10
Biocatalysis with Immobilized Cells

CAVIT AKIN

Amoco Corporation, Amoco Research Center, Naperville, Illinois 60566, USA

Introduction

In its broadest sense, biocatalysis comprises all forms of catalysis in which the activating species is a biological entity or a combination of such. Included among these are enzymes, subcellular organelles, whole cells and multicellular organisms.

In some phase of their life cycles, most cells tend to locate or attach themselves to a solid surface (Messing, 1985). In nature, cell immobilization occurs by the formation of microbial or algal mats, as well as by adhesion to available surfaces. In some fermenters and in waste treatment systems flocculation, pellet formation and surface attachment can be viewed as forms of immobilization. For formal definition, immobilized cell biocatalysts can be designated as 'cells which are physically confined or localized in a defined region or space with retention of their catalytic activities or selected portions thereof, for repeated and continuous use' (compare with Klein and Wagner, 1983).

Throughout history, man has used biocatalytic capabilities in nature for his well-being by collecting, screening, selecting and domesticating living organisms, as well as by modifying them and their environment. Today, molecular biology is creating powerful new tools to design, engineer and modify biocatalytic entities. Immobilization can provide, in addition, a felicitous environment so that the biocatalytic activities of cells can be further enhanced. With the realization of this potential as a goal, during the last 15 years (particularly during 1980–83) investigators have focused increased attention on the practical exploitation of immobilized cell biocatalysis (Rosevear, 1984a). Such researches have led to several industrial applications of

Abbreviations: ATP, adenosine 5'-triphosphate; DEAE-cellulose, diethyl aminoethyl-cellulose; i.d., internal diameter; NAD(H) nicotinamide adenine dinucleotide (reduced form); NMR, nuclear magnetic resonance; o.d., outside diameter; P_i, inorganic phosphate; RBS, rotating biological surface; UDPG, uridine 5'-diphosphate D-glucose.

Biotechnology and Genetic Engineering Reviews—Vol. 5, September 1987

immobilized cells: continuous production of L-aspartic acid with immobilized *Escherichia coli* (Chibata, Tosa and Sato, 1974; Sato *et al.*, 1975); production of L-malic acid with immobilized *Brevibacterium ammoniagenes* (Yamamoto *et al.*, 1976); isomerization of glucose to fructose by immobilized cells of *Streptomyces* spp., of *Actinoplanes missouriensis*, or of *Arthrobacter* spp. (Hupkes and van Tilburg, 1976; Bucke, 1977), and production of L-alanine by immobilized *Pseudomonas dacunhae* (Yamamoto, Tosa and Chibata, 1980; Takamatsu *et al.*, 1981). These achievements and other aspects of biocatalysis with immobilized cells have been described in review articles (*Table 1*) and in books that are devoted exclusively or partly to this subject (Chibata and Wingard, 1983; Mattiasson, 1983a; Laskin, Tsao and Wingard, 1984; Tramper, van der Plas and Linko, 1985). This paper presents an overview of the most recent publications on various aspects of biocatalysis with immobilized cells. Features examined include (1) the advantages and disadvantages of immobilized cells; (2) supports, matrices and procedures for cell immobilization; (3) reactions of, and products from, cells; (4) performance characteristics of immobilized cells; (5) the design and function of bioreactors.

Advantages and disadvantages of immobilized cells

The major expected advantage of immobilized cells, in contrast to free cells or immobilized enzymes, is reduction of the cost of bioprocessing. This should follow from the repeated and continuous use of the biocatalyst, the maintenance of a high cell density, and the provision of a system with minimal cost for cell separation. In addition, immobilization may provide resistance to shear for shear-sensitive cells such as those from plants and

Table 1. Selected review articles on biocatalysis with immobilized cells

Topic	Reference
Preparation of chemicals	Chibata, Tosa and Sato (1983); Rao and Hall (1984), Vorlop (1985)
Industrial applications	Linko and Linko (1984)
Methods of cell immobilization	Klein and Wagner (1983); Nilsson and Mosbach (1984); Fukui and Tanaka (1984)
A critical review	Rosevear (1984a)
Microbial and plant cells	Shuler, Pyne and Hallsby (1984); Brodelius (1985a,b); Knorr, Miazga and Teutonico (1985); Dainty *et al.* (1985)
Animal cells	Rosevear and Lambe (1985)
Ethanol production	Margaritis (1984)
Production of vinegar	Mori (1985)
Production of antibiotics	Karube, Suzuki and Vandamme (1984)
Solvent production	Kolot (1984)
Reactions in organic solvents	Fukui and Tanaka (1985)
Hydrolysis of lactose	Gekas and Lopez-Leiva (1985)
Biosensors	Corcoran and Rechnitz (1985)
Biocatalysis business	Midler (1985)

animals. Furthermore, growth of live cells, when immobilized, can be of value in some instances (*see* page 355) but in other circumstances can be disastrous, in that unrestrained growth can burst soft or thin-walled containment structures to release previously entrapped organisms which then either are lost or contaminate the product. Disadvantages may also arise from the diffusion barrier created by the immobilization matrix as well as by the high cell density. A further disappointment has been the failure of efforts to create a versatile, general matrix capable of holding a variety of cells and functioning in differing bioprocesses. In actual practice, therefore, a large number of immobilization supports and matrices have been prepared and their characteristics examined under varied circumstances.

Supports, matrices and procedures for cell immobilization

Supports and matrices for cell immobilization ideally should meet the following criteria (compare Klein and Wagner (1983) and Brodelius (1985a)):

1. Not reduce the desired biocatalytic activity of the cell;
2. Not react with the substrates, nutrients or products;
3. Retain their physical integrity and be insoluble under the bioprocess reaction conditions;
4. Be permeable to reactants and products;
5. Have large specific surface;
6. Have high diffusion coefficients for substrates, nutrients and products;
7. Provide appropriate hydrophilic–hydrophobic balance for nutrients, reactants and products;
8. Be resistant to microbial degradation;
9. Retain chemical and thermal stability under bioprocess and storage conditions;
10. Be elastic enough to accommodate growing cells;
11. Have functional groups for cross-linking;
12. Be generally recognized as safe for food and pharmaceutical bioprocess applications;
13. Be generally available in adequate quantities with consistent quality and acceptable price;
14. Be easy and simple to handle in the immobilization procedure;
15. Be environmentally safe to dispose of, and/or be capable of recycling.

The immobilization procedures should also satisfy certain other requirements. They must:

1. Have minimal (if any) adverse effects on the desired biocatalytic properties;
2. Be safe and simple to use;
3. Require few process steps and ingredients;
4. Generate no by-products requiring special disposal procedures;

5. Be amenable to scale-up;
6. Be adaptable to automation;
7. Be economical.

Not all of these requirements may be satisfied by any one immobilization support, matrix or procedure. The individual nature of each cell line means that even a well-characterized immobilization matrix may require modification to fit the cell and the process involved. Specific problems must be solved to obtain the most suitable system for a particular biocatalysis application, and compromises made regarding certain requirements. For this reason, suggestions for numerous supports, matrices and procedures for cell immobilization have been documented (*see* reviews by Klein and Wagner, 1983; Mattiasson, 1983b; Fukui and Tanaka, 1984; Hartmeier, 1985 and Tramper, 1985). The supports and matrices described in the recent publications fall into seven groups:

1. Solid supports or matrices for adhesion or adsorption of cells;
2. Solid supports or matrices for cross-linking with cells;
3. Directly cross-linked cells;
4. Gels and other polymers for entrapment or encapsulation of cells;
5. Combinations of gels and other polymers for encapsulation or entrapment of cells;
6. Composite immobilization matrices;
7. Hollow structures, fibres and plates (membrane reactors) for physical retention of cells.

Representative examples from the recent literature for each group, with related immobilization procedures, are described below.

SOLID SUPPORTS OR MATRICES FOR ADHESION OR ADSORPTION OF CELLS

Immobilization by adhesion and adsorption relies on the inherent tendencies of the cells to adhere to particular surfaces, or to flocculate, or to settle in the pores of the framework. Such immobilization is usually achieved by keeping the support material and the actively growing cells in contact for a specific length of time. A variety of frameworks for immobilization of cells by adhesion have been reported in the literature (*Table 2*). For example, to immobilize methanogenic bacteria, Nishio, Kayawake and Nagai (1985) employed granular materials such as zeolites, glass beads, baked clay, and foamed slag, or fibrous supports such as sponge rubber, cellulose acetate fibre and activated carbon fibre. These materials were packed in column reactors, concentrated methanogenic bacteria were added, and static cultivation with fresh medium was begun. When the generation of methane and carbon dioxide was observed, continuous operation of the reactor was initiated. In these packed-bed reactors high concentrations of methanogenic bacteria could be maintained (20 – 44 g/l compared with 0·1 – 0·5 g/l in

unpacked chemostat cultures). In another example, Kautola *et al.* (1985) immobilized *Aspergillus terreus* mycelia by growing the culture in shake flasks in the presence of Celite for 3 days.

In some cases, pretreatment such as starving and washing the cells (Van Haecht, De Bremaeker and Rouxhet, 1984) or activation of the surface of the support material or of the cells my be beneficial. For example, absorption of aluminium ions on the surface of *Saccharomyces cerevisiae* cells neutralized the surface charge and thus facilitated cell adhesion to glass plates (Mozes and Rouxhet, 1984, 1985; Van Haecht, Bolipombo and Rouxhet, 1985). For the immobilization of *Zymomonas mobilis* (Daugulis, Krug and Choma, 1985) an anionic microreticular resin was used. The resin was packed into the reactor columns, conditioned with hydrochloric acid and washed with distilled water to remove excess acid. A suspension of *Zymomonas mobilis* cells was then forced through the column to achieve immobilization.

Del Borghi *et al.* (1985) immobilized *Saccharomyces cerevisiae* on commerical synthetic sponge discs without any pretreatment. In this immobilization the yeast cells were grown in a rotating disc reactor under aerobic conditions. Uniform colonization of the sponge cross-section was observed. Immobilization of *Saccharomyces cerevisiae* on cotton cloth was accomplished by submerging the cloth in growing yeast culture for 1 h, removing it and then drying it in air for 17 h (Joshi and Yamazaki, 1984). Cheetham, Garrett and Clark (1985) immobilized *Erwinia rhapontici* on DEAE cellulose by mixing 2 g cells with 10 ml of a very thick slurry of DEAE cellulose at pH 7. *Gluconobacter oxydans* cells were immobilized by circulating the culture broth through column reactors packed with fibrous nylon (Seiskari, Linko and Linko, 1985). Noll (1985) recommended a monolithic ceramic support, in the form of a honeycomb structure consisting of a multiplicity of parallel channels of square cross-section, for use as a culturing support for plant and animal cells. The monolith was composed of cordierite and had the form of a cylinder about 2 cm in diameter and 6 cm in length. It contained about 300 channels per square inch of cross-sectional area transversing the length of the structure. Potts and Bowman (1985) immobilized *Nostoc commune* cells by filtering the cell suspension through a nylon filter, 2 cm in diameter and 0·04 mm^2 pore size, blotting the filter to remove excess water and desiccating the cells rapidly at −99·5 MPa in an incubator with constant light, temperature, and relative humidity. Robertson and Philips (1984) suggested immersing ribbons of polytetrafluoroethylene or polyethylene in an aqueous nutrient medium to serve as the adhesion immobilization surface for hydrocarbon-utilizing micro-organisms. Okuhara (1985) immobilized *Acetobacter* by putting a pellicle of the cells in a column reactor packed with polypropylene fibres of 40 μm diameter and feeding the fermentation medium through the column.

Schwanniomyces castelli, Endomycopsis fubiligera and *Saccharomyces diastaticus* were immobilized on 1 cm^3 reticulated polyurethane cubes by growing the cells in contact with the cubes for 20 h (Amin *et al.*, 1985). *Capsicum frutescens* was fixed on 1 cm^3 polyurethane foam cubes by invasive growth of the cells within a 23-day growth period (Lindsey and Yeoman, 1984a,b).

Table 2. Supports for immobilization of cells by adhesion or adsorption

Supports	Cells	Cell concn (g/l)	Support diam. (mm)	Catalyst life	Reference
Activated carbon fibre	Methanogens	5·8	*	—	Nishio, Kayawake and Nagai (1985)
Anionic microreticular (microporous) resin (XE-352)	*Zymomonas mobilis*	—	—	232 h**	Daugulis, Krug and Choma (1985)
Baked clay	Methanogens	20·1	2·9–4·4	—	Nishio, Kayawake and Nagai (1985)
Celite	*Aspergillus terreus*	—	—	2 weeks**	Kautola *et al.* (1985)
Cellulose acetate fibre	Methanogens	5·8		—	Nishio, Kayawake and Nagai (1985)
Commercial synthetic sponge	*S. cerevisiae*	***		—	Del Borghi *et al.* (1985)
Cotton cloth	*S. cerevisiae*	—	—	—	Joshi and Yamazaki (1984)
Cubes of reticulated polyester foam	*S. cerevisiae; S. uvarum*	—	—	123 days	Black *et al.* (1984)
DEAE cellulose	*Erwinia rhapontici*	—	—	400 h	Cheetham, Garrett and Clark (1985)
Fibrous nylon	*Gluconobacter oxydans*	—	—		Seiskari, Linko and Linko (1985)
Glass beads	Methanogens	—	64·0–65·5	—	Nishio, Kayawake and Nagai (1985)
	Arthrobacter simplex		2		Mozes and Rouxhet (1984)
Glass or polycarbonate plates	*S. cerevisiae*	—	—	—	Van Haecht, De Bremaecker and Rouxhet (1984); Van Haecht, Bolipombo and Rouxhet (1985)
Glass plates	*S. cerevisiae*	—	—	—	Mozes and Rouxhet (1985)
Glass wool		—	—	—	Mozes and Rouxhet (1985)
Macroporous clay	*Nitrobacter winogradskyi*	—	1·8	—	Audic, Faup and Navarro (1984)
Magnetite	*Rhodopseudomonas sphaeroides*			—	Mac Rea (1985)
Melted slag	Methanogens	14·9	6·6–10·3	—	Nishio, Kayawake and Nagai (1985)
Monolithic ceramic support in the form of honeycomb	Mammalian, animal and insect cells			—	Noll (1985)
Needle-punched polyester	Propane-using methanogenic bacteria				Khan and Meek (1985)
Nylon filter	*Nostoc commune*				Potts and Bowman (1985)

(*contd*)

Table 2 (*contd*)

Supports	Cells	Cell concn (g/l)	Support diam. (mm)	Catalyst life	Reference
Polyethylene ribbons	Hydrocarbon-utilizing micro-organisms				Robertson and Philips (1984)
Polypropylene fibres	*Acetobacter*		0·04		Okuhara (1985)
Polyurethane foam	*Schwanniomyces castelli; Endomycopsis fubiligera; Saccharomyces diastaticus; Capsicum frutescens*				Amin *et al.* (1985)
	Chlorogloea fritschii; Nostoc muscorum; Mastigocladus laminosus;				Lindsey and Yeoman (1984a,b)
	Porphyridium cruentum				Muallem, Bruce and Hall (1983)
	Pseudomonas dacunhae				Thepenier, Gudin and Thomas (1985)
Spheres of stainless steel	*S. cerevisiae*		31–105	6 months	Fusee and Weber (1984)
	S. uvarum		14–55		Black *et al.* (1984)
Sponge rubber	Methanogens	—	—	—	Nishio, Kayawake and Nagai (1985)
Titanium (IV)	*Aeromonas hydrophila*	—	—	—	Willetts (1985)
Zeolite	Methanogens	19·2	2·5–12·6	—	Nishio, Kayawake and Nagai (1985)

* Specific area 1400 m^2/g
** Reactor operation period
*** 1273 mg cells dry weight/g support

Similarly, *Chlorogloea fritschii, Nostoc muscorum* and *Mastigocladus laminosus* were maintained on polyurethane by growing these cultures in presence of the support material (Muallem, Bruce and Hall, 1983). *Porphyridium cruentum* cells were immobilized by vigorously mixing wet cell paste with urethane prepolymer at room temperature for 1 min and then letting the mixture stand at room temperature for 60 min (Thepenier, Gudin and Thomas, 1985). *Pseudomonas dacunhae* cells also were immobilized by using the polyurethane prepolymer procedure (Fusee and Weber, 1984). Willetts (1985) fixed *Aeromonas hydrophila* cells on titanium (IV) hydroxide by mixing the cells and the support material, agitating them gently for 5 min, allowing the mixture to stand for 60 min and then consolidating the contents by centrifugation.

The ease and mildness of cell adhesion and adsorption and the availability of inert support surfaces make an immobilized cell procedure attractive for biocatalysis with living and growing cells. Thus, Messing (1985) observed very rapid reproduction of *E. coli, B. subtilis* and *S. marcescens* immobilized on an inorganic support of controlled pore size. He estimated that each *E. coli* parent cell in a plug flow system generated a new progeny every 36 seconds. Laboratory-scale continuous bioreactors can be operated for extended periods with cells immobilized by adhesion. For example, Black *et al.* (1984) maintained their ethanol bioreactors for up to 113 days with no loss of immobilized biomass or of cell viability. Some cell movement and washout of the newly grown cells may take place during operation. Where the cell washout needs to be reduced or eliminated, other immobilization procedures may be useful, for instance cross-linked matrices and cells.

