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Immobilized Cell and Enzyme Technology [and Discussion]

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Immobilized cell and enzyme technology

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The development of immobilized enzyme and cell technology is summarized. Industrial processes for sucrose inversion, penicillin deacylation and glucose isomerization using immobilized enzymes are described. An alternative process for glucose isomerization using immobilized cells, and some other industrial applications of immobilized cells are indicated. Recent developments in immobilized enzyme and cell technology are assessed and the relative merits of the different biochemical catalyst forms are considered.

Introduction

The modern fermentation industry has a $£3 \times 10^9$ worldwide market built upon the catalytic activity of microbial cells growing freely in nutrient solutions. The much smaller enzyme catalysis industry has classically employed a few soluble enzymes that are excreted by micro-organisms. Biological catalysts can be used in other forms, and, in the last 10 years, these have begun to make their own practical impact. The alternative forms share the feature that the enzyme or cell is deliberately associated with another, larger structure. Cells trapped in slime layers and enzymes bound to soil particles are examples of natural association that are well known and have their own uses in waste treatment and in agriculture. However, for 70 years people have sought to immobilize enzymes and, recently, cells by better defined and more permanent interactions.

The first conscious attempt to immobilize an enzyme was evidently made by Michaelis & Ehrenreich (1908). The enzyme, invertase, was adsorbed on charcoal, but preparations of this and other immobilized enzymes were of variable and often very low activity. Better results had to await the dramatic advances in enzyme chemistry that occurred after World War II. With use of this new knowledge, enzymes were immobilized by covalent binding, adsorption or ion exchange. Alternatively, they were entrapped in gels, encapsulated or simply cross linked with other enzyme molecules to form particles (Mosbach 1976; Wingard *et al.* 1976; Chibata 1978*a*; Ghose *et al.* 1978; Broun *et al.* 1978). In the same post-war period, a few pioneers applied entrapment, in particular, to the immobilization of microbial cells, but there were far fewer studies of immobilized cells than of immobilized enzymes (Jack & Zajik 1977). For both the immobilized enzymes and the immobilized cells, the main technical objective was more readily to retain or retrieve the catalyst. With newer, refined techniques it was found that immobilized enzymes could be more operationally stable than the corresponding free enzyme. This was a bonus, added to the ease of retention. Catalyst half lives of many days and sometimes many weeks are now possible with immobilized enzymes. In some instances, long catalyst half lives are also observed with immobilized cells, but the reasons for this can be quite complicated.

Today, with over 1000 publications and more than 100 patents on immobilized enzymes and cells, the tangible practical results and the prospects may be assessed. The results can be illustrated best by a few examples of the commercial application of immobilized enzymes and cells.

Industrial immobilized enzyme technology

The first industrial application of immobilized enzymes was evidently with the same charcoal-adsorbed invertase that appeared in the original Michaelis & Ehrenreich (1908) paper. In the 1940s the Tate and Lyle Company used adsorbed invertase for sucrose inversion on a reactor scale still unsurpassed in any application (British Patent 564,270). Bone char columns 10 ft (3.05 m) in diameter and 20 ft (6.10 m) deep contained 40 t of charcoal and were used to decolorize golden syrup in the conventional process. In a modified process the columns were partially preloaded with invertase produced by simple plasmolysis of yeast. Six columns decolorized and inverted 32 000 gal (145.5×10^3 l) of syrup during each 6-day working week. The process gave significant cost economies over the normal batch inversion, but continuous operation was difficult to link to batch steps downstream. There were product variations resulting from lack of reproducible immobilization, microbial growth and the absence of process control with the non-purpose-built reactors. These types of biochemical engineering problems, which are not resolved fully even today, contributed to the gap of 20 years before a second generation of commercial reactors was operated in a related field.

