Enzymreaktoren: Gegenwärtiger Stand und Zukunftsentwicklungen

In den Tagen vom 10. bis 14. September 1974 veranstaltete der Schweizerische Chemiker-Verband in der Mustermesse in Basel die ILMAC 74, d. h. die 6. Internationale Fachmesse für Laboratoriums- und Verfahrenstechnik und Meßautomatik in der Chemie. Gleichzeitig fand in Sälen der Mustermesse das 4. Schweizerische Treffen für Chemie statt, das zugleich die 146. Veranstaltung der Europäischen Föderation für Chemie-Ingenieur-Wesen war. Der Nachmittag des 10. September war einem Symposium über Enzymreaktoren gewidmet, das von der Schweizerischen Chemischen Gesellschaft und der Schweizerischen Gesellschaft für Biochemie organisiert worden war.

Die 4 Vorträge, die an diesem Symposium von eingeladenen Spezialisten über das aktuelle und zukunftsreiche Gebiet des Einsatzes von Enzymen in der Industrie gehalten wurden, finden sich nachfolgend ungekürzt abgedruckt.

Enzymes as Industrial Catalysts*

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Summary

This paper outlines the progress which has been made in extending the utility of enzymes as catalysts by developing efficient methods for their production and isolation and by immobilizing them on solid carriers. The properties of immobilized enzymes are discussed in relation to technological and economic requirements.

Introduction

Enzymes are outstanding macromolecular catalysts notable for their high activity under mild conditions and their specificity. These two properties are essential for the efficient and orderly course of reactions in living cells and may be used to advantage in the laboratory and in industry. Thus specific transformations can be effected in mixtures as complex as biological fluids, or in molecules which offer more than one site of attack to less selective reagents. The use of enzymes makes it possible to avoid high reaction temperatures and corrosive conditions and to carry out stereospecific reactions and other transformations which are difficult to effect by other means.

Of the two thousand known enzymes[1] about 150 are available commercially in milligram to kilogram quantities, and are used mainly in analysis, medical diagnosis and research. Only a few are produced and used on an industrial scale[2] as shown in *Table 1*. A large fraction of these proteins are extracellular hydrolases of microbial origin. They are excreted by selected strains of microorganisms into their environment in copious quantities, are easy to isolate and therefore relatively cheap[2].

The broad catalytic potential of microbial cells resides in the large number of intracellular enzymes which individually represent only a small fraction of the biomass. Extending their utility hinges on reducing their production and isolation costs and designing methods for their recovery and efficient use. This paper outlines the progress which has been made in these areas with emphasis on the immobilization of enzymes on solid carriers and on the industrial utility of these novel heterogeneous catalysts.

The Nature of Enzymes and their Properties

Enzymes are classified into six major groups according to the type of reaction they carry out: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases[1]. They are composed of one or more macromolecular chains $H(-NH\cdot CHR\cdot CO-)_nOH$ made up 21 different L-α-amino acids. These chains are folded in a specific way into compact globular shaped molecules, with the hydrophobic groups inside, and the hydrophilic groups ($R = CH_2OH$, $-CH_2CO_2H$, $-CH_2C_6H_5OH$, $CH_2CH_2CH_2CH_2NH_2$, for example) on the external surface. This surface has a cleft or cavity which accommodates the substrate during the transformation, the active site. Three dimensional models of lysozyme and other proteins are shown in the work of Dickersen and Geis[3]. The exposed groups outside this active center are nonessential for activity and may be used to bind proteins to other molecules and solid carriers. Since enzymes carry acidic and basic groups on their surface, they are positively or negatively charged, depending on the *p*H of the medium, and are adsorbed on ion exchangers and other substances. Hydrophobic adsorbents and organic solvents with a high affinity for the burried hydrophobic

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* Presented at the Symposium on Enzyme Reactors, organized by the Swiss Chemical Society and the Swiss Biochemical Association at the 4th Swiss Chemical Convention, September 10, 1974 in Basel.