SOLID SUPPORTS OR MATRICES FOR CROSS-LINKING WITH CELLS

Cell walls contain functional groups such as $-NH_2$, $-OH$, and $-COOH$ that lend themselves to covalent coupling. These groups can be used advantageously to immobilize cells by cross-linking them to solid supports. Cross-linking reduces cell washout and improves the mechanical strength of the biocatalyst, yet usually does not create a significant diffusion barrier. Two examples of covalent coupling of micro-organisms to solid support may be cited from recent publications. *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Azotobacter vinelandii*, *E. coli* and *Micrococcus luteus* have been attached to cellulose particles using cyanuric chloride as the coupling agent (Okita, Bonham and Gainer, 1985). To assemble this system, 'Solka Floc' was derivatized with cyanuric chloride, mixed with the cell suspension and stirred on a gyratory shaker or roller bottle apparatus. Cell attachment was observed to be more effective if the stirring speeds were low. The ethanol productivity with immobilized yeast was determined by using a 1 litre chemostat. Nutrient broth was supplied continuously at a dilution rate of $1\,h^{-1}$, which was above the washout rate in this fermenter. Maximum productivity, 2·1 g ethanol/l/h, was achieved in 14 h. This productivity was about the same as that seen in chemostat runs with free cell suspensions. The technique of whole-cell immobilization offered an advantage over gel entrapment by eliminating the resistance of gel to the transfer of nutrients. In a second example, Wood and Calton (1984a,b) used polyazetidine prepolymers for entrapment and covalent attachment of *Escherichia coli* containing aspartase (aspartate ammonia-lyase; EC 4.3.1.1). They took advantage of cross-linking of polyazetidine with various functional groups on the cell surface during drying of the cell–polyazetidine mixtures. For supports they used molecular sieves, ion-exchange resins, alumina, silica gel, Celite and polymer latex beads. The immobilized cells retained 97% of the aspartase activity of the free cells in the production of aspartic acid from fumarate. At 25°C, this immobilized system was stable for more than 2 years.

DIRECTLY CROSS-LINKED CELLS

In this immobilization method, whole cells are attached to each other with bifunctional chemicals. For example, Doppner and Hartmeier (1984) co-immobilized added catalase (EC 1.11.1.6) and permeabilized *Aspergillus niger* cells, containing high intracellular glucose oxidase (EC 1.1.3.4) and catalase activity, by using ovalbumin and glutaraldehyde. They observed that low cost per unit activity was a major advantage of the co-immobilizate compared with immobilized enzyme. Melelli and Bisso (1985) used albumin (1%) together with glutaraldehyde (0·25%) to immobilize permeabilized yeast cells. This biocatalyst was used for the production of fructose 1,6-diphosphate. In this immobilization, glutaraldehyde served both for cross-linking the cells and modulating the degree of permeabilization. Cheetham, Garrett and Clark (1985) immobilized *Erwinia rhapontici* cells with glutaraldehyde and compared the performance of this system in isomaltulose production with that of biocatalysts prepared by other immobilization procedures. They concluded that the biocatalyst immobilized in calcium alginate pellets had the best operational stability compared with all other biocatalysts prepared, including glutaraldehyde cross-linking.

One disadvantage of cross-linking is that the bifunctional agents used in this procedure may react with the enzymes in the cells in such a way as to reduce the biocatalytic activity of the cells. Therefore, in some applications, entrapment in a natural or synthetic gel or polymer matrix may be preferable.

GELS AND OTHER POLYMERS FOR ENTRAPMENT OR ENCAPSULATION OF CELLS

Natural gels (such as agar, agarose, alginate, carrageenan, collagen, glucan, and gelatin), chemically modified natural polymers (such as cellulose acetate), and synthetic gels and polymers (such as polyacrylamide, polyazetidine and polyhydroxyethylmethacrylate) have been employed for immobilization of cells by entrapment or encapsulation.

The most popular cell entrapment matrix in recent studies is calcium alginate (*Table 3*). Alginate is a linear co-polymer of two uronic acids, D-mannuronic acid and L-guluronic acid, linked together by β-1,4 and α-1,4 glucosidic bonds in sequence. The two monomers are arranged in homopolymeric blocks, interspaced with sequences containing both monomers. Although viscosity depends mainly on the molecular size, the affinity for ions and the gel-forming properties are related to the content of guluronic acid. When two guluronic acid residues are in neighbouring positions in the polymer, they form a binding site for calcium ions. Consequently, the content of guluronic acid blocks is the main determinant of the strength and stability of the gel. The popularity of alginate for cell immobilization by entrapment is attributable to the following features: it is not toxic; production is rapid; it is easy to sterilize and the cells stay alive for a long time. The immobilization is carried out under very mild conditions with little loss of viability for most cells. No chemical coupling is required and the cells are not exposed

Table 3. Uses of cells immobilized by calcium alginate

Type of cells	Reaction or product	Alginate (%)	Bead diameter (mm)	References
Alkene-utilizing bacteria	Epoxides	3	0·8	Habets-Crutzen *et al.* (1984)
Arthrobacter simplex	Steroid transformation	—	—	Kloosterman and Lilly (1985)
Aspergillus niger	Citric acid	—	—	Eikmeier and Rehm (1984)
Aspergillus niger	Glucoamylase and α-amylase	3–10	2–3	Li, Linko and Linko (1984)
Aspergillus terreus	Itaconic acid	6	—	Kautola *et al.* (1985)
Chlorella emersonii	Glycollate	3	2	Day and Codd (1985)
Claviceps purpurea	Alkaloid	2, 4, 8	—	Kopp and Rehm (1984)
Clostridium sp.	Ethanol	4–16	—	Largier *et al.* (1985)
Lavandula vera	Pigments	3	5	Nakajima *et al.* (1985)
Mortierella isabellina	Biotransformation of dihydroabietic acid	3	—	Kutney *et al.* (1985)
Pediococcus halophilus; Saccharomyces rouxii; Torulopsis versatilis; Rat erythrocytes	Soy sauce	2	4	Osaki *et al.* (1985)
	Aflatoxicol	—	—	Chang, Lin and Hsiung (1985)
Saccharomyces cerevisiae	Ethanol	1	4–5	McGhee, Carr and St Julian (1984)
Saccharomyces uvarum	Beer	1	3	Onaka *et al.* (1985)
Thiobacillus ferrooxidans	Ferrous ion oxidation	2	3	Lancy and Tuovinen (1984)
Zymomonas mobilis	Ethanol	1·5	2	Bajpai and Margaritis (1985c)

to any harmful reagents. The immobilization can be performed in an isotonic, buffered medium at neutral pH and in a range of temperature from 0 to 100°C. Reaction times, including the hardening of the gel-beads in calcium chloride solution, range from a few minutes up to 30 min. Gel entrapment can easily be performed under sterile conditions, either by autoclaving or by sterile filtration of the sodium alginate solution before mixing with cells. Once immobilized, the cells are protected from contaminating organisms.

Calcium alginate gels seem to be compatible with most living cells and, in several cases, the viability and/or the enzyme activities have been stabilized over extended periods. Calcium alginate gel beads are easy to produce on a large scale without any sophisticated equipment. Usually, the cell suspension is mixed with a solution of the water-soluble anionic polymer sodium

alginate, and the mixture is dripped into a solution containing a multivalent cation such as Ca^{2+}, Sr^{2+} or Al^{3+}. By ionotropic gelation the droplets instantaneously form a gel-sphere, entrapping the cells within the three-dimensional lattice of the ionically linked polymer. Calcium alginate gels are thermostable in the temperature range 0–100°C. Alginate is non-toxic and meets the requirements for additives in pharmaceutical and food applications. The beads can easily be dissolved under mild conditions and the cells regenerated.

A few limitations of calcium alginate gels should be kept in mind. As the gel is cross-linked with calcium ions, substances with high affinity for calcium will destabilize the gel: this precludes the use of buffers containing citrate or phosphate. Destabilization may also occur from exchange of calcium with sodium, potassium and magnesium ions if these ions are present in high concentrations. It is therefore recommended that 3 mM calcium should be included in the medium, or that the sodium:calcium ratio should be maintained at less than 25:1 (Anonymous, 1983). Recent publications indicate a broad range of sodium alginate concentrations employed in the immobilization of cells: the lowest concentration was 1% (McGhee, Carr and St Julian, 1984; Onaka *et al.*, 1985); the highest was 4–16% (Largier *et al.*, 1985).

Bead diameters also varied, from 0·8 mm (Habets-Crutzen *et al.*, 1984) to about 5 mm (Nakajima *et al.*, 1985). Most investigators used syringes or tubes for extrusion and dropwise addition of aqueous sodium alginate/cell slurries into calcium chloride solution to form the immobilized cell beads. In one typical example *Saccharomyces uvarum* was immobilized in calcium alginate by suspending a 380 g sample of pressed yeast in 2400 ml 1% (w/v) sodium alginate and extruding this suspension slowly, as drops, from a tube with a 1 mm diameter tip, into 0·05 M calcium chloride solution at room temperature (Onaka *et al.*, 1985). The drops formed beads upon contact with the $CaCl_2$ solution. These beads, approximately 3 mm in diameter, were stored at 0°C for 24 h to allow their complete gelation before use. In a variation introduced by Chang, Lin and Hsiung (1985), an erythrocyte–alginate mixture was first added to a 50 mM zinc chloride solution to form beads which were then rapidly transferred into a calcium chloride solution. Cheetham, Garrett and Clark (1985) obtained fibrous particles instead of beads by rapidly extruding the cell alginate slurry into calcium chloride solution vigorously mixed in a food blender.

Scanning electron microscopic examination by Kuek and Armitage (1985), of calcium alginate beads with immobilized growing mycelia of *Aspergillus phoenicus*, revealed that mycelial growth was restricted to the subsurface and that the degree of agitation was a factor in surface stability of beads. In general, however, some cell washout is expected with most growing cells.

Most of the preceding investigators used an injection dropwise-addition procedure for the immobilization of alginate. Hulst *et al.* (1985) studied a new technique for immobilization of biocatalysts in large quantities. This method breaks up a jet of biocatalyst–presupport mixture into uniform droplets by resonance techniques. In this procedure, bead diameters between 1 and 2 mm are obtained, with standard deviations of 3–5% for immobilized

yeast and 10–15% for plant cells immobilized in calcium alginate. Resonance techniques increased the production capacity by two orders of magnitude compared with the conventional injection through a nozzle.

κ-Carrageenan is another popular gel for immobilization of cells by entrapment (*Table 4*). This polymer is an anionic polysaccharide extracted from seaweed. When a carrageenan solution is warm, the chains exist as random coils. On cooling, double helices form and produce a cross-linked gel. Such a gel can be 'cured' or strengthened if it is soaked in solutions containing cations such as potassium. For example, for immobilization of *Lavandula vera* cells, Nakajima *et al.* (1985) first removed the calcium ions present in the cell suspension by washing with 3% sucrose solution, then mixed the suspension with 5% κ-carrageenan solution at 50°C, and finally dropped the mixture rapidly through a wide-mouthed pipette into 0·3 M KCl solution cooled on ice. Alternatively, Habets-Crutzen *et al.* (1984) extruded a carrageenan–cell mixture into cold 2% $CaCl_2$ solution first and then after 20 minutes replaced the $CaCl_2$ solution with cold 2% KCl solution. Karkare, Dean and Venkatasubramanian (1985) prepared immobilized yeast beads with κ-carrageenan by the following procedure: κ-carrageenan, 1·78% (w/w), was dissolved at 60°C in deionized triple-distilled water with NaCl (8·76 g/l) and silica gel (150 g/l) and then cooled to 40°C; *Saccharomyces cerevisiae*

Table 4. Use of cells immobilized by κ-carrageenan

Type of cells	Reaction or product	Carrageenan (%)	Bead diam. (mm)	References
Acetobacter aceti	Vinegar	4	5	Osuga, Mori and Kato (1984)
Alkene-utilizing bacteria	Epoxides	2·5	—	Habets-Crutzen *et al.* (1984)
Erwinia herbicola	L-Dopa	3	2–4	Para, Rifai and Baratti (1984)
Erwinia rhapontici	Isomaltulose	—	—	Cheetham, Garrett and Clark (1985)
Leuconostoc oenos; Lactobacillus brevis	Malolactic fermentation	3	—	McCord and Ryu (1985)
Lavandula vera	Pigments	5	5	Nakajima *et al.* (1985)
Rhodopseudomonas capsulata	Hydrogen	3·4	1–3	Francou and Vignais (1984)
Saccharomyces cerevisiae	Ethanol	1·78	0·5	Karkare, Dean and Venkatasubramanian (1985)
Scenedesmus quadricauda	Ammonium and orthophosphate removal	2·5	3	Chevalier and de la Noue (1985)
Zymomonas mobilis	Ethanol	5	2–3	Jain, Toran-Diaz and Baratti (1985a,b)

inoculum was added at approximately 5×10^7 cells/ml in growth medium, and beads were immediately cast under sterile conditions by extrusion of the mixture from a vibrating needle into 0·3 M KCl solution held at 10°C. This procedure produced beads with diameters as small as 0·3 mm.

Polyacrylamide is another gel commonly used for the immobilization of microbial cells (*Table 5*). Rossi and Clementi (1985) immobilized *Pichia membranaefaciens* in polyacrylamide gel by the following steps: cells (0·4 g wet weight) were suspended in the polymerization solution containing 1·0 ml of phosphate buffer pH 7·0, 1·0 ml of 40% acrylamide and 2·5 ml of 2·3% *N,N'*-methylene-bis-acrylamide (both prepared in phosphate buffer pH 7·0) and the components were thoroughly mixed; then the catalyst mixture, consisting of 0·1 ml of 12·5% ammonium persulphate and 0·01 ml of *N,N,N',N'*-tetramethylethylenediamine (Temed) was added: polymerization, under bubbling nitrogen, proceeded within 10 minutes; the gel was then washed several times with 0·2 M KH_2PO_4 pH 5·6 and cut into small cubes of about 0·3 cm^3. Variations of this procedure can be found in references listed in *Table 5*.

Several other gels and polymers for the immobilization of cells by entrapment have been reported recently. Agar was used by Cheetham, Garrett and Clark (1985) for immobilization of *Erwinia rhapontici*. This was accomplished by mixing cells with 3% agar solution at 50°C and rapidly extruding the mixture into ice-cold water. Agar was also used by Tuli *et al.* (1985) for the immobilization of *Lactobacillus casei*. In this case, the cells were suspended in 2% agar at 50°C, the agar was cooled to 37°C to solidify it and gel cubes of $4 \times 2 \times 2$ mm were cut out. Vogel and Brodelius (1984) immobilized *Catharanthus roseus* cells in 5% agarose. Cheetham, Garrett and Clark (1985) used cellulose acetate and collagen for immobilization of *Erwinia rhapontici*, and Mohite and SivaRaman (1984) immobilized *Saccharomyces uvarum* in open-pore gelatin. An immobilization matrix comprising a water-insoluble β-1,3-glucan gel in the shape of beads was prepared by dispersing an alkaline aqueous solution of a water-soluble β-1,3-glucan in a water-immiscible organic solvent, and adding an organic acid to the resultant dispersion (Miyashiro *et al.*, 1985). The soluble glucan used in this

Table 5. Uses of cells immobilized by polyacrylamide

Type of cells	Reaction or product	References
Aspergillus niger	Citric acid	Horitsu *et al.* (1985)
Aspergillus ochraceus	11 α-Hydroxylation	Bihari *et al.* (1984)
Clostridium absonum	Ursodeoxycholic acid	Kole and Altosaar (1985)
Corynebacterium fascians	Limonoid debittering	Hasegawa *et al.* (1985)
Enterobacter aerogenes	Chorismic acid	Keller and Lingens (1984)
Escherichia intermedia	L-Tyrosine	Para, Lucciardi and Baratti (1985)
Humicola spp.	Rifamycin S	Lee *et al.* (1984)
Lactobacillus casei	Lactic acid	Tuli *et al.* (1985)
Pichia membranaefaciens	L-Malic acid	Rossi and Clementi (1985)

study was produced by *Alcaligenes fecalis*, *Agrobacterium radiobacter* or *Poria cocos*.

