr[59] used low molecular weight compounds which were immobilized in complex coacervate capsules consisting of water-soluble chitosan salts or acid-soluble chitosan cross-linked with κ-carrageenan or alginate. This type of coacervate capsules could be used for cell immobilization and simultaneously the presence of chitosan salts in the capsule will affect permeabilization of the cells. Low temperature radiation-induced polymerization of glass-forming monomers were used for cell immobilization[60] with considerable success. One of the advantages of radiation-induced polymerization is that it produces a macromolecular matrix of a purity that could not be attained with conventional polymerization. Several microbial cultures such as *Streptomyces haechromogenes*[61], yeast cells[62] and *Trichoderma resei*[63] were successfully immobilized in the above matrices for obtaining microbial metabolites. In a recent study, yeast cells were entrapped in sol-gel layers on glass surfaces and used for sucrose degradation. It has been reported that immobilized yeast cells exhibited high mechanical strength without affecting the metabolic functions of the cells[64].

Antibiotic production by immobilized microbial cells

Production of antibiotics is one of the key areas in the field of applied microbiology. The conventional method of production is in stirred tank batch reactors. Since it is a nongrowth associated process, it is difficult to produce the antibiotic in continuous fermentations with free-cells. But it is a suitable case for cell immobilization, since growth and metabolic production can be uncoupled without affecting metabolite yields. Therefore, several attempts have been made to immobilize various microbial species on different support matrices for antibiotic production. The most widely studied system is the production of penicillin G using immobilized cells of *Penicillium chrysogenum*. Gaucher group entrapped the conidia of the fungi in κ-carrageenan and used for batch and continuous production of penicillin, and compared it with fungi adsorbed on celite[65–67]. It has been reported that the adsorption on celite was five times more productive than entrapment in κ-carrageenan. The adsorbed cells exhibited maximum specific reaction rates compared to the free-cells. This may be due to better oxygen availability to the cells. In another study, Mahmoud *et al.*[68,69] tried entrapment of *P. chrysogenum* in calcium alginate and used in bubble column reactors with limited success. Ogaki *et al.*[70] demonstrated the use of immobilized cells of *Streptomyces rimosus* for the continuous production of oxytetracycline. Among the various methods of immobilization, adsorption on hydrophilic urethane prepolymer was found to be superior to the other methods. But Farid *et al.*[71] have attempted entrapment of *S. rimosus* in calcium alginate gels and reported good level of antibiotic production in repeated batch fermentations for a period of 28 days. Literature is also available on the production of bacitracin[72], patulin[73–75], nikkomycin[76], neomycin[77], candicidin[78], cephalosporin[79–81], erythromycin[82], actinomycin D (ref. 83), and cyclosporin[84].

Many of the reports on antibiotic production are confined to shake-flask level, however there are a few reports on attempts at using different types of bioreactors. Park *et al.*[77] have tried air-lift bioreactor to improve the oxygenation of the culture. In an another study it was demonstrated that a three-phase fluidized bed reactor, using immobilized *P. urdicae* for the production of patulin, could operate continuously for 360 h with 35% higher antibiotic production[85]. Recently, Sarra *et al.*[86] reported continuous production of hybrid antibiotic by a transformed strain of *Streptomyces lividans* TK 21 in a three-phase fluidized bed. Self-immobilized cell aggregates were developed and used continuously in the reactor for a period of 85 days.

As antibiotic fermentations are not growth associated, the requirements of nutrients are different from growth and metabolite synthesis. In principle, proliferation of immobilized cells is prevented. Hence, it is possible to use a very dilute medium for the synthesis of the metabolite. The decoupling of growth with the product synthesis can be achieved by manipulating key nutrients for growth. Reports are available on using glucose-free medium for oxytetracycline production[70] and phosphate limitation for hybrid antibiotic production. Such limitations of nutrients were reported to be effective in case of bacitracin, patulin, cephalosporin C, and cephamycin.

Though entrapment of microbial cells in polymer gels is the choice for many, it might not be the best. Since most of the antibiotic producers are highly aerobic, the limitation of oxygen supply in gel matrix adversely affect their performance. The adsorption of the microbial species to a great extent eliminates diffusional problems and encourages metabolite synthesis. Antibiotics produced by immobilized microbial cells are given in Table 1.

Organic acids production by immobilized cells

Organic acids are important microbial products used in a variety of applications such as food and medicines. Among the various organic acids, citric acid occupies predominant position as a commercial biochemical. *Aspergillus niger* is the widely used microorganism for the synthesis of citric acid. In conventional practices, batch fermentation of *A. niger* is employed. The fungal fermentations have serious disadvantage of rising viscosity during growth, leading to poor oxygen supply to the cells. Therefore, it becomes necessary to aerate the cultures with large volumes of sterile air. In case of immobilized cells, since growth is restricted, it is possible to operate the fermentor without affecting the viscosity, thereby facilitating good oxygen transfer rates with minimal cause. There are several investigations on citric acid production using immobilized cells of *A. niger*[100–109].

The methods most widely used for immobilization of *A. niger* cells are the entrapment in alginate gels[105,110], agarose[111], and polyacrylamide[104]. In addition, adsorption on various supports, such as polyurethane foam[112,113], and entrapment in hollow fibres[102] have also been attempted. Fujii *et al.*[106] tried porous cellulose carrier as support matrix for *A. niger* and observed that the immobilized cells enhanced the productivity substantially. Unlike conventionally used sucrose as the substrate, Khare *et al.*[101] successfully utilized soy whey as substrate for citric acid production.

A few reports are available on the use of yeast, *Yarrowia lipolitica,* in immobilized phase for the production of citric acid[114–116]. Working with this yeast, Kautola *et al.*[114] evaluated several carriers such as alginate, κ-carrageenan, polyurethane gel, nylon web, and polyurethane foam for active and passive immobilization of the cells. Amongst these various carriers, entrapped cells in alginate exhibited highest citric acid productivity, 155 mg/l/h. It has been observed[117] that the immobilized cells of *A. niger* required a lower initial sucrose concentration than the free-cells for maximum productivity. High sucrose concentrations led to reduced yields and increased polyol formation.

In a detailed study of immobilized *A. niger* in alginate, Omar *et al.*[107] examined the morphological alterations in immobilized cells, using electron microscope. Similar studies were also carried out by others[118,119]. All these

Table 1. Antibiotic production by immobilized microbial cells

Antibiotic	Microorganism	Support	Reference
Actinomycin D	*Streptomyces parvullus*	Ca-alginate	83, 87
Bacitracin	*Bacillus* sp.	Polyacrylamide	72
Candicidin	*S. griseus*	κ-carrageenan	78
Cephalosporins	*S. clavuligerus*	Polyacrylamide hydrazide, cotton cloth	79, 88
Chlortetracycline	*S. aureofacino*	Ca-alginate	89, 90
Cyclosporin A	*Tolypocladium inflatum*	κ-carrageenan Celite	84, 91
Daunorubicin	*S. eucetius*	Ca-alginate	92
Erythromycin	*S. erythreus*	Alginate	82
Hybrid antibiotic	*S. lividans*	Self aggregate pellets	86
6APA	*Escherichia coli*	Ca-alginate	93
Mithramycin	*S. iverini*	Agar	94
Neomycin	*S. fradiae*	Cellulose beads	77
Nikkomycin	*S. tendae*	Ca-alginate	76, 95, 96
Patulin	*Penicillium urticae*	κ-carrageenan	73–75
Penicillin G	*P. chrysogenum*	Polyacrylamide celite, κ-carrageenan	65–68, 97, 98
Rifamycin B	*S. rimosus* Pf12ER 182.34–2	Glass wool	99
Thienamycin	*S. cattleya*	Celite	74
Tylosin	*Streptomyces* sp.	Ca-alginate	76

studies revealed altered cell morphology characterized by short, stubby, forked and bent mycelia with numerous swollen spherical vesicles and bulbous hyphae.

The use of immobilized cell technology to improve lactic acid fermentation processes has been tried by several research workers[120–128]. *Lactobacillus helveticus*, *Streptococcus salivarius*, *L. delbrueckii* sub sp. *bulgaricus*, *L. casei*, *Rhizopus oryzae*, and *Pedioccus halophilus* are the important microorganisms used for the production of lactic acid by immobilized cells. Similar to other microbial fermentations, entrapment in calcium alginate or κ-carrageenan is the method of immobilization widely followed. Since many workers have reported shrinkage and decreased mechanical strength of alginate beads during lactic acid fermentation, Audet *et al.*[129] suggested a cell entrapment process using κ-carrageenan and locust bean gum, which significantly modified the mechanical properties of the gel. Using the above mixed gel, several studies were carried out with various lactic acid-producing microorganisms[130–134]. The κ-carrageenan locust-bean-gum-mixed gel matrix showed significant stability for 3 months in continuous fermentation in a stirred-tank reactor. In another study, Guoqiang *et al.*[122] compared the performance of alginate-immobilized *L. casei* cells in stirred-tank and packed-bed reactors. The total productivity of 1.6 g/l/h was achieved in stirred-tank reactor with total utilization of glucose. A novel method of extractive fermentation of lactic acid with immobilized cells has been proposed by few workers[121,126,127]. Since the product inhibition could be eliminated by adsorption of lactic acid by selective resin, this method offers higher productivity in bioparticle-continuous fermentation.