The largest tonnage, and probably the most widely known current application of immobilized enzymes, is for the partial isomerization of glucose to fructose to give a low-cost sweetener (Bucke 1977). This is a reaction not satisfactorily brought about by chemical means. The development is less favoured in Europe, where beet sugar is the major local sweetening commodity, than in the U.S.A., with its great starch-producing capability. Despite the extraordinary range of published methods of immobilization, a rather small number of simple ones have been used to immobilize glucose isomerase and other industrially applied enzymes. This is principally because of Government regulatory and cost considerations. Early immobilized glucose isomerase processes employed ion exchange binding to cellulosic supports. Other processes have centred on the use of glutaraldehyde cross linking of crude enzymes. Glucose isomerization is notable for the high concentration of the glucose feed (up to 45 % dry solids), which illustrates that immobilized enzyme conversions are not inherently limited to dilute feedstocks. A single plant, that of A. E. Staley in the U.S.A., has capacity of 450 000 t/a of high fructose syrup and is said to be the most highly computer-controlled food plant in the world (Homan *et al.* 1978). Such developments, together with the increasingly stringent American regulations on additives (Roland 1979), including free enzymes, are creating considerable interest in immobilized enzymes for the food industry.

The major application of immobilized enzyme technology in the pharmaceutical industry has been in the conversion of benzyl penicillin, produced by fermentation, to 6-aminopenicillanic acid, for the preparation of semi-synthetic penicillins. This conversion was originally made by the use of free *E. coli* cells containing the necessary enzyme. They were used once and then discarded. This was a rather cumbersome procedure, and material from the cells could be carried through into the product stream. In the early 1970s procedures for the large-scale isolation and immobilization of the single enzyme involved, penicillin acylase, were developed (Balasingham *et al.* 1972), and the kinetics of immobilized enzyme reactors were studied

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(Warburton *et al.* 1973). Following much subsequent industrial research and development, Beecham Ltd and other companies now use immobilized enzyme processes for commercial production of semi-synthetic penicillins. The released acid must be neutralized in a stirred reactor in such a way that the alkali used does not damage the penicillin or the enzyme. Equally, the powerful mixing employed must not lead to destruction of the enzyme support. The immobilized enzyme process now competes effectively with a chemical process that had begun to displace biochemical conversion, and figure 1 illustrates some of the reasons. To achieve the conversion chemically, a several-stage process under low temperature, strictly anhydrous conditions, with the use of a number of chemical reagents, is required. The virtues of a mild one-stage enzymic conversion are well illustrated here.

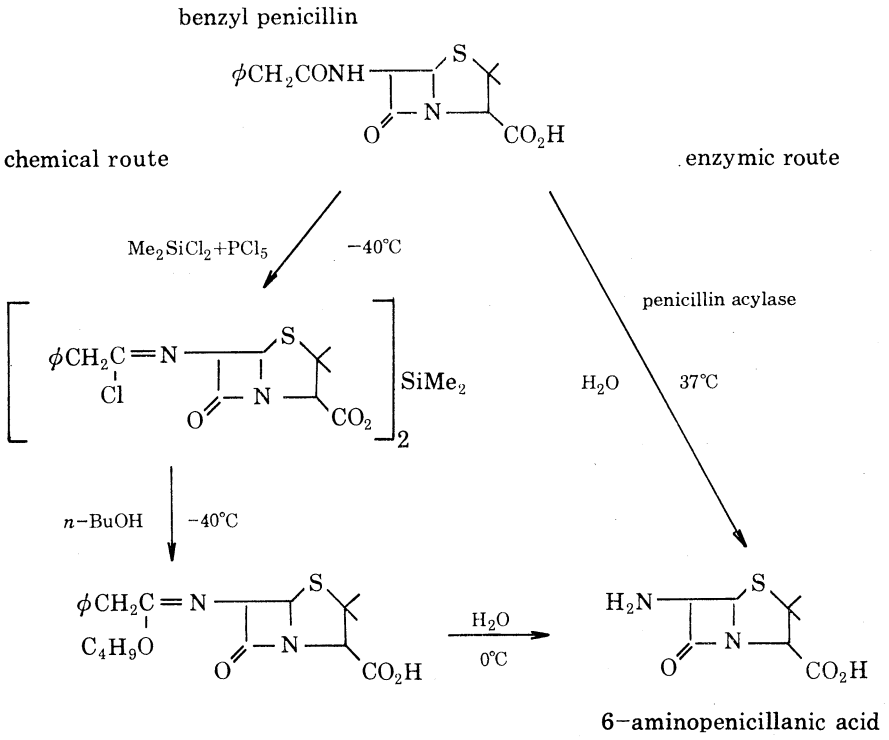

FIGURE 1. Chemical and enzymic methods for the conversion of benzyl penicillin to 6-aminopenicillanic acid.