COMBINATIONS OF GELS AND OTHER POLYMERS FOR ENTRAPMENT OR ENCAPSULATION OF CELLS

Various combinations of gels with other polymers have been studied in order to improve the quality and performance of immobilized cell biocatalysts (*Table 6*). For example, Kuu (1985) observed that the gel strength of agar or κ-carrageenan could be at least doubled by introducing polyacrylamide within these gels. Growing yeast cells for use in ethanol fermentation could be immobilized in such combination gels. Activation periods of 90 h and 35 h were required before the maximum ethanol fermentation rate could be achieved with cells immobilized in agar–polyacrylamide and in κ-carrageenen–polyacrylamide combination gels, respectively. Mitani, Nishizawa and Nagai (1984a) observed that the gel strength of calcium alginate or κ-carrageenen could be improved by combining this gel with a photo-cross-linkable resin, but the permeability of the combined immobilization matrix was low: addition of Celite improved the permeability. A combination of κ-

Table 6. Combination of gels and polymers for cell immobilization

Gels and other polymers	References
Agar and polyacrylamide	Kuu (1985)
Polyethylenimine, glutaraldehyde and chitosan	Lantero (1985)
Calcium alginate and polyamine-sulphone	Sakata and Imai (1985a,b)
Calcium alginate and photo-cross-linkable polymer	Mitani, Nishizawa and Nagai (1984a,b); Hasegawa, Iida and Sakamoto (1985)
Calcium alginate and polyamino acids	Posillico (1985)
Carrageenan and locust bean gum	Mitani, Nishizawa and Nagai (1984a,b)
Carrageenan and photo-cross-linkable polymer	Mitani, Nishizawa and Nagai (1984a,b); Hasegawa, Iida and Sakamoto (1985)
Carrageenan and polyacrylamide	Kuu (1985)
Carrageenan and poly(4-vinyl-1-methylpyridinium chloride)	Sakata and Imai (1985a)
Co-polymers of hydrophilic and hydrophobic monomers	Kumakura and Kaetsu (1984a,b)
Co-polymer of hydroxyethyl acrylate (or methacrylate) and *N*-vinyl-2-pyrrolidone (highly hydrophilic polymer)	Kumakura and Kaetsu (1984a,b)
Polyacrylamide and poly(vinylbenzyltrimethylammonium chloride)	Sakata and Imai (1985a,b)
Polyacrylamide and 2-acrylamido-2-methyl-propanesulphonic acid	Sakata and Imai (1985b)
Poly(maleic anhydride/styrene)-co-polyethylene (graft co-polymer)	Beddows, Gil and Guthrie (1985)
Poly(maleic anhydride/vinyl acetate)-co-polyethylene (graft co-polymer)	Beddows, Gil and Guthrie (1985)
Xanthan–locust bean gum	Cheetham, Garrett and Clark (1985)
Viscosity agents/gels	Rosevear (1984b)

carrageenan, locust bean gum and Celite imparted the strength and permeability needed for use in ethanol production with forced substrate supply. Sakata and Imai (1985a) prepared hydrogels by combining polyanions with polycations: calcium alginate with polyamine sulphone, or κ-carrageenan with poly(4-vinyl-1-methylpyridinium chloride), for *Serratia marcescens* immobilization, and polyacrylamide with poly(vinylbenzyltrimethylammonium chloride), for yeast immobilization. They observed the formation of colonies of bacteria or yeast in the vicinity of the surfaces and/or in the interior of the hydrogel particles.

Kumakura and Kaetsu (1984a,b) studied the immobilization of *Streptomyces phaeochromogenes* in matrices obtained by radiation co-polymerization at low temperatures of hydrophilic and hydrophobic monomers, such as hydroxyethyl methacrylate, trimethylpropane triacrylate, hydroxybutyl methacrylate, tetraethyleneglycol diacrylate, methyl methacrylate, hydroxyethyl acrylate, methoxytetraethyleneglycol methacrylate, and *N*-vinyl-2-pyrrolidone. They observed that the glucose isomerase (D-xylose isomerase, EC 5.3.1.5) activity of the cells was affected by the monomer concentration, the hydrophilicity and the chemical structure of the polymer matrix. The enzyme activity within the cells immobilized in the co-polymer of hydroxyethyl acrylate (or methacrylate) and *N*-vinyl-2-pyrrolidone monomer increased with increased content of the latter due to increased hydrophilicity of the co-polymer. Graft co-polymers of poly(maleic anhydride/styrene) and polyethylene, and of poly(maleic anhydride/vinyl acetate) and polyethylene, were prepared by Beddows, Gil and Guthrie (1985) for immobilization of enzymes and *Bacillus stearothermophilus*. Cell immobilization at levels of 0·2 mg active cells per gram of styrene-based co-polymer and 0·3 mg active cells per gram of vinyl acetate-based co-polymer were achieved. Rosevear (1984b) claimed beneficial effects from the use of graft polymers of gel precursors such as acrylic, acrylate and bisacrylate monomers with viscosity-enhancing agents such as xanthan gum, sodium alginate, carboxymethylcellulose-sodium salt, cellulose ethers, polyvinyl alcohol, agarose, cold-water-soluble starch, cellulose paste or carrageenan. *Catharanthus roseus* (Vinca) cells were immobilized in a composite obtained from acrylamide, sodium alginate and cloth sheet, to improve alkaloid production by these plant cells (Lambe and Rosevear, 1985). Xanthan–locust bean gum combination as an immobilization matrix for *Erwinia rhapontici* producing isomaltulose was compared with several other immobilization systems by Cheetham, Garrett and Clark (1985). The activity half-life of the cells immobilized in xanthan–locust bean gum was very low (8 h) compared with that of calcium alginate-immobilized cells (8500 h).

Lantero (1985) mixed an aqueous suspension of cells with polyethylenimine and then added to this mixture glutaraldehyde and an aqueous solution of chitosan to obtain a cross-linked immobilized biocatalyst. Manecke and Klussmann (1985a,b) immobilized *Arthrobacter simplex*, *Aspergillus ochraceus*, *Bacillus sphaericus*, *Curvularia lunata*, *Flavobacterium dehydrogenans*, *Mycobacterium* spp. and *Corynebacterium simplex* on a co-polymer of acrolein and 1-vinyl-2-pyrrolidone, cross-linked with an alkylenedioxyamide

(H_2NO-$(CH_2)_n ONH_2$) for application in steroid transformation. Hybridoma cells have been encapsulated in a semi-permeable membrane (Posillico, 1985). The encapsulation was accomplished in two steps: first, the hybridoma cells were captured in beads with calcium alginate and a semi-permeable membrane was layered on to the periphery of the gel beads by inserting them into a polyamino acid solution; the intracapsular alginate subsequently was liquefied by addition of chelating agent. In these capsules hybridoma cells could be otained in high density. Decleire *et al.* (1985) mixed *Kluyveromyces bulgaricus* with hen egg white and treated the composite with glutaraldehyde; the hard gel so obtained was shattered by passage through a syringe and excess glutaraldehyde was washed off with distilled water. These immobilized cells were then used for the hydrolysis of whey lactose.

COMPOSITE IMMOBILIZATION MATRICES

These systems include combinations of gels, polymers, cross-linking agents and solid particles, and are employed to improve the mechanical properties or diffusion characteristics of the biocatalyst (*Table 7*). Chibata, Tosa and Takamatsu (1985) treated *Escherichia coli*, which had aspartase activity, and *Pseudomonas dacunhae*, which had L-aspartate 4-decarboxylase (EC 4.1.1.12) activity, with glutaraldehyde before the cells were immobilized in carrageenan gel; this treatment stabilized the enzyme activity. Bettmann and Rehm (1984, 1985) immobilized *Pseudomonas putida* in calcium alginate and

Table 7. Composite immobilization matrices

Matrices	References
Bone char and cross-linking with tannic acid and glutaraldehyde	Cheetham, Garrett and Clark (1985)
Calcium alginate and polyacrylamide hydrazide cross-linked with glyoxal	Bettmann and Rehm (1984, 1985)
Calcium alginate and gelatin hardened with glutaraldehyde cross-linking, or hexamethylenediamine and glutaraldehyde, or polyethylenimine and glutaraldehyde	Bajpai and Margaritis (1985a,b)
Carrageenan and glutaraldehyde	Chibata, Tosa and Takamatsu (1985)
Carrageenan, photo-cross-linkable polymer and Celite	Mitani, Nishizawa and Nagai (1984a,b)
Carrageenan, photo-cross-linkable polymer, locust bean gum and Celite	Mitani, Nishizawa and Nagai (1984a,b)
Egg white and glutaraldehyde	Decleire *et al.* (1985)
Vermiculite and polysaccharide	Swann (1985)
Vermiculite, polyethylenimine and glutaraldehyde	Swann (1985)
Vermiculite and alginate	Swann (1985)
Vermiculite and polyacrylamide	Swann (1985)
Vermiculite and polyurethane	Swann (1985)

polyacrylamide hydrazide cross-linked with glyoxal, and observed protection of the organism from phenol. The stability of the inulinase (2,1-β-D-fructan fructanohydrolase, EC 3.2.1.7) of immobilized *Kluyveromyces marxianus* was improved by treatment with hardening agents, such as glutaraldehyde, hexamethylenediamine plus glutaraldehyde and polyethyleneimine plus glutaraldehyde (*see* pages 340, 342) (Bajpai and Margaritis, 1985a,b). Mitani, Nishizawa and Nagai (1984a,b) observed that a mixture of photo-cross-linkable resin, κ-carrageenan, locust bean gum and Celite together had the best mechanical and diffusion characteristics compared with those of any other combination of these components in the matrix. Decleire *et al.* (1985) immobilized *Saccharomyces cerevisiae* cells by mixing with hen egg white and then treating the mixture with glutaraldehyde, as described previously (page 334). Cheetham, Garret and Clark (1985) immobilized *Erwinia rhapontici* cells on bone char by suspending it and the cells in water and adding tannic acid and glutaraldehyde to the mixture. In studies by Swann (1985), *Escherichia coli* cells were absorbed on vermiculite particles, and glutaraldehyde and polyethyleneimine were then added to the mixture to immobilize the cells through covalent linkages.

HOLLOW STRUCTURES, FIBRES AND PLATES (MEMBRANE REACTORS) FOR PHYSICAL RETENTION OF CELLS

Membrane reactors combine three desirable characteristics of a bioreactor: retention of the cells; separate introduction of various components of the substrate to the biocatalyst; and separation and recovery of the desired products. Matson (1985) has presented a general analysis of the trends and opportunities in membrane reactors. In addition to cost reduction in existing bioprocesses, he foresaw potential applications in production of agricultural chemicals, flavours and fragrances, speciality organic chemicals and pharmaceuticals. As described on page 333, Beddows, Gil and Guthrie (1985) studied immobilization of enzymes and of *Bacillus stearothermophilus* on to poly(maleic anhydride/styrene)-co-polyethylene and poly(maleic anhydride/vinyl acetate)-co-polyethylene polymers: both of the graft co-polymers immobilized *B. stearothermophilus* cells to the extent of 0·2–0·3 mg/g. Inloes *et al.* (1985) immobilized yeast cells for ethanol fermentation in polysulphone ultrafiltration hollow fibres of 440 μm i.d. and 860 μm o.d. and a nominal molecular weight cut-off of 10 000. They used a nitrogen-deficient medium to control cell growth in these reactors. In vinegar-production studies, Nanba, Kimura and Nagai (1985) selected polypropylene hollow fibre as a hydrophobic membrane and fixed *Acetobacter rancens* cells on the surface of these devices. In these studies, liquid medium was supplied from the outside, and oxygen was supplied from the inside of the fibres in order to provide a larger interfacial area between the liquid and the gas than that available in conventional fermenters. Tobacco cell culture has been immobilized in a flat plane reactor for the production of phenolics (Shuler, Pyne and Hallsby, 1984): in this system, nutrient medium could flow both over and under the cell layer, and the effluent could be recycled with continuous addition of

fresh medium. Brouers and Hall (1985) reported that the photosynthetic stability of an alginate-immobilized thermophilic cyanobacterium, *Phormidium laminosum*, could be enhanced by a drying pretreatment.

Cells, reactions and products

A variety of functional and product-formation applications of immobilized-cell biocatalysis have been investigated. An alphabetical listing of such studies reported in the recent literature is given in *Table 8*. Selected products or groups of products, and functional applications are summarized in the following sections.

ETHANOL PRODUCTION

Interest in production of ethanol from renewable raw materials has prompted intensive research during the last decade. A continuous fermentation process for ethanol production with immobilized growing cells would be advantageous because of low operating and maintenance costs, high productivity and high ethanol yield (with resultant low capital investment), and it would be applicable to a variety of sugar substrates (Nagashima *et al.*, 1984). Many immobilized-cell fermentation systems for ethanol production have been described recently (*Table 9*). Ethanol concentrations produced range from 2·24% in rotating-disc, sponge-immobilized yeast (Del Borghi *et al.*, 1985) to as high as 12·6% for *Zymomonas mobilis* immobilized on calcium alginate (Bajpai and Margaritis, 1985c). Very high ethanol yields, 97% and 98%, were achieved by Black *et al.* (1984), McGhee, Carr and St Julian (1984), Grote and Rogers (1985) and Jain, Toran-Diaz and Baratti (1985a,b). Mohite and SivaRaman achieved a very high fermenter productivity, 168 g ethanol/l/h, but the ethanol concentration was low, 2·4%. Another high fermenter productivity, 55 g/l/h, with an ethanol concentration of 6·8% was reported by Grote and Rogers (1985). Nagashima *et al.* (1984) achieved 25 g/l/h productivity and 8·5% ethanol concentration with *Saccharomyces cerevisiae* cells immobilized on calcium alginate in a column fermenter of 4 m^3 total volume. In this study, diluted cane molasses was used as the substrate. Consistent high-level bioreactor performance was observed for 113 days by Black *et al.* (1984), for 105 days by Karkare, Dean and Venkatasubramanian (1985), and for over 90 days by Nakajima *et al.* (1985). Direct alcoholic fermentation of starch or dextrin with *Saccharomyces diastaticus* was studied by Amin *et al.* (1985), and 7·7% ethanol concentration at 75% yield and a fermenter productivity of 7·6 g/l/h were achieved. Most of these investigators considered that immobilized cell bioreactors were an improvement compared with conventional batch ethanol fermenters.

The use of starch, cellulose, cellobiose and inulin as raw materials for hydrolysis to fermentable sugars to produce alcohol has also been considered. Production of the starch-cleaving enzymes glucoamylase (glucan 1,4-α-glucosidase, EC 3.2.1.3) and α-amylase (EC 3.2.1.1) by *Aspergillus niger* mycelia or spores immobilized in calcium alginate beads has been investigated by Li,

Table 8. Products from or reactions with immobilized cell biocatalysts

Product or reaction	Cells	Support or matrix	References
Acetone	*Clostridium acetobutylicum*	Calcium alginate	Largier *et al.* (1985)
Aflatoxicol	Rat erythrocytes	Calcium alginate	Chang, Lin and Hsiung (1985)
L-Alanine	*Pseudomonas dacunhae*	Polyurethane	Fusee and Weber (1984)
Alkaloid	*Claviceps purpurea*	Calcium alginate	Kopp and Rehm (1984)
Ammonium and orthophosphate removal	*Scenedesmus quadricauda*	κ-Carrageenan	Chevalier and de la Noue (1985)
Aspartic acid	*Escherichia coli*	Polyazetidine	Wood and Calton (1984a,b)
Beer	*Saccharomyces uvarum*	Calcium alginate	Onaka *et al.* (1985)
Butane 2,3-diol	*Aeromonas hydrophila*	Titanium (IV) hydroxide	Willetts (1985)
Butanol	*Clostridium acetobutylicum*	Calcium alginate	Largier *et al.* (1985)
	Clostridium beyerinckii	Calcium alginate	Schoutens, Nieuwenhuizen and Kossen (1985)
Capsaicin	*Capsicum frutescens*	Reticulate polyurethane	Lindsey and Yeoman (1984a,b)
Cellobiose hydrolysis	*Pichia etchellsii*	Calcium alginate, cellulose acetate membrane, aluminium hydroxide, or bentonite granules	Jain and Ghose (1984)
Cellulose hydrolysis	*Trichoderma reesei*	Plastic fibrous substances + polyhydroxethyl methacrylate	Kumakura, Tamada and Kaetsu (1984)
Chorismic acid	*Enterobacter aerogenes*	Polyacrylamide	Keller and Lingens (1984)
Citric acid	*Aspergillus niger*	Calcium alginate	Eikmeier and Rehm (1984)
	Aspergillus niger	Polyacrylamide	Horitsu *et al.* (1985)
Dehydroabietic acid (biotransformation)	*Mortierella isabellina*	Calcium alginate	Kutney *et al.* (1985)
Dihydroxyacetone	*Gluconobacter oxydans*	Calcium alginate	Adlercreutz, Holst and Mattiasson (1985); Holst, Lundback and Mattiasson (1985)
L-Dopa	*Erwinia herbicola*	Carrageenan	Para, Rifai and Baratti (1984)
Epoxides	Alkene-utilizing bacteria	Calcium alginate or κ-carrageenan	Habets-Crutzen *et al.* (1984)
Ethanol	*Clostridium acetobutylicum*	Calcium alginate	Largier *et al.* (1985)
	Saccharomyces cerevisiae or Saccharomyces uvarum	Knitted SS mesh or reticulated polyester foam	Black *et al.* (1984)

(*contd*)

Table 8 (*contd*)

Product or reaction	Cells	Support or matrix	References
Ethanol (*contd*)	*Saccharomyces cerevisiae*	κ-Carrageenan and silica	Karkare, Dean and Venkatasubramanian (1985)
	Saccharomyces cerevisiae	Calcium alginate	McGhee, Carr and St Julian (1984)
	Saccharomyces cerevisiae	Synthetic commercial sponge	Del Borghi *et al.* (1985)
	Saccharomyces cerevisiae var. *elipsoidus*	Cellulose ('Solka Floc')	Okita, Bonham and Gainer (1985)
	Saccharomyces cerevisiae or *Kluyveromyces marxianus* or *Kluyveromyces fragilis*	Cotton cloth	Joshi and Yamazaki (1984)
	Saccharomyces formosensis	Radiation polymers of the mixtures of methoxypolyethylene glycol methacrylate and 2-hydroxy-ethyl-acrylate	Fujimara and Kaetsu (1985)
		Photo-cross-linkable resin	Yamada *et al.* (1985)
	Saccharomyces uvarum	Open-pore gelatin	Mohite and SivaRaman (1984)
	Zymomonas mobilis	Calcium alginate	Bajpai and Margaritis (1985c)
	Zymomonas mobilis	κ-Carrageenan	Jain, Toran-Diaz and Baratti (1985a,b)
	Zymomonas mobilis	Calcium alginate	Grote and Rogers (1985)
	Saccharomyces diastaticus	Polyurethane foam	Amin *et al.* (1985)
Ferrous ion oxidation	*Thiobacillus ferrooxidans*	Calcium alginate	Lancy and Tuovinch (1984)
Glucoamylase and α-amylase	*Aspergillus niger*	Calcium alginate	Li, Linko and Linko (1984)
Gluconic acid	*Aspergillus niger*	Mycelium + ovalbumin and catalase + catalase crosslinked with glutaraldehyde	Doppner and Hartmeier (1984)
	Gluconobacter oxydans	Fibrous nylon	Seiskari, Linko and Linko (1985)
Glycollate	*Chlorella emersonii*	Calcium alginate	Day and Codd (1985)
Hydrocarbons	*Botryococcus braunii*	Calcium alginate	Bailliez, Largeau and Casadevall (1985)
Hydrogen	*Rhodopseudo-monas capsulata*	Agar or carrageenan	Francou and Vignais (1984)
	Rhodospirillum rubrum	Agar	Von Felten, Zurrer and Bachofen (1985)