Fungal fermentation for lactic acid production has also been studied[124,135]. Using *R. oryzae* cells, Tamada *et al.*[124] tried immobilization of the cells with polymer supports prepared from polyethylene glycol (No 400) and dimethyl-acrylate as monomers by γ-ray induced polymerization. The specific rate of lactic acid production from immobilized cells reached a value of 1.8 times higher than the free-cells. Kurosawa *et al.*[136] have employed mixed cultures of *A. awamori* and *Streptococcus lactis* in alginate for the production of L-lactic acid from starch. They demonstrated that the immobilized mixed cultures could degrade starch and produce lactic acid with a yield coefficient of 0.66.

Several groups have investigated the production of acetic acid by the cells of *Acetobactor* sp. immobilized in hollow fibres[137], ceramic supports[138–140], and carrageenan[141,142]. It has been reported that the addition of α-alumina (2%) to the aqueous solution of sodium alginate (4%) for immobilization of viable cells of *Acetobacter* sp. allows the production of denser particles than the ones obtained with sodium alginate alone[143]. These particles allow for greater oxygen transfer resulting in enhanced volumetric production rate of acetic acid, since oxygen is the limiting factor of acetic acid production. Sun and Furasaki[144] attempted a three-phase fluidized-bed bioreactor for acetic acid production, using *Acetobacter acetie* cells in alginate matrix. A steady state operation for over 30 days was observed. Sueki *et al.*[145] suggested a porous ceramic carrier (Aphrocell cartsidge) for cell immobilization. In continuous fermentation, the productivity increased to 17.25 g/l/h.

Various acids produced by immoblized cells are listed in Table 2. Cell-immobilized fermentations for the production of other acids such as itaconic acid[146,147], malic acid[148,149], propionic acid[150], gluconic acid[151], fumaric acid[152], gibberelic acid[153], succinic acid[154], and butyric acid[155] have also been reported.

Production of enzymes by immobilized cells

Microorganisms are the best sources for the production of useful enzymes. Cell immobilization technology is aptly suited to produce extracellular enzymes. There is growing interest in applying cell immobilization techniques for the continuous production of enzymes. Among the microbial enzymes, starch degrading enzymes α-amylase and glucoamylase have been studied extensively. Several researchers have attempted the production of α-amylase by immobilized cells[162–173]. Entrapment of cells in polyacrylamide, calcium alginate, agar, and several other polymer supports have been tried. Ramakrishna *et al.*[173] reported the immobilization of *Bacillus cereus* in calcium alginate and employed packed-bed and fluidized-bed reactors to continuously synthesize thermostable α-amylase. They spun alginate fibres by pultrusion technique to reduce the diffussional resistances in the gel matrix, and thereby a 24-fold increase in the productivity compared to batch fermentation with free-cells was attained. In a novel technique, Chevalier and Noue[169] tried co-immobilizing of microalgae *Scenedesmus obliquus* along with α-amylase-producing *B. subtilis* strain to overcome oxygen diffusion problem, resulting in 20% increase in enzyme activity. There are also reports on production of thermophilic α-amylase from transformed cells of *E. coli*[170] immobilized in κ-carrageenan with supplementation of glycine. Among the various supports tried, silicone foam was reported to be superior for entrapment of the recombinant *E. coli* EC147 to produce thermostable α-amylase, resulting in a five-fold increase in enzyme production[174].

Similarly, *Aspergillus* strains have been immobilized for the production of glucoamylase[175–178]. Emili Abhraham *et al.*[178] demonstrated continuous production of glucoamylase by immobilizing mycelial fragments of *A. niger*. Among the several polymer matrices tried for immobilization, κ-carrageenan and alginate were the most effective. The authors reported the operation of aerated-packed-bed reactor for a period 360 h, attaining maximum

productivity of 1638 IU/l/h. Fiedurek and Szczodrak[175] attempted passive immobilization of *A. niger* cells by physical adsorption on wheat, whey, barley and muster seeds. Among the above support materials, mycelium grown on muster exhibited highest enzyme activity, and could be used repeatedly.

Cyclodextrin glycosyl transferase (CGTase) is a unique transglycosidase enzyme used for the production of cyclodextrins. Jamuna *et al.*[179] and Saswathi *et al.*[180] demonstrated continuous production of CGTase, using *B. cereus* and *B. circulans*. The alginate-entrapped cells were employed in a fluidized-bed reactor and operated over a period of 360 h with high dilution rate of 0.88 h^{-1}, resulting in 23 kU l^{-1} h^{-1}.

In recent years, enzymatic hydrolysis of cellulosic materials has been the focus of attention, since these materials can be converted into fuels. There are several investigations on immobilized *Tricoderma reesei* for the continuous production of cellulase. Immobilization of the fungi on polyester cloth[181], nönwoven material[182], and cellulosic fabric[183] has been attempted. Xin and Kumakura[184] studied the effect of surface property of the matrix for the immobilization of *T. reesei*. The carrier materials were precoated with hydrophilic and hydrophobic monomers, using co-polymerization technique, and these treated materials were used for passive immobilization of fungi. Their study has demonstrated that pretreatment with hydrophilic matrices allows increased growth of immobilized organisms, thereby resulting in higher activity[184].

In recent years, lipase-catalysed inter esterifications have been considered superior to chemical processes. Several papers have appeared on the immobilization of various microbial cells, namely *Rhizopus chinensis*[185], *A. niger*[186], *Candida rugosa*[187], and *Sporotrichum thermophile apinis*[188] for the production of lipases. Both entrapment methods[186–188] as well as passive immobilization[185,189–190] on the support have been attempted successfully. Using *Rhizopus* sp., various biomass support particles (BSPs) made from urethane, nylon, polyester, and stainless steel were tried to enhance the activity of lipase several folds, compared to free-cells[185].

Immobilization of *Phanerochaete chrysosporium* for their production of lignin peroxidases is a subject of great interest and several reports are available on immobilization of white rot fungi. Passive immobilization on the surface of various support matrices such as nylon web, polyurethane foam, silicon tubing, stainless steel, sintered glass, porous poly-styrene-di-vinylbenzene[191–195] have been examined. The immobilized cells were used in packed-bed reactor for continuous production of the enzyme. Regulating the rate-limiting nutrients, Dosoretz *et al.*[196] cultured immobilized cells of white rot fungi to maximize the production of lignin peroxidases with considerable success.

Penicillin acylase is one of the most important enzymes used commercially for the production of 6APA and phenyl acetic acid (PAA) (ref. 93). The 6APA is further utilized to prepare semi-synthetic antibiotics, such as amphiciin and amoxycilin. *E. coli* or *Bacillus megaterium*

Table 2. Various acids production by Immobilized cells

Acid	Microbe	Support	Reference
Citric	*Aspergillus niger*	Na-alginate	105
Citric	*A. niger*	Agarose	101
Citric	*A. niger*	Dual hallow fibre	102
Citric	*A. niger*	Polyacrylamide	104
Citric	*A. niger*	Polyurethane foam	112
Citric	*Yarrowia lipolitica*	Various supports	114
Citric	*Y. lipolitica*	Na-cellulose sulphate	115, 116
Lactic	*Lactobacillus delbreuckii*	Resin	126
Lactic	*L. casei*	Resin	121
Lactic	*L. lactis* ssp. *lactis*	Ca-alginate	156
Lactic	*L. helveticus*	Locust bean gum gel	130
Lactic	*Streptococcus faecalis*	Polypropylene	157
Lactic	*Sporolactobacillus cellulosolvens*	Na-alginate	128
Lactic	*Pediococcus halophius*	Porous alumina beads	125
Lactic	*Rhizopus orizae*	Polyethylene glycol dimethylacrylate	124
Lactic	*Aspergillus awamori*	Ca-alginate	136
Acetic	*Acetobacter* sp. K-1024	κ-carrageenan	158
Gluconic	*A. niger*	Ca-alginate	151
Kojic	*A. orizae*	Alginate	150
Malic	*Brevibacterium flavum*		148
Fumaric	*Rhizopus arrhizus*	Cork, clay	152
Succinic	Yeast	Na-alginate	154
Gibberellic	*Gibberella fujikuroi*	Ca-alginate	159
Vanillic	*Pseudomonas fluorescens*	Na-alginate	160
Itaconic	*A. terreus*	Polyurethane	161

are known to be potential producers of this enzyme. There are few reports on production of penicillin acylase biocatalyst, using whole-cells[197–198]. All the commercially available catalysts have been elaborated with a partially purified enzyme mainly by covalent linkage. Hence, biocatalyst prepared from whole-cells would bring down the production cost due to elimination of enzyme separation and purification steps. Cells are usually immobilized by entrapment in various polymers, but the specific activity is rather low. Rodriguez *et al.*[199] attempted a biocatalyst preparation using *E. coli* cells by the gel-entrapment technique and demonstrated the stability of the biocatalyst, loosing only 15% of the original activity after 40 runs.