The great advantage of stereoselectivity, which enzyme catalysts exhibit, is beginning to be made use of in the chemical industry, particularly in the production of specific optical isomers, for example, in the industrial conversion of racemic mixtures of amino acids, produced chemically, to L-isomers. This has been followed by production of derivatives such as D-phenyl glycine and D-parahydroxyphenylglycine. These D,L-amino acids are converted, via methyl esters, to the amides, the L-form of which can be selectively reconverted to the L-acid by an enzyme. The D-amide and L-acid may then be separated. The D-amide can be hydrolysed to the desired product, while the L-acid is racemized for further conversion (J. J. Dahlmans, personal communication). In another chemical industry process, producing urea, residual urea in the waste water is converted to recoverable ammonia by immobilized urease. By using countercurrent catalyst flow through multiple fluidized beds, the urea level in the effluent water is reduced to

10 parts per million (J. J. Dahlmans, personal communication). Munnecke (1977) has immobilized a pesticide-hydrolysing enzyme produced from a mixed bacterial culture grown in the presence of the insecticide parathion. It hydrolyses eight other insecticides and an assessment of the requirements for industrial treatment of pesticide residues in waste water was made. Several industrial processes using immobilized enzymes exploit the advantage of being able to produce supported enzymes by fermentation and subsequent enzyme isolation and immobilization in one country and then to use them in a simpler facility in another country.

Immobilized enzymes are beginning to have an impact upon clinical analysis, where their multiple reuse offers the prospect of reducing the considerable cost of high-purity enzymes and of those reagents that can be generated enzymically (Guilbault 1976). Immobilization of enzymes is also promising for the therapeutic use of enzymes, where it can reduce the problems of allergic response and short half-life *in vivo* that are observed with free enzymes (Chang 1977).

Industrial immobilized cell technology

Of the current industrial processes using immobilized cells, that for the production of fructose-enriched glucose is again a major one. I.C.I. Ltd uses *Arthrobacter* cells flocculated by polyelectrolyte. The cells are supplied in the form of dry cylindrical pellets and hydrated prior to transfer to a column reactor. Each kilogram of catalyst converts more than 2000 kg of glucose-syrup (both expressed as dry solids). This illustrates the need to look at the mass and the value of the product, not the catalyst, during assessment of the importance of immobilized cell or enzyme technology.

The best known industrial immobilized cell process is that of the Tanabe Seiyaku company, for the stereoselective conversion of fumaric acid to L-aspartic acid. It employs *E. coli* cells trapped in polyacrylamide and subsequently treated so as to destroy the normal cell permeability barrier. The half-life of the immobilized cell catalyst is 120 days, and the cost of production of aspartic acid by this method has been estimated to be 40 % less than that by the conventional process employing freely suspended cells (Chibata 1978*a*). Another process originating in Japan has been described as involving immobilized cells. In fact, it uses pellets of the mould *Mortierella vinacea* excreting α-galactosidase to hydrolyse raffinose, which contaminates beet sugar (McGinnis 1975). It is true that the size of the pellets permits their retention and reuse, but this system is on the borderline between the novel and the conventional. The process, operating with 2000 t of beet per day, does not differ greatly from processes using autoflocculative yeasts in a tower fermenter. The approach is restricted to conversions where the intrinsic diffusional limitations associated with large aggregates can be tolerated.

These are some of the immobilized enzyme and cell processes in commercial operation. Recent research developments and prospects for the future will be examined next.

Enzyme technology research

Classical microbial genetics has been a most important tool in industrial enzyme production. Recently, more advanced techniques have begun to be developed. For example, Silhavy *et al.* (1976) have fused the *E. coli* β-galactosidase gene to a gene for outer membrane protein. They have produced a membrane-associated enzyme activity and it may eventually be possible to

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create an extracellular β-galactosidase from *E. coli*. Excreted microbial enzymes are easier to recover and to isolate in a useful form.

The difficulty and cost of enzyme isolation is obviously one of the major obstacles to the use of immobilized enzyme catalysts. Recent studies have established methods for the production of the majority of water-soluble cytoplasmic enzymes from microorganisms (Charm & Matteo 1971; Atkinson 1973; Dunnill 1978). The cost of these enzymes need not be greatly different now from those of excreted enzymes such as the amylases. In processes such as penicillin deacylation, the intracellular enzyme cost is not a dominant factor now that considerable catalyst half-lives are achieved with the immobilized enzyme. However, many of the conversions such as hydroxylations are catalysed by membrane-associated microbial enzyme complexes. Their large-scale isolation is proving to be a currently intractable problem, but one that may yield to study during the 1980s (Dunnill 1979).

