CURRENT TRENDS IN ENZYME TECHNOLOGY

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INTRODUCTION

In 65 years since Michaelis and Menten (1) postulated their classic mechanism of enzymatic reactions, much progress has taken place in the rapidly-expanding area of Enzymology. At present the latter is no longer the exclusive domain of chemists or biochemists but has attracted the intensive efforts of chemical engineers, medical doctors, agriculturists and other professionals. The wide array of enzyme applications in industry, chemical analysis, medical therapy and agriculture has given rise to the new discipline of Enzyme Technology (2) as well as the more specialized branch of Enzyme Engineering (3).

Enzyme Technology is the application of Enzymology in solving practical problems such as those relating to industrial processing and chemical analysis. It requires a thorough knowledge of Enzyme Chemistry as well as some familiarity with basic engineering and microbiological principles. Although it is often considered a branch of Biological Technology, which also includes Fermentation Technology, it is actually an interdisciplinary study which requires the collaborative efforts of chemists, biochemists, engineers, microbiologists and other scientists.

IMMOBILIZED ENZYMES

The accelerated growth of Enzyme Technology is due notably to the development of techniques in enzyme immobilization. The latter allows large-scale enzyme applications since immobilized enzymes can be easily prepared, recovered and reused. Furthermore, enzyme immobilization permits the design and operation of industrial enzyme reactors (4).

Enzyme immobilization techniques which are diagrammatically shown in Figure 1, may be classified as (a) covalent bonding, (b) adsorption and ionic bridge formation, (c) physical entrapment and microencapsulation, and (d) aggregation and other techniques (5, 6). The choice of immobilization technique depends on several factors such as the nature of substrates, products and desirable support material, enzyme properties and cost considerations. A wide array of support materials has been reported in the literature. These include inorganic materials such as porous glass and magnetite as well as organic polymers such as agar, dextran, cellulose, polyacrylamide and collagen (5).

Typical immobilization reactions are presented in Figure 2. Three commonly used cross-linking reagents are given namely glutaraldehyde, cyanogen bromide and γ-amino-propyltriethoxysilane. The first-named reagent is a bifunctional dialdehyde which can be used for covalent coupling of an enzyme

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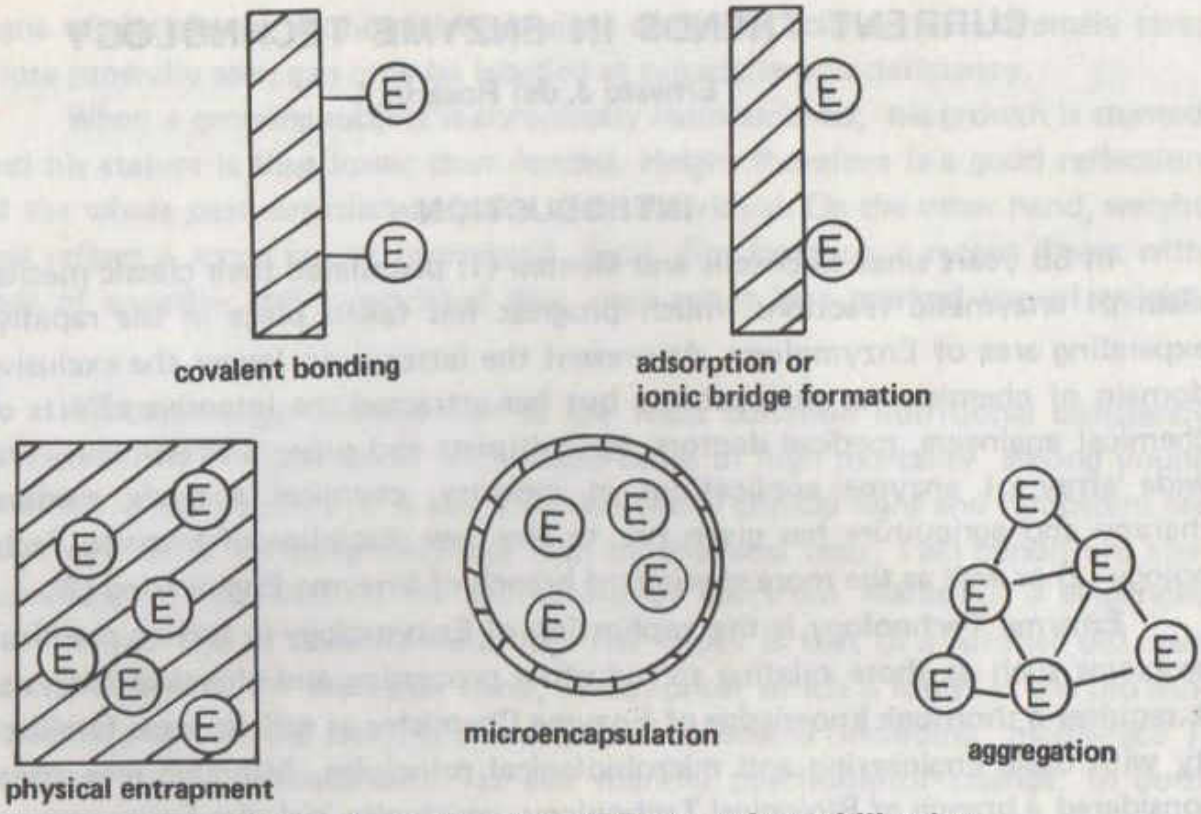