Table 1. Enzymes Produced and Used on an Industrial Scale[a]

Enzyme	Principal source	Principal application
Carbohydrases		
Bacterial amylase[b]	*B. subtilis*	Starch liquifaction for glucose production, textile desizing and paper industry
Amyloglucosidase[b]	*Asp. niger*	Glucose production from liquefied starch
Fungal amylase[b]	*Asp. oryzae*	Corn syrup production, digestive aid
Invertase	*Sacharomyces cerevisiae*	Production of invert sugar from sucrose
Pectinase[b]	*Asp. niger*	Clarification of fruit juice
Cellulase[b]	*Asp. niger* *Trichoderma viridae*	Improvement of wood pulp, removal of vegetable matter from wool
Proteases		
Pancreatin	Mamalian pancrease	Digestive aid
Bromelain	*Ananas comosus*	Digestive aid, antiinflamatory preparations
Papain	Papaya fruit	Meet tenderizing, chill proofing of beer
Fungal protease[b]	*Asp. oryzae*	Bread baking
Pepsin	Pig stomach	Milk clotting, digestive aid
Rennin	Calve stomach *Rhizopus sp.*	Cheese manufacture
Bacterial protease[b]	*B. subtilis*	Additive to detergents, bread baking
Aminoacylases		
L-Aminoacylase[b]	*Asp. oryzae*	Resolution of D,L-amino acids into the stereoisomers
Penicillin acylase	*E. coli*	Production of 6-aminopenicillanic acid from penicillin G
Isomerases		
Glucose isomerase	*Streptomyces sp.*	Isomerization of glucose to fructose
Oxidoreductases		
Glucose oxidase	*Asp. niger*	Food preservation

[a] Source: Wolnak and Associates[2], [b] extracellular microbial enzyme

groups may break up the conformation of the folded chain with an irreversible loss of activity. Heat, acids and bases have a similar effect and enzymes are therefore stable at only moderate or low temperatures and within a restricted *p*H range. Chemical modification of the essential groups in the active site also leads to a loss of activity.

The kinetics of many enzymatic reactions follow the Michaelis-Menten equation

$$-\frac{dS}{dt} = \frac{k\cdot E\cdot S}{K_m + S} = \frac{V_{\max}\cdot S}{K_m + S}, \qquad (1)$$

where E is the concentration of enzyme and S of the substrate; $V_{\max}$ or kE represent the reaction rate when the substrate concentration is much higher than K_m, a constant characteristic of the reaction. The values of both $V_{\max}$ and K_m depend on *p*H.

Many enzymes utilize coenzymes, such as ATP (adenosine triphosphate, the "fuel" of living cells) and NADH, reduced nicotinamide adenine dinucleotide, as auxiliary reagents.

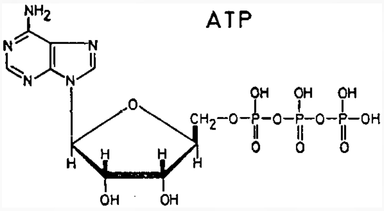
NADH, for example, acts as a hydrogen donor and, in its oxidized state NAD^+ as a hydrogen acceptor. It is involved in many reactions in living cells, where it cycles between the two oxidation states.

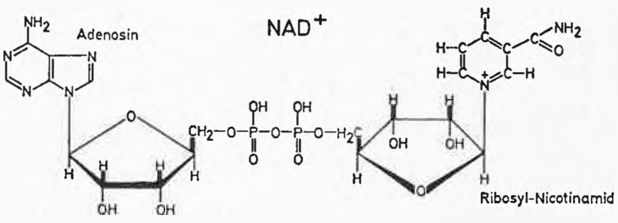
Sources and Isolation

Enzymes can be obtained from animal tissue and plants but the preferred source are microorganisms because they reproduce fast and can be grown under controlled industrial conditions.

A typical bacterial cell contains 1000 to 2000 different proteins which constitute a major part of the dry biomass. Thus in wild strains a particular enzyme usually represents only a fraction of a percent of total protein[4]. These levels can be increased by strain selection, mutation and environmental manipulation, in favorable cases by as much as two orders of magnitude[5, 6]. Recent developments in microbiology indicate that it may become possible to develop superproductive strains[7], but enzyme levels of the order of 10% of total protein are still an exception at the present time.