Even when membrane-associated enzymes become available, they, like some of the larger water-soluble enzymes, probably will be of more limited stability than the lower-molecular-mass enzymes now in commercial use. The immobilization of enzymes does generally confer greater operational stability upon them; however, it would be even better if they could be further stabilized in a step distinct from immobilization, and this may be possible in several ways (Klibanov 1979). Methods are available by which all the amino acid residues of one type on an enzyme surface can be modified. For example, the lysine residues may be modified by acetamidination, which evidently stabilizes several enzymes by altering the condition of lysine ionization (Tuengler & Pfleiderer 1977). Alternatively, an enzyme may be stabilized by cross-bridging. At present, bridging is mostly done between the active site and a neighbouring point. However, techniques such as combined thermochemical and photochemical linkage can, in principle, be applied successfully at other sites on the enzyme surface (Guire 1978), particularly when full three-dimensional structures are available as a guide.

A radical alternative to the isolation and stabilization of the more complex enzymes is suggested by the recent studies of Kaiser and his coworkers (see, for example, Levine & Kaiser 1978). They took a simple and cheap enzyme, papain, and covalently attached a flavin molecule to the edge of the active site. The objective was to retain the stereoselectivity and reactant binding properties of the active region while creating a flavin oxido-reductive enzyme. Their most recent work shows that one such semi-synthetic flavopapain binds reduced nicotinamide substrates with considerable specificity. It catalyses oxidation with modest but significant rate accelerations compared to simple flavin compounds. The preparation of semi-synthetic enzymes is now well documented (Offord & Di Bello 1978). Together with derivatives of the above-mentioned type or of protein complexes of metal coordination compounds (Wilson & Whitesides 1978), this may lead to the development of ultrastable semi-synthetic catalysts of a form that at present can only be hinted at.

Natural or semi-synthetic flavin enzymes can perform their function without the involvement of a complex dissociable cofactor. However, many enzymes do carry out their catalytic role with the aid of such cofactors. It is not clear yet whether these enzymes and their cofactors isolated from the cell can provide the basis of economic processes. Synthesis of the decapeptide antibiotic Gramacidin S requires the expenditure of one ATP molecule for each of the ten peptide bonds formed. Gramacidin S has been synthesized in gram quantities by means of a pair of synthesizing enzymes and an enzymic ATP regeneration system. Subsequent economic analysis indicated that, at present, a product would have to be extremely valuable to justify

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this approach commercially (Wang *et al.* 1977). However, it is now possible to coimmobilize a pair of oxido-reductive enzymes with a cofactor that they share and recycle. This approach, when refined, may give better cofactor stability and utilization (Mansson *et al.* 1979).

It is increasingly realized that immobilized enzyme or cell catalysis need not be restricted to water-soluble materials. For example, the interesterification of fats in organic solvents and the dehydrogenation of steroids in mixed aqueous–organic solvents (Carrea *et al.* 1979) have both been achieved by means of immobilized enzymes.

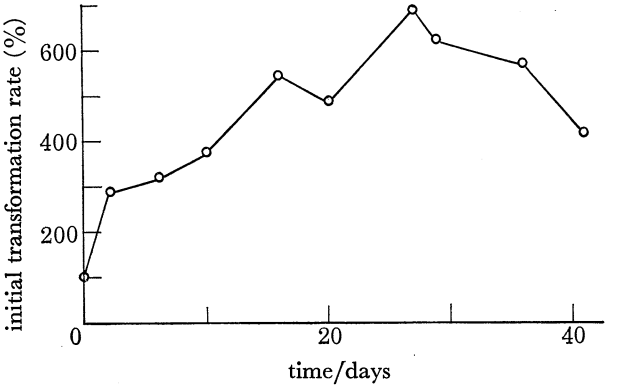

FIGURE 2. Transformation of cortisol to prednisolone by immobilized *Arthrobacter simplex* cells in 10 g/l peptone medium. Initial 3-ketosteroid Δ^1-dehydrogenase activity is set at 100 %.