In addition to the above enzymes (Table 3), several other important enzymes such as proteases[200], β-galactadases[201], xylanases[202], dextron sucrases[203], and invertases[204] have also been produced using immobilized cells.

Production of alcohols by immobilized cells

Ethanol fermentation using immobilized cells of yeast, is one of the widely studied systems. In fact, almost all the methods of immobilization, namely, gel entrapment, adsorption on the surfaces of the various carriers, crosslinking were tried for alcohol fermentation. There are specialized reviews on ethanol fermentation with immobilized *Saccharomyces cerevisiae*[217–218]. There are also several reports on *Zymomonas mobilis*[219,220] for alcohol production. Many reactor systems are being operated on continuous basis with encouraging results. However, except for one or two attempts on large scale, the remaining investigations are confined to laboratory scale. One of the problems usually encountered in alcohol fermentation is the *in situ* generation of CO_2 in large quantities[221], resulting in severe operational difficulties in continuous fermentation. Several studies have been carried out to overcome the problems associated with gas generation such as bed compaction, resulting higher pressure drop, breakage of the gel particles, and improper CO_2 ventilation[222–225]. Gil *et al.*[226] have used ceramic-like matrix material constructed of aluminum silicate composition as a carrier for yeast immobilization. It has been reported that the continuous process was operated over a period of 2 years producing 98 g/l of alcohol[226]. Similarly, another research group used polyurethane foam to entrap *Z. mobilis* to produce high concentrations of ethanol[227]. Nojima[228] reported for the first time a large-scale continuous alcohol fermentation system by immobilized living cells of yeast. The yeast cells were mixed with photocrosslinkable resin, and were polymerized by light sources. A pilot-plant-unit, producing 250 litres of alcohol/day, was operated for 8000 h continuously. Nagashima *et al.*[229] operated a pilot plant of 4.0 kl capacity, using alginate-entrapped cells of yeast for alcohol fermentation for a period of 4000 h with a constant alcohol production rate of 8.5 to 9.0% by volume. Using Ca-alginate-entrapped cells of *S. cerevisiae*, Jamuna and Ramakrishna[225] reported rapid fermentation of high concentration sugar solution, thereby obtaining 20% (w/v) alcohol in 30 h.

Yamauchi *et al.*[230] operated multistage reactors for 6–7 months continuously for beer production using immobilized yeast. There are several studies on physiology of the yeast under immobilized state[231,232], which have enriched our knowledge on process intensification during immobilization. In a recent study, it has been reported that

Table 3. Enzyme production by immobilized cells

Enzyme	Organism	Support	Reference
Peptidase	Yeast	ENT-2000	143
β-amylase	*Bacillus megaterium*	Acrylamide	205
Glucoamylase	*Aureobasidium pullulans*	Ca-alginate	206, 207
Ribonuclease	*Aspergillus clavatus*	PVA	178
Protease	*Humicola lutea*	Wort agar	200
Alkaline phosphatase	*Escherichia coli*	κ-carrageenan	208
Glucose oxidase	*A. niger*	Wheat, rye	209
Chitinase	*Micromonospora chalcae*	Ca-alginate	210
Hydantinase	*Pseudomonas* sp.	Polyacrylamide	211
Pectinase	*A. niger*	Wheat	212
Invertase	Yeast	Polymer	213
α-amylase	*E. coli*	κ-carrageenan	214
Amyloglucosidase	*A. niger*	Ca-alginate	178
Cellulase	*Trichoderma reesei*	Cellulosic fibre	183
Penicilin acylase	*E. coli*	Polyacrylamide agar oil	215
CGTase	*Bacillus cereus, B. circulans*	Ca-alginate	179, 180
Chloroperoxidase	*Caldariomyces fumago*	κ-carrageenan	216
Lipase	*Sporotrichum thermophile*	Alginate	188
Lignin peroxidase	*Phanerochaete chysogenum*	Polystyrene-divinylbenzene	193
Pullulanase	*Clostridium* sp.	Ca-alginate	165
Xylanase	*A. sydowii*	Ca-alginate	202

alcohol can be produced from starch directly in a single-step process by co-immobilizing an aerobic amylolytic fungus, *A. awamori*, with an obligatory anaerobic bacterium, *Z. mobilis*[233]. The O_2 gradients in the gel bifurcates the growth of mixed cultures into two zones: the peripheral region of the gel bead is occupied by the fungi; and the core-central region by the bacteria. This is a typical case of exploiting the diffusional behaviour of gel matrix.

Apart from ethanol, several other alcohols such as sorbitol[234], glycerol[235,236], propane-di-ol[237], 2,3-butane-di-ol[238], solvents (acetone, butanol, ethanol)[239,240], mannitol[241], and xylitol[242] have been tried with immobilized microorganisms (Table 4). Employing immobilized *S. cerevisiae*, Bisping *et al.*[243] attempted semicontinuous-fed batch fermentation of glycerol. The authors reported highest glycerol yield of 85 g/l without product inhibition.

The acetone, butanol, ethanol (ABE) fermentation process continues to receive great attention as a source of chemical feed stock[239]. Cells of *Clostridium acetobutylicum* were immobilized by adsorption on bonechar and successfully employed in packed-bed and fluidized-bed reactors for continuous production of ABC from whey. In a similar report, Park *et al.*[244] adsorbed *C. acetobutalicum* cells on polyester sponge sheets and utilized them in a trickle-bed reactor for long-term operation of ABE production.

Biotransformations by immobilized microbial cells

The application of immobilized cells for steroid conversion has been described for several particular cases. Some of these exhibited higher conversion rates and longer half life. The field of biotransformations using immobilizing cells has been expanding in recent years. Schmauder *et al.*[260] reviewed the state of art of this area and have summarized the research output so far available. The commercial interest in steroid biotransformations for the production of pharmaceutical compounds has triggered renewed interest in this field. Some of the recent reports indicate the potential of immobilized *Arthrobacter simplex* for δ-1-dehydrogenation of cartisol[261–263]. Among the various support materials tried, entrapment in polyacrylamide, alginate, and κ-carrageenan are included. Several reactions such as 11-α-hydroxylation, etc. have been mediated successfully using immobilized cells of *Nocardia* sp., *Cornybacterium* sp., *Mycobacterium* sp., and various fungal strains of *Rhizopus*[260]. In many cases, since the substrates were insoluble in water, new methods of transport of substrates and products between the matrix-embedded cells and bulk phase had to be developed. The reverse-micellar technology may provide some answers to the above problems.