The isolation of a desired enzyme from the crude extract, obtained by disrupting the cells, requires mild conditions





and usually involves various combinations of the following steps[8]: Precipitation with salts and solvents, batch adsorption and desorption on ion exchangers and column chromatography on ion exchangers and various gels. The properties utilized in the separation are differences in solubility, stability, charge and molecular weight. A relatively new and powerfull technique is affinity chromatography, which exploits the high affinity of an enzyme for its substrate or inhibitor. The latter is attached covalently to the chromatographic support by means of a flexible chain.

As an illustrative example the isolation of penicillin acylase[9] from a high producing strain of *E. coli* is shown in *Table 2*.

Table 2. Purification of Penicillin Acylase[a]

Step	Cumulative yield, %	Purity %
1. Sludge of disrupted cells	100	–
2. Centrifuged supernatant	72	1.0
3. Ammonium sulfate precipitation	65	2.2
4. SE-Sephadex column chromatography	37	28
5. DEAE-Sephadex column chromatography	35	51
6. Ammonium sulfate precipitation	25	100

[a] Source: C. Kutzbach and E. Rauenbusch[9]

Procedures convenient in the laboratory may be impractical or uneconomical on a larger scale. In particular, column chromatography on soft gels can be scaled up only to a certain point. Considerable strides in rationalizing the separations have been made[4, 10–15] and new promising chromatographic materials have recently been described[16]. The objective is always to eliminate the bulk of the undesirable proteins in a few fast and simple steps and thus to reduce the scale of subsequent operations by one or two orders of magnitude. The enrichment which can be obtained in three of four operations[4] is shown in *Table 3*.

Immobilization of Enzymes

The core of the new technology is the immobilization of enzymes on solid carriers. The procedures employed have been described in several reviews[17–22] and one monograph[23]. The principal methods are shwon schemaitcally in Fig. 1 where the drawings in the middle illustrate the larger surface area and correspondingly higher binding capacity of macroporous carriers.

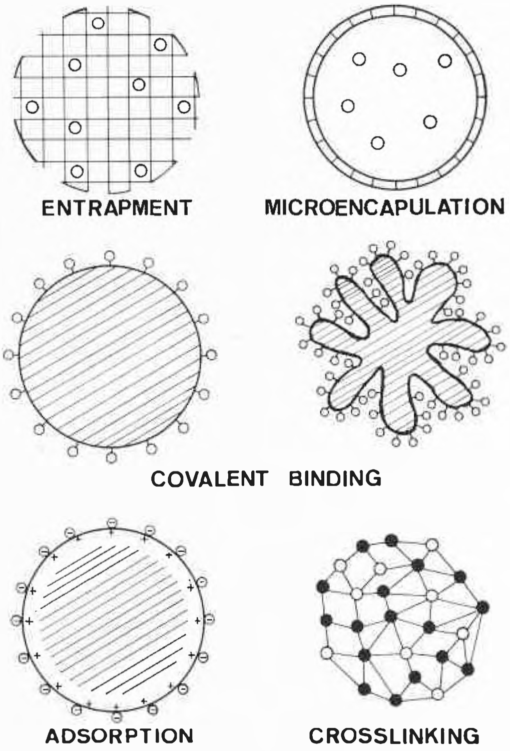


Fig. 1. Principal immobilization methods

Entrapment is a simple procedure involving the polymerization of a solution of the enzyme, momomer and crosslinking agent by a chemical initiator or radiation. A frequently employed monomer is acrylamid. The dimensional stability of the soft gels can be improved by increasing the concentration of the crosslinking agent[24] and by employing other monomers[25–27]. The advantage of the method is its wide applicability and its low requirements on enzyme purity. Preparations containing a large fraction of foreign proteins can be used. However, diffusion limitations, which will be discussed in more detail later on, are more severe when the enzyme is surrounded

Table 3. Purification Factors on Chromatography of Extracts from 5-kg Cells[a]

Enzyme[b]	DEAE-cellulose *p*H 7.0	Hydroxylapatite *p*H 6.8	Ammonium sulfate precipitation	DEAE-Sephadex *p*H 8.0	Total factor
E. coli:					
Met tRS	3	11	1.5	8	400
TIM	3	12	–	4	148
B. stearothermophilus:					
Leu, Met tRS	4	5	–	10	200
tryp tRS	3	14	–	13	546
Rhodanese	3	4	2	11	198
LAP II	4	5	–	5	100