IMMOBILIZED CELL RESEARCH

As indicated earlier, conscious efforts to immobilize cells are not new. Nevertheless, serious study only followed the intensive period of work, in the late 1960s, on enzyme immobilization. In 1973 there were 220 publications on immobilized enzymes, but only 7 on immobilized cells. In 1977 there were over 50 publications on immobilized cells and several on immobilized organelles (Kierston & Bucke 1977; Yang *et al.* 1976). Most of the published work has described techniques for entrapping cells within gels. To develop immobilized cell technology further it will be necessary to understand the nature of the cells after this type of procedure. It is already clear that what are described as 'immobilized cells' vary widely in their nature. In some instances the cells have been lysed before addition of entrapping agent and are really cell homogenates. In other cases, reagents that destroy the integrity of the cell membrane are added after entrapment. However, the cells can remain viable, and the consequences of this are strikingly illustrated in figure 2. Entrapped *Arthrobacter simplex* cells were used over a period of days for the continuous dehydrogenation of the steroid cortisol. Their activity does not fall initially; indeed it rises steadily, provided that nutrient is supplied with the steroid feed. Tests (Ohlson *et al.* 1978) confirmed that the cells were replicating within the gel. So the catalytic activity observed was partially from newly synthesized enzyme. For some days this more than compensates for any enzyme inactivation occurring with time, and this behaviour has recently permitted the establishment of a commercial process (Ger. Offen 2,729,490 1978). Information on why the activity finally declines is provided by the work of Somerville *et al.* (1977). They took electron micrographs of gel-entrapped *Pseudomonas putida* mutant cells that were carrying out a limited oxidation of benzene. They repeated the micrographs after 21 days passage of benzene and nutrient. The sequence of micrographs indicated that cell replication occurs, but

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reaches a point at which crowding leads to the implosion of cells under the considerable force of cell osmotic pressure. Their work suggested that regulation of nutrients fed with the reactant could delay the self-destructive phase. However, it may be necessary to bear this cell crowding effect in mind when selecting entrapping polymers. Table 1 shows the relative compressive strengths of some of the polymer beads used for entrapment (Klein & Wagner 1978). Those at the lower end of the table were prepared to counter the mechanical weakness of polyacrylamide, the most widely used entrapping support. This weakness is a disadvantage in industrial reactor operation. On the other hand, the strong gels may permit very little replication prior to cell implosion.

TABLE 1. CRITICAL FORCE (RELATIVE UNITS) FOR THE RUPTURE OF A SINGLE POLYMERIC BEAD IN A COMPRESSIVE EXPERIMENT (KLEIN)

polymer	force
polyacrylamide	10
carrageenan	10
alginate	100–800
epoxide	600–1000
polystyrene–divinylbenzene resin	$\geqslant$ 900

One of the potential advantages if immobilized cells do not have to replicate is that they can function under conditions that inhibit growth and functioning of metabolizing free cells. For example, Buckland *et al.* (1975) showed that wet packed *Nocardia* cells could act upon cholesterol dissolved in water-immiscible organic solvents such as hexane and carbon tetrachloride. This permitted conversion of cholesterol to cholestenone at feed concentrations 100-fold greater than that possible with an aqueous cell suspension. After seven reuses the cells still retained 50% of their initial catalytic activity, evidently because the cellular enzymes were still in an aqueous environment. Recently, Yamane *et al.* (1979) have extended this approach by entrapping the *Nocardia* cells in gels by photo-crosslinking techniques and have observed an extended life of the cells for a related steroid conversion. Since many of the reactants of interest to the chemical industry are water-insoluble, developments in this direction could open up quite new applications for cell catalysis.

The use of mixed cultures of growing microorganisms has found only limited industrial application. Satisfying the requirements for cell growth and product formation with more than one metabolizing microorganism is not easy, but it may be more feasible to take the cells, once grown, and to find conditions where mixtures of immobilized cells can be used. Martin & Perlman (1976) developed a mixed *Gluconobacter–Pseudomonas* immobilized cell process to convert sorbose to 2-ketogulonic acid, a precursor of ascorbic acid. The presence of the *Pseudomonas* prevented inhibition of the conversion by an intermediate, sorbosone.