(*contd*)

Table 8 (*contd*)

Product or reaction	Cells	Support or matrix	References
	Chlorogloea fritschii, *Nostoc muscorum* and *Mastigocladus laminosus*	Polyurethane foam	Muallem, Bruce and Hall (1983)
Inulin hydrolysis	*Kluyveromyces marxianus*	Calcium alginate and gelatin hardened by glutaraldehyde, or hexamethylenediamine + glutaraldehyde, or polyethylenimine + glutaraldehyde	Bajpai and Margaritis (1985a,b)
Lactic acid	*Lactobacillus casei*	Agar or polyacrylamide	Tuli *et al.* (1985)
Limonoid debittering	*Corynebacterium fascians*	Polyacrylamide	Hasegawa *et al.* (1985)
Malolactic fermentation	*Leuconostoc oenos* and *Lactobacillus brevis*	κ-Carrageenan	McCord and Ryu (1985)
Mead	*Saccharomyces cerevisiae*	Calcium alginate	Qureshi and Tamhane (1985)
Methane	Methanogenic bacteria	Baked clay or melted slag	Nishio, Kayawake and Nagai (1985)
Milk acidification	*Streptococcus lactis*	Calcium alginate	Kim, Naveh and Olson (1985)
NADPH	*Chlorogloea fritschii*, *Nostoc muscorum* and *Mastigocladus laminosus*	Polyurethane foam	Muallem, Bruce and Hall (1983)
Pesticide removal	*Rhodopseudomonas sphaeroides* and *Alcaligenes eutrophus*	Magnetite	Mac Rea (1985)
Phenol degradation	*Pseudomonas putida*	Polyacrylamide-hydrazide	Bettman and Rehm (1985)
Phenolic compounds	*Daucus carota* and *Petunia hybrida*	Calcium alginate	Hamilton, Pedersen and Chin (1984)
Photosynthetic stability	*Phormidium laminosum*	Calcium alginate	Brouers and Hall (1985)
Pigments	*Lavandula vera*	Agar, calcium alginate or κ-carrageenan	Nakajima *et al.* (1985)
Polysaccharides	*Porphyridium cruentum*	Polyurethane foam	Thepenier, Gudin and Thomas (1985)
Prednisolone	*Corynebacterium simplex*	Poly[acrolein-co-(vinyl-2-pyrrolidone)]	Manecke and Klussmann (1985a,b)
	Curvularia lunata and *Arthrobacter simplex*	Photo-cross-linkable polymers	Mazumder *et al.* (1985)
Progesterone (11 α-hydroxylation)	*Aspergillus ochraceus* spores	Calcium alginate, epoxy resin, collagen, or polyacrylamide	Bihari *et al.* (1984)
Rifamycin S	*Humicola* spp.	Polyacrylamide	Lee *et al.* (1984)
Soy sauce	*Pediococcus halophilus*, *Saccharomyces rouxii* and *Torulopsis versatilis*	Calcium alginate	Osaki *et al.* (1985)

(*contd*)

Table 8 (*contd*)

Product or reaction	Cells	Support or matrix	References
Steroid transformation	*Arthrobacter simplex*	Calcium alginate	Kloosterman and Lilly (1985)
L-Tyrosine	*Escherichia intermedia*	Polyacrylamide	Para, Lucciardi and Baratti (1985)
Ursodeoxycholic acid	*Clostridium absonum*	Polyacrylamide	Kole and Altosaar (1985)
Vinegar	*Acetobacter*	Polypropylene fibres	Okuhara (1985)
	Acetobacter aceti	κ-Carrageenan	Osuga, Mori and Kato (1984)
	Acetobacter rancens	Hollow fibre	Nanba, Kimura and Nagai (1985)
Weed control	Fungi	Calcium alginate	Connick, Walker and Quimby (1985)
Whey hydrolysis	*Kluyveromyces bulgaricus*	Calcium alginate or egg white	Decleire *et al.* (1985)

Linko and Linko (1984). Immobilized mycelia generated lower enzyme activities than did immobilized spores germinated in a growth medium and subsequently cultured in an enzyme-production medium. In repeated batch experiments, free cells could be used for only four 4-day runs, whereas immobilized spores were active for at least 11 4-day runs, and gradual increases in enzyme activity in each successive batch were possible. Immobilization altered the ratio of glucoamylase to α-amylase activity.

Production of cellulase (1,4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase, EC 3.2.1.4) and hydrolysis of cellulose by immobilized *Trichoderma reesei* has been investigated by Kumakura, Tamada and Kaetsu (1984). They observed that the enzyme activity was dependent on the irradiation dosages employed during immobilization. Cellulase activity is subject to end-product inhibition by cellobiose. The rate of cellulose digestion was increased on hydrolysis of cellobiose to dextrose by the β-glucosidase (EC 3.2.1.21) activity of *Pichia etchellsii* cells immobilized in various matrices (Jain and Ghose, 1984). Bentonite granules, aluminium hydroxide gel, cellulose acetate membrane and calcium alginate beads were tested as the cell immobilization matrices: immobilization by entrapment in calcium alginate gel was found to be the most simple and efficient method. A retention of 96·5% of the initial activity after 10 sequential batch uses of the immobilized preparation was observed. Beads stored at 4°C for 6 months retained 80% of their activity. A half-life of over 7 days was observed in continuous operation in a packed-bed reactor at 45°C. The half-life increased to 17 days at 40°C and fell to 2 days at 50°C.

Inulinase production and the hydrolysis of inulin to fructose by immobilized *Kluyveromyces marxianus* cells has been investigated by Bajpai and Margaritis (1985a,b). The activity and stability of inulinase could be improved by treating the immobilized cells with polycations and cross-linking agents. For instance, the inulinase stability of immobilized cells hardened with

Table 9. Ethanol production

Cells	Immobilization matrix	Ethanol (%)	Yield (%)	Productivity (g/l/h)	Stability (days)	References
S. diastaticus	Polyurethane	7·7	75	7·6	—	Amin *et al.* (1985)
S. cerevisiae or *S. uvarum*	Knitted SS mesh or reticulated polyester foam	8	98	11·2	113	Black *et al.* (1984)
S. cerevisiae	Calcium alginate	5·7**	97	—	11	McGhee, Carr and St Julian (1984)
S. cerevisiae	Calcium alginate	8·5	95	25	>90	Nagashima *et al.* (1984)
S. cerevisiae	Sponge	2·24	—	7·1	—	Del Borghi *et al.* (1985)
S. cerevisiae; K. marxianus; K. fragilis	Cotton cloth	4·6	90	11	—	Joshi and Yamazaki (1984)
S. cerevisiae	Carrageenan and silica	4·2*	—	—	105	Karkare, Dean and Venkatasubramanian (1985)
S. cerevisiae var. *elipsoidus*	Cellulose ('Solka Floc')	—	—	2·1	—	Okita, Bonham and Gainer (1985)
S. diastaticus	Polyurethane	8·06	78·8	7·6	18	Amin *et al.* (1985)
S. formosensis	Radiation polymers of the mixtures of methoxypolyethylene glycolmethacrylate and 2-hydroxy-ethyl-acrylate	—	40*	—		Fujimara and Kaetsu (1985)
S. formosensis	Photo-cross-linkable resin	9·3	91*	9·3	—	Yamada *et al.* (1985)
S. uvarum	Gelatin	2·4	—	168	—	Mohite and SivaRaman (1984)
S. cerevisiae	Cellulose	—	—	2·1	—	Okita, Bonham and Gainer (1985)
Z. mobilis	Calcium alginate	12·6	95	—	—	Bajpai and Margaritis (1985c)
Z. mobilis	Calcium alginate	6·8	98	55	—	Grote and Rogers (1985)
Z. mobilis + *Schw. castelli*	Carrageenan	4	97	28·4	55	Jain, Toran-Diaz and Baratti (1985a,b)

* Estimated from author's data
** w/w Estimated from author's data.

glutaraldehyde increased twofold, while treatment with hexamethylenediamine plus glutaraldehyde, or polyethylenimine plus glutaraldehyde increased stability sixfold compared with that of unhardened cells. The hardening treatment resulted in only little change in reactor productivity. In a packed-bed column reactor, the maximum volumetric reactor productivity was achieved with the unhardened immobilized cells (103 g/l/h). This was obtained at a dilution rate of 3 h^{-1} and 60% substrate conversion. With immobilized cells hardened with glutaraldehyde, hexamethylenediamine plus glutaraldehyde, or polyethylenimine plus glutaraldehyde, maximum productivities were 99, 72 and 89 g/l/h, respectively. These were achieved at a dilution rate of 3 h^{-1} and with 57%, 41% and 48% substrate conversion, respectively. Cell loss through leaching decreased by a factor of 1·5 with glutaraldehyde-hardened cells, and five- and sixfold, respectively, with cells hardened with hexamethylenediamine plus glutaraldehyde and polyethylenimine plus glutaraldehyde.

PRODUCTION OF SOLVENTS

The production of acetone, butanol and ethanol by immobilized, sporulation-deficient *(spo) Clostridium acetobutylicum* P262 mutants was investigated by Largier *et al.* (1985). In a continuous-bed immobilized-cell reactor the concentration of total solvents was 15·4 g/l at a productivity of about 3 g/l/h, and the concentration of butanol was 8·37 g/l at a productivity of about 1·6 g/l/h. Calcium alginate was used as the immobilization matrix. Continuous production of butanol from whey by *Clostridium beyerinckii* LMD 27.6 immobilized on calcium alginate was studied by Schoutens, Nieuwenhuizen and Kossen (1985). With the immobilized cells in the continuous reactor the productivity was 0·5–1 g (butanol + isopropanol)/l/h (16 times higher than that found in the batch reactor with free cells). Butane 2,3-diol production from soluble starch by *Aeromonas hydrophila* immobilized on titanium (IV) hydroxide has been investigated by Willetts (1985). A maximum level of 0·49 g/l diol was obtained. This level of production was lower than the maximum of 1·83 g diol/l that could be obtained in free cell suspensions under equivalent conditions.

PRODUCTION OF AMINO ACIDS

Aspartic acid

Ammonium fumarate was converted to aspartic acid by immobilized *Escherichia coli* cells with high aspartase activity (Wood and Calton, 1984a,b). The cells were immobilized by covalent attachment and entrapment with polyazetidine. In a continuous bioreactor an average aspartate concentration of 19% was achieved. The fumarate conversion ranged from 63% to 96%.

L-Alanine

L-Aspartic acid was converted to L-alanine by whole cells of *Pseudomonas dacunhae* immobilized in polyurethane and containing L-aspartate 4-decarboxylase activity (Fusee and Weber, 1984). As much as 100% retention of enzyme activity was observed when cell lysis was allowed to occur before immobilization. An L-alanine concentration of about 1·6 mol/l could be achieved within 3–4 hours in a batch reactor. Under conditions of semicontinuous use of a batch reactor, a 2·5% loss in L-aspartate 4-decarboxylase activity of the immobilized cells was observed over a 31-day period.

L-Tyrosine

This amino acid was produced from phenol, pyruvate and ammonia by *Escherichia intermedia* cells immobilized by entrapment in polyacrylamide gel (Para, Lucciardi and Baratti, 1985). Synthesis of L-tyrosine up to 10 g/l was demonstrated. High conversion yield (95–100%) and a maximal productivity of 2 g/l/h were achieved in batch reactors.

PRODUCTION OF ORGANIC ACIDS

Several teams have investigated the possibilities of production of organic acids by immobilized cells.

Acetic acid (see 'Vinegar', p. 349)

Chorismic acid

A mutant of *Enterobacter aerogenes* immobilized in polyacrylamide gel can accumulate chorismic acid, one of the key intermediates in the synthesis of aromatic amino acids, in amounts comparable to those obtained from free cells (Keller and Lingens, 1984). Within 15–16 h of incubation the concentration of chorismic acid reached 330 mg in 2 l of medium. After the initial cycle the amount of the excreted chorismate was reduced by 50%. Addition of chorismate precursors or cell membrane permeabilizing agents did not increase the level of activity of the immobilized cells.

Citric acid

Eikmeier and Rehm (1984) immobilized spores of *Aspergillus niger* by entrapment in calcium alginate beads, and precultivated this system in growth media with various amounts of nitrogen to obtain immobilized mycelia. During a 24-day period in an air-lift fermenter, the citric acid concentration reached 42·7 g/l and the yield was 50%. The productivity of the immobilized mycelia was 1·5 times higher than that of free pellets. Horitsu *et al.* (1985) entrapped living *Aspergillus niger* cells in polyacrylamide gels and followed

the production of citric acid from sucrose in both replacement batch and continuous column bioreactors. With the former bioreactor, an increase of citric acid was observed under conditions of higher aeration and of wider surface of immobilized cells. With the continuous bioreactor, the maximum citric acid yield was 39·3 mg/h for 40 g gels. The biocatalyst half-life was 105 days. Thin slices of the immobilized cell gel had 1·4 times higher productivity than the corresponding cube shape, a reflection of a diffusion problem.

Gluconic acid

Sometimes it is essential to increase the permeability of the cell membrane of the micro-organism to permit entry of the substrate and/or escape of the desired metabolite(s). This can often be done by treatment with solvents or gentle heat. A new strain of *Aspergillus niger* containing high levels of intracellular glucose oxidase and catalase activity was permeabilized in this way by treating with iso-propanol at 5°C, drying under vacuum at 30°C, suspending in an ovalbumin/catalase solution and cross-linking with glutaraldehyde (Doppner and Hartmeier, 1984). This co-immobilizate was tested for glucose oxidation in stirred-tank reactors where oxygen was supplied by controlled addition of hydrogen peroxide. Additional bound catalase provided a cover for the glucose oxidase–catalase system in the mycelium against the added hydrogen peroxide, at the same time producing oxygen from the hydrogen peroxide. Thus, oxygen became available in high concentrations and in direct proximity to the mycelium. In a stirred reactor, up to 90% glucose could be converted into gluconic acid in less than 2 h at 25°C. A 20% loss of glucose oxidase activity and a 30% loss of catalase activity was observed at this temperature. This loss of activity, however, was lower than that observed in non-immobilized mycelia under the same conditions.

Seiskari, Linko and Linko (1985) immobilized living *Gluconobacter oxydans* cells by attachment to nylon fibres. In an aerated tubular immobilized-cell bioreactor these cells produced free gluconic acid continuously for at least 6 months, with a volumetric productivity of at least 5 g/l/h at 100 g/l glucose substrate and a yield of 80 g/l product gluconic acid concentration.

Itaconic acid (methylene succinic acid)

Aspergillus terreus spores were entrapped in calcium alginate gel beads or, alternatively, the fungal mycelium was immobilized either on Celite R-626 or in agar gel cubes, and the biocatalyst was employed both in repeated batch and in continuous column reactors to produce itaconic acid from D-xylose or D-glucose (Kautola *et al.*, 1985). The highest itaconic acid yield obtained in a submerged culture batch fermentation was 54·5% based on total initial glucose (55 g/l) with a volumetric productivity of 0·32 g/l/h, and was 44·8% from xylose (67 g/l) with a productivity of 0·20 g/l/h. In a repeated batch fermentation, mycelium immobilized in agar gel had a productivity of 0·12 g/l/h, and mycelium grown from spores immobilized in calcium alginate gel 0·06 g/l/h, both from xylose (60 g/l). With the best immobilized biocatalyst

system, employing Celite R-626 as a carrier, volumetric productivities of 1·2 g/l/h from glucose and of 0·56 g/l/h from xylose (both at 60 g/l) were obtained in continuous column operation for more than 2 weeks.

Lactic acid

Agar and polyacrylamide matrices have been assessed for the immobilization of *Lactobacillus casei* cells for production of lactic acid from whey permeate (Tuli *et al.*, 1985). The performance of agar-immobilized cells was better than that of polyacrylamide-immobilized cells. In three successive runs with the agar-immobilized cells, the lactic acid concentrations were 3·3% 2·6% and 1·4% and the corresponding productivities were 0·70, 0·54 and 0·29 g/l/h. In contrast, with the polyacrylamide-immobilized cells, the highest lactic acid concentration was 2·6% and the highest productivity was 0·54 g/l/h.