[a] Source: A. Atkinson[4], [b] Abbreviations: tRS = tRNA synthetase, TIM = triose phosphate isomerase, LAP = leucine aminopeptidase

by a polymer matrix, rather than held on the surface of a carrier. Catalytic efficiency, as compared with that of the free enzyme, decreases with increasing particle size[27] and increasing concentration of the entrapped enzyme[28] as shown in Figs. 2 and 3. At a given particle size, the retardation of the reaction by diffusion depends on the porosity of the matrix and the diffusion rates of the species involved in the reaction. These in turn are a function of the concentrations of the species and their molecular dimensions and charge. The reported activities range from a few to several thousand international enzyme units IU/g carrier[a]. An ingenious method for entrapping enzymes as a finely dispersed solution in fibres of cellulose triacetate has been developed[28–33].

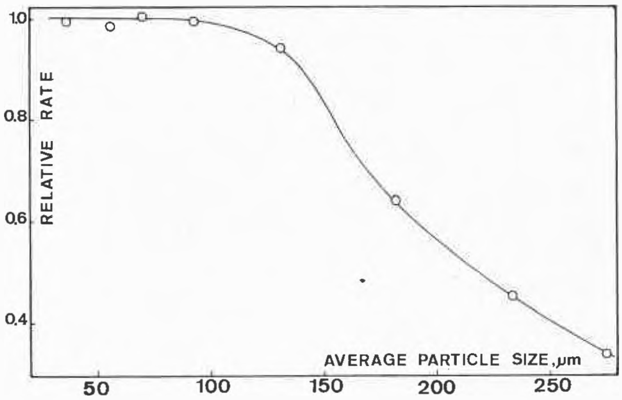


Fig. 2. The activity of β-galactosidase entrapped in crosslinked 2-hydroxyethyl methacrylate as a function of the particle size of the catalyst. Activity of 100 μ particles 4 IU/g gel. Source: I. Hinberg et al.[27]

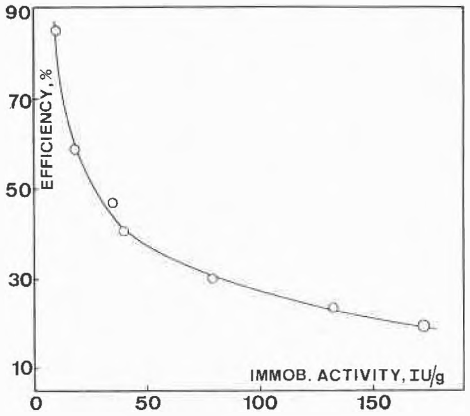


Fig. 3. The activity of penicillin acylase entrapped in cellulose triacetate fibres as a function of the enzyme concentration. Source: W. Marconi et al.[28]

Adsorption has the outstanding advantage that the support is regenerable, at least with ion exchangers as carriers. Furthermore, since no chemical reactions are involved—which may destroy the active site or freeze the molecule in an unfavorable orientation on the support—yields tend to be higher than with covalent binding[34]. The method is, however, restricted to enzymes with adequate affinity for the support and to reaction mixtures having a relatively low salt content. (This problem may perhaps be overcome by the covalent binding of the enzyme to a soluble polyionic molecule having a high affinity for the ion exchanger). The effect of ionic strength on the adsorption of a *B. subtilis* esterase on three different ion exchangers[35] is shown in Fig. 4. At low ionic strengths 4000 IU of the esterase were immobilized on 1 g dry DEAE cellulose with 75% retention of activity. Promising new materials for column chromatography[16] and enzyme immobilization[36, 37] are controlled-pore ceramics, like TiO_2.

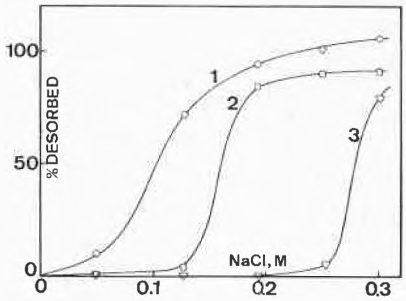


Fig. 4. Adsorption equilibrium of a *B. subtilis* esterase as a function of ionic strength at 25° and pH 8. Carriers: (1) aminoethyl cellulose, (2) diethylaminoethyl cellulose, (3) Dowex 1 X-1. Source: J. Konecny[35]