Figure 1. Techniques of Enzyme Immobilization

to an amino-containing carrier and for enzyme aggregation through cross-linking of the lysine amino groups. Cyanogen bromide allows enzyme immobilization on a polyhydroxy carrier such as dextran, cellulose or glass while the silane reagent permits the covalent coupling of enzyme and glass. Other reagents for immobilizing an enzyme to a variety of organic and inorganic carriers are described in the literature (5, 6).

ENZYMES IN INDUSTRIAL AND FOOD PROCESSING

An abbreviated list of enzyme applications is given in Table 1 and includes both soluble and immobilized enzymes. One of the most promising industrial enzyme processes, which is actively studied at present, is the production from starch of invert sugar. The latter is a large-volume feedstock used in the soft drink and food industries and is presently manufactured by inverting cane- or beet-derived sucrose. Sweet syrup containing an equilibrium mixture (roughly equal proportion) of glucose and fructose can be produced from raw starch using the sequential action of glucoamylase and glucose isomerase (7, 8).

The first industrial plant based on an immobilized enzyme process was started in 1969 in Japan (9). The process used *Aspergillus oryzae* aminoacylase, which was ionically bound on DEAE-Sephadex, to selectively hydrolyze the L-enantiomer in a synthetic racemic mixture of amino acid acylesters (see Table 1). The resulting L-amino acid was separated, using solubility differences, from the unhyrolyzed D-acylester and the latter was racemized for further processing.