Recently, Kokubu *et al.* (1978) have reported the production of α-amylase by immobilized cells of *Bacillus subtilis*. Thus, there is now a prospect of immobilized cell catalysis yielding enzyme catalysts over long periods. The latter may then be used in situations in which macromolecular or particulate reactants preclude effective action of immobilized enzymes or cells (Dunnill 1979).

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Immobilized cell technology *versus* fermentation

In assessing the importance of immobilized cells, it is necessary to bear in mind that as much as 60% of the present industrial fermentation market is associated with antibiotics. Few significant studies of secondary metabolite production by immobilized cells have yet been published (Egerov *et al.* 1978; Morikawa *et al.* 1979*a*, *b*), and maintenance of the proper physiological state of the immobilized cell in such a situation will probably be more difficult than for simple conversions. In addition, fermentation technology is advancing rapidly. For example, some antibiotics companies use fed-batch operation, where a proportion of the cells of a fully developed fermentation is repeatedly discharged and replaced by concentrated nutrient. The maximum rate of antibiotic synthesis can be increased up to twofold and the synthesis maintained for a much longer period. Such fermentations can be very unstable, but modern, computer-assisted control may ease this problem. Adequate oxygen transfer is required to utilize fully the concentrated nutrient and this may also become a limiting factor. There is little experience of how dissolved oxygen levels affect immobilized cells utilizing nutrients, though, in the steroid dehydrogenation mentioned earlier, it was found that cortisol conversion to prednisolone was increased from 45 to 70% with increased dissolved oxygen in the nutrient feed (Ohlson *et al.* 1978).

It may be that attachment of metabolizing cells to larger particles, combined with conventional fermentation, will be significant (Atkinson *et al.* 1979). In continuous culture it can prevent washout. In batch culture it may permit more efficient operation. Immobilization allows the use of dense microbial populations by altering the rheological properties of the suspension. When microorganisms are agglomerated or attached to a relatively large solid support, fluid viscosity is lower than when comparable numbers of cells are freely suspended in solution. Lower viscosities contribute to better mixing and mass transfer properties in the fermenter (Abbott 1977).

Some key processes that are traditionally associated with conventional fermentation may yet feel the impact of immobilized cell technology. Recently, there have been a number of publications on the use of immobilized yeast cells for ethanol production. In one of these, Chibata (1978*b*) reports a yeast cell concentration of 10^9 ml^{-1} in the gel, about 50% yeast cells, giving a continuous 10% ethanol output, undiminished after a month of operation.

Immobilized cells and waste water treatment

Just as cell immobilization has interesting relations with industrial fermentation, so it does with biological waste treatment. Cells trapped in the slime layers of trickle-bed filters have already been mentioned. In the complementary activated sludge method of waste treatment, it is the flocculation of cells that permits their economic recycling. The enhancement of natural flocculation by the use of synthetic polyelectrolytes can further improve this partial cell immobilization. Several organizations have immobilized cells with a view to removing particular substances from waste water. At East Hyde Sewage Works, waste water is forced through a sand bed on which species such as *Hyphomicrobium* are deliberately grown with added methanol to cause nitrate reduction. Another plant at Rye Meads, processing 81000 m^3 of water per day, uses a similar technique, but without methanol addition. Nitrate reductions between 50% and 90% are obtained (Anon 1977). Mohan & Li (1974) examined the use of *Micro-*

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coccus dentrificans cells encapsulated in liquid membranes for the reduction of nitrate and nitrite. The procedure, involving emulsification in the presence of cells, oil, surfactant and membrane-strengthening additives, gave capsules containing 500–600 cells, which retained 78 % of their activity for 120 h, compared with zero remaining activity in 16 h for the free cells. The immobilized cells also tolerated 10^{-4} M mercuric chloride, in strong contrast to the sensitive free cells. It remains to be seen whether the requirement of very low cost can be met by this approach. Immobilized cells have recently been used in a sensor continuously to evaluate the biological oxygen demand of waste water (Karube *et al.* 1977).