Covalent binding is the most widely explored technique. The most common approach is to introduce reactive groups onto hydrophylic carriers like cellulose, Sephadex, and their derivatives and on glass. The ZrO_2-coated[a] controlled-pore glass manufactured by the Corning Glass Co., for example, contains free amino groups which can be transformed to $-N{=}CH{-}R{-}CHO$, $-NH{-}CO{-}C_6H_4{-}N_2^+$, $-NH{-}CO{-}CH_2{-}CH_2{-}COCl$ and $-NCS$ for protein binding. Active carriers have been prepared by copolymerization and, more recently, by polymerizing diaminobenzene on porous inorganic solids[38]. As yet another approach is to make a derivative of the enzyme with a compound containing a vinyl group and then to copolymerize this derivative with suitable monomers. The yields of activity vary widely[34, 39] from 1% or less to values higher than 50%, depending on the method, carrier and enzyme. The reported activities range from a few to several thousand units per gram carrier[34, 39].

Enzymes can be crosslinked with other polymers or proteins either in solution, or after prior adsorption or entrapment, by means of bifunctional reagents, like glutaraldehyde.

Some of the carriers which have been used are shown in *Table 4*.

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[a] One IU is that quantity of enzyme which transforms 1 μmole/min of substrate under specified experimental conditions.

[a] The ZrO_2-coated surface is more resistant to hydrolysis at neutral and alkaline pH than silica.

Table 4. Examples of Supports Employed in the Immobilization of Enzymes

Method	Carrier
Entrapment and microencapsulation	Polyacrylamide, poly-2-hydroxyethyl methacrylate, cellulose triacetate, nylon
Adsorption	DEAE and CM cellulose, DEAE Sephadex, clays, glass, TiO_2
Covalent binding	Cellulose, AE-, DEAE- and CM-cellulose, Sephadex, Sepharose, glass, ZrO_2-coated glass, TiO_2, NiO, Fe_3O_4, celite, nylon; ethylene-maleic anhydride, methacrylate-vaniline and other copolymers
Crosslinking	Silica, phenol-formaldehyde resin, nylon

Properties of Immobilized Enzymes

The kinetic properties of immobilized enzymes differ from those of free enzymes, mainly because the *p*H and substrate concentration at the site of action differ more or less from the values in the bulk of the solution[17, 20c]. These differences are due to the short or long range forces exerted by the support and to diffusion, across the stagnant interfacial film and through the bulk of the carrier matrix or through the stagnant liquid regions in macroporous supports.

Since the thickness of the interfacial diffusion film decreases as the flow of liquid relative to the catalyst surface increases, reaction rates change with stirring rates in suspensions[40] or with flow rates[41] in reactor columns. These effects disappear at high turbulence and may be conveniently studied and eliminated in a differential recirculation reactor[42].

Unless the mass transfer rate exceeds the transforming capacity of enzyme, the concentration of substrate at the site of action is lower than its concentration in the bulk of the solution. On the other hand, forces exerted by the support, such as the electrical field of polyionic carriers, may increase or decrease the concentration of electrically charged substrates in relation to their values in the solution. These electrostatic forces also lead to differences between the external and internal concentration of hydrogen ions, which manifest themselves in the displacement of the *p*H-activity profiles of enzymes to lower or higher *p*H[43] and the dependence of the reaction rates on ionic strength[44].

While equation (1) usually[a] still describes the relation between the substrate concentration and reaction rate at constant *p*H, V_{max} and K_m are now empirical parameters the values of which depend not only on the nature of the enzyme and substrate and external *p*H, but many other factors as well: the nature of the carrier and its particle size, enzyme concentration, and also the concentration, charge and molecular dimensions of all species involved directly or indirectly in the reaction. For example, while the rate of hydrolysis of (2-methoxy ethyl) acetate by the free *B.subtilis* esterase is unambiguously specified by the *p*H, the reaction rate of the immobilized enzyme with the substrate depends also on the concentration, *p*K and nature of the buffer species[35], as shown in Fig. 5a + b. Since the reaction produces acid the internal *p*H, and thus the reactivity of the esterase, is also a function of the buffer and its diffusion rate.