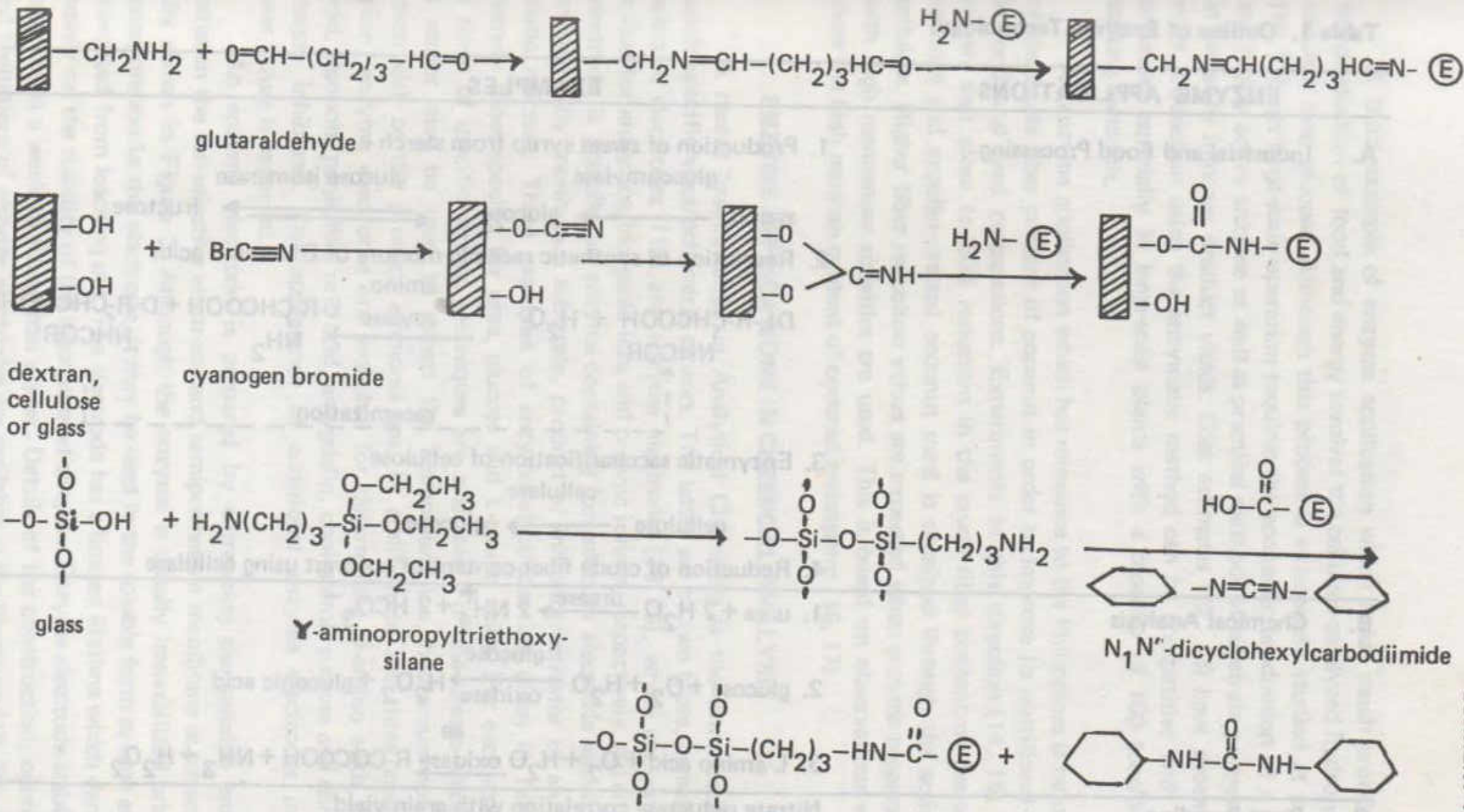

Figure 2. Some Enzyme Immobilization Reactions

Table 1. Outline of Enzyme Technology.

ENZYME APPLICATIONS		EXAMPLES
A.	Industrial and Food Processing	1. Production of sweet syrup from starch starch $\xrightarrow{\text{glucoamylase}}$ glucose $\underset{}{\overset{\text{glucose isomerase}}{\rightleftharpoons}}$ fructose 2. Resolution of synthetic racemic mixture of DL-amino acids DL-R-CHCOOH (NHCOR) + H_2O $\xrightarrow{\text{amino-acylase}}$ L-R-CHCOOH (NH_2) + D-R-CHCOOH (NHCOR′) racemization 3. Enzymatic saccharification of cellulose cellulose $\xrightarrow{\text{cellulase}}$ glucose 4. Reduction of crude fiber content of coconut using cellulase
B.	Chemical Analysis	1. urea + 2 H_2O $\xrightarrow{\text{urease}}$ 2 NH_4^+ + 2 HCO_3^- 2. glucose + O_2 + H_2O $\xrightarrow[\text{oxidase}]{\text{glucose}}$ H_2O_2 + gluconic acid 3. L-amino acid + O_2 + H_2O $\xrightarrow[\text{oxidase}]{\text{aa}}$ R-COCOOH + NH_3 + H_2O_2
C.	Plant Breeding	Nitrate reductase correlation with grain yield
D.	Medical Therapy and Clinical Analysis	Tumor inhibition by asparaginase Lactate dehydrogenase isozymes in therapy

A third example of enzyme application which holds much promise for the production of food and energy involves the cellulase-catalyzed hydrolysis of cellulose into glucose. Although this process is extensively studied at present (10, 11) its large-scale operation requires the economic production of a cheap and highly active enzyme as well as practical methods of substrate pretreatment in order to improve product yields. Cost estimates (12, 13) have shown that sugar production using the enzymatic method can be competitive with other processes especially in large-scale plants with a capacity of 100 tons/day of starting materials.