Co-immobilization

Though cell immobilization technology is mainly confined to potential monocultures, it is prudent to examine the benefits of mixed cultures under immobilized state to accelerate the fermentation processes. There are several reports of co-immobilization of two or more cultures to derive the benefit of both cultures under monoculturing state. For example, direct alcoholic

Table 4. Various alcohols production by immobilized cells

Alcohol	Organism	Support	Reference
Ethanol	*Zymomonas* sp.	Resin gel	245
Ethanol	Yeast	Polymer	246
Ethanol	Yeast	Ca-alginate	247
Ethanol	*Zymomonas mobilis*	Fibrous matrix	248
Ethanol	Yeast	Ca-alginate	249
Ethanol	Yeast	Alginate	250
Ethanol	*Kluyveromyces marxianus*	Alginate	251
Ethanol	Yeast	PVA	252
Ethanol	*S. cerevisiae*	Microcarriers	253
Ethanol	Yeast	Ca-alginate	254
Acetone, butanol, ethanol	*Clostridium acetobutylicum*	Bonechar	239
Butanol	*C.acetobutylicum*	Carrageenan	255
2,3-butanediol	*Klebsiella pneumoniae*	Ca-alginate	238
2,3-butanediol	*Klebsiella oxytoca, Kluyveromyces lactis*	Chitosan + glasswool	256
Butanol	*Clostridium* sp.	Ca-alginate	257
Glycerol	*S.cerevisiae*	Sintered glass	236
Glycerol	*Citrobacter fraundii*	Polymethane	237
Acetone, butanol	*C. acetobutylicum*	Carrageenan chitosan	258
Methanol	*Methylosinus trichosporium*	DEAE cellulose	259
Sorbitol	*Z. mobilis*	κ-carrageenan glutaraldehyde	234
Xylitol	*Candida pelliculosa, Methanobacterium* sp.	Photocrosslinkable resin ENT 4000	242

fermentation of soluble starch and dextrin uses amylotic yeasts *Saccharomyces diastaticus*, *Schwanniomyces castellii*, and *Endomycopsis fibuligera*. Reilly and Scott[264] reviewed several cases of mixed-culture fermentations to derive various biochemicals. Dincbas *et al.*[265] co-immobilized mixed cultures of plasmid-free and plasmid-containing *E. coli* HB101 in alginate matrix to study the stability of plasmid for long-time cultivation. This study has indicated that the stability of the plasmid-recombinant cells was enhanced in co-immobilized state. The co-immobilization of mixed cultures of algae and aerobic bacteria by encapsulation has solved the problems of oxygen limitation under high cell density conditions in the hydrogels[266,267]. Wikstrom *et al.*[268] have co-immobilized *Chlorella vulgaris* with *Providencia* sp. in agarose, and employed them in a photoreactor for the production of α-keto-isocaproic acid from L-leucine. It has been proved that the algae in the gel matrix acted as an *in situ* oxygen generator. In a similar study, *Cephalosporium acrymonium*, an oxygen-consuming fungus, and *Chlorella pyrenoidosa*, an oxygen-generating alga, were co-immobilized for the cephalosporin C production. It was noticed that the co-immobilized microbial system enhanced the production of cephalosporin C by 370% (ref. 267). Similar co-immobilization protocols were worked out for *in situ* enzyme regeneration[269], complementation[270], multistep biotransformation[271], exploitation of oxygen gradient in the polymer gel and photoproduction of hydrogen[272].

Emerging trends

Whole-cell immobilization as a tool to intensify microbiological processes has been well established. Several examples of production of a variety of biochemicals by immobilized cells have been successfully demonstrated. Though initially our knowledge on physiology of immobilized cells was limited and hypothetical, the use of microelectrodes and development of non-invasive techniques to study the immobilized cells under microenvironment have revealed significant information pertaining to metabolic structural alterations occurring in the cell under immobilized phase. One of the difficulties experienced to evaluate various carriers, process conditions, and operating conditions is the nonuniformity of reporting the information. For example, the volumetric productivity of the bioreactor system with immobilized cells can be determined by considering total volume of the reactor, or the void volume. But researchers calculate and report either one of them, which is sometimes misleading. It is necessary to evolve a common protocol to assess the performance of a given system. Though a variety of carrier materials have been tried, there are very few reports comparing these in terms of their performance, long-term stability, and cost. The observations made with immobilized cells and altered morphology indicate the influence of anchorage on cell metabolism. Perhaps this may lead to a separate study of solid state fermentation, which can be considered as microbial proliferation on solid surfaces, and its influence on bioprocess acceleration in some cases. An important area of research requiring greater focus is the bioreactor design and its long-term operation. Except for a couple of experimental ventures, most of the experiments have been carried out on a very small scale, and hence very difficult to scale up. The future research should centre around not only for developing feasible microbiological processes with immobilized cells but also for carrying out extensive research in bioreacter design to solve some of the engineering problems, specially the ones that are connected with diffusional limitations. It is important to generate adequate data with larger systems for longer times to enable the design engineers to translate these results into commercial realities. It is also very important that future research should focus on microbial physiology under immobilized state to enrich our knowledge on process intensification. The recent reports on enhanced plasmid stability of genetically engineered microorganisms[265,273] under immobilized conditions, and the viability of microbial cells over a period of 18 months[274] under entrapped conditions, are few of the many potential new applications of immobilized cells.

1. Dsouza, S. F., *Indian J. Microbiol.*, 1989, **29**, 83–117.
2. Furusaki, S., *J. Chem. Eng. (Japan)*, 1988, **21**, 219–230.
3. Groboillot, A., Boadi, D. K., Poncelet, D. and Neufeld, R., *Critical Rev. Biotechnol.*, 1994, **14**, 75–107.
4. Ogbonna, C., Matsumura, M. and Kotaoka, H., *Process Biochem.*, 1991, **26**, 109–121.
5. Klein, J. and Ziehr, H., *J. Bacteriol.*, 1990, **16**, 1–16.
6. Furusaki, S., in *Bioproducts Bioprocesses* (eds Fiechter, Okada and Tanner), Springer Verlag, Berlin, Heidelberg, 1989, pp. 71–85.
7. Westrin, B. A., Axelsson, A. and Zacchi, G., *J. Controlled Release.*, 1994, **30**, 189–199.
8. Phillips, C. R. and Poon, Y. C., *Biotechnology Monograms*, Springer Verlag, Berlin, Heidelberg, 1988.
9. de Bont *et al.* (eds), Proceedings of the International Symposium on Physiology of Immobilized cells, Elsevier, Amsterdam, 1990.
10. Hottori, T. and Furusaka, C., *J. Biochem.*, 1960, **48**, 831–837.
11. Hottori, T. and Furusaka, C., *J. Biochem.*, 1961, **50**, 312–315.
12. Kolot, F. B., *Process Biochem.*, 1981, 2–9.
13. Horisberger, M., *Biotechnol. Bioeng.*, 1976, **18**, 1647
14. Novarro, J. M. and Durand, G., *Eur. J. Appl. Microbiol.*, 1977, **4**, 243–254.
15. Kennedy, J. F. and Cabral, J. M. S., in *Immobilized Cells and Enzymes* (ed. Woodward, J.), IRL Press, 1985, 19–37.
16. Chibata, I., Tosa, T. and Sato, T., Japanese Patent No. 74,25,189, 1974.
17. Lartigue, D. J. and Weetall, H., U.S. Patent No. 3,939,041, 1976.
18. Lee, G. K. and Long, M. E., U.S. Patent. No. 3,821,086, 1974.
19. Mcginis, R., *Sugar J.*, 1985, **38**, 8.
20. Bernfeld, P. and Wan, J., *Science*, 1963, **142**, 678–679.
21. Mosbach, K. and Mosbach, R., *Acta. Chem. Scand.*, 1966, **20**, 2807–2810.
22. Vieth, W. R. and Venkatasubrahmanian, K., *Chem. Technol.*, 1974, **4**, 42.