Technological Requirements

The technological utility of immobilized enzymes depends on a number of factors, including the type and size of the reactor[20d] foreseen for the process. Many of the supports which have been used in laboratory work are soft, compressible matierials unsuitable for industrial use. The carriers to be employed in reactor columns should meet the following requirements: 1) They should be physically and chemically stable and preferably resistant to microbial attack. 2) The particles should be dimensionally stable under pressure. 3) The pore structure should be large enough to accomodate the enzyme, but not so large as to reduce the surface area for protein binding. 4) The particles should be large enough to permit adequate flow rates at moderate pressures but small

Fig. 5a

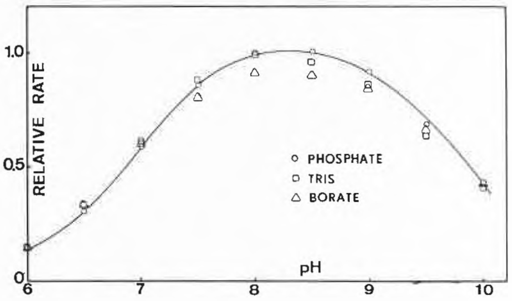


Fig. 5b

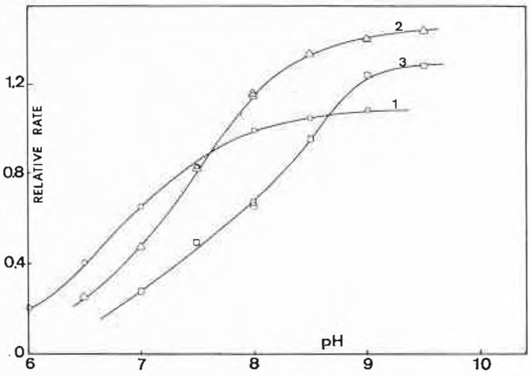


Fig. 5. The *p*H-activity profile of a *B.subtilis* esterase determined in 100 mM NaCl and 10 mM (1) Na phosphate, (2) Tris/HCl and (3) Na borate. a) Free enzyme, b) enzyme bound covalently to aminoethyl cellulose with glutaraldehyde (activity 126 IU/g dry carrier measured in phosphate *p*H 8.0) in a differential recirculation reactor. Source: J. Konecny[35]

[a] A departure from this equation, expected on theoretical grounds, has been reported recently[27].

enough so that the reduction of catalytic efficiency by diffusion is not excessive. 5) The material should be cheap or regenerable.

The controlled-pore ceramics, developed recently by the Corning Glass Company[36], represent an improvement on the ZrO_2-coated porous glass, which does not meet the first and the last requirement. The best particle size lies presumably between 25 and 80 mesh.

The results of pore size studies[21], with controlled pore glass as a support, glucoamylase as the enzyme and dextrin as substrate are shown in *Table 5*.

Table 5. Results of Pore Size Study [a]

Average Pore Diameter Å	Surface Area m^2/g	Activity units/g [b]
2300	8	643
1380	12	925
875	29	2500
550	60–80	3840
248	80–120	64

[a] Particle size 100–120 mesh, source: H.H. Weetall[21], [b] μmole glucose $\cdot min^{-1} \cdot g^{-1}$

The fibre entrapped enzymes, mentioned earlier, are employed in tubular reactors in which the fibres are strung parallel to the axis of the column[28]. The relatively loose packing has the advantage that the columns can be operated at high flow rates in recirculation reactors. An alternative to reactor columns are fluidized beds or stirred tanks. The factors which must be taken into account in this case are the density of the particles, their mechanical desintegration and the retention of the fines. Cellulose and its derivatives are too fragile to be used in such reactors[45]. The use of magnetic supports like Fe_3O_4[46, 47] or of iron coated with polymer[48] has been tried as a means for retaining the particles but no process studies have been reported so far.

Economic Considerations

The reduction of catalyst costs associated with the use of immobilized enzymes depends on the yield of immobilized activity, the cost of the carrier and the operational life of the catalyst. The lower the retention of avtivity in the immobilization, the higher are the demands on the operational life. The level of activity (IU/ml bed volume) determines the volume of the reactor for a given throughput and the expenditures for the support. It also determines the residence time of the substrate required for the specified conversion and therefore the yields, if the substrate or product undergo non-enzymatic hydrolysis or other side reactions.