Immobilized cells *versus* immobilized enzymes

Comparison of the merits of immobilized cells versus immobilized enzymes can be made according to several criteria. As already indicated, immobilized cells at present appear more suited to multi-step conversions, especially with dissociable cofactors. In the laboratory, at least, reports of reactions such as the five stage synthesis of coenzyme A from pantothenic acid by means of immobilized cells (Shimizu *et al.* 1975) are becoming increasingly common. Only time will tell whether industrial technology can be built up from these demonstrations of principle. Industrial users will be very concerned with the purity and yield of products that they can obtain by biological catalysis. As the reaction time is increased to raise yield, there will be a tendency for cell catalysts to produce secondary products. This can only partially be countered by genetic selection of the cell type and is a weakness of fermentation. With the reuse of immobilized cells, it is possible to contemplate treating the cell with reagents that will inhibit or destroy unwanted enzymes. This approach has been adopted by Chibata *et al.* (1978*a*), who added detergents to destroy enzymes producing succinic acid in the immobilized *Brevibacterium* that they employed for commercial malic acid synthesis. A different problem of product purity that troubles those contemplating the use of immobilized enzymes and cells in the food and pharmaceutical industries is the possibility of leaching of catalyst or support into the product. This is potentially more serious with cells than with enzymes and with the monomers used in gel entrapment than with other supports.

In any catalytic reactor, it is convenient to have a high catalyst site density, which will permit a large conversion flux per unit reactor volume. This is more readily achieved with a purified enzyme than with a cell containing relatively low concentrations of the enzyme. Immobilized cells maintaining any cell integrity will also exhibit diffusional resistance to reactant access. However, because of very high catalyst site densities, enzymes immobilized in porous supports can suffer diffusional resistance to an even more severe extent (Dunnill 1979).

The balance of advantage between immobilized enzymes and immobilized cells can only be determined with certainty by a very detailed study of any particular conversion. More broadly, there will still be circumstances in which free enzymes are used and are intrinsically lost in the product, as with amylases in bread. There will be situations in which the cost of immobilized cells will not be justified and conventional fermentation will be appropriate, but it is an exciting prospect that the reuse of elegant enzyme catalysts or their even more sophisticated cellular complexes can be extended. With man-made alterations to enzymes it may be possible to develop quite new catalytic processes. In this field the horizons of microbiology and microbial enzymology are rapidly widening.

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Discussion

B. W. Langley (*I.C.I. Corporate Laboratory, P.O. Box* 11, *The Heath, Runcorn WA*7 4*QE, Cheshire, U.K.*). How dry are these non-aqueous systems?

P. Dunnill. The enzymes still function in an aqueous environment, while the organic phase provides a sink for the reactant and products, which diffuse rapidly across the thin aqueous layer. If the system is totally dry the enzyme will not function.

B. S. Hartley, F.R.S. (*Department of Biochemistry, Imperial College, London S.W.*7). Surely it would be a good idea to develop methods of genetic manipulation for an organism that behaves well

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in your entrapment system, as it should be a relatively easy task to raise the level of a specific enzyme to account for from 2% to 5% of the soluble cell protein?

P. Dunnill. Yes, I agree that it would be a good idea. In fact, when we worked with a β-galactosidase super-producing strain of *E. coli*, we found that on scale-up to the 1000 l stage low cell densities were obtained; the total flux through the system was therefore less than that obtained for a simple constitutive organism. Moreover, the β-galactosidase super-producers were also less mechanically robust than the parent strain.

P. Gould (*BP Research Centre, Chertsey Road, Sunbury-on-Thames, Middlesex TW*16 7*LN, U.K.*). Has there been any progress in developing immobilized enzyme systems that can introduce functional groups (e.g. COOH, CO, OH or NH_2) into specific sites on alkyl chains? While chemical methods do exist for introducing these groups, they are so non-specific as to be very wasteful of the substrate.

P. Dunnill. Most of these enzyme systems are membrane-bound, and we do not at present have the technology to prepare them in immobilized form; however, work on such systems is in progress.

P. Gould. Since the cost of purification of intracellular enzymes has in the past proved prohibitive, could Dr Dunnill tell us about the new methods of large-scale purification?

P. Dunnill. There have been no dramatic improvements in techniques. A few years ago affinity techniques looked exciting, but the results have been disappointing, although reverse affinity chromatography (i.e. the removal of impurities) can be of considerable use at a late stage in purification. Groups in Germany believe that two phase liquid–liquid fractionation may be a promising technique. The cost of enzyme purification often does not constitute a large percentage of the final cost of the bulk chemical product.

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