23. Chibata, I., in *Enzyme Engineering* (ed. Brown, G. B.) Plenum Press, New York, vol. 4, 1978.
24. Kierstan, M. and Bucke, C., *Biotechnol. Bioeng.*, 1977, **29**, 387–397.
25. Nishida, Y., Sato, T., Tosa, T. and Chibata, I., *Enzyme Microbial Tech.*, 1979, **1**, 95.
26. Takata, I., Tosa, T. and Chibata, I., *Appl. Biochem. Biotechnol.*, 1983, **8**, 31.
27. Castillo, E., Rodriguez, M., Casas, L., Quintero, R. and Lopez-Munguia, A., *Enzyme Microbial Tech.*, 1991, **13**, 127–133.
28. Chotani, G. K. and Constantindius, A., *Biotechnol. Bioeng.*, 1984, **26**, 217.
29. Klein, J., Wagner, P. and Washausen, P., Proceedings of the First European Congress on Biotechnology, Interlaken, 1978.
30. Lorenz, D., Hautena, F. and Rose, G., *Biotechnol. Bioeng.*, 1987, **29**, 388–391.
31. Yamad, T., *Yuki Gosei Kagaku Kyokai Shi*, 1983, **41**, 1098–1107.
32. Nojima, S., *Chem. Econ. Eng. Rev.*, 1983, **15**, 4.
33. Martinsen, A., Skjak-Break, G. and Smidsrod, O., *Biotechnol. Bioeng.*, 1989, **33**, 79–89.
34. Skjak-Break, G., Murans, E. and Paoletti, S., *Biotechnol. Bioeng.*, 1989, **33**, 90–94.
35. Martinsen, A., Storro, I. and Skjak-Break, G., *Biotechnol. Bioeng.*, 1992, **39**, 186–194.
36. Nguyen, A. L. and Luong, J. H. T., *Biotechnol. Bioeng.*, 1986, **28**, 1261–1267.
37. Kurosawa, H., Matsumura, M. and Tanaka, H., *Biotechnol. Bioeng.*, 1989, **34**, 926–932.
38. Mignot, L. and Junter, G. A., *Appl. Microbiol. Biotechnol.*, 1990, **33**, 167–171.
39. Axelsson, A., Sisak, C., Westrin, B. A. and Szajani, B., *Chem. Eng. J.*, 1994, **55**, B35–39.
40. Westrin, I. A., *Appl. Microbiol. Biotechnol.*, 1990, **34**, 189–190.
41. Willaert, R. G. and Baron, G. V., *Biotechnol. Tech.*, 1994, **8**, 695–700.
42. Teixeira, J. A., Mota, M. and Venancio, A., *Chem. Eng. J.*, 1994, **56**, B9–B14.
43. Jamuna, R., Vora, S., Sai, P. S. T. and Ramakrishna, S. V., *J. Ferment. Bioeng.*, 1992, **73**, 319–322.
44. Ogbonna, J. C., Amuno, Y. and Nakamura, K., *J. Ferment. Bioeng.*, 1989, **67**, 92–96.
45. Vorlop, K. D. and Klein, J., *Biotechnol. Lett.*, 1981, **3**, 9–14.
46. Nishida, Y., Sato, T., Tosa, T. and Chibata, I., *Enzyme Microb. Technol.*, 1977, **1**, 95–99.
47. Takata, I., Tosa, T. and Chibata, I., *J. Solid. Phase Biochem.*, 1978, **2**, 255–366.
48. Yamagiwa, K., Shimizu, Y., Kozawa, T., Onodera, M. and Ohkawa, A., *J. Chem Eng. Japan*, 1992, **25**, 723–728.
49. Yamagiwa, K., Shimizu, Y., Kozawa, T., Onodera, M. and Ohkawa, A., *J. Chem. Eng. Japan*, 1993, **26**, 449–450.
50. Ruggeri, B., Sassi, G., Specchia, V., Bosco, F. and Marzona, M., *Process Biochem.*, 1991, **21**, 331–335.
51. Gemeiner, P., Kurillova, L., Markovic, O., Malovikova, A., Uhrin, D., Ilavsky, M., Stefuca, V., Polakovic, M. and Bales, V., *Biochem. Biotechnol. Appl.*, 1991, **13**, 335–345.
52. Gemeiner, P., Nahalka, J., Vikartovska, A., Nhalkova, J., Tomaska, M., Sturdik, Markovic, O., Malovikova, A., Zatkova, I. and Ilavsky, M., in *Immobilized Cells: Basics and Applications (Progress in Biotechnology)*, vol. II, 1996.
53. Tomaska, M., Gemeiner, P., Materlin, I., Sturdik, E. and Handrikova, G., *Biotechnol. Appl. Biochem.*, 1995, **21**, 347–356.
54. Hanki, K., Hirunmasuwan. and Matsuo, T., *Waters Res.*, 1994, **28**, 993–997.
55. Chotiyani, G. K. and Constantinidus, A., *Biotechnol. Bioeng.*, 1984, **26**, 217–224.
56. Chu, C. H., Kumagai, H. and Nakamura, K., *J. Appl. Polymer Sci.*, 1996, **60**, 1041–1047.
57. Sakiyama, T., Chu, C.H., Fujii, T. and Yano, T., *J. Appl. Polymer Sci.*, 1993, **59**, 717–721.
58. Chu, C. H., Sakiyama, T. and Yano, T., *Biosci. Biotech. Biochem.*, 1995, **59**, 717.
59. Pandya, Y. and Knorr, D., *Process Biochem.*, 1991, **26**, 75–81.
60. Carenza, M., Fujimura, T. and Kaetsu, I., *Chimicaoggi*, 1990, 41–49.
61. Kumakura, M. and Keatsu, I., *J. Chem. Tech. Biotechnol.*, 1984, **B34**, 39.
62. Fujimura, T. and Kaetsu, I., *Biotechnol. Bioeng.*, 1987, **29**, 171.
63. Kumakura, M., Kaetsu, I. and Nisizawa, K., *Biotechnol. Bioeng.*, 1984, **26**, 17.
64. Inama, L., Due, S., Carturan, G. and Cavazza, A., *J. Biotechnol.*, 1993, **30**, 197–210.
65. Jones, A., Wood, D. N., Razniewska, T. and Gaucher, G. M., *Can. J. Chem. Eng.*, 1986, **64**, 547–552.
66. Kalogerakis, N., Linarda, T., Behie, L. A., Svrcek, W. Y. and Gaucher, G. M., *Can. J. Chem. Eng.*, 1986, **64**, 581–587.
67. Deo, Y. M. and Gaucher, G. M., *Biotechnol. Bioeng.*, 1984, **26**, 285–295.
68. Mahmoud, A. H., El Sayed, M. and Rehm, H. J., *Appl. Microbiol. Biotechnol.*, 1987, **26**, 215–218.
69. Mahmoud, A. H., El Sayed, M. and Rehm, H. J., *Appl. Microbiol. Biotechnol.*, 1987, **26**, 211–214.
70. Ogaki, M., Sonomoto, K., Nakajima, H. and Tanaka, A., *Appl. Microbiol. Biotechnol.*, 1986, **24**, 6.
71. Farid, M. A., El Diwany, A. I. and El Enshasy, H. A., *Acta Biotechnol.*, 1994, **14**, 303–309.
72. Morikawa, Y., Karube, I. and Suzukis, S., *Biotechnol. Bioeng.*, 1980, **22**, 1015.
73. Berk, D., Behie, L. A., Jones, A., Lesser, B. H. and Gaucher, M., *Can. J. Chem. Eng.*, 1984, **62**, 112.
74. Berk, D., Behie, L. A., Jones, A., Lesser, B. H. and Gaucher, M., *Can. J. Chem. Eng.*, 1984, **62**, 120.
75. Deo, Y. M. and Gaucher, M., *Appl. Microbiol. Biotechnol.*, 1985, **21**, 220.
76. Veelken, M. and Pape, H., *Eur. J. Appl. Microbiol. Biotechnol.*, 1982, **15**, 206.
77. Park, S. O., Ohta, N. and Okabe, M., *J. Ferment. Bioeng.*, 1994, **78**, 265–268.
78. Constantinides, A. and Mehta, N., *Biotechnol. Bioeng.*, 1991, **37**, 1010–1020.
79. Freeman, A. and Aharonowitz, Y., *Biotechnol. Bioeng.*, 1981, **23**, 2747–2759.
80. Khang, Y. H., Shankar, H. and Senatore, F., *Biotechnol. Lett.*, 1988, **10**, 719–724.
81. Khang, Y. H., Shankar, H. and Senatore, F., *Biotechnol. Lett.*, 1988, **10**, 867–872.
82. Bandyopadhyay, A., Das, A. K. and Mandal, S. K., *Biotechnol. Lett.*, 1993, **15**, 1003–1006.
83. Dalili, M. and Chau, P. C., *Biotechnol. Lett.*, 1988, **10**, 331–336.
84. Chun, G. T. and Agathos, S. N., *J. Biotechnol.*, 1993, **27**, 283–294.
85. Berk, D., Behie, L. A., Jones, A., Lesser, B. H. and Gaucher, G. M., *Can. J. Chem. Eng.*, 1984, **62**, 112–119.
86. Sarra, M., Casas, C. and Godia, F., *Biotechnol. Bioeng.*, 1997, **53**, 601–610.
87. Dalili, M. and Chau, P. C., *Biotechnol. Lett.*, 1988, **10**, 331.
88. Joshi, S. and Yamazaki, H., *Biotechnol. Tech.*, 1987, **1**, 45–48.
89. Mahmoud, W. and Rehm, H. J., *Appl. Microbiol. Biotechnol.*, 1987, **26**, 333–337.
90. Mahmoud, W. and Rehm, H. J., *Appl. Microbiol. Biotechnol.*, 1987, **26**, 338–341.
91. Foster, B. C., Coutts, R. T., Pasutto, F. M. and Dossetor, J. B., *Biotechnol. Lett.*, 1983, **5**, 693
92. Takashima, Y., Nakajima, H., Sonomoto, K. and Tanaka, A., *Appl. Microbiol. Biotechnol.*, 1987, **27**, 106–109.
93. Nagalakshmi, V. and Pai, J. S., *Biotechnol. Tech.*, 1994, **8**, 431–434.
94. Egresi, A., Sisak, G. S., Harasanyi, I. and Szajani, B., *Biotechnol. Tech.*, 1992, **6**, 261–264.