L-*Malic acid*

The conversion of fumaric acid to L-malic acid by the fumarase (fumarate hydratase, EC 4.2.1.2) activity of *Pichia membranaefaciens* cells immobilized in polyacrylamide gel was studied by Rossi and Clementi (1985). Cetylpyridinium chloride was used in the reaction mixture in order to increase cell permeability. Synthesis of L-malic acid at a rate of about 0·15 mmol/h/g of immobilized cells was achieved. L-Malic acid production was directly proportional to the immobilized cell concentration from 4% up to 8%. When higher amounts of entrapped cells were used, a non-linear increase in the fumarase activity was observed. At about 20% immobilized cell concentration the fumarase activity reached a plateau. The immobilized cells could be stored at 5°C for repeated use. After storage an increase in the fumarase activity was observed, perhaps due to the modification of the gel under storage conditions resulting in higher diffusion rates.

ALKALOIDS, STEROIDS, ANTIBIOTICS AND L-DOPA

Kopp and Rehm (1984) investigated the production of chanoclavine, agroclavine, elymoclavine, ergometrine and total alkaloid production by immobilized mycelia of *Claviceps purpurea*. In semicontinuous operation, the beads were separated from the production medium every 14 days, washed with 0·85% NaCl and cultured in 100 ml fresh medium. Alkaloid production of 16 consecutive incubations was assessed: that from free cells declined after 60 days, whereas immobilized cells retained their activity for 200 days. The cumulative alkaloid production for all fermentation cycles using mycelia immobilized with 8% calcium alginate was 25 times higher than that from free cells. The cells immobilized in 2% and 4% calcium alginate produced elymoclavine as the main product, whereas higher gel concentrations caused a shift in alkaloid biosynthesis towards high clavine alkaloid production. The best yields of the ergopeptide, ergometrine, were reached with mycelia immobilized in 4% gels.

The bioconversion of chenodeoxycholic acid to ursodeoxycholic acid by immobilized *Clostridium absonum* cells was studied by Kole and Altosaar (1985). Polyacrylamide gel was used as the immobilization matrix. If the bacteria were grown in the presence of chenodeoxycholic acid before immobilization, conversion to ursodeoxycholic acid took place in 2 days. If the cells were grown in the absence of chenodeoxycholic acid before immobilization, conversion to the 3-α-7-keto derivative took place. The activity of the immobilized cells was stable up to 10 days.

Transformation of cortisol to prednisolone by *Arthrobacter simplex* immobilized by adhesion on glass has been tested under no-growth conditions in the absence of nutrients (Mozes and Rouxhet, 1984). In a closed-circuit reactor, the transformation level ranged between 61% and 89% in ten runs, and over 80% in eight of these runs. The specific activity of the immobilized cells was not significantly different from that of the free cells. Manecke and Klussmann (1985a,b) observed that in the production of prednisolone from cortisol the activity was dependent on the hydrophobicity of the immobilization matrix, and could be increased about tenfold by enzyme induction and incubation in peptone-containing media. They could obtain 0·57 g prednisolone per gram of biocatalyst per day. Two-step bioconversion of cortexolone to prednisolone was successfully performed by the combined use of immobilized mycelia of *Curvularia lunata* and immobilized cells of *Arthrobacter simplex* (Mazumder *et al.*, 1985). Kloosterman and Lilly (1985) observed that the Δ^1-dehydrogenation of a hydrocortisone suspension by *Arthrobacter simplex* immobilized in calcium alginate was diffusion limited.

Hydroxylation in the 11-α postition of progesterone was achieved employing *Aspergillus ochraceus* spores immobilized in alginate, epoxy resin, collagen or polyacrylamide beads (Bihari *et al.*, 1984). Spores entrapped in polyacrylamide gel were found to be the most active and most stable.

The biotransformation of rifamycin B to rifamycin S by acetone-dried whole cells of a *Humicola* sp. immobilized in polyacrylamide gel was studied by Lee *et al.* (1984). In a fluidized-bed reactor a linear relationship between loading of the immobilized cells and conversion was exhibited in both batch and continuous operations. In batch operations, the conversion of rifamycin B to rifamycin S increased with increased rate of aeration. In continuous operations, steady-state conversion reached a maximum with an aeration rate between 5·8 and 12·5 vvm; beyond this range the steady-state conversion decreased because of cell leakage. The half-life for the operational stability at 40°C was about 13 h.

Immobilized cells of *Erwinia herbicola* were used for the production of L-dopa from pyrocatechol and DL-serine (Para, Rifai and Baratti, 1984). The optimum conditions for L-dopa synthesis were 37°C, 130 mM ammonium acetate, 100 mM DL-serine and 64 mM pyrocatechol. In reactor studies, the rate of L-dopa formation was proportional to the gel concentration up to 40%. The initial specific productivity of L-dopa was 0·02 g/g cells per hour, and a maximum concentration of 1·6 g/l was obtained in 5 h (productivity 0·3 g/l/h). Increase in the pyrocatechol and DL-serine concentrations raised the initial productivity and final L-dopa concentration. The volumetric productivities in the substrate concentration studied ranged between 0·33 and

0·48 g/l/h. However, the conversion yields were low (16–18%) probably due to the inactivation of the biocatalyst by pyrocatechol. An increase in temperature from 15°C to 37°C had a significant effect on L-dopa production. The initial productivity increased from 0·2 to 1·2 g/l/h and the final L-dopa concentration changed from 1 to 2·3 g/l. The best results were observed at 37°C with a volumetric productivity of 0·46 g/l/h and conversion yield of 18%. In a packed-bed reactor containing 20 g of gel, at 0·1 h^{-1} space velocity and 5 mM pyrocatechol and DL-serine concentrations in the feed, the L-dopa concentration in the effluent increased slowly to 0·13 g/l within 50 h. Conversion yield was 14%, and the productivity was 0·013 g/l/h. After this peak, the activity declined gradually and became zero 150 h after the start.

FOOD AND RELATED PRODUCTS

Production of foods and beverages, food ingredients and bioconversion of food and agricultural by-products by immobilized cell biocatalysis, has been an active area of research.

Beer

Development of an acceptable flavour is a very significant parameter in the production of foods and beverages. The process improvements introduced by new techniques should not have an adverse effect on the flavour of such products. Diacetyl is a flavour ingredient in beer and its concentration has to be maintained at an acceptable level by proper fermentation management. Onaka *et al.* (1985) studied the rapid production of beer by *Saccharomyces uvarum* immobilized in calcium alginate. They observed that when the wort contained 8·5 mg/l of dissolved oxygen, 2·25 mg/l of total diacetyl was formed after a short period of fermentation. However, when the wort contained 0·04 mg/l of dissolved oxygen, only 0·05 mg/l of total diacetyl was formed. On the basis of these results, Onaka *et al.* (1985) developed an immobilized yeast reactor for continuous rapid production of beer with a low, acceptable level of diacetyl precursors.

Wine

Malolactic fermentation is a secondary process that occurs in production of some wines, and involves mainly the conversion of L-malate to L-lactate and carbon dioxide. Proper control of the malolactic fermentation is necessary to achieve development of the appropriate wine flavour. McCord and Ryu (1985) studied malolactic fermentation with *Leuconostoc oenos* and *Lactobacillus brevis* immobilized in κ-carrageenan. They observed lower rates of malate conversion with the immobilized cells than with the free cells.

Mead

Mead is a drink produced by fermentation of a honey and malt mash. Qureshi and Tamhane (1985) investigated the continuous production of mead

by continuous fermentation of honey mash with *Saccharomyces cerevisiae* of high tolerance to alcohol and sugar immobilized in calcium alginate. The ethanol production rate in the immobilized cell reactor was 4·09 g/h compared with 0·09 g/h in the free cell reactor. Immobilized cells produced mead for over 3 months.

Limonoid debittering

In citrus juices, limonoids cause an undesirable bitter taste and should therefore be removed entirely or reduced in concentration. *Corynebacterium fascians* cells capable of metabolizing limonoids were immobilized in polyacrylamide gel and employed for debittering citrus juice (Hasegawa *et al.*, 1985). The process significantly reduced the limonin and nomilin contents of citrus juices, being particularly effective in the reduction of nomilin. This debittering process did not have any adverse effect on other citrus constituents such as citric, malic and ascorbic acids, or fructose, glucose and sucrose.

Capsaicin

This principle of chilli pepper fruits can be produced as a pungent secondary metabolic product of *Capsicum frutescens* Mill. cv. annuum cells immobilized in reticulate polyurethane foam (Lindsey and Yeoman, 1984a,b). The immobilized cells retained a high level of viability after a 23-day period of entrapment, as determined by respiratory activity, esterase activity, nutrient uptake and secondary metabolic activity (capsaicin production). They were capable of producing capsaicin for at least 12 consecutive batches, although the rate of production decreased after seven or eight batches. In a continuous column reactor, the concentration of capsaicin produced fluctuated between 2·20 and 8·80 mg/g dry weight/l.

Milk acidification

In the manufacture of cheddar cheese, it is desirable to acidify milk to pH 6·2–6·4 before coagulation to reduce calcium retention in the curd. Kim, Naveh and Olson (1985) developed a plug flow and a stirred tank basket reactor for the continuous acidification of milk by *Streptococcus lactis* cells immobilized in calcium alginate.

Whey hydrolysis

Lactose must be hydrolysed in the gut to glucose and galactose, before it can be absorbed. However, many people are unable to secrete lactase (β-galactosidase, EC 3.2.1.23) in their alimentary tract and, also, glucose and galactose are sweeter than lactose, so the hydrolysis of lactose in milk or whey is often desirable (Moulin and Galzy, 1984). Decleire *et al.* (1985) achieved 80% conversion of lactose in whey with cells of a lactose producer, *Kluyveromyces bulgaricus*, immobilized in calcium alginate or egg white in

a column reactor. At 4°C, the rate of hydrolysis was decreased by 10% after 13 days and by 20% after 17 days.

Isomaltulose

Honey contains about 1% isomaltulose as a natural component. Cheetham, Garrett and Clark (1985) investigated the production of isomaltulose by immobilized *Erwinia rhapontici*. Whole cells were fixed in a number of different matrices and tested in a packed-bed column bioreactor. The best results (0·325 g product/g wet cells/h productivity, 8500 h half-life and 99% conversion) were obtained with cells immobilized in calcium alginate. The immobilized cells produced 1500 times their weight of isomaltulose during one half-life of use (*c.* 1 year). The activity and the stability of the immobilized cells were greatest when 55% (w/v) sucrose was used as substrate.

Soy sauce

This condiment was produced continuously for 80 days in 280 l column-type reactors containing immobilized *Pediococcus halophilus*, *Saccharomyces rouxii* and *Torulopsis versatilis* entrapped in calcium alginate gels (Osaki *et al.*, 1985). The products obtained by fermentation in these reactors with immobilized cells resembled soy sauce but had quantitatively different patterns of organic acid and aroma compounds. With this immobilized-cell process, the total production time for soy sauce could be reduced to 2 weeks compared with up to 6 months in traditional koji fermentations.

Vinegar

A number of immobilization techniques and immobilized cell processes have been documented recently for the production of vinegar (Mori, 1985). Steady acetic acid production was achieved at 4 mg/ml liquid/h for 120 days in a fluidized-bed reactor of *Acetobacter aceti* immobilized in κ-carrageenan (Osuga, Mori and Kato, 1984). In rice fermentation, vinegar was also produced by *Acetobacter rancens* cells fixed on the surface of hollow polypropylene fibres as an alternative surface culture (Nanba, Kimura and Nagai, 1985). In continuous operation with an oxygen partial pressure of 840 mmHg (111·72 kPa), a retention time of 100 h, an ethanol concentration of 39·2 g/l, and seed vinegar concentration of 7·9 g acetic acid/l, 30 g/l of acetic acid was produced — a productivity of 0·2 g/l/h compared with the 0·02–0·08 g/l/h observed in traditional surface culture of vinegar production (at 50 g acetic acid/l). Polypropylene fibres of approximately 40 μm diameter were used as the support material for *Acetobacter* in the production of vinegar (Okuhara, 1985). The fibres were packed in a column and inoculated with *Acetobacter*. Fermentation was continued in a closed system by recycling the wort and the air between the column and the wort tank until most of the ethanol in the wort was converted into acetic acid. The fermenter productivity was 25-fold greater than that observed in submerged culture. Nutrient depri-

vation was used to synchronize a culture of *Acetobacter suboxydans* immobilised in κ-carrageenan and micronized silica (Bailey, Venkatasubramanian and Karkare, 1985). In a fluidized-bed reactor, the dilution rate was decreased to limit the availability of nutrients. Oscillations in cell, substrate and product concentrations were observed.

HYDROCARBONS, HYDROGEN, PHENOLS AND EPOXIDES

Hydrocarbons

Bailliez, Largeau and Casadevall (1985) investigated hydrocarbon formation by *Botryococcus braunii* immobilized in calcium alginate. They observed that decreased cell growth rates were accompanied by a shift of the metabolism of the organism to the production of hydrocarbons. Rapid methane production from formic acid or acetic acid was attempted in fixed-bed bioreactors (Nishio, Kayawake and Nagai, 1985). Baked clay and foamed slag were useful to fix the methanogen cells. In the fixed-bed reactors, the cell concentration could be retained at high values of 20 and 44 g cell/l compared with 0·1 and 0·5 g cell/l in the usual chemostat cultures. Hence, methane formation increased up to 1·7 mol/day in formate and up to 0·4 mol/day in acetate, compared with 0·037 mol/day in formate and 0·013 mol/day in acetate in the usual chemostat cultures without fixed beds.

Hydrogen

Cells of the photosynthetic bacterium *Rhodopseudomonas capsulata* immobilized in agar or carrageenan beads (1–3 mm diameter) produced hydrogen from lactate at a rate of 54 ml/h/g dry weight (Francou and Vignais, 1984). The efficiency of hydrogen formation with the immobilized cells was similar to that of free cells and was 60–65% that of the theoretical maximum from lactate. Carrageenan-entrapped cells produced hydrogen steadily over a 16-day period. Cyanobacterial cells *Chlorogloea fritschii*, *Nostoc muscorum* and *Mastigocladus laminosus* were grown in polyurethane foams of the polyester and polyvinyl type and remained immobilized in the foams (Muallem, Bruce and Hall, 1983). The immobilized cells were used for continuous photoproduction of hydrogen from ascorbate, with methyl viologen and hydrogenase (EC 1.18.99.1) or platinum catalysts, for periods in excess of 9 days. Spinach chloroplasts immobilized in 2% agar gel were used to prepare a film which was then attached to a SnO_2 optically transparent electrode, and employed as a photoanode in a photoelectrochemical cell. On illumination in the presence of methyl viologen water was photolysed with evolution of hydrogen from a smooth platinum cathode. In this way, Okano *et al.* (1984) succeeded in producing hydrogen by photolysis of water. The pH and the temperature of the anolyte were kept at 7·8 and 25°C. Optimization of the concentration of methyl viologen and of chlorophyll in the immobilized chloroplast film were studied. The optimum thickness was about 0·8 mm. The immobilized

chloroplasts had higher storage stability than isolated chloroplasts. When they were stored at 4°C in the dark they retained more than 50% of the initial activities of Photosystem I and Photosystem II after 10 days (*see* Barber and Marder, 1986).

Epoxides

Alkene-utilizing bacteria immobilized in calcium alginate or κ-carrageenan retained 60–100% of their epoxide production activity (Habets-Crutzen *et al.*, 1984).

Phenolic compounds

Growth and phenolic-compound productivity of gel-entrapped *Daucus carota* and of *Petunia hybrida* cells were analysed by Hamilton, Pedersen and Chin (1984). Comparisons were made with corresponding free cell suspensions. It was found that the growth and productivity were optimal in a temperature range between 22° and 28°C and that the levels of formation of phenolic compounds were dependent upon the auxin levels in the reaction media.

APPLICATIONS TO ENVIRONMENTAL PROBLEMS AND AGRICULTURAL USES

Phenol degradation

The continuous degradation of phenol by cells of *Pseudomonas putida* entrapped in polyacrylamide-hydrazide was investigated in a columnar fluidized-bed reactor (Bettmann and Rehm, 1985). Under sterile conditions and with phenol as the sole carbon source a degradation rate of 7·2 g/l/day was obtained; in non-sterile waste water, only 3·12 g phenol/l/day were degraded. The immobilized cells showed a stable phenol-degradation activity, and if cresols or 4-chlorophenol were added simultaneously they were also utilized.

Pesticide removal

The removal of lindane (γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane), 2,4-D ((2,4-dichlorophenoxy)acetic acid) and 2,4,5-T((2,4,5-trichlorophenoxy) acetic acid) from water by microbial cells immobilized on magnetite was studied by Mac Rae (1985). *Rhodopseudomonas sphaeroides* had the highest sorption factor of the yeast, bacterial and algal cells tested. Four sorption stages employing fresh cells of *Rhodopseudomonas sphaeroides* on magnetite removed 90% of lindane from the water sample, whereas magnetite alone removed 70% of the lindane. *Alcaligenes eutrophus* removed 81% of 2,4-D, 21·4% of the lindane and 12·6% of the 2,4,5-T added to water samples.

Biotransformation of dehydroabietic acid

Dehydroabietic acid occurs in certain pulp mill effluents and is toxic to fish. It can be converted to innocuous metabolites by mycelia of *Mortierella isabellina*, whether free or immobilized in calcium alginate (Kutney *et al.*, 1985). Immobilization of *M. isabellina* increased the stability of its hydroxylase activity.