An enzyme application which has relevance to the Philippines is reduction of the crude fiber content of coconut in order to improve its nutritional value in food and feed preparations. Experiments in this direction (14, 15) have shown that close to 50% reduction in the crude fiber content of desiccated coconut and expeller-pressed coconut meal is obtained through the action of cellulase. Higher fiber reduction values are expected when enzyme preparations with high mannanase activities are used. This is based on observations which show the high mannan content of coconut endosperm (16, 17)

ENZYME APPLICATIONS IN CHEMICAL ANALYSIS

A recent breakthrough in Analytical Chemistry is the introduction of solute-specific electrochemical sensors. The latter are of two types, namely ion-selective electrodes (18), and enzyme electrodes (19, 20), which are generally suited for analyzing inorganic ions and organic solutes, respectively. An enzyme electrode is actually an enzyme-containing ion-selective electrode which electrometrically monitors a substrate, product or inhibitor/activator of an enzymatic reaction. Three examples of enzyme electrodes are given in Table 1, namely those specific for urea, glucose and L-amino acids. For each example a range of specific sensing techniques are available. For the urease electrode, a sensor may be used to detect the ammonium ion, hydronium ion (pH electrode) or the product ammonia gas. In addition to the three examples given, enzyme electrodes are available for determining D-amino acids, lactic acid, alcohols, penicillin, uric acid, amygdalin, cholesterol, sucrose and cholinesterase inhibitors. The appearance of additional enzyme electrodes in the near future is expected.

An enzyme electrode is prepared by entrapping the suitable enzyme between the ion-selective electrode and semipermeable membrane as schematically shown in Figure 3. Although the enzyme is usually immobilized prior to incorporation in the electrode, it may be used in the soluble form as long as it is prevented from leaching out. The electrode has a limited lifetime which depends mainly on the stability of the incorporated enzyme. Enzyme electrode stabilities range from a week to more than a year. Details of the construction, operation and limitations of enzyme electrodes are available in the literature (19, 20).