95. Truck, H. U., Chmiel, H., Hammes, W. P. and Trosch, W., *Appl. Microbiol. Biotechnol.*, 1990, **33**, 1–4.
96. Truck, H. U., Chmiel, H., Hammes, W. P. and Trosch, W., *Appl. Microbiol. Biotechnol.*, 1990, **33**, 139–144.
97. Deo, Y. M. and Gaucher, M., *Biotechnol. Bioeng.*, 1984, **26**, 285.
98. Keshavarz,T., Eglin, R., Walker, E., Bucke, C., Holt, G., Bull, A. T. and Lilly, M. D., *Biotechnol. Bioeng.*, 1990, **36**, 763–770.
99. Farid, M. A., El Diwany, A. I. and El Enshasy, H. A., *Acta Biotechnol.*, 1994, **14**, 67–74.
100. Honecker, S., Bisping, B. and Rehm, H. J., in Conference on DECHEMA Biotechnology, VCH, Veriagsgesellschaff, 1989.
101. Khare, S. K., Jha, K. and Gandhi, A. P., *Appl. Microbiol. Biotechnol.*, 1994, **41**, 571–573.
102. Chung, B. H. and Chang, H. N., *Biotechnol. Bioeng.*, 1988, **32**, 205–212.
103. Honecker, S., Bisping, B., Yang, Z. and Rehm, H. J., *Appl. Microbiol. Biotechnol.*, 1989, **31**, 17–24.
104. Mittal, Y., Mishra, I. M. and Varshney, B. S., *Biotechnol. Tech.*, 1993, **15**, 41–46.
105. Gupta, S. and Sharma, C. B., *Biotechnol. Lett.*, 1994, **16**, 599–604.
106. Fujii, N., Yasuda, K. and Sakakibara, M., *J. Ferment. Bioeng.*, 1994, **78**, 389393.
107. Omar, S. H., Honecker, S. and Rehm, H. J., *Appl. Microbiol. Biotechnol.*, 1992, **36**, 518–524.
108. Honecker, S., Bisping, B. and Rehm, H. J., in Conference on DECHEMA Biotechnology, VCH, Veriagsgesellschaff, 1990.
109. Eikmeier, H. and Rehm, H. J., *Appl. Microbiol. Biotechnol.*, 1987, **26**, 105–111.
110. Bisping, B., Hellfors, H., Honecker, S. and Rehm, H. J., *Food Biotechnol.*, 1990, **4**, 17–23.
111. Khare, S. K., Jha, K. and Gandhi, A. P., *Appl. Microbiol. Biotechnol.*, 1994, **41**, 571–573.
112. Sanroman, A., Pintado, J. and Lema, J. M., *Biotechnol. Tech.*,1994, **8**, 389–394.
113. Lee, Y. H., Lee, C. W. and Chang, H. N., *Appl. Microbiol. Biotechnol.*, 1989, **30**, 141–143.
114. Kautola, H., Rymowicz, W., Linko, Y-Y. and Linko, P., *Appl. Microbiol. Biotechnol.*, 1991, **35**, 447–449.
115. Forstr, M., Mansfeld, J., Schellenberger, A. and Dautzenberg, H., *Enzyme Microbiol. Technol.*, 1994, **16**, 777–784.
116. Mansfeld, J., Forster, M., Hoffmann, T. and Schellenberg, H., *Enzyme Microbiol. Technol.*, 1995, **17**, 11–17.
117. Honecker, S., Bisping, B., Yang, Z. and Rehm, H. J., *Appl. Microbiol. Biotechnol.*, 1989, **31**, 17–24.
118. Honecker, S., Bisping, B., Yang, Z. and Rehm, H. J., *Appl. Microbiol. Biotechnol.*, 1989, **31**, 17–24.
119. Legisa, M. and Muttey, M., *Enzyme Microbiol. Technol.*, 1988, **10**, 32–36.
120. Norton, S., Lacroix, C. and Vuillemard, J. C., *Enzyme Microbiol. Technol.*, 1994, **16**, 457–466.
121. Jianlong, W., Ping, L. and Ding, Z., *Biotechnol. Tech.*, 1994, **8**, 905–908.
122. Guoqiang, D., Kaul, R. and Mattiasson, B., *Appl. Microbiol. Biotechnol.*, 1991, **36**, 309–314.
123. Krischke, W., Schroder, M. and Trosch, W., *Appl. Microbiol. Biotechnol.*, 1991, **34**, 573–578.
124. Tamada, M., Begam, A. A. and Sadi, S., *J. Ferment. Bioeng.*, 1992, **74**, 379–383.
125. Iwasaki, K. I., Nakajima, M. and Sasahara, J., *J. Ferment. Bioeng.*, 1992, **74**, 375–379.
126. Kaufman, E. N., Cooper, S. P. and Davison, B. H., *Appl. Microbiol. Biotechnol.*, 1994, **45/46**, 545–554.
127. Davison, B. and Scott, C. D., *Biotechnol. Bioeng.*, 1992, **39**, 365–368.
128. Kanwar, S. S., Chadha, B. S., Tewari, H. K. and Sharma, V. K., *World J. Microbiol. Biotechnol.*, 1995, **11**, 687–688.
129. Audet, P., Paquin, C. and Lacroix, C., *Appl. Microbiol. Biotechnol.*, 1988, **29**, 11–18.
130. Norton, S., Lacroix, C. and Vuillemard, J. C., *Enzyme Microbiol. Technol.*, 1994, **16**, 457–466.
131. Norton, S., Lacroix, C. and Vuillemard, J. C., *J. Dairy Sci.*, 1994, **77**, 249–2508.
132. Audet, P., Lacroix, C. and Paquin, C., *Int. Dairy J.*, 1991, **1**, 1–15.
133. Arnaud, J. P. and Lacroix, C., *Biotechnol. Bioeng.*, 1991, **38**, 1041–1049.
134. Audet, P., Paquin, C. and Lacroix, C., *Appl. Microbiol. Biotechnol.*, 1990, **32**, 662–668.
135. Hang, Y. D., Hamamci, H. and Woodams, E. E., *Biotechnol. Lett.*, 1989, **11**, 119–120.
136. Kurosawa, H., Ishikawa, H. and Tanaka, H., *Biotechnol. Bioeng.*, 1988, **31**, 183–187.
137. Nanba, A., Kimura, K. and Nagi, S., *J. Ferment. Technol.*, 1985, **63**, 175.
138. Ghommidh, C., Navaro, J. M. and Durand, G., *Biotechnol. Lett.*, 1981, **3**, 93–
139. Ghommidh, C. and Navaro, J. M., *Biotechnol. Bioeng.*, 1982, **24**, 1991.
140. Kondo, M., Suzuki, Y. and Kato, H., *Hakko Kogaku Kaishi*, 1988, **66**, 393.
141. Osuga, J., Mori, A. and Kato, J., *J. Ferment. Technol.*, 1984, **62**, 139.
142. Mori, A., *Process Biochem.*, 1985, **20**, 67.
143. Paz, D. E. D., Aantana, M. H. A. and Eguchi, S. Y., *Appl. Biochem. Biotechnol.*, 1993, **39/40**, 455–466.
144. Sun, Y. and Furusaki, S., *J. Ferment. Bioeng.*, 1990, **69**, 102–110.
145. Sueki, M., Kobayashi, N. and Suzuki, A., *Biotechnol. Lett.*, 1991, **13**, 185–190.
146. Vassilev, N., Kautola, H. and Linko, Y. Y., *Biotechnol. Lett.*, 1992, **14**, 201–206.
147. Kautola, H., Vassilev, N. and Kinko, Y. Y., *Biotechnol. Lett.*, 1989, **11**, 313–318.
148. Furui, M., Sakata, N., Otsuki, S. and Tosa, T., *Biocatalysis.*, 1988, **2**, 69–77.
149. Fialova, M., Zahradnik, J. and Kucerova, H., *Collect. Czech. Chem. Commun.*, 1990, **55**, 2192–2198.
150. Kqak, M. Y. and Rhee, J. S., *Appl. Microbiol. Biotechnol.*, 1992, **36**, 578–583.
151. Rao, D. S. and Panda, T., *Bioprocess Eng.*, 1994, **11**, 209–212.
152. Buzzini, P., Gobbetti, M. and Rossi, J., *Ann. Microbiol. Enzimol.*, 1993, **43**, 53–60.
153. Kumar, P. K. R. and Lonsane, B. K., *Appl. Microbiol. Biotechnol.*, 1988, **28**, 537–542.
154. Shindo, S., Sahara, H. and Koshino, S., *Biotechnol. Lett.*, 1993, **15**, 51–56.
155. Qureshi, N. and Moddox, I. S., *Enzyme Microb. Technol.*, 1987, **9**, 668–675.
156. Cachon, R., Molin, P. and Divies, C., *Biotechnol. Bioeng.*, 1995, **47**, 567–574.
157. Ohara, H., Hiyama, K. and Yashida, T., *J. Ferment. Bioeng.*, 1993, **76**, 73–75.
158. Mori, A., Matsumoto, N. and Imai, C., *Biotechnol. Lett.*, 1989, **11**, 183–188.
159. Saucedo, J. E. N., Barbotin, J. N. and Thomas, D., *Appl. Microbiol Biotechnol.*, 1989, **30**, 226–233.
160. Bare, G., Delaunois, V., Rikir, R. and Thonart, P. H., *Appl. Biochem. Biotech.*, 1994, **45/46**, 599–610.
161. Kautola, H., *Appl. Microbiol Biotechnol.*, 1990, **33**, 7–11.
162. Kokubu, T., Karube, I. and Suzuki, S., *Eur. J. Appl. Microbiol. Biotechnol.*, 1978, **5**, 233.
163. Tonkova, A., Ivanova, V., Dobreva, E., Stefanova, M. and Spasova, D., *Appl. Microbiol. Biotechnol.*, 1994, **41**, 517–522.
164. Monolov, R. J., Kambourova, M. S. and Emanuilova, E. I., *Process Biochem.*, 1995, **30**, 141–144.