Ammonium and orthophosphate removal from waste waters

Hyperconcentrated *Scenedesmus quadricauda* cells (up to 3·29 g dry weight/l) immobilized in κ-carrageenan beads can efficiently remove nitrogen and phosporus from urban secondary effluent (Chevalier and de la Noue, 1985).

Aflatoxicol production

Rat erythrocytes were immobilized in calcium alginate and tested for the conversion of aflatoxin B_1 to aflatoxicol, a potent mutagen (Chang, Lin and Hsiung, 1985). The half-life of such a preparation was shown to be about 10 days. The immobilized erythrocytes could be repeatedly used at 37°C for the batch-wise production of aflatoxicol without substantial loss of enzyme activity. Haemolysis of these erythrocytes was not observed upon prolonged storage at 4°C. The immobilized cells were more resistant than free erythrocytes to incubation at 40°C.

Glycollate production

Day and Codd (1985) immobilized *Chlorella emersonii* in calcium alginate and observed undiminished capacity of photosynthetic activity and glycollate production.

Pigments

Blue pigments were synthesized *de novo* in the presence of L-cysteine by free and gel-entrapped *Lavandula vera* cells (Nakajima *et al.*, 1985). The entrapped cells grew well in the gel matrix as indicated by the increases in oxygen uptake, cell number and chlorophyll content. Cells immobilized in calcium alginate were employed for repeated production of the pigments for over 7 months in alternating growth and production phases. Entrapment in a suitable gel stabilized markedly the viability and pigment productivity of the plant cells.

Photosynthetic stability

Brouers and Hall (1985) observed that immobilization of the thermophilic cyanobacterium, *Phormidium laminosum*, in alginate after a drying pretreatment stabilized Photosystem II water-splitting activity for at least 3 months.

Polysaccharides

During the stationary phase of growth, *Porphyridium cruentum* produces large amounts of sulphated polysaccharide. Thepenier, Gudin and Thomas (1985) observed that *Porphyridium cruentum* cells immobilized in polyurethane foam grew and colonized the foam, producing polysaccharide for at least 8 weeks. The production rate was 0·7 g polysaccharide/m^2/day with the immobilized cells, compared with 2 g/m^2/day observed with the free cells under identical conditions.

Nitrification

Biomass collected from a continuous culture of activated sludge was immobilized in calcium alginate and the performance of this mixed culture was compared with that of an immobilized pure culture of *Nitrosomonas europaea* (Tramper, Suwinska-Borowiec and Klapwijk, 1985). The mixed culture was more difficult to work with than the pure strain. The general trends in the nitrifying capacities of the pure culture and the mixed culture systems were similar, except for operational stability which was poorer in the case of the immobilized mixed culture. Biological nitrification was optimized by using *Nitrobacter winogradskyi* immobilized by attachment on granular media (Audic, Faup and Navarro, 1984). A 130% increase was observed in the respiratory activity of the attached cells compared with that of the free cells.

Water stress

A capacity to tolerate water stress and desiccation is a feature of many cyanobacteria. An immobilization technique was applied to the study of the sensitivity of *Nostoc commune* (cyanobacteria) to water stress (Potts and Bowman, 1985). Cells in a 4 ml aliquot of liquid culture were immobilized on a circular nylon filter support of 2 cm diameter and pore size 0·04 mm^2. On this support the filamentous aggregates were retained efficiently, yet excess liquid could be removed rapidly by blotting. Immobilized cells were subjected to varying rates of equilibration to the desired water potential. When the cells were exposed to matrix water potentials between −0·10 and −99·5 MPa, they exhibited an increase in nitrogenase (EC 1.18.6.1) activity. Cells incubated at −0·10 MPa maintained increased activity for at least 48 h following immobilization. At water potentials below −23·1 MPa, the increase was transitory. Nitrogenase activity decreased rapidly when cells were incubated at lower water potentials.

Chlorella

The physiology of immobilized and free cells of *Chlorella* was studied by Robinson *et al.* (1985). *Chlorella emersonii* Shihira and Karaus var. *emersonii* cells entrapped in calcium alginate displayed reduced respiratory and growth rates, and a higher chlorophyll-*a* content, than free cells. Respiratory rate

per cell was reduced as cell loading density increased. Cell leakage from the matrix was closely related to cell growth within the matrix and occurred throughout the 20-day test period, but was highest during the first 5–6 days.

Euglena gracilis

The cytological and physiological behaviour of *Euglena gracilis* cells entrapped in a calcium alginate gel was studied by Tamponnet *et al.* (1985). These cells maintained respiratory and photosynthetic activities and ultrastructural integrity. Moreoever, immobilization did not prevent *Euglena* cells from becoming green inside the gel beads. Electron microscopy demonstrated that the immobilized cells remained in the cellular state which existed at the time of immobilization. Long-term storage tests on *E. gracilis* immobilized in calcium alginate have shown that the algae can be maintained in this state for up to 2 years.

Plant cell protoplasts

Schnabl and Youngman (1985) investigated membrane degradation as a possible result of the enzymatic lipid peroxidation. They studied the activities of some key enzymes in this process, using plant cell protoplasts which were suspended in a mannitol solution or immobilized in alginate. They concluded that immobilization of plant cell protoplasts appeared to lead to an increased stability, which thus prolonged the life of the cell.

Plasmid stability

In continuous culture, *Escherichia coli* BZ 18 cells immobilized in carrageenan gel beads provided better conditions for stability of the plasmid PTG 201 than free cells, with an approximately equal production of biomass (de Taxis du Poet, Dhulster and Thomas, 1984). A similar observation was reported by Shuler (1985) for β-lactamase (EC 3.5.2.6) production by *E. coli* immobilized in calcium alginate. Production levels of at least 390 units/l/h have been sustained for at least 120 days in a continuous reactor.

Performance characteristics of immobilized cell biocatalysts

DIFFUSION

Diffusion coefficients of solutes in immobilized cell catalysts were studied by Furui and Yamashita (1985a). The effective diffusion coefficients for ammonium fumarate, ammonium L-aspartate, sodium fumarate, sodium L-malate, L-malic acid, L-alanine and potassium chloride in *Escherichia coli*, *Brevibacterium flavum* and *Pseudomonas dacunhae* immobilized in polyacrylamide or κ-carrageenan gel were much lower than those in aqueous solution. Mitani, Nishizawa and Nagai (1984a) applied forced substrate supply (*see* page 355) to eliminate the diffusion dependency of the substrate supply in

ethanol production with immobilized cells. Karkare, Dean and Venkatasubramanian (1985) observed that for ethanol fermentation by κ-carrageenan-immobilized yeast cells, the productivity per cell was only about 60% of that of free cells. They were of the opinion that the reduction in productivity per cell in immobilized bioreactors was primarily due to diffusion resistance causing increased product concentration and lowered substrate concentration around the cells deep inside the beads. Cantarella *et al.* (1984) entrapped *Saccharomyces cerevisiae* in polymers of 2-hydroxyethyl methacrylate and observed that immobilization did not introduce diffusional resistance to mass transfer of reactant and product in sucrose hydrolysis catalysed by yeast invertase (β-fructofuranosidase, EC 3.2.1.26).

CELL DENSITY

Investigation of immobilized yeast beads by scanning electron microscopy revealed that the cells tended to grow in chains and were smaller than those commonly found in non-immobilized cell fermentations (Karkare, Dean and Venkatasubramanian, 1985). In addition, the growth rate of the immobilized cells was about one-third that observed in the free-cell controls. A κ-carrageenan matrix permitted the release of the excess cells into the fermentation liquor. In this study, after a few days of incubation, the yeast cells packed the gel matrix to more than 50% of maximum theoretical density. The concentration of the cells in the beads was about 8×10^9 cell/ml whereas that in the liquor was $2–10 \times 10^7$ cells/ml. The cell viability, as determined by staining with erythrosin B, was about 85% in the beads and 75% in the liquor. These investigators could operate the immobilized cell fermenters for up to 15 weeks with steady ethanol production. The cell density distribution of *Saccharomyces cerevisiae* in an immobilized cell layer has also been analysed during ethanol fermentation with forced substrate supply (Mitani, Nishizawa and Nagai, 1984a). The cells were immobilized in a mixed gel composed of κ-carrageenan, locust bean gum and Celite (2 : 0·5 : 40 wt/vol %) with an initial cell concentration of 4×10^8 cells/ml gel. When a medium containing 200 g/l of dextrose was forced through an immobilized cell layer of 14 mm thickness, with a retention time of 1·5 h, the yeast cells grew homogeneously, reaching 2×10^9 cells/ml gel in the first 24 hours. After 24 h, the cells continued to grow and a cell density gradient formed in the vertical direction of the gel layer. Finally, a steady state was attained at 250 h of operation, with $1·7 \times 10^{10}$ cells/ml at the surface and 4×10^9 cells/ml at the bottom of the gel layer.

MOLECULAR MOTION

The dependence of activity of immobilized yeast cells on the molecular motion of the trapping agent was investigated by Fujimara and Kaetsu (1985) using carriers which had been polymerized by radiation. Molecular motion of the polymeric carriers was determined by the line width of a nuclear magnetic resonance spectrum. In this study, *Saccharomyces formosensis* cells

were immobilized inside the polymer and the surface modified by irradiation of mixtures of two glass-forming monomers, methoxypolyethyleneglycol methacrylate and 2-hydroxy-ethacrylate. Higher ethanol production was observed to be related to the higher population density achieved by cells immobilized in the synthetic polymers with higher molecular motion. It was concluded that yeast cells inside a polymer carrier can multiply only if space is made by excluding the surrounding polymer. High molecular motion facilitates polymer exclusion and thus cell multiplication, leading to high cell density and a corresponding increase in production rate.

HYDROPHILICITY

The enzyme activity in immobilized cell composites could be increased by using highly hydrophilic polymer matrices (Kumakura and Kaetsu, 1984b). *Streptomyces phaeochromogenes* cells were immobilized by radiation polymerization of hydrophilic polymers. Higher levels of glucose isomerase activity were achieved in composites from co-polymerization of hydroxyethyl acrylate (or methacrylate) and *N*-vinyl-2-pyrrolidone monomer. As the content of the latter was increased, so activity increased almost to that observed in non-immobilized cells.

HYDROPHILIC–HYDROPHOBIC BALANCE

Fukui and Tanaka (1985) reviewed enzymatic reactions in organic solvents and reported that the hydrophilic–hydrophobic balance of the immobilization matrix, together with the substrate hydrophobicity and the polarity of the reaction solvents, are important factors in the application of immobilized biocatalysts in organic solvents.

BACTERIAL FILAMENTS

Filament formation by *Zymomonas mobilis* in absorbed cell bioreactors for ethanol fermentation was observed by Daugulis, Krug and Choma (1985). Bacterial filament formation took place after several days of continuous operation and resulted in excessive pressure increases across the reactor bed. When the fermentation temperature was controlled at 20–25°C it was possible to eliminate the filament formation, and to operate the reactor continuously for 232 h with an ethanol productivity of 50 g/l/h.

PLANT CELLS

In vivo ^{31}P NMR spectra obtained for *Catharanthus roseus* plant cells entrapped in agarose or alginate were compared with those obtained for freely suspended cells (Vogel and Brodelius, 1984). Essentially no differences were observed in the levels of major metabolites such as ATP, NAD(H), UDPG, cytoplasmic P_i, and sugar phosphates. Although the rate of uptake of P_i from the medium and the utilization of P_i from the vacuolar pool were

lower in gel-entrapped cells, the same pattern of P_i uptake, storage and utilization was observed in all cases. Thus, the results obtained by the non-invasive ^{31}P NMR technique suggested that entrapment in agarose or alginate did not adversely affect cell metabolism, as the phosphate metabolism and the cytoplasmic pH appeared to be unaltered.

Bioreactors

In most applications of immobilized cells, air-lift or plug-flow-type bioreactors have been used, although other types have been employed.

A rotating biological surface (RBS) reactor was studied by Del Borghi *et al.* (1985). In this system *Saccharomyces cerevisiae* cells were immobilized on synthetic commercial sponge. The reactor assembly consisted of a rotating disc shaft mounted on a glass tank (two superimposed semicylinders). The sponge discs were assembled on the shaft. A low rotation rate of 3·5 rev/min was used to reduce energy consumption. The working volume of the reactor was 2·4 l and the external surface area of the discs was 2044 cm^2. Cell immobilization was achieved by filling the lower part of the fermenter with a thin suspension of cells in the presence of the rotating discs. Periodic aseptic renewals of the medium allowed aerobic growth of the yeast within the cavities of the support. In the presence of the RBS, an ethanol productivity of 7·1 g/l/h was achieved at a dilution rate of 0·3 h^{-1}. This productivity rate is 2·5 times higher than that achieved without the support discs.

Karkare, Dean and Venkatasubramanian (1985) used a cascade of three fluidized bioreactor cells, each of which was homogeneous. The cascade was expected to simulate plug flow. In order to aerate the broth, a side loop was used for each bioreactor cell. This side loop also served for the stripping of CO_2. κ-Carrageenan concentrations in the gel were increased to reduce the biocatalyst attrition in long-term continuous operations, even though it was known that this increase would reduce the diffusion rates.

In their studies of rapid beer production, Onaka *et al.* (1985) used a cylindrical polycarbonate column (8 cm i.d. and 500 cm long) as the bioreactor. The immobilized yeast was introduced into this reactor, which had five cylindrical baskets (each 7 cm o.d. and 10 cm long). The baskets were made of stainless steel wire netting of 9 mesh. Seventy per cent of the volume of these baskets and 54% of the total reactor volume were occupied by the immobilized yeast. This reactor is used as the third stage of a new rapid fermentation process, the first stage being used for free cell fermentation with aeration and the second stage for free cell carbonation, while the third stage using the continuous immobilized cell reactor yielded beer with low diacetyl content.

A basket type of reactor was also developed by Kim, Naveh and Olson (1985) for the continuous acidification of milk by immobilized *Streptococcus lactis* before coagulation.

Furui (1985) used a heat-exchange column for exothermic immobilized cell reactions. A stainless steel column, 64 × 78 cm in cross-section and 200

cm in height, was equipped with 96 horizontal tubes to circulate water. The efficiency of the column was tested with immobilized *E. coli* for aspartase and immobilized *Brevibacterium flavum* for fumarase activity. The horizontal tubes prevented a rise in temperature as well as compaction of the bed. The column showed ideal plug flow-type behaviour.

Compaction may be a problem in immobilized cell bioreactors. Furui and Yamashita (1985b) developed a column equipped with horizontal baffles in order to avoid compaction of soft immobilized cell beds. To confirm the baffle effect, they compared the void fraction, pressure drop and liquid flow characteristics of columns with and without baffles. The void fraction in the column with baffles was little affected by superficial liquid velocity compared with the control. The pressure drop was smaller and the liquid flow was closer to plug flow in the column with the baffles.

An airlift loop reactor for the transformation of steroids by immobilized cells was built by Kloosterman and Lilly (1985). The reactor had a working volume of 4·3 l and riser i.d. of 75 mm. The ratio of the internal diameters of the riser and the downcomer was 3. A perforated disc at the bottom plate facilitated harvesting the reaction liquids while retaining the immobilized cell beads in the reactor. The stability of the beads in the loop reactor was a significant improvement over stirred-tank immobilized cell reactors.

Joshi and Yamazaki (1984) immobilized *Saccharomyces cerevisiae*, *Kluyveromyces marxianus* and *Kluyveromyces fragilis* cells on cotton cloth for use as a film fermenter for ethanol production. In this study a cylindrical film fermenter and a rectangular film fermenter was assembled. With the cylindrical film fermenter, a rectangular cotton cloth was placed on a bronze screen of the same dimensions (7 cm × 15 cm). The doubled layer was rolled along the longer side and the roll was vertically fitted into the jacketed column (2·8 cm i.d.; 25 cm height). The average distance between loops of the coil was 0·4 cm. In the rectangular film fermenter, three rectangular cloths (5 cm × 30 cm) were stretched lengthwise, mounted in parallel (0·7 cm apart) in a stainless steel stand, and placed in a plexiglass container (2·2 cm width, 32 cm length, 20 cm height, inside dimensions). The colonization of yeast on the cloth was initiated by adding an inoculum of late log-phase yeast culture to the fermenter together with fresh growth medium.

In a continuous fermentation of dextrose to butanol and isopropanol by immobilized *Clostridium beyerinckii* cells, Groot *et al.* (1984) observed that both the dextrose conversion and the reactor productivity were 65–70% higher if the products were continuously removed by pervaporation. Oxygen supply to immobilized *Gluconobacter oxydans* with *p*-benzoquinone as a substitute for oxygen was studied by Adlercreutz and Mattiasson (1984). In the oxidation of glycerol to dihydroxyacetone, the reaction rate was at least fourfold higher when *p*-benzoquinone was used in place of oxygen. The operational stability of the process was also good. When *p*-benzoquinone was used, productivity decreased from 60 to 10 mmol/h/g over an 8-day period. In a similar experiment with oxygen the productivity decreased from 14 to 6 mmol/g/h. The hydroquinone that formed as the by-product of this reaction could be reoxidized and reused repeatedly if hydrogen peroxide was added as an oxidizing agent.

Prospects

During the past two years, the applications of immobilized cells have continued to be refined and broadened. It is abundantly evident that these biocatalysts are adaptable to many different systems and are very versatile in the range of transformations to which they can be applied. Applications to classic fermentations have become well established. Exciting future challenges lie in coupling immobilized cell technology with the novel creations of recombinant DNA manipulations. In particular, if specially engineered plant and animal cells can be entrapped in matrices, it should be possible to design efficient reactors for the continuous production of commercially viable materials.