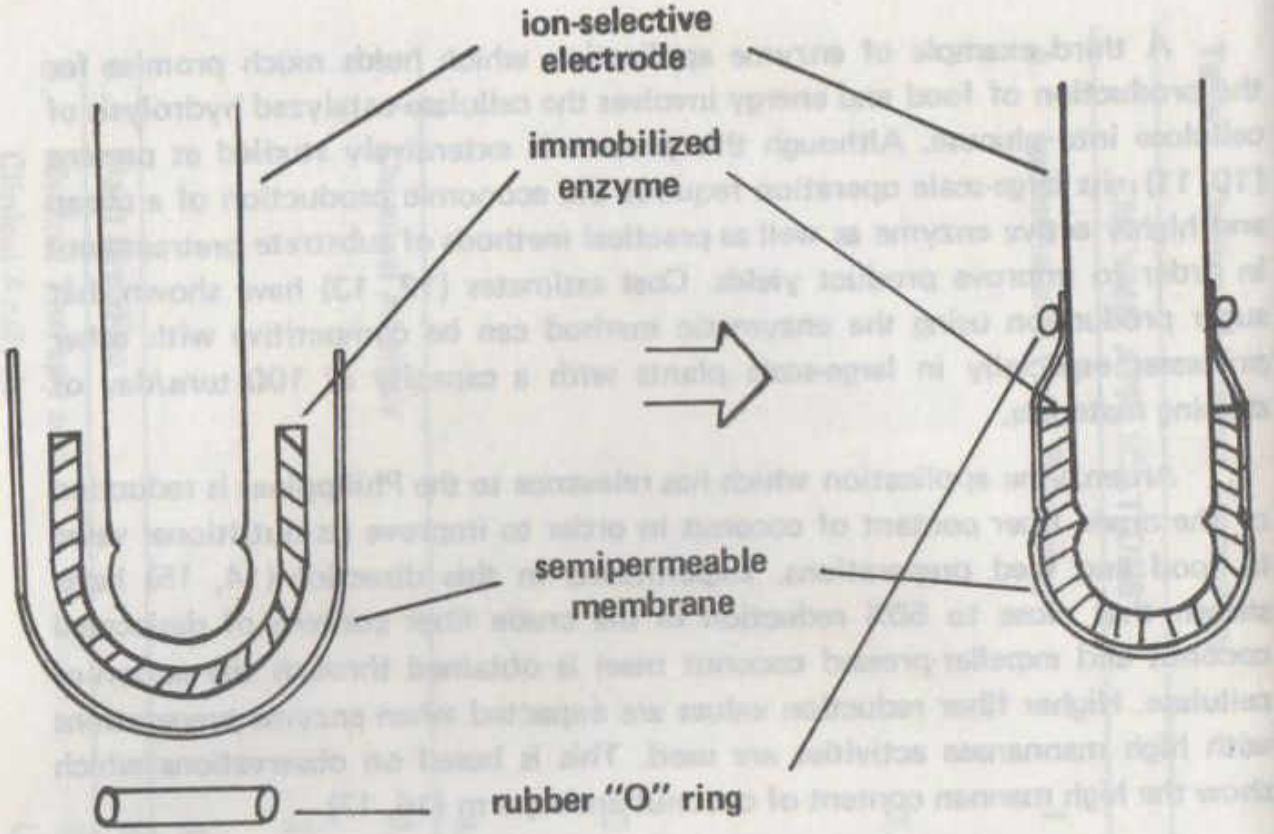

Figure 3. Construction of an Enzyme Electrode (Schematic)

ENZYMOLOGY AND PLANT BREEDING

An interesting area for the enzyme technologist is Phytogenetics and Plant Breeding. Although Enzymology and Genetics are intimately related through the "one-gene one-enzyme hypothesis" (see, e.g., ref. 21), the correlation of the activities of certain enzymes with crop yield and plant characteristics has only recently been observed. One of these enzymes is nitrate reductase which is involved in nitrate assimilation by the plant. Studies by Johnson et al. (22) indicated the possibility of using nitrate reductase activity as a predictor of grain yield. Furthermore, various studies especially by Hageman and co-workers on cereals such as wheat (23, 24) and corn (25) showed a highly significant correlation between the amount of nitrogen supplied to the plant, as estimated by the induced nitrate reductase activity, and the actual amount of nitrogen accumulated by the plant. However, large-scale use of the technique is speculative and needs further research in order to have wide applicability. Further work is justified by the tremendous potential of the technique, especially for long maturing plants such as the coconut.

ENZYMES IN MEDICAL THERAPY AND CLINICAL ANALYSIS

The number of enzymological applications in Medicine has recently increased at a rapid pace. In addition to the popular enzyme-based metabolite assays, such as that for glucose using glucose oxidase and peroxidase (26),

electrophoretic isozyme patterns and immobilized enzymes have been introduced in clinical analysis and medical therapy. The diagnosis and treatment of diseases using enzymes is only logical since enzymes are the catalysts which regulate metabolic processes.

One of the key enzymes employed in medical diagnosis is lactate dehydrogenase (LDH) which consists of multiple forms called isozymes (or isoenzymes). Each isozyme shows a characteristic electrical charge and mobility during electrophoresis and the five LDH isozymes in human serum are revealed as distinct bands in the electrophoretograms. Homogenates of human tissues display characteristic electrophoretic patterns showing differences in the distribution of the five isozymes. By comparing the electrophoretograms of healthy and diseased tissues, the latter can be identified since it will show variations in LDH isozyme patterns relative to the healthy tissue. Generally, damage to a tissue causes the release of its LDH isozymes into the serum so that changes in the serum isozyme pattern is traceable to the injured tissue. Furthermore, specific disease states such as myocardial infarction, chronic granulocytic leukemia and infectious hepatitis show distinct LDH isozyme patterns. The latter serve as direct and valuable diagnostic tools for these diseases (27).

Immobilized enzymes may be used in medical therapy either in extracorporeal perfusion systems, such as one employing a microencapsulated urease system to remove blood urea (28), or as liposome-entrapped enzymes for intracorporeal application (29). The latter offers many exciting prospects such as in tumor inhibition by asparaginase, which is immobilized in liposomes (lipid vesicles) to prevent antigenic reactions in the body and allow assimilation of the active enzyme by the target tissues. Although problems still prevent clinical applications of these 'missile-type' enzymes, intensive researches in this area are expected to provide the breakthroughs which shall benefit mankind.

In summary, the present review of selected enzyme applications is only a capsule coverage of the vast and expanding area of Enzyme Technology. Detailed discussion of the specific applications would require familiarity with multidisciplinary areas. It is clear that the synergistic cooperation of different professionals is required to allow Applied Enzymology to help provide solutions to some of man's important problems.

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