A comparison of the costs for the enzymatic resolution of (acetylated) D,L-methionine into the stereoisomers[49] with a soluble aminoacylase and with the immobilized enzyme is shown in Fig. 6. The continuous plant utilizing the immobilized enzyme has a capacity for the production of 260 tons L-methionine/year. The enzyme is fixed by adsorption on an expensive but regenerable carrier, DEAE-Sephadex, with 47 % retention of activity. The specific activity is low (3 IU/ml bed volume) and the reactor volume correspondingly large (1000 l). The column looses about 30 % of its activity after one month of continuous operation and is reactivated by the simple expedient of adding fresh enzyme. As is apparent from Fig. 6, very substantial savings were achieved in labor and catalyst costs. The consumption of enzyme was reduced by at least one order of magnitude but these savings were partly offset by the cost of the carrier.

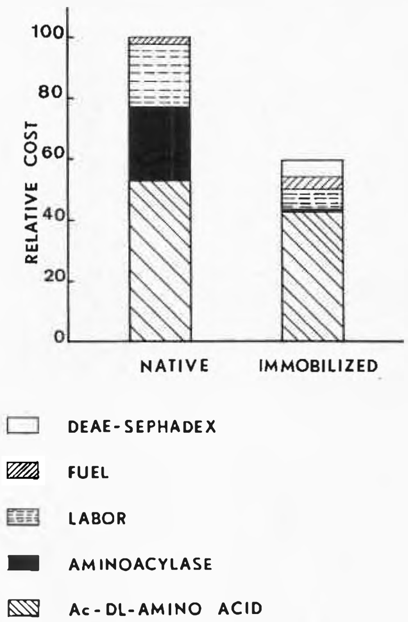


Fig. 6. Comparison of costs for the production of L-amino acids with the free and immobilized aminoacylase. Source: I. Chibata et al.[49]

The estimated operational half lives of a number of immobilized enzymes, taken from a recent review[20c], are summarized in *Table 6*. Other examples of fairly long operational lives have been reported[21, 28–31, 50–53].

It should perhaps be noted that the half-lives do not necessarily reflect the stability of the enzymes. Desorption, hydrolysis of the chemical bonds holding the protein, leakage through mechanically damaged membranes or the clogging of their pores may also lead to a loss of activity. Conversely, bleeding of enzyme from the carrier or its denaturation may take place with little loss of activity, if the efficiency of the catalyst is diffusion limited.

Industrial Processes Utilizing Enzymes

The discussion does not include the enzymatic upgrading of foods[54] or the uses of whole microbial cells for effecting chemical transformations and for biosyntheses. The interested reader is referred to the extensive literature

Table 6. Estimated Half-Lives of Immobilized Enzymes[a]

Enzyme/Carrier	Temperature °C	Estimated half-life days
Glucoamylase/ZrO_2-porous glass	45	645
Glucoamylase/porous glass	60	4.2
Invertase/porous glass	23	42.5
Invertase/cellulose	23	3.7
Acylase/DEAE Sephadex	50	65
Carbamyl phosphokinase/alkaline glass	40	55
Glucoamylase/DEAE cellulose	45	61
Glucose isomerase/porous glass	60	14.4
Glucoamylase/polyacrylamide	50	29
β-Galactosidase/DEAE cellulose	45	10.4
Amyloglucosidase/DEAE cellulose	50	30
Urease/collagen	25	300
Invertase/collagen	30	200

[a] Source: W.R.Vieth and V.Venkatasubramarian[20c]. Activity is assumed to decay exponentially with time, the longest operational time being 46 days

on the uses of microbial transformations in the laboratory[55, 56] and in industry[57, 58].

The largest current application for free enzymes is the hydrolysis of starch to glucose: About 600000 tons of the sugar were produced in the U.S.A. in 1971[2]. This batch reaction is carried out in large (160000 l) stirred tanks in two steps:

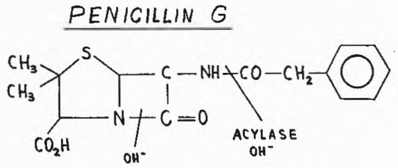
$$\text{Starch} \xrightarrow[\text{or } \alpha\text{-amylase}]{\text{hydrolysis by acid}} \text{Dextrin} \xrightarrow[\text{glucosidase}]{\text{amylo-}} \text{Glucose}$$

The α-amylase route, which solubilizes starch under milder conditions than acid hydrolysis and which gives a better endproduct, now accounts for at least 40% of the glucose produced.