165. Klingeberg, M., Vorlop, K. D. and Antranikian, G., *Appl. Microbiol. Biotechnol.*, 1990, **33**, 494–500.
166. Kikuchi, S., Shibayama, J., Yasui, M., Takeuchi, T., Yamamoto, I. and Sato, M., *Biosci. Biotech. Biochem.*, 1993, **57**, 845–846.
167. Guo, Y., Lou, F., Peng, Z. Y. and Yuan, Z. Y., *Biotechnol. Bioeng.*, 1990, **35**, 99–102.
168. Mohandass, C. M. and Chandrasekaran, M., *Indian J. Exp. Biol.*, 1994, **32**, 912–913.
169. Chevalier, P. and Noue, J., *Enzyme Microbiol. Technol.*, 1988, **10**, 19–23.
170. Ariga, O., Andoh, Y., fujishita, Y., Watari, T. and Sano, Y., *J. Ferment. Bioeng.*, 1991, **71**, 397–402.
171. Argirakos, G., Thayanithy, K., and Wase, D. A. J., *J. Chem. Tech. Biotechnol.*, 1992, **53**, 33–38.
172. Chevalier, P., and Noue, J., *Enzyme Microbiol. Technol.*, 1987, **9**, 53–56.
173. Ramakrishna, S. V., Jamuna, R. and Emery, A. N., *Appl. Biochem. Biotechnol.*, 1993, **37**, 275–282.
174. Oriel, P., *Biotechnol. Lett.*, 1988, **10**, 113–116.
175. Fiedurek, J. and Szczodark, J., *Starch/Starke*, 1995, **47**, 196–199.
176. Bon, E. and Webb, C., *Enzyme Microb. Technol.*, 1989, **11**, 495–499.
177. Kuek, C., *Appl. Microbiol. Biotechnol.*, 1991, **35**, 466–470.
178. Emili Abraham, T., Jamuna, R., Vijay Bansilal, C. and Ramakrishna, S. V., *Starch/Starke*, 1991, **43**, 113–116.
179. Jamuna, R., Saswathi, N., Sheela, R. and Ramakrishna, S. V., *Appl. Biochem. Biotechnol.*, 1993, **43**, 163–176,
180. Saswathi, N., Sheela, R., Jamuna, R. and Ramakrishna, S. V., *Bioprocess Eng.*, 1995, **12**, 283–286.
181. Sheldon, J. B. D., *Biotechnol. Bioeng.*, 1988, **31**, 345–348.
182. Tamada, M., Kasai, N. and Kaetsu, I., *Biotechnol. Bioeng.*, 1989, **33**,1343–1346.
183. Kumakura, M., Tamada, M., Kasai, N. and Kaestu, I., *Biotechnol. Bioeng.*, 1989, **33**, 1358–1362.
184. Xin, L. Z., *Chem. Tech, Biotechnol.*, 1992, **54**, 129–133.
185. Nakashima, T., Kyotani, H., Izumoto, E. and Fukuda, H., *J. Ferment. Technol.*, 1990, **70**, 85–89.
186. Jamil, N. I. N. and Omar, I. C., *J. Biosci.*, 1992, **3**, 31–41.
187. Ferrer, A. and Sola, C., *Appl. Microbiol. Biotechnol.*, 1992, **37**, 737–741.
188. Johri, B. N., Alurralde, J. D. and Klein, J., *Appl. Microbiol. Biotechnol.*, 1990, **33**, 367–371.
189. Nakashima, T., Fukuda, H., Kyotani, S. and Morikawa, H., *J. Ferment. Technol.*, 1988, **66**, 441–448.
190. Kyotani, S., Nakashima, T., Izumoto, E. and Fukuda, H., *J. Ferment. Bioeng.*, 1991, **71**, 286–288.
191. Rogalski, J., Dawidowicz, A. L. and Wojtas-Wasilewska, M., *Acta Biotechnol.*, 1992, **12**, 191–201.
192. Feijoo, G., Dosoretz, C. and Lema, J. M., *Biotechnol. Lett.*, 1994, **8**, 363–368.
193. Ruckenstein, E. and Wang, X. B., *Biotechnol. Bioeng.*, 1994, **44**, 79–86.
194. Krikpatrick, N. and Palmer, J. M., *Appl. Microbiol. Biotechnol.*, 1987, **27**, 129–133.
195. Jager, A. G. and Wandrey, C., in Symposium on Physiology of Immobilized Cells, Wageningen, The Netherlands, 10–13 December 1989.
196. Dosoretz, C. G., Rothschild, N. and Hadar, Y., *Appl. Environ. Microbiol.*, 1993, **59**, 1919–1926.
197. Ospina, S., Lopez-Munguia, A., Gonzalez, R. L. and Quintero, R., *J. Chem. Ech. Biotechnol.*, 1992, **53**, 205–214.
198. Klein, J. and Wagner, F., *Enzyme Eng.*, 1984, **5**, 335–345.
199. Rodriguez, M. E., Quintero, R. and Lopez-Munguia, A., *Process Biochem.*, 1994, **29**, 213–218.
200. Grozeva, L., Tchorbanov, B. and Aleksieva, P., *Appl. Microbiol. Biotechnol.*, 1993, **39**, 512–514.
201. Siso, M. I. G. and Doval, S. S., *Enzyme Microb. Technol.*, 1994, **16**, 303–310.
202. Ghosh, M. and Nanda, G., *Biotechnol. Lett.*, 1991, **13**, 807–808.
203. El-Sayed, A. M. M., Abdul-Wahid, K. and Coughlin, R. W., *Biotechnol. Bioeng.*, 1992, **40**, 617–624.
204. Hasal, P., Vojtisek, V., Cejkova, A., Kleczek, P. and Kofronova, O., *Enzyme Microb. Technol.*, 1992, **14**, 221–229.
205. Ray, R. R., Jana, S. C. and Nanda, G., *J. Basic Microbiol.*, 1995, **2**, 113–116.
206. Federici, R. G., Federici, F. and Petruccioli, M., *Biotechnol. Lett.*, 1990, **12**, 661–666.
207. Federici, F., Petruccioli, M. and Miller, M. W., *Appl. Microbiol. Biotechnol.*, 1990, **33**, 407–409.
208. Mannin, C., Barbotin, J. N., Thomas, D., Lazzaroni, J. C. and Portaier, R., *Appl. Microbiol. Biotechnol.*, 1989, **32**, 143–147.
209. Federici, J., Gromada, A. and Szczodrak, J., *Microbiol. Res.*, 1994, **149**, 425–428.
210. O'Riordan, A., McHale, M. L., Gallagher, J. and McHale, A. P., *Biotechnol. Lett.*, 1989, **11**, 735–738.
211. Kim, D. M., Kim, G. J. and Kim, S. K., *Biotechnol. Lett.*, 1994, **16**, 11–16.
212. Fiedurek, J., Szczodrak, J. and Rogalski, J., *J. Appl. Bacteriol.*, 1995, **78**, 409–412.
213. Aykut, G., Hasirci, V. N. and Alaeddinoglu, G., *Biomaterials*, 1988, **9**, 168–172.
214. Ariga, O., Andoh, Y., Fujishita, Y., Watari, T. and Sano, Y., *J. Ferment. Bioeng.*, 1991, **71**, 397–402.
215. Prabhune, A. A., Rao, B. S., Pundle A. V. and Sivaraman, H., *Enzyme Microbiol. Technol.*, 1992, **14**, 161–163.
216. Carmichael, R. D., Jones, A. and Pickard, M. A., *Appl. Environ. Microbiol.*, 1986, **51**, 276–280.
217. Godia, F., Casas, C. and Sola, C., *Process Biochem.*, 1987, 43–48.
218. Ramakrishna, S. V., in *Biotechnology for Development* (eds Das, M. R. and Satish, M.), SCSTE, Kerala, 1996, pp. 162–174.
219. Nellaiah, H. and Gunasekaran, P., *Indian J. Microbiol.*, 1992, **32**, 435–442.
220. Gunasekaran, P. and Kamini, N. R., *World J. Microbiol. Biotechnol.*,1991, **7**, 551–556.
221. Ramakrishna, S. V., Sreedharan, V. P. and Prema, P., in *Bioreactor Immobilized Enzymes and Cells: Fundamentals and Applications*. (ed. Moo-Young), Elsevier Appl. Sci., Amsterdam, 1988, pp. 251–260.
222. Jamuna, R. and Ramakrishna, S. V., *Bioprocess Eng.*, 1992, **8**, 61–66.
223. Ramakrishna, S. V., Prema, P. and Sai, P. S. T., *Bioprocess Eng.*, 1991, **6**, 117–121.
224. Ramakrishna, S. V., Ratnakumari, T. S. and Sai, P. S. T., *Bioprocess Eng.*, 1991, **6**, 141–144.
225. Jamuna, R. and Ramakrishna, S. V., *Biomass Bioenergy*, 1992, **3**, 117–119.
226. Gil, G. H., Jones, W. J. and Tornabene, T. G., *Enzyme Microb. Technol.*, 1991, **13**, 390.
227. Amin, G. and Doelle, H. W., *Enzyme Microbiol. Technol.*, 1990, **12**, 443.
228. Nojima, S., *Chemical Economy Eng. Rev.*, 1983, **15**, 17–22.
229. Nagashima, M., Azuma, M., Noguchi, S. and Inuzuka, K., *Biotechnol. Bioeng.*, 1984, **26**, 992–997.
230. Yamauchi, Y., Okamoto, T., Murayama, H., Nagara, A. and Kashihara, T., *Appl. Biochem. Biotechnol.*, 1995, **53**, 277–283.
231. Jamuna, R. and Ramakrishna, S. V., in *Physiology of Immobilized Cells* (eds de Bont, J. A. M., Visser, J., Mattiasson, B. and Tramper, J.), Elsevier Sci. Publ., Amsterdam, 1989, pp. 522–538.
232. Ghose, T. K., in *Horizons of Biochemical Engineering* (ed. Aiba, S.), Univ. Tokyo Press, Tokyo, 1987, pp. 59–73.
233. Lee, S. W., Ebata, T., Liu, Y. C. and Tanaka, H., *J. Ferment. Bioeng.*, 1993, **75**, 6–42.
234. Jang, K. H., Park, C. J. and Chun, U. H., *Biotechnol. Lett.*, 1992, **14**, 311–316.