References

Adlercreutz, P. and Mattiasson, B. (1984). Oxygen supply to immobilized cells. 4. Use of *p*-benzoquinone as an oxygen substitute. *Applied Microbiology and Biotechnology* **20**, 296–302.

Aldercreutz P., Holst, O. and Mattiasson, B. (1985). Characterization of *Gluconobacter oxydans* immobilized in calcium alginate. *Applied Microbiology and Biotechnology* **22**, 1–7.

Amin, G., Mot, R. de, Van Dijck, K. and Verachtert, H. (1985). Direct alcoholic fermentation of starchy biomass using amylolytic yeast strains in batch and immobilized cell systems. *Applied Microbiology and Biotechnology* **22**, 237–245.

Anonymous (1983). *Alginates for immobilization and encapsulation. Protan Biopolymers Technical Brochure*. Protan A/S. P.O.Box 420, N-3001 Drammen, Norway.

Audic, J.M., Faup, G.M. and Navarro, J.M. (1984). Specific activity of *Nitrobacter* through attachment on granular media. *Water Research* **18**, 745–750.

Bailey, K.M., Venkatasubramanian, K. and Karkare, S.B. (1985). Immobilized live cell reactor dynamics following dilution rate shift to growth conditions: Cell synchrony effects. *Biotechnology and Bioengineering* **27**, 1208–1213.

Bailliez, C., Largeau, C. and Casadevall, E. (1985). Growth and hydrocarbon production of *Botryococcus braunii* immobilized in calcium alginate gel. *Applied Microbiology and Biotechnology* **23**, 99–105.

Bajpai, P. and Margaritis, A. (1985a). Improvement of inulinase stability of calcium alginate immobilized *Kluyveromyces marxianus* cells by treatment with hardening agents. *Enzyme and Microbial Technology* **7**, 34–36.

Bajpai, P. and Margaritis, A. (1985b). Immobilization of *Kluyveromyces marxianus* cells containing inulinase activity in open pore gelatin matrix: 1. Preparation and enzymatic properties. *Enzyme and Microbial Technology* **7**, 373–376.

Bajpai, P. and Margaritis, A. (1985c). Kinetics of ethanol production by immobilized cells of *Zymomonas mobilis* at varying D-glucose concentrations. *Enzyme and Microbial Technology* **7**, 462–464.

Barber, J. and Marder, J.B. (1986). Photosynthesis and the application of molecular genetics. In *Biotechnology and Genetic Engineering Reviews* (G.E. Russell, Ed.), pp. 355–404. Intercept, Ponteland, Newcastle upon Tyne.

Beddows, C.G., Gil, H.G. and Guthrie, J.T. (1985). The immobilization of enzymes and cells of *Bacillus stearothermophilus* onto poly(maleic anhydride/styrene)-co-polyethylene and poly(maleic anhydride/vinyl acetate)-co-polyethylene. *Biotechnology and Bioengineering* **27**, 579–584.

Bettmann, H. and Rehm, H.J. (1984). Degradation of phenol by polymer entrapped microorganisms. *Applied Microbiology and Biotechnology* **20**, 285–290.

Bettmann, H. and Rehm, H.J. (1985) Continuous degradation of phenol(s) by *Pseudomonas putida* P8 entrapped in polyacrylamide-hydrazide. *Applied Microbiology and Biotechnology* **22**, 389–393.

BIHARI, V.B., GOSWAMI, P.P., RIZVI, S.H.M., KHAN, A.W., BASU, S.K. AND VORA, V.C. (1984). Studies on immobilized fungal spores for microbial transformation of steroids: 11 α-hydroxylation of progesterone with immobilized spores of *Aspergillus ochraceus* G8 on polyacrylamide gel and other matrices. *Biotechnology and Bioengineering* **26**, 1403–1408.

BLACK, G.M., WEBB, C., MATTHEWS, T.M. AND ATKINSON, B. (1984). Practical reactor systems for yeast cell immobilization using biomass support particles. *Biotechnology and Bioengineering* **26**, 134–141.

BORGHI, M. DEL, CONVERTI, A., PARISI, F. AND FERRAIOLO, G. (1985). Continuous alcohol fermentation in an immobilized cell rotating disk reactor. *Biotechnology and Bioengineering* **27**, 761–768.

BRODELIUS, P. (1985a). Immobilized microbial and plant cells: techniques and applications. In *Biotech '85 (Europe)* pp. 573–585. Online Publications, Pinner, Middlesex.

BRODELIUS, P. (1985b). The potential role of immobilization in plant cell biotechnology. *Trends in Biotechnology* **3**, 280–285.

BROUERS, M. AND HALL, D.O. (1985). Drying pretreatment enhances the photosynthetic stability of alginate immobilized *Phormidium laminosum*. *Biotechnology Letters* **7**, 567–572.

BUCKE, C. (1977). Industrial glucose isomerase. *Topics in Enzyme and Fermentation Technology* **1**, 147–171.

CANTARELLA, M., MIGLIARESI, C., TAFURI, M.G. AND ALFANI, F. (1984). Immobilization of yeast cells in hydroxymethylmethacrylate gels. *Applied Microbiology and Biotechnology* **20**, 233–237.

CHANG, W-M., LIN, J-K. AND HSIUNG, K-P. (1985). Immobilization of rat erythrocytes for aflatoxicol production. *Applied Microbiology and Biotechnology* **21**, 361–364.

CHEETHAM, P.S.J., GARRETT, C. AND CLARK, J. (1985) Isomaltulose production using immobilized cells. *Biotechnology and Bioengineering* **27**, 471–481.

CHEVALIER, P. AND NOUE, J. DE LA (1985). Efficiency of hyperconcentrated algae for ammonium and orthophosphate removal. *Biotechnology Letters* **7**, 395–400.

CHIBATA, I. AND WINGARD, L.B. JR (EDS) (1983). Immobilized microbial cells. In *Applied Biochemistry and Bioengineering* (L.B. Wingard, Jr, L. Goldstein and E. Katchalski-Katzir, Eds), volume 4. Academic Press, New York.

CHIBATA, I., TOSA, T. AND SATO, T. (1974). Immobilized aspartase containing microbial cells: preparation and enzymatic properties. *Applied Microbiology* **27**, 878–885.

CHIBATA, I., TOSA, T. AND SATO, T. (1983). Immobilized cells in preparation of fine chemicals. In *Advances in Biotechnological Processes* (A.Mizrahi and A.L. van Wezel, Eds), volume 1, pp. 203–222. Alan R. Liss, Inc., New York.

CHIBATA, I., TOSA, T. AND TAKAMATSU, S. (1985). *Process for Preparing Immobilized Microorganisms*. US Patent 4,526,867.

CONNICK, W.C. JR, WALKER, H.L. AND QUIMBY, P.C., JR (1985). Granular formulations for biological control of weeds. *SIM News* **35** (1), 7–9.

CORCORAN, C.A. AND RECHNITZ, G.A. (1985). Cell-based biosensors. *Trends in Biotechnology* **3**, 92–96.

DAINTY, A.L., GOULDING, K.H., ROBINSON, P.K., SIMPKINS, I. AND TREVAN, M.D. (1985). Effect of immobilization on plant cell physiology — real or imaginary? *Trends in Biotechnology* **3**, 59–60.

DAUGULIS, A.J., KRUG, T.A. AND CHOMA, C.E.T. (1985). Filament formation and ethanol production by *Zymomonas mobilis* in absorbed cell bioreactors. *Biotechnology and Bioengineering* **27**, 626–631.

DAY, J.G. AND CODD, G.A. (1985). Photosynthesis and glycollate excretion by *Chlorella emersonii*. *Biotechnology Letters* **7**, 573–576.

DECLEIRE, M., VAN HUYNH, N., MOTTE, J.C. AND CAT, W. DE (1985). Hydrolysis of whey by whole cells of *Kluyveromyces bulgaricus* immobilized in calcium alginate gels and in hen egg white. *Applied Microbiology and Biotechnology* **22**, 438–441.

Doppner, T. and Hartmeier, W. (1984). Glucose oxidation by modified mould mycelium. *Starch* **36**, 283–287.

Eikmeier, H. and Rehm, H.J. (1984). Production of citric acid with immobilized *Aspergillus niger*. *Applied Microbiology and Biotechnology* **20**, 365–370.

Felton, P. von, Zurer, H. and Bachofen, R. (1985). Production of molecular hydrogen with immobilized cells of *Rhodospirillum rubrum*. *Applied Microbiology and Biotechnology* **25**, 15–20.

Francou, N. and Vignais P.M. (1984). Hydrogen production by *Rhodopseudomonas capsulata* cells entrapped in carrageenan beads *Biotechnology Letters* **6**, 639–644.

Fujimara, T. and Kaetsu, I. (1985). Nature of yeast cells immobilized by radiation polymerization — activity dependence on the molecular motion of the polymer carriers. *Zeitschrift für Naturforschung* **40**, 576–579.

Fukui, S. and Tanaka, A. (1984). Application of biocatalysts immobilized by prepolymer methods. *Advances in Biochemical Engineering and Biotechnology* **29**, 1–33.

Fukui, S. and Tanaka, A. (1985). Enzymatic reactions in organic solvents. *Endeavour, New Series*, **9**, 10–17.

Furui, M. (1985). Heat-exchange column with horizontal tubes for immobilized cell reactions with generation of heat. *Journal of Fermentation Technology* **63**, 371–375.

Furui, M. and Yamashita, K. (1985a). Diffusion coefficients of solutes in immobilized cell catalysts. *Journal of Fermentation Technology* **63**, 167–173.

Furui, M. and Yamashita, K. (1985b). Horizontal baffle effects on compaction of immobilized cell catalyst beds. *Journal of Fermentation Technology* **63**, 73–78.

Fusee, M.C. and Weber, J.E. (1984). Immobilization by polyurethane of *Pseudomonas dacunhae* cells containing L-aspartate β-decarboxylase activity and application to L-alanine production. *Applied and Environmental Microbiology* **48**, 694–698.

Gekas, V and Lopez-Leiva, M. (1985). Hydrolysis of lactose: A literature review. *Process Biochemistry*, February 1985, 2–12.

Groot, W.J., Schoutens, G.H., Van Beelen, P.N., Van Den Oever, C.E. and Kossen, N.W.F. (1984). Increase of substrate conversion by pervaporation in the continuous butanol fermentation. *Biotechnology Letters* **6**, 789–792.

Grote, W. and Rogers, P.L. (1985). Ethanol production from sucrose-based raw materials using immobilized cells of *Zymomonas mobilis*. *Biomass* **8**, 169–184.

Habets-Crutzen, A.Q.H., Brink, L.E.S., van Ginkel, C.G., Bont, J.A.M. de and Tramper, J. (1984). Production of epoxides from gaseous alkenes by resting-cell suspensions and immobilized cells of alkene-utilizing bacteria. *Applied Microbiology and Biotechnology* **20**, 245–250.

Hamilton, R., Pedersen, H. and Chin, C-K. (1984). Immobilized plant cells for the production of biochemicals. *Biotechnology and Bioengineering Symposium* **14**, 383–396.

Hartmeier, W. (1985). Immobilized biocatalysts — from simple to complex systems. *Trends in Biotechnology* **3**, 149–153.

Hasegawa, E., Iida, T. and Sakamoto, M. (1985) *Process for Producing Granular Fixed Enzymes and/or Microorganism Strains*. UK Patent 2 149 816 A.

Hasegawa, S., Vandercrook, C.E., Choi, G.Y., Herman, Z. and Ou, P. (1985). Limonoid debittering of citrus juice sera by immobilized *Corynebacterium fascians*. *Journal of Food Science* **50**, 330–332.

Holst, O., Lundback, H. and Mattiasson, B. (1985). Hydrogen peroxide as an oxygen source for immobilized *Gluconobacter oxydans* converting glycerol to dihydroxyacetone. *Applied Microbiology and Biotechnology* **22**, 383–388.

Horitsu, H., Adachi, S., Takahashi, Y., Kawai, K. and Kawano, Y. (1985). Production of citric acid by *Aspergillus niger* immobilized in polyacrylamide gels. *Applied Microbiology and Biotechnology* **22**, 8-12.

Hulst, A.C., Tramper, J., van 'T Riet, K. and Westerbeek, J.M.M. (1985). A new

technique for the production of immobilized biocatalyst in large quantities. *Biotechnology and Bioengineering* **27**, 870–876.

HUPKES, J.V. AND VAN TILBURG, R. (1976). Production and properties of an immobilized glucose isomerase. *Staerke* **28**, 356–360.

INLOES, D.S., MICHAELS, A.S., ROBERTSON, C.R. AND MATIN, A. (1985) Ethanol production by nitrogen-deficient yeast cells immobilized in a hollow-fiber membrane bioreactor. *Applied Microbiology and Biotechnology* **23**, 85–91.

JAIN, D. AND GHOSE, T.K. (1984). Cellobiose hydrolysis using *Pichia etchellsii* cells immobilized in calcium alginate. *Biotechnology and Bioengineering* **26**, 340–346.

JAIN, V.K., TORAN-DIAZ, I. AND BARATTI, J. (1985a). Continuous production of ethanol from fructose by immobilized growing cells of *Zymomonas mobilis*. *Biotechnology and Bioengineering* **27**, 613–620.

JAIN, V.K., TORAN-DIAZ, I. AND BARATTI, J. (1985b). Preparation and characterization of immobilized growing cells of *Zymomonas mobilis* for ethanol production. *Biotechnology and Bioengineering* **27**, 273–279.

JOSHI, S. AND YAMAZAKI, H. (1984). Film fermentor for ethanol production by yeast immobilized on cotton cloth. *Biotechnology Letters* **6**, 782–797.

KARKARE, S.B., DEAN, R.C. JR AND VENKATASUBRAMANIAN, K. (1985). Continuous fermentation with fluidized slurries of immobilized microorganisms. *Biotechnology* **3**, 247–251.

KARUBE, I., SUZUKI, S. AND VANDAMME, E.J. (1984). Antibiotic production with immobilized living cells. *Drugs and the Pharmaceutical Sciences* **22**, 761–780.

KAUTOLA, H., VAHVASELKA, M., LINKO, Y-Y. AND LINKO, P. (1985). Itaconic acid production by immobilized *Aspergillus terreus* from xylose and glucose. *Biotechnology Letters* **7**, 167–172.

KELLER, E. AND LINGENS, F. (1984). Synthesis of chorismic acid by immobilized cells of *Enterobacter aerogenes* 62–1. *Applied Microbiology and Biotechnology* **20**, 3–5.

KHAN, A.W. AND MEEK, E. (1985). Immobilization of syntrophic culture of propionate degrading and methanogenetic bacteria present in cellulose-enriched culture. *Biomass* **8**, 195–204.

KIM, K., NAVEH, D. AND OLSON, N.F. (1985). Continuous acidification of milk before ultrafiltration by an immobilized cell bioreactor. I. Development of the bioreactor. *Milchwissenschaft* **40**, 605–607.

KLEIN, J. AND WAGNER, F. (1983). Methods for the immobilization of microbial cells. In *Applied Biochemistry and Bioengineering* (L.B. Wingard, Jr., L. Goldstein and E. Katchalski-Katzir, Eds.), *Volume 4: Immobilized Microbial Cells* (I. Chibata and L.B. Wingard, Eds), pp. 12–51. Academic Press, New York..

KLOOSTERMAN, J., IV AND LILLY, M.D. (1985). An airlift loop reactor for the transformation of steroids by immobilized cells. *Biotechnology Letters* **7**, 25–30.

KNORR, D., MIAZGA, S.M. AND TEUTONICO, R.A. (1985). Immobilization and permeabilization of cultured plant cells. *Food Technology* **39**, 135–142.

KOLE, M.M. AND ALTOSAAR, I. (1985). Conversion of chenodeoxycholic acid to ursodeoxycholic acid by *Clostridium absonum* in culture and by immobilized cells. *FEMS Microbiology Letters* **28**, 69–72.

KOLOT, F.B. (1984). Immobilized cells for solvent production. *Process Biochemistry* **19**, (1) 7–13.

KOPP, B. AND REHM, H.J. (1984). Semicontinuous cultivation of immobilized *Claviceps purpurea*. *Applied Microbiology and Biotechnology* **19**, 141–145.

KUEK, C. AND ARMITAGE, T.M. (1985). Scanning electron microscopic examination of calcium alginate beads immobilizing growing mycelia of *Aspergillus phoenicus*. *Enzyme and Microbial Technology* **7**, 121–125.

KUMAKURA, M. AND KAETSU, I. (1984a) Immobilization of cells by radiation copolymerization of hydrophilic and hydrophobic monomers. *Acta chimica hungarica* **116**, 345–351.

KUMAKURA, M. AND KAETSU, I. (1984b). Increased enzyme activity in immobilized cell composites with highly hydrophilic polymer matrix. *Journal of Chemical Technology and Biotechnology* **34B**, 39–44.

KUMAKURA, M., TAMADA, M. AND KAETSU, I. (1984). Properties of *Trichoderma reesei* cells immobilized by the irradiation technique. *Enzyme and Microbial Technology* **6**, 411–414.