A highly active catalyst (3800 μmole glucose/min/g carrier) has been prepared by immobilizing glucoamylase on ZrO_2-coated porous glass, and it is estimated that a 70 l reactor column of this material would suffice for the production of 5000 tons of glucose per year[21]. However, glucosamylase is cheap, the carrier expensive and the half life of the catalyst is only 19 days at the operational temperature of 60°C. The realization of the continuous process hinges, among other factors, on the availability of cheap supports.

Of the three processes utilizing immobilized enzymes, the resolution of D,L-amino acids into their stereoisomers has already been mentioned. In this process the racemate is first acetylated and then desacetylated by a stereospecific aminoacylase, which attacks only the L-isomer. The flow scheme and references to the literature describing the development of the process are given in the paper cited earlier[49].

Another process of major industrial importance is the conversion of penicillin G to 6-aminopenicillanic acid, an intermediate for the production of semisynthetic penicillins. While nonspecific reagents, like hydrogen and hydroxide ions cleave the β-lactam ring faster than the side chain, penicillin acylase leaves the ring intact. Since the development of the original process, involving the use of whole *E.coli* cells, the immobilization of penicillin acylase and its use has been described in several publications[28, 51, 53] and a number of patents. The immobilized enzyme is said to be in industrial use in Europe and in U.S.A., but no details of the process have so far been released.



Other enzymatic processes of major importance in the pharmaceutical industry are the hydroxylations and dehydrogenations of steroids. They are carried out with the live cells as catalysts.

A major advance in enzyme technology is the realization of a process for the isomerization of glucose to the sweeter fructose with immobilized glucose isomerase[59]. In 1971 about 10% of all sucrose used in Europe and U.S.A. has been converted to liquid "invert sugar" composed of about 40% fructose, 40% glucose and 20% sucrose by means of the enzyme invertase[2]. A similar mixture can be obtained from glucose by means of glucose isomerase, a relatively expensive intracellular enzyme, the advantage of this route being that glucose is a much cheaper substrate than sucrose. The core of the process is a stacked reactor consisting of shallow beds containing DEAE cellulose on which the enzyme is immobilized by adsorption. This design presumably reduces the pressure drop in the reactor and minimizes channelling. It is reported[59] that Corning Glass Co. has a ceramic carrier for the enzyme and that plans for building a commercial plant utilizing this catalyst are in progress.

An enzyme which has a major industrial potential and which is currently attracting much attention is cellulase[60]. A process leading from waste paper and other cellulose waste to cheap glucose would provide a valuable raw material for the production of unicellular protein and fat, alcohol and other materials[61, 62].

An interesting new development is the enzymatic conversion of starch syrups (dextrins and oligosacharides) to pullulan[63], a viscous polysacharide which can be used as a film for coating and packaging food-stuffs, and can be made into an adhesive, laminates or nylon-like fibres and fabrics. Construction of a plant in Japan to produce 300 tons/year of pullulan is scheduled for 1975, with a larger plant to follow.

Future Outlook

As is apparent from these recent developments, the effort in enzyme engineering is beginning to bear fruit. In particular, considerable progress toward developing satisfactory carriers and immobilization methods has been made in the short time which has elapsed since the technological and economic requirements on the supports became fully appreciated. Large forward strides may be expected in the near future in this area.

Work on the utilization of enzymes requiring coenzymes and of enzyme systems is, technologically, in its infancy. One possible solution of the coenzyme problem is the attachment of the coenzyme to a soluble macromolecule[64] and microencapsulation of this derivative together with the enzyme or enzymes required for the reaction. Whether such systems will be able to compete with whole cells as catalysts, is still an open question.

Extending the range of enzyme applications is a matter of finding reactions in which their use is advantageous and economically feasible and of developing new uses for the products of enzymatic reactions. Since the technological potential of natural products is still largely untapped and since the incentives for using regenerable resources are increasing, progress may be expected also in this area. However, this progress will be gradual and the broader impact of enzyme technology therefore still lies in the future.

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