235. Leon, R. and Galvan, F., *J. Biotechnol.*, 1995, **42**, 61–67.
236. Benito, G. G., Ozores, M. and Pena, M., *Bioresources Tech.*, 1994, **49**, 209–212.
237. Pflugmacher, U. and Gottschalk, G., *Appl. Microbiol. Biotechnol.*, 1994, **41**, 313–316.
238. Lee, H. K. and Maddox, I. S., *Enzyme Microbiol. Technol.*, 1986, **8**, 409–411.
239. Qureshi, N. and Maddox, I. S., *J. Ferment. Bioeng.*, 1995, **80**, 185–189.
240. Friedl, A., Qureshi, N. and Maddox, I. S., *Biotechnol. Bioeng.*, 1991, **38**, 518–527.
241. Soetaert, W. and Vandamme, E. J., *Bioorg. Che. Healthcare Technol.*, 1991, 249–250.
242. Nishio, N., Sugawa, K., Hayase, N. and Nagai, S., *J. Ferment. Bioeng.*, 1989, **67**, 356–360.
243. Bisping, B., Hecker, D. and Rehm, H. J., *Appl. Microbiol. Biotechnol.*, 1989, **32**, 119–123.
244. Park, C. H., Okos, M. R. and Wankat, P. C., *Biotechnol. Bioeng.*, 1990, **36**, 207–217.
245. Iida, T., Hitoshi, I., Yoshimi, A. and Masahiro, S., *J. Ferment. Bioeng.*, 1993, **75**, 32–35.
246. Lu, Z. and Fujimura, T., *Radiat. Phys. Chem.*, 1993, **42**, 923–926.
247. Kazuaki, Y., Yukan, S., Taro, K., Masayuki, O. and Akira, O., *Biotechnol. Tech.*, 1994, **8**, 271–274.
248. Castellar, M. R., Borrego, F., Canovas, M. and Iborra, J. L., *Process Biochem.*, 1994, **29**, 569–574.
249. Vorlop, K., Di, E. and David, G. F., *Biotechnol. Tech.*, 1993, **7**, 287–292.
250. Gelson, C. D. and Thomas, A., *Biotechnol. Tech.*, 1993, **7**, 397–400.
251. Nolan, A. M. and McHale, A. P., *Biotechnol. Lett.*, 1994, **16**, 849–852.
252. Yang, P. D. J. and Liu, S., *Gongye Weishengwu*, 1992, **22**, 12–15.
253. Mebin, E. and Shich, W. K., *Chem. Eng. J.*, 1992, **50**, B17–B22.
254. Cheong, S. H., Park, J. K., Kun, B. S. and Chang, H. N., *Biotechnol. Tech.*, 1993, **12**, 879–884.
255. Davison, B. H. and Thompson, J. E., *Appl. Biochem. Biotehcnol.*, 1993, **39/40**, 415426.
256. Champluvier, B., Francart, B. and Rouxhet, P. G., *Biotechnol. Bioeng.*, 1989, **34**, 844–853.
257. Groot, W. J. and Luyben, K. Ch. A. M., *Biotechnol. Lett.*, 1987, **9**, 867–870.
258. Frick, Ch. and Schugerl, K., *Appl. Microbiol. Biotechnol.*, 1986, **25**, 186–193.
259. Mehta, P. K., Mishra, S. and Ghose, T. K., *Biotechnol. Bioeng.*, 1991, **37**, 551–556.
260. Schmauder, H. P., Schlosser, D., Gunther, T., Hattenbach, A., Sauerstein., Jungnickel, F. and Augsten, H., *J. Basic Microbiol.*, 1991, **6**, 453–477.
261. Prevost, H. and Divies, C., *Biotechnol. Lett.*, 1987, **9**, 789–794.
262. Martin, C. K. A. and Perlman, D., *Eur. J. Appl. Microbiol.*, 1976, **3**, 91–95.
263. Yuan, Z. Y., Wei, D. Z., Yin, G. L. and Yuan, W. K., *Ann. N. Y. Acad. Sci.*, 1992, **672**, 628–633.
264. O'Reilly, A. M. and Scott, J. A., *Enzyme Microbiol. Technol.*, 1995, **17**, 636–646.
265. Dincbas, V., Hortacsu, A. and Camurdan, A., *Biotechnol Prog.*,1993, **9**, 218–220.
266. Adlercreutz, P., Holst, O. and Mattiasson, B., *Enzyme Microbiol. Technol.*, 1982, **4**, 395–400.
267. Khang, Y. H., Shankar, H. and Senatore, F., *Biotechnol. Lett.*, 1988, **10**, 867–872.
268. Wikstrom, P., Szwajcer, E., Brodelius, P., Nilsson, K. and Mosbach, K., *Biotechnol. Lett.*, 1982, **4**, 153–158.
269. Murata, K., Tani, K., Kato, J. and Chibata, I., *Eur. J. Appl. Microbiol. Biotechnol.*, 1980, **10**, 11–21.
270. Zache, G. and Rehm, H. J., *Appl. Microbiol. Biotechnol.*, 1989, **30**, 426–432.
271. Ghanem, K. M., El-Aassar, S. A. and Yusef, H. H., *J. Chem. Technol. Biotechnol.*, 1992, **54**, 115–121.
272. Weetall, H. H., Sharma, B. P. and Detar, C. C., *Biotechnol. Bioeng.*, 1981, **23**, 605–614.
273. Nasri, M., Sayadi, S., Barbotin, J-N., Dhulster, P. and Thomas, D., *Appl. Environ. Microbial.*, 1987, **53**, 740–744.
274. Romo, S. and PerezMartinez, C., *J. Phycol.*, 1997, **33**, 1073–1076.