KUTNEY, J.P., CHOI, L.S.L., HEWITT, G.M., SALISBURY, P.J. AND SINGH, M. (1985). Biotransformation of dehydroabietic acid with resting cell suspensions and calcium alginate-immobilized cells of *Mortierella isabellina*. *Applied and Environmental Microbiology* **49**, 96–100.

KUU, W-Y. (1985). *Immobilized Biocatalysts*. US Patent 4,518,693.

LAMBE, C. AND ROSEVEAR, A. (1985). *Improvements in or relating to the Production of Chemical Compounds*. UK Patent 2,096,169.

LANCY, E.D. AND TUOVINEH, O.H. (1984). Ferrous ion oxidation by *Thiobacillus ferrooxidans* immobilized in calcium alginate. *Applied Microbiology and Biotechnology* **20**, 94–99.

LANTERO, O.J., JR (1985). *Immobilization of Biocatalysts*. European Patent 0 133 531.

LARGIER, S.T., LONG, S., SANTANGELO, J.D., JONES, D.T. AND WOODS, D.R. (1985). Immobilized *Clostridium acetobutylicum* P262 mutants for solvent production. *Applied and Environmental Microbiology* **50**, 477–481.

LASKIN, A.I., TSAO, G.T. AND WINGARD, L.B., JR (EDS) (1984). *Enzyme Engineering 7. Annals of the New York Academy of Sciences* volume 434. NY Academy of Sciences, New York.

LEE, G.M., CHOI, C.Y., PARK, J.M. AND HAN, M.H. (1984). Biotransformation of Rifamycin B to Rifamycin S using immobilized whole cells of *Humicola* spp. in a fluidised bed reactor. *Journal of Chemical Technology and Biotechnology* **55B**, 3–10.

LI, G-X., LINKO, Y-Y. AND LINKO, P. (1984). Glucoamylase and α-amylase production by immobilized *Aspergillus niger*. *Biotechnology Letters* **6**, 645–650.

LINDSEY, K. AND YEOMAN, M.M. (1984a). The viability and biosynthetic activity of cells of *Capsicum frutescens* Mill. cv. annuum immobilized in reticulate polyurethane. *Journal of Experimental Botany* **35**, 1684–1696.

LINDSEY, K. AND YEOMAN, M.M. (1984b). The synthetic potential of immobilized cells of *Capsicum frutescens*, *Planta* **162**, 495–501.

LINKO, P. AND LINKO, Y-Y. (1984). Industrial applications of immobilized cells. *Critical Reviews in Biotechnology* **1**, 289–338.

MCCORD, J.D. AND RYU, D.Y. (1985). Development of malolactic fermentation process using immobilized whole cells and enzymes. *American Journal of Enology and Viticulture* **36**, 213–218.

MCGHEE, J.E., CARR, M.E. AND ST JULIAN, G. (1984). Continuous bioconversion of starch to ethanol by calcium-alginate immobilized enzymes and yeasts. *Cereal Chemistry* **61**, 446–449.

MAC RAE, I.C. (1985). Removal of pesticides in water by microbial cells adsorbed to magnetite. *Water Research* **19**, 825–830.

MANECKE, G. AND KLUSSMANN, U. (1985a). Immobilization of *Corynebacterium simplex* by entrapment in synthetic cross-linked gels — biocatalysts for steroid transformations. *Die Angewandte Makromolekular Chemie* **134**, 125–149.

MANECKE, G. AND KLUSSMANN, U. (1985b). *Polymer Containing Biocatalyst*. US Patent 4,546,078.

MARGARITIS, A. (1984). Advances in ethanol production using immobilized cell systems. *Critical Reviews in Biotechnology* **1**, 339–393.

MATSON, S. L. (1985). Membrane bioreactors—trends and opportunities. In *Biotech '85 (Europe)*, pp. 597–610. Online, Pinner, Middlesex.

MATTIASSON, B. (ED.) (1983a). *Immobilized Cells and Organelles*, volumes 1 and 2.

CRC Press, Inc., Boca Raton, Florida.

MATTIASSON, B. (1983b). Immobilization methods. In *Immobilized Cells and Organelles* (B. Mattiasson, Ed), volume 1, pp. 3–25. CRC Press, Inc., Boca Raton, Florida.

MAZUMDER, T.K., SONOMOTO, K., TANAKA, A. AND FUKUI, S. (1985). Sequential conversion of cortexolone to prednisolone by immobilized mycelia of *Curvularia lunata* and immobilized cells of *Arthrobacter simplex*. *Applied Microbiology and Biotechnology* **21**, 154–161.

MELELLI, F. AND BISSO, G.M. (1985). Production of fructose 1,6-diphosphate by permeabilized yeast cells crosslinked with glutaraldehyde-albumin. In *Biotech '85 (Europe)*, pp. 663–664. Online, Pinner, Middlesex.

MESSING, R.A. (1985). Bioreactors and immobilized cells. *SIM News* **35** (3), 4–7.

MIDLER, O.G. (1985). Building a business in biocatalysis. In *Biotech '85 (Europe)*, pp. 639–648. Online, Pinner, Middlesex.

MITANI, Y., NISHIZAWA, Y. AND NAGAI, S. (1984a). Ethanol production by immobilized cells with forced substrate supply. *Journal of Fermentation Technology* **62**, 249–253.

MITANI, Y., NISHIZAWA, Y. AND NAGAI, S. (1984b). Growth characteristics of immobilized yeast cells in continuous ethanol fermentation with forced substrate supply. *Journal of Fermentation Technology* **62**, 401–406.

MIYASHIRO, Y., MASAO, O., YAMAZAKI, Y. AND IGARASI, S. (1985). *Polysaccharide Beads*. US Patent 4,493,894.

MOHITE, U. AND SIVARAMAN, H. (1984). Continuous conversion of sweet sorghum juice to ethanol using immobilized yeast cells. *Biotechnology and Bioengineering* **26**, 1126–1127.

MORI, A. (1985). Production of vinegar by immobilized cells. *Process Biochemistry*, **20**, (3), 67–74.

MOULIN, G. AND GALZY, P. (1984). Whey, a potential substrate for biotechnology. In *Biotechnology and Genetic Engineering Reviews* (G.E. Russell, Ed.), volume 1, pp. 347–374. Intercept, Ponteland, Newcastle upon Tyne.

MOZES, N. AND ROUXHET, P.G. (1984). Dehydrogenation of cortisol by *Arthrobacter simplex* immobilized as supported monolayer. *Enzyme and Microbial Technology* **6**, 497–502.

MOZES, N. AND ROUXHET, P.G. (1985). Metabolic activity of yeast immobilized as supported monolayer. *Applied Microbiology and Biotechnology* **22**, 92–97.

MUALLEM, A., BRUCE, D. AND HALL, D.O. (1983). Photoproduction of H_2 and $NADPH_2$ by polyurethane-immobilized cyanobacteria. *Biotechnology Letters* **5**, 365–368.

NAGASHIMA, M., AZUMA, M., NOGUCHI, S. AND INUZUKA, K. (1984). Continuous alcohol production with immobilized growing microbial cells. *Arab Gulf Journal of Scientific Research* **2**, 299–314.

NAKAJIMA, H., SOSNOMOTO, K., USUI, N., SATO, F., YAMADA, Y., TANAKA, A. AND FUKUI, S. (1985). Entrapment of *Lavandula vera* cells and production of pigments by entrapped cells. *Journal of Biotechnology* **2**, 107–117.

NANBA, A., KIMURA, K. AND NAGAI, S. (1985). Vinegar production by *Acetobacter rancens* cells fixed on hollow fiber module. *Journal of Fermentation Technology* **63**, 175–179.

NILSSON, K. AND MOSBACH, K. (1984). A general method for entrapment of cells in beaded polymers. In *Biotech '84 (Europe)*, pp. 217–231. Online, Pinner, Middlesex.

NISHIO, N., KAYAWAKE, E. AND NAGAI, S. (1985). Rapid methane production from formate or acetate in fixed bed bioreactors. *Journal of Fermentation Technology* **63**, 205–209.

NOLL, L.A. (1985). *Cell Culture Using a Monolithic Support*. US Patent 4,514,499.

OKANO, M., IIDA, T., SHINOHARA, H., KOBAYASHI, H. AND MITAMURA, T. (1984). Water photolysis by a photoelectrochemical cell using an immobilized

chloroplast–methyl viologen system. *Agricultural and Biological Chemistry* **48**, 1977–1983.

OKITA, W.B., BONHAM, D.B. AND GAINER, J.L. (1985). Covalent coupling of microorganisms to a cellulosic support. *Biotechnology and Bioengineering* **27**, 632–637.

OKUHARA, A. (1985). Vinegar production with *Acetobacter* grown on fibrous support. *Journal of Fermentation Technology* **63**, 57–60.

ONAKA, T., NAKANISHI, K., INOUE, T. AND KUBO, S. (1985). Beer brewing with immobilized yeast. *Biotechnology* **3**, 467–470.

OSAKI, K., OKAMOTO, Y., AKAO, T., NAGATA, S. AND TAKAMATSU, H. (1985). Fermentation of soy sauce with immobilized whole cells. *Journal of Food Science* **50**, 1289–1292.

OSUGA, J., MORI, A. AND KATO, J. (1984). Acetic acid production by immobilized *Acetobacter aceti* cells entrapped in a κ-carrageenan gel. *Journal of Fermentation Technology* **62**, 139–149.

PARA, G., LUCCIARDI, P. AND BARATTI, J. (1985). Synthesis of L-tyrosine by immobilized *Escherichia intermedia* cells. *Applied Microbiology and Biotechnology* **21**, 273–279.

PARA, G., RIFAI, S. AND BARATTI, J. (1984). Production of L-dopa from pyrocatechol and DL-serine by bioconversion using immobilized *Erwinia herbicola* cells. *Biotechnology Letters* **6**, (11), 703–708.

POSILLICO, E.G. (1985). Microencapsulation technology for large-scale antibody production. *Bio/Technology* **4**, 114–117.

POTTS, M. AND BOWMAN, M.A. (1985). Sensitivity of *Nostoc commune* UTEX 584 (cyanobacteria) to water stress. *Archives of Microbiology* **141**, 51–56.

QURESHI, N. AND TAMHANE, D.V. (1985). Prodction of mead by immobilized whole cells of *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology* **21**, 280–281.

RAO, K.K. AND HALL, D.O. (1984). Photosynthetic production of fuels and chemicals in immobilized systems. *Trends in Biotechnology* **2**, 124–129.

ROBERTSON, B.W. AND PHILIPS, G.T. (1984). *Process for Immobilisation of Microorganisms on a Plastic Carrier on which Microorganisms have been Immobilized*. European Patent 0 112 597.

ROBINSON, P.K., DAINTY, A.L., GOULDING, K.H., SIMPKINS, I. AND TREVAN, M.D. (1985). Physiology of alginate-immobilized *Chlorella*. *Enzyme and Microbial Technology* **7**, 212–216.

ROSEVEAR, A. (1984a). Immobilized biocatalysts — a critical review. *Journal of Chemical Technology and Biotechnology* **34B**, 127–150.

ROSEVEAR, A. (1984b). *Immobilization of Biologically Active Material in a Hydrogel on a Support*. US Patent 4,452,892.

ROSEVEAR, A. AND LAMBE, C.A. (1985). Animal cells — a suitable case for immobilization? In *Biotech '85 (Europe)*, pp. 559–571. Online, Pinner, Middlesex.

ROSSI, J. AND CLEMENTI, F. (1985). L-Malic acid production by polyacrylamide gel entrapped *Pichia membranaefaciens*. *Biotechnology Letters* **7**, 329–334.

SAKATA, K. AND IMAI, K. (1985a). *Methods of Immobilizing Enzymatically Active Materials*. US Patent 4,525,457.

SAKATA, K. AND IMAI, K. (1985b). *Method of Immobilizing Microorganisms*. US Patent 4,547,463.

SATO, T., MORI, T., TOSA, T., CHIBATA, I., FURUI, M., YAMASHITA, K. AND SUMI, A. (1975). Engineering analysis of continuous production of L-aspartic acid by immobilized *Escherichia coli* cells in fixed beds. *Biotechnology and Bioengineering* **17**, 1797–1804.

SCHNABL, H. AND YOUNGMAN, R.J. (1985). Immobilization of plant cell protoplasts inhibits enzymatic lipid peroxidation. *Plant Science* **40**, 65–69.

SCHOUTENS, G.H., NIEUWENHUIZEN, M.C.H. AND KOSSEN, N.W.F. (1985). Continuous butanol production from whey permeate with immobilized *Clostridium beyerinckii* LMD 27.6. *Applied Microbiology and Biotechnology* **21**, 282–286.

SEISKARI, P., LINKO, Y-Y. AND LINKO, P. (1985). Continuous production of gluconic acid by immobilized *Gluconobacter oxydans* cell bioreactor. *Applied Microbiology and Biotechnology* **21**, 356–360.

SHULER, M.L. (1985). Immobilized *E. coli* produces rDNA products continuously. *Genetic Technology News* **5** (3) 4–5.

SHULER, M.L., PYNE, J.W. AND HALLSBY, G.A. (1984). Prospects and problems in the large scale production of metabolites from plant cell tissue culture. *Journal of American Oil Chemists Society* **61**, 1724–1728.

SWANN, W.E. (1985). *Vermiculite as a Carrier Support for Immobilized Biological Materials*. US Patent 4,504, 582.

TAKAMATSU, S., YAMAMOTO, K., TOSA, T. AND CHIBATA, I. (1981). Stabilization of L-aspartate β-decarboxylase activity of *Pseudomonas dacunhae* immobilized with carrageenan. *Journal of Fermentation Technology* **59**, 486–493.

TAMPONNET, C., COSTANTINO, F., BARBOTIN, J-N, AND CALVAYRAC, R. (1985). Cytological and physiological behaviour of *Euglena gracilis* cells entrapped in a calcium alginate gel. *Physiologia plantarum* **63**, 277–283.

TAXIS DU POET, P. DE, DHULSTER, P. AND THOMAS, D. (1984) Stability of the plasmid pTG 201 during continuous culture of *Escherichia coli* BZ 18 immobilized in a gel of carrageenan, without selection pressure. *Comptes rendus des séances de l'Académie des sciences, Paris* **229** Ser III (11) 481–486.

THEPENIER, C., GUDIN, C. AND THOMAS, D. (1985). Immobilization of *Porphyridium cruentum* in polyurethane foams for the production of polysaccharide. *Biomass* **7**, 225–240.

TRAMPER, J. (1985). Immobilizing biocatalysts for use in syntheses. *Trends in Biotechnology* **3**, 45–50.

TRAMPER, J., SUWINSKA-BOROWIEC, G. AND KLAPWIJK, A. (1985). Characterization of nitrifying bacteria immobilized in calcium alginate. *Enzyme and Microbial Technology* **7**, 155–160.

TRAMPER, J., VAN DER PLAS, H.C. AND LINKO, P. (1985). Biocatalysis in organic synthesis. *Studies in Organic Chemistry, Vol. 22. International Symposium on Biocatalysts in Organic Syntheses, Noordwijkerhout, Netherlands, 14–17 April 1985*. Elsevier, Amsterdam.

TULI, A., SETHI, R.P., KHANNA, P.K., MARWAHA, S.S. AND KENNEDY, J.F. (1985). Lactic acid production from whey permeate by immobilized *Lactobacillus casei*. *Enzyme and Microbial Technology* **7**, 164–168.

VAN HAECHT, J.L., BREMAEKER, M. DE AND ROUXHET, P.G. (1984). Immobilization of yeast by adhesion to support without use of a chemical agent. *Enzyme and Microbial Technology* **6**, 221–227.

VAN HAECHT, J.L., BOLIPOMBO, M. AND ROUXHET, P.G. (1985). Immobilization of *Saccharomyces cerevisiae* by adhesion: Treatment of cells by Al ions. *Biotechnology and Bioengineering* **27**, 217–224.

VOGEL, H.J. AND BRODELIUS, P. (1984). An *in vivo* P NMR comparison of freely suspended and immobilized *Catharanthus roseus* plant cells. *Journal of Biotechnology* **1**, 159–170.

VORLOP, K-D. (1985). Preparation and application of single enzyme systems in immobilized non-living cells. In *Biotech '85 (Europe)*, pp. 587–596. Online, Pinner, Middlesex.

WILLETTS, A. (1985). Butane 2,3-diol production by immobilized *Aeromonas hydrophila*. *Biotechnology Letters* **7**, 261–266.

WOOD, L.L. AND CALTON, G.J. (1984a). A novel method of immobilization and its application in aspartic acid production. *Biotechnology* **2**, 1081–1084.

WOOD, L.L. AND CALTON, G.J. (1984b). *Immobilized Microbial Cell Composition for Making L-Aspartic Acid*. US Patent 4,436,813.

YAMADA, T., SAZANAMI, T., WATANABE, K., IIDA, T., HASEGAWA, E. AND SAKAMOTO, M. (1985). *Continuous Alcohol Fermentation Process Using Immobilized Yeast*. US Patent 4,546,081.

YAMAMOTO, K., TOSA, T. AND CHIBATA, I. (1980). Continuous production of L-alanine using *Pseudomonas dacunhae* immobilized with carrageenan. *Biotechnology and Bioengineering* **22**, 2045–2054.

YAMAMOTO, K., TOSA, T., YAMASHITA, K. AND CHIBATA, I. (1976). Continuous production of L-malic acid by immobilized *Brevibacterium ammoniagenes* cells. *European Journal of Applied Microbiology* **3**, 